Nutrition and Health
Series Editor: Adrianne Bendich

Michael F. Holick
Jeri W. Nieves *Editors*

Nutrition and Bone Health

Second Edition

Humana Press

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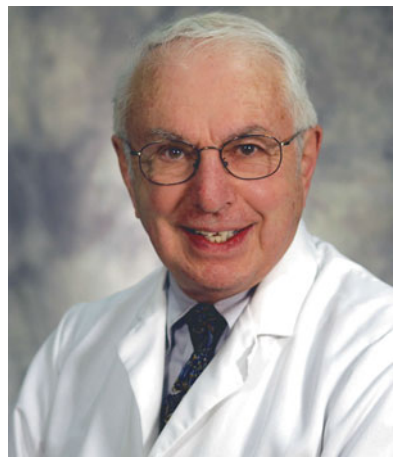

The second edition of Nutrition and Bone Health *is fondly dedicated to the memory of Dr. Larry Raisz (1925–2010). Larry was one of the most influential and productive physician-scientists the field of bone metabolism has ever seen. He was an outstanding bench scientist, clinician, educator, mentor and advocate for basic and clinical research. Although less well-known than his scientific papers, Larry also wrote poems and, as with everything else he undertook, he was a good poet. Here are his musings on biomedical researchers and the currently sorry state of research funding:*

The Charge of the White Coat Brigade

They come by their hundreds in order to ease
The suffering of illness, the pain of disease
They teach us all about healthy behavior
For in the long run, prevention is saviour.
But to maintain the progress of this fine brigade
New knowledge must be sought, new discoveries made
Government and foundations, which once were there
Are finding their cupboards ever more bare.
But if we all work and plan together
This is a storm we all can weather.
And make better health our next port of call.
But this is a gala so let's have a ball.

Larry Raisz
April 2010

Larry led that charge valiantly all his life and his passing has left a void. Older readers of this volume will fondly remember the first person at the microphone after almost every scientific presentation in our field. "Raisz, Connecticut!" would echo around the auditorium by way of introduction and there would then follow a series of insightful and constructive questions, never aggressive or combative, but always seeking and providing enlightenment. Sadly, Larry's voice and pen are stilled now, but he leaves a legacy that will serve as an inspiration for generations of researchers to continue the charge.

David W. Dempster
New York, July 2014

Foreword

A rational approach to understanding the skeleton, its physiology, and pathology requires an integrated approach. Often in textbooks on metabolic bone disease, nutrition is given short shrift. In 41 chapters, *Nutrition and Bone Health* is a comprehensive review of all aspects of nutrition and the skeleton, and the interrelationships between nutrition and skeletal homeostasis. From a teleological perspective, prevention of phosphate deficiency in our saltwater ancestors represented an early environmental adaptation. The move to terrestrial hunter-gatherers changed the requirements, imposing the need to find large quantities of food of low caloric density to satisfy energy requirements. Today's challenges are different. Now a surfeit of high-calorie foods and a corollary obesity has become epidemic. In this environment are we achieving a diet that is adequate enough to allow us to build and maintain a healthy skeleton? To answer this question we need to understand the nutritional requirements of the skeleton, and how these requirements interact with, for example, genetic control of bone growth and remodeling. It is most propitious that this volume, which addresses those very issues, should be published at a time when there is much discussion about the various fad diets that potentially could modify skeletal behavior.

It has become commonplace to think of the skeleton only in terms of calcium nutrition. Indeed, calcium is stressed to be the "building block" of the skeleton and the backbone (so to speak) of all pharmacological interventions. But it is much more complex than that simple view. The skeleton is required to be strong, but flexible. It must support the everyday stresses placed on it, but must also resist sudden traumatic forces. The skeleton is also the depository for potentially harmful metals, and of course it is the major source for ions required in carefully controlled concentrations in serum. That it can achieve these three functions is one of nature's miracles, requiring a carefully controlled remodeling process to maintain its health and vigor, to provide one means for ion storage and release, and to repair stress-related damage. Because we are told that we are what we eat, it is not surprising that the skeleton requires a variety of nutrients for its own health. This remarkable volume tries to place into context the role of nutrition, both good and bad, in the overall health of the skeleton, and consequently of the organism. In putting together a unique group of internationally respected authors, the editors, themselves international experts in vitamin D and calcium homeostasis, have synthesized for the reader the wide variety of impacts that nutrition can have on the skeleton.

The achievement of adequate skeletal mass and strength requires complex interactions among genetics, health, nutrition, and physical stress during growth, and toward the end of growth, a normal transition through puberty. We often forget the many interactions required for this process. From early fetal development, nutrients supplied by the mother provide the basis for bone growth. Maternal nutrition is key here. Recent data suggest that a maternal diet inadequate in protein may result in a deficient stem cell population in the developing fetal skeleton, implying that we may have much more to learn about

the role of maternal nutrition and its interactions with genetics in determining the mass of the skeleton at birth. Why might that be important? It is suggested, but by no means proven, that even early in life, a skeleton that has failed to develop adequately may set the stage for osteoporosis in later life.

The growth of the skeleton in the child is no less important. The interplay between genetics and environment creates an adult skeleton sufficient to withstand the stresses placed on it in everyday life. Big people grow big bones (simplistically put) and, by the tests that we use to measure bones, have "denser" bones as an artifact of the test rather than a biological fact. Nevertheless, in the absence of proper nutrition, clearly the skeleton cannot respond to the variety of endocrine factors stimulating its growth and expansion, nor to the stress of childhood play and sport. Here again, nutrition means much more than assuring optimal calcium intake. It is not commonly recognized that the period of transition through puberty is a period of adaptation by the growing organism to increased needs to sustain the accelerated phase of growth. It is only then that the recognized gender differences in the skeleton become evident. A not infrequent disaster at this point is the appearance of an eating disorder, which can have catastrophic effects on the final maturation of the skeleton. Anorexia, coupled as it often is with failure of the hypothalamic–pituitary–ovarian axis, can lead to significant fracture risk at a young age, as the organism steals from the skeleton the essential nutrients it is failing to get otherwise.

Maintaining an adequate skeleton during adult life is equally complex. Here the effects of poor nutrition more often result in obesity, a highly prevalent feature of our adult society (and increasingly of our pediatric population). Although there is some suggestion that bone density, at least at some sites, may be increased in obese individuals, this by no means offsets the other multiple health problems that besiege the obese. Consequently, efforts at controlling weight abound, largely because of the relative ineffectiveness (usual among individuals not fully committed to the concept, but perhaps not always). Several of these will have detrimental effects on the skeleton. For example, gastric surgery and ketogenic diets will induce nutritional effects that result in excess bone loss. It is transition through menopause that alters the relationship between skeletal homeostasis and nutrition, by rendering the organism less efficient at absorbing and retaining calcium. The consequence is increased bone turnover and loss, with osteoporosis and fractures being the outcome.

At an even later stage in life, the efficiency of calcium absorption across the intestine declines and may be accompanied by vitamin D insufficiency. Secondary hyperparathyroidism ensues, with further loss of bone mass. Nutritional requirements thus change with age, with a need for higher vitamin D intakes, since skin synthesis declines at the same time. Finally, in old age, when hip fractures are common, protein nutrition assumes an important role, and it is clear that recovery from hip fracture, perhaps repair of the bone, and reduction in risk of a second hip fracture can be mediated by improved protein nutrition.

Nutrition and Bone Health, crafted by two international experts on calcium and vitamin D, brings together in one place the nutritional aspects of skeletal health and integrates them with other aspects of the control of mineral homeostasis. It begins with teleology, traverses genetics and the control of bone growth and metabolism, and includes discussion of other factors (such as medications) that might alter the nutritional requirements of bone. As the chapters unwind, they interweave the fundamental importance of good nutrition in maintaining the health of the skeleton. The editors have chosen their authors with care and have created a volume that should be read by all interested in bone health and nutrition.

West Haverstraw, NY, USA Robert Lindsay, M.B.C.H.B., P.H.D., F.R.C.P.

Preface

The adage "you are what you eat" is certainly true for skeletal health from birth until death. The skeleton is often perceived as an inert structure that simply acts as the scaffolding for the musculature and to house the brain and other essential organs. Thus, the skeleton is taken for granted. However, just as the intricate scaffolding of a suspension bridge requires constant maintenance, so too does the skeleton require nutritional maintenance. It has a voracious appetite for calcium and other macro- and micronutrients in order for it to maximize its size and to maintain its maximum structural strength.

The consequences of not providing the skeleton with its nutritional requirements can be quite severe. Infants and young children who do not get an adequate amount of calcium and vitamin D in their diet suffer from growth retardation and bony deformities of their skull, rib cage, arms, and legs. For adolescents and young adults, inadequate nutrition results in not being able to attain their genetically prescribed maximum peak bone mineral density. For middle-aged and older adults, inadequate calcium, vitamin D, protein, and macro- and micronutrient nutrition leads to a more rapid loss of bone that can precipitate and exacerbate osteoporosis. Twenty-five million Americans and an equal number of Europeans and an untold hundreds of millions of adults worldwide are at risk for osteoporosis and its unfortunate consequences. In the USA, approximately one in two women and up to one in four men age 50 and older will break a bone due to osteoporosis. Osteoporosis is responsible for two million broken bones and $19 billion in related costs in the USA every year. Approximately 300,000 of these fractures will be of the hip. Twenty-five percent of women and fifteen percent of men will suffer a hip fracture by the age of 80. It is estimated that between $10–20 billion a year is expended for the acute and chronic care of patients suffering hip fracture. However, the most serious consequences of a hip fracture is that 50 % of patients will never have the quality of life they once had and often become infirm, and 20 % die within the first year after the fracture owing to complications. Therefore, prevention of these devastating fractures becomes very important, and nutrition is a cornerstone of prevention.

The first objective of the second edition of *Nutrition and Bone Health* is to provide practicing health professionals, including physicians, dietitians, nutritionists, dentists, pharmacists, health educators, policymakers, research investigators, graduate students, and medical students with comprehensive, well-balanced reviews of the newest clinical findings as well as up-to-date research discoveries regarding the role of nutrition in maintaining a healthy skeleton. It is a given that adequate calcium and vitamin D are important for skeletal health. However, the skeleton craves other nutrients that are equally essential for bone health.

This second edition of *Nutrition and Bone Health* explores how our earliest ancestors evolved in a relatively calcium-rich environment that served them well in providing a structurally sound skeleton in a hostile environment. Chapters describing the role of genetics, bone physiology, hormones, and biomechanics of bone provide background to the reader as they delve deeper into the role of nutrition

in bone health. The tools used to study nutrition and bone health, including nutritional epidemiology, nutritional assessment and counseling, and dietary patterns, are described and set the stage for the chapters that provide up-to-date reviews of nutritional requirements during pregnancy, for fetal, neonatal, childhood, adolescent, young, middle-aged, and older adult's skeletal health presented in extensively referenced individual chapters. The effects of race and ethnicity on nutrition and bone health are described in detailed chapters.

The second edition of *Nutrition and Bone Health* includes several chapters devoted to examining the effects of specific dietary components on bone health: macronutrients (protein and fat), minerals, and micronutrients. Additionally, dietary components such as food groups, and special diets such as vegetarian diets as well as nutraceuticals are discussed in separate chapters. As examples, the importance of proper acid-based balance and the effect of minerals such as calcium, sodium, potassium, phosphorus, and magnesium, as well as micronutrients including fat-soluble vitamins, zinc, and selenium, are reviewed. The chapter on vitamin K provides an expert perspective on the role of vitamin K and its various forms on bone health and cardiovascular disease. Another goal of the second edition of *Nutrition and Bone Health* is to put into perspective the impact of eating disorders, body weight, exercise, and body weight change on bone health. There are a multitude of diseases and drugs and other environmental and behavioral factors that negatively affect bone health. Among these are cystic fibrosis, celiac disease, HIV/AIDS smoking, and alcohol abuse. The relationship between nutrition, inflammation, and bone health are explored. In addition, the chapter on medications and nutrients provides important information as to the importance of proper nutrition for expensive bone active drugs to have favorable effects on the skeleton.

The role of dietary factors, exercise, and sun exposure on bone cell function and bone mineral density are reviewed in detail to assure that the totality of the evidence presented to the reader provides up-to-date information on these topical, controversial subjects.

As editors, we are very excited about the expanded contents of the second edition of *Nutrition and Bone Health*. Chapters are written by experts who provide not only an overview of the subject, but also specific recommendations for how this information can be effectively utilized for practical application by health care professionals. The volume includes numerous tables and figures to help the reader quickly glean the essentials of each chapter. There is even an app that provides guidance for sensible sun exposure in the chapter on Vitamin D. There is an extensive index that also helps provide a road map to easily cross-reference how particular nutrients, diseases and drugs, environmental factors, race, and age affect bone health.

Metabolic bone diseases, such as rickets and osteomalacia, as well as osteoporosis, are diseases of neglect. Vigilance for satisfying the nutrient requirements of the skeleton is a small price to pay for remaining erect and fracture free throughout life. The second edition of *Nutrition and Bone Health* should serve as a critical resource for health care professionals interested in utilizing nutrition, exercise, and other positive lifestyle factors to enhance the overall health and well-being for skeletal health throughout life, minimizing the need for bone active medications which are expensive and associated with many unwanted side effects.

Boston, MA, USA — Michael F. Holick
New York, NY, USA — Jeri W. Nieves

Series Editor Page

The great success of the Nutrition and Health Series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers and clinicians in their respective fields; (3) extensive, up-to-date fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and the consequences; (7) virtually no overlap of information between chapters, but targeted, interchapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patient as well as health professionals' questions which are based upon the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter and in the choice of chapter authors. The international perspective, especially with regard to public health initiatives, is emphasized where appropriate. The editors, whose trainings are both research and practice oriented, have the opportunity to develop a primary objective for their book; define the scope and focus; and then invite the leading authorities from around the world to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

Nutrition and Bone Health, 2nd Edition, edited by Michael F. Holick and Jeri W. Nieves, is a very welcome addition to the Nutrition and Health Series and fully exemplifies the series' goals. The first edition was published a decade ago and it is very timely to have this comprehensive update. The explosion of clinical research over the last decade also warrants the inclusion of five new chapters resulting in this revised 41 chapter volume. The book is designed as a valuable resource for nutritionists and dietitians, internists and endocrinologists who treat patients with potential bone loss, public health scientists, epidemiologists, and health care professionals from various disciplines who interact with clients, patients, and/or family members. The volume is also a unique resource for graduate and medical students who have an interest in how diet affects bone health. This important volume includes objective, relevant information provided in extensive, up-to-date literature reviews, instructive tables and figures, and excellent references on the critical aspects of clinical research on bone health and diseases that are affected by dietary nutrients.

The editors of this volume are experts in their respective fields and represent the medical profession as well as the academic research community. Moreover, Drs. Holick and Nieves have included in-depth chapters by the leading researchers and clinicians in fields including, but not limited to, nutrition, exercise, space travel, infant and childhood growth, molecular biology, endocrinology, genetics,

skeletal health, and osteoporosis. The overall expertise of the editors and chapter authors, who have made significant discoveries as well as serving as outstanding educators, cannot be matched by any other text in the field of nutrition and bone health to date.

Dr. Michael F. Holick, Ph.D., M.D., is Professor of Medicine, Physiology, and Biophysics; Director of the General Clinical Research Unit, Director of the Bone Health Care Clinic; and Director of the Heliotherapy, Light, and Skin Research Center at Boston University Medical Center. Dr. Holick has made numerous contributions to the field of the biochemistry, physiology, metabolism, and photobiology of vitamin D. He was the first to isolate and identify 25-hydroxyvitamin D3 in human blood and also determined that the active form of vitamin D is 1,25-dihydroxyvitamin D3. He participated in the first chemical synthesis of 1,25-dihydroxyvitamin D3 and its analog 1alpha-hydroxyvitamin D3 that was used to treat vitamin D-dependent rickets and hypoparathyroidism. Dr. Holick is a Diplomat of the American Board of Internal Medicine, a Fellow of the American College of Nutrition, and the recipient of the American College of Nutrition Award, the Robert H. Herman Memorial Award in Clinical Nutrition from the American Society for Clinical Nutrition, the Annual General Clinical Research Centers' Program Award for Excellence in Clinical Research, the Linus Pauling Functional Medicine Award from the Institute for Functional Medicine, Linus Pauling Prize, DSM Innovation In Nutrition Award, American Association of Clinical Chemist's Van Slyke Award, American College of Nutrition's Communication Media Award, the Delbert Fisher Research Scholar, Best Doctors in America 2011–2014, and the American Society of Bone and Mineral Research Louis Avioli Award. Dr. Holick is editor-in-chief for the *Journal for Clinical Laboratories and Laboratories Related to Blood Transfusion* and Associate Editor for *Dermato-Endocrinology*. He has authored more than 500 peer-reviewed publications and written more than 250 review articles as well as numerous book chapters. He has served as editor and/or coeditor on 13 books including *Vitamin D*, both the first and second editions, and the first edition of *Nutrition and Bone Health* which are also part of the Nutrition and Health Series. Dr. Holick was selected by Thompson Reuters as one of the world's most influential scientific minds in the field of clinical medicine in 2014.

Dr. Jeri Wanzor Nieves is a Research Scientist at the Helen Hayes Hospital in New York, where she is investigating various aspects of osteoporosis and serves as the Principal Investigator for the New York State Osteoporosis Prevention and Education Program (NYSOPEP). She also serves on several committees for the National Osteoporosis Foundation. Dr. Nieves is an Associate Professor of Clinical Epidemiology and Nutrition at Columbia University. Dr. Nieves has coauthored over 100 journal articles, reviews, and book chapters on nutrition, epidemiology, and osteoporosis. She is an Associate Editor of *Osteoporosis International*. Dr. Nieves' research has focused on various aspects of bone health and osteoporosis including peak bone mass, stress fractures, vitamin D, anabolic and antiresoptive treatments, and fracture healing. In addition, her research includes aspects of nutritional epidemiology and determining the role of nutrition in various disease states.

Part 1: The ten introductory chapters in the first part, entitled "Basics of Nutrition and Bone Biology," provide readers with an introduction to the current understanding of skeletal evolution, genetic factors in bone biology that are affected by diet, a review of the key hormones that control bone growth and health, the current status of bone measurement technologies, and the current mechanisms used to determine dietary intakes. Oral health and nutrition counseling during osteoporosis therapy are each reviewed in separate chapters. The first chapter examines the evolutionary aspects of bone health that are preserved in the fossil record. We learn that developments in genetic analyses have yielded insights into our evolutionary history and breakthroughs have allowed genome sequencing of fossil bones from populations that lived 50,000 years ago. The chapter reviews the evolution of human skeletal adaptation and external factors including diet, which may have affected skeletal health in human ancestors and form the basis for skeletal conditions seen in modern human populations. Chapter 2 describes the gene–nutrient interactions that may affect bone health. The nutritional and genetic factors that interact to influence bone modeling and mineral homeostasis during the years of

peak bone mass acquisition, bone remodeling, and the maintenance of bone mass are described in detail. Candidate gene and genome-wide association studies with bone mineral density and fractures and gene–dietary interaction studies in osteoporosis are also reviewed. The third chapter, coauthored by the late Lawrence Raisz, explores the importance of bone physiology with an emphasis on how bones are formed during growth and how bone tissue continues to be a very active tissue throughout life. The term "modeling" is used to describe bone changes during growth or in response to a change in mechanical loading. Remodeling is defined as bone resorption and formation that occurs essentially at the same site and at the same time. The bone cells, hormones, and nutrients required for successful maintenance of bone density and structure are described in detail and the included figures help the reader to visualize these complex processes.

Chapter 4, authored by Robert Lindsay, who also provides the Foreword critique of this volume, explores the effects of menopause or other causes of estrogen loss on bone remodeling in women. The author reminds us that in all situations of estrogen deprivation there is an increase in skeletal remodeling that in many, but not necessarily all women, results in a loss of bone mass and disruption of skeletal architecture that increases fracture risk significantly. The chapter includes an extensive review of the clinical trials with estrogen treatment and contains discussions of alternatives for preservation of the female skeleton postmenopause. Chapter 5 continues to examine the effects of estrogen loss and also looks at the current tools available to measure the effects of aging on the skeleton of both sexes. The chapter reviews the etiology of age-related fractures from a biomechanics point of view and evaluates the structural failures in aging bone. Whole bone strength is determined by the amount of bone, the spatial distribution of the bone mass, and the intrinsic properties of the materials that make up bone. Factors that influence bone strength, including dietary factors, are examined as potential determinants of bone strength. Chapter 6 explains in detail the benefits and limitations of the current methodologies commonly used to determine bone mineral density (BMD). Bone densitometric techniques can provide quantitative measurement of BMD and are commonly divided into central and peripheral. Central methods measure BMD in the spine and proximal femur and include dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT). Peripheral methods measure BMD in the phalanges, forearm, tibia, or calcaneus and include peripheral dual X-ray absorptiometry (pDXA) and peripheral quantitative computed tomography (pQCT). Quantitative ultrasound (QUS) does not measure BMD.

The seventh chapter examines the role of nutritional epidemiology and its use of nutrient assessment tools to determine population intakes of nutrients affecting bone health. The 24-h diet recall, diet record, and food frequency questionnaires are compared. Diet–bone linkages have been determined for calcium, phosphorus, and vitamin D. The potential skeletal effects of the two forms of vitamin K, menaquinones (vitamin K_2) and phylloquinone (vitamin K_1), phytochemicals, nutrient supplements, protein, vitamin C, certain B vitamins, magnesium, potassium and carotenoids, and other dietary components require randomized controlled trials to test their efficacy. There is a growing awareness that dietary patterns can help us to understand the relationship between certain nutrients and risk of bone fractures. Chapter 8 includes a detailed outline of how to generate dietary patterns with commonly available statistical software and examines the data on the association of dietary patterns and BMD and fractures measured by DEXA and fracture risk in postmenopausal women. Informative tables review the data from survey studies of dietary patterns that are associated with reduced fracture risk. The next chapter looks at the association between dietary patterns and oral health. The chapter includes an extensive review of the literature that indicates that the prevalence of periodontal disease is lower among persons with healthy eating patterns and ideal body weight. There is an in-depth discussion of tooth and jaw structure, inflammatory mediators, and foods associated with decreased risk of tooth loss and diseases, such as diabetes and obesity that are associated with greater risk of tooth loss. The final chapter in this part is of great value to health practitioners as it concentrates on nutrition

counseling for skeletal health. There are evidence-based nutrition recommendations as well as several helpful tables that list the major sources of nutrients associated with bone health.

The second part of the volume examines the effects of life stages and race on the interactions between nutrition and bone health. The seven chapters follow human development and begin with Chapter 11, which concentrates on the major effects of pregnancy and lactation on maternal and fetal bones. The chapter summarizes the literature on the effects of human pregnancy, lactation, and weaning on calcium metabolism and maternal bone health; the role of maternal dietary calcium and vitamin D on maternal calcium metabolism and bone health; the epidemiological evidence relating parity and lactation to maternal osteoporosis and fracture risk later in life; and the role of maternal calcium and vitamin D intake on calcium homeostasis, growth, and bone development of the offspring. The maternal physiological responses to the fetal calcium demands are illustrated in the excellent figures included in the chapter. Chapter 12 follows logically and examines the many factors affecting fetal and neonatal bone growth and development. There is a discussion of "developmental programming" that suggests that metabolic events during critical time periods of antenatal and postnatal development have moderating effects on peak bone mass achieved in late adolescence and osteoporosis risk. This long-term programming of bone growth and bone mass accretion may be influenced by exposures during pregnancy including maternal body composition, diet and lifestyle factors and early infant nutrition, physical activity and growth patterns. The status of the research on the genetic variants responsible for diversity in bone mass between individuals and populations is also reviewed.

The changes in bone growth and development during childhood and adolescence are described in Chapter 13. The chapter includes 17 figures that illustrate that bone accretion during childhood is proportional to the rate of growth. During this age interval height velocity is relatively slow for both boys and girls. Retention of calcium in the body of an average child is lower than the calcium retention in an adolescent. Calcium needs are greater during adolescence (pubertal growth spurt) than in either childhood or adulthood. According to calcium balance studies the threshold intake for adolescents is about 1,500 mg/day. Inadequate calcium intake during growth may increase the risk of childhood fractures and predispose certain individuals to a lower peak bone mass in adulthood and later in life. Dr. Bess Dawson-Hughes, who coedited the first edition of *Nutrition and Bone Health*, reviews the calcium and vitamin D needs of adults for optimal bone health. Chapter 14 includes discussions of studies that span the years between young adulthood and menopause in women and includes an examination of the physiological changes that precede the seventh decade. There is an emphasis on the effects of calcium and vitamin D3 intakes on fracture risk and risk of falls. Chapter 15 explores the relationships between protein, calcium and vitamin D, and fracture risk in the elderly. The antifracture efficacy of nutritional intervention in the elderly such as supplementation, fortification, and dietary changes are reviewed, with a particular focus on elderly in institutional care. Future research directions are also examined in an attempt to offset the growing fracture burden due to the rapidly increasing aged population.

Chapters 16 and 17 review the nutritional and skeletal health of non-white racial groups and certain ethnic groups. US blacks have a reduced risk for osteoporosis compared with US whites and others racial groups. The prevalence of osteoporotic fractures and all nonvertebral fractures among US blacks is about half that of US whites. Blacks also have greater BMD compared to age, sex, and BMI-matched whites which is unexpected given that the skin pigmentation in blacks sharply reduces the amount of vitamin D that is produced during sunlight exposure and calcium intakes are significantly lower than matched white populations. Intervention studies with calcium and vitamin D in black postmenopausal women suggest that supplementation may reduce fracture risk; however, the studies have not reached statistical significance. Chapter 17, the last chapter in this part, looks at data from several non-white racial groups in the USA. Adult Asians living in Asia or the USA have lower areal BMD (aBMD) in comparison to whites and other racial/ethnic groups. Most studies indicate that Hispanic Americans have similar or slightly higher aBMD as compared to non-Hispanic whites although there

are inconsistencies between studies. Areal BMD data in American Indian and Native Alaskan women is limited but indicates similar aBMD compared to white women. There are almost no published data in Native Hawaiians/Pacific Islanders in the USA. Hawaiian women tended to have higher aBMD compared to white, Japanese, and Filipino women. Men usually have higher BMD than women of the same race. With regard to fracture risk, Asian and African Americans share a similarly low risk of fractures despite low aBMD. Detailed review of published data concerning specific population groups and potential fracture sites is included in this comprehensive chapter.

Part 3 includes five chapters that examine the effects of dietary macronutrients on bone health. There is an overview of food groups followed by separate chapters on vegetarian diets, protein, fat, and acid-base balance. Chapter 18 looks at the data concerning the effects of food groups and whole foods on bone health. The whole diet and food-based approach has yielded insights into the relationship between nutrition and bone health and suggests that diets that are higher in fruit, vegetables, milk, and cereal are associated with increased bone mass as compared with diets high in processed and snack foods. Chapter 19 provides an insightful review of the possible associations between certain types of vegetarian diets in women and beneficial or negative effects on aspects of bone health. The literature review indicates that following a lacto-ovo-vegetarian diet with adequate calcium, protein, and vitamin D may favorably affect BMD. Long-term adherence to a vegan diet, however, is associated with lower bone density and increased fracture risk. Chapter 20 examines the associations between protein intake levels throughout the life span from bone growth in children to the benefits of adequate protein post-osteoporotic fracture. This comprehensive chapter includes eight informative figures that illustrate the interactions between cellular factors and bone cell responses, production of bone-specific proteins, and other components of bone with an emphasis on the importance of adequate protein intake for synthesis and functioning of the growth factor, IGF1. The next chapter examines the new research on the role of dietary fat and body fat stores on bone size and strength and concentrates on the unexpected effects of obesity on fracture risk. Chapter 21 includes discussions of the increased risk of cardiovascular disease and cancer with increased body mass index and includes a review of the female athlete triad where fat intake is very low, exercise levels are very high, yet BMD is unexpectedly low. Also included is a review of the different effects of saturated versus unsaturated dietary fats on bone. The last chapter examines the strong link between kidney function and bone calcium levels. The chapter contains 18 figures that are of great value in understanding the complex balance that must be maintained to keep bone calcium levels at their optimum. There is an in-depth discussion of the changes in kidney function with aging that results in the potential for increased risk of osteoporosis and fracture.

Part 4 includes three chapters that review the role of key minerals in bone accretion and loss. The first chapter in this part, coauthored by Connie Weaver who coedited the series volume entitled *Calcium and Human Health* with Robert Heaney, the author of Chapter 24, reviews the techniques that enable the accurate calculation of calcium status in bone. Metabolic balance studies using a crossover design can help to determine the effect of one dietary change on net calcium retention. Balance studies using stable isotopes of calcium are described and the tables and figures included in the chapter illustrate the parameters of calcium metabolism including absorption, endogenous secretion, excretion, bone formation rates, and bone resorption rates. As 99 % of the body's calcium resides in the skeleton, calcium metabolism is directly reflective of bone metabolism. Chapter 24 looks at the dietary sources and metabolism of several minerals that can affect bone health. The chapter reviews the consequences of the variations in intakes of sodium, potassium, phosphorus, and magnesium and their potential effects on the adult and aging skeleton. Chapter 25 describes the effects of trace mineral status on bone health and provides an overview of the importance of adequate copper especially in preterm infants or adults given parenteral nutrition as their only source of nutrients. The author reviews both animal and human studies of zinc deficiency, boron status, strontium supplementation, silica status, and fluoride functions.

The fifth part's four chapters examine the role of the fat-soluble vitamins A, D, and K on skeletal health. Chapter 26 examines the full data set on the role of vitamin A in bone formation and resorption. This chapter includes a review of laboratory animal studies of the vitamin as well as its metabolites that have been used as potent drugs that can also affect bone. The authors conclude that there are insufficient data to determine the high dose of vitamin A that can consistently cause adverse bone effects, and at the same time acknowledge that low vitamin A intakes especially in utero and in children have proven adverse effects. Critical issues involve the age of the subjects as extreme age, either young or old, appear to be more sensitive to high doses of vitamin A; duration of intake; concomitant vitamin D intake; and other lifestyle habits all appear to affect the safety of vitamin A. Relevant studies are tabulated.

Michael Holick, who was also coeditor of the first edition of this volume and editor of the *Vitamin D* volume for the Nutrition and Health Series, has aptly provided the comprehensive review of vitamin D. The chapter, which includes 24 figures and 1 table, examines the formation and metabolism of vitamin D, its role in bone during the lifetime, and the newest research linking vitamin D status with many aspects of health beyond bone. There is an emphasis on the realization of the potential for vitamin D deficiency which was not appreciated until fairly recently. The author indicates that vitamin D deficiency is extremely common and needs to be recognized. Vitamin D deficiency in children and teenagers can result in poor bone health and the inability to attain the genetically predetermined peak bone mass. In young, middle-aged, and older adults, vitamin D deficiency causes osteomalacia and can precipitate and exacerbate osteoporosis. Maintenance of an adequate serum 25(OH) vitamin D level throughout life may help reduce the risk of developing many chronic diseases, including type 1 diabetes, hypertension, multiple sclerosis, infectious diseases, and cancers of the breast, prostate, colon, and ovary. The purpose of the unique and very relevant Chapter 28 is to examine the role of the vitamin D response element binding protein first identified in subhuman primates and other associated intracellular proteins that are involved in the regulation of the expression of vitamin D-controlled genes in nonhuman and human primates. The chapter includes a comprehensive review of the metabolism of vitamin D by New World primates, several of whom have been shown to develop severe rickets when in captivity that is cured by high concentrations of vitamin D and/or significant exposure to sunlight. These primates have helped us to understand the intracellular movements of vitamin D metabolites and this knowledge is of value in understanding the molecular changes in vitamin D in humans.

Vitamin K has been examined closely in the decade since the first edition of this volume was published. Chapter 29 reviews the findings of vitamin K's role in age-related bone loss. The chapter includes an extensive discussion of the different forms of vitamin K and concentrates on the forms found in food and dietary supplements. The non-bone and the bone-related functions of vitamin K are reviewed. Several vitamin K-dependent proteins are present in bone, including osteocalcin, which is one of the most abundant non-collagenous proteins in bone and is the most extensively studied vitamin K-dependent bone protein. The emerging evidence that vitamin K may have roles in skeletal tissue independent of currently accepted functions is also discussed. Of greatest value to the reader, there is a comprehensive review of the vitamin K-related studies in osteoporotic women, mainly from Asia, including survey and intervention studies with several types of vitamin K. At present, the results remain inconsistent.

Part 6 describes the associations between lifestyle factors and use of dietary supplements on bone health parameters. Chapter 30 reviews the effects of cigarette smoking and/or alcohol consumption on bone health in young and older adults. The effects of nicotine and alcohol on bone cells in vitro are reviewed. The author discusses studies that show that smoking adversely affects bone density and increases hip fracture risk in postmenopausal women. In men and younger women the evidence is not conclusive. Recent studies on the role of alcohol on the skeleton suggest a "J"-shaped curve with benefits associated with 1–2 drinks/day and adverse effects seen at higher intakes. Moderate ingestion

of alcohol may be associated with some benefit to the skeleton. Both ethanol and non-ethanol components of alcohol can have effects on skeletal health.

The next two chapters examine the importance of exercise for enhancing peak bone mass and reducing the loss of bone with aging. The first chapter describes the literature on exercise and bone and the second chapter examines the role of diet, especially calcium intake in enhancing the beneficial effects of exercise. Chapter 31 reviews the important role of exercise in maintaining bone health and provides over 200 relevant references for the reader. The author outlines the goals of exercise for fracture prevention that change over the course of the life span. In childhood and adolescence, the emphasis is on achievement of peak bone mass; in middle age, exercise is important for the preservation of bone and muscle strength; and in aging, exercise can be of great help in keeping one's balance and skeletal muscle strength. Intervention studies in young and older population groups of exercisers versus sedentary controls that assessed bone mineral density and fracture risk are tabulated. Clinical studies of aerobic versus resistance training programs are also reviewed. Chapter 32 examines the requirement of adequate calcium to get the full benefits of exercise on bone mineral density. The beneficial effects of weight-bearing exercise during adolescence are stressed and the role of bone-building hormones is reviewed. Each of the important nutritional factors including dietary calcium, vitamin D, protein, total caloric intake, phosphorus, vitamins C and K, copper, zinc, and manganese is discussed. Calcium and phosphorus make up 80–90 % of the mineral content of bone. Many nutrients interact with other nutrients, genetics, and environmental factors. The complexity of these interactions and with physical activity are also examined.

Body weight has a direct effect on bone mineral density and loss of weight can result in loss of bone. Chapter 33 begins with an evaluation of the effects of obesity on bone health. Even though the greater body weight of the obese person should be associated with greater bone strength, there is evidence that obesity and osteoporosis are not mutually exclusive and that obesity does not protect against osteoporosis. Of great interest are the studies that show a higher fracture incidence in obese children and greater fracture risk at certain bone sites in obese versus normal weight, age-matched adults. In contrast, lean older women have an increased annual rate of bone loss compared to heavier women which is linked to the higher rate of bone turnover in leaner women. The chapter also reviews the effects of weight loss, either voluntary or involuntary on bone loss, and also includes a discussion of the effects of bariatric surgery on subsequent bone loss; almost 200 references are included. The last chapter in this part, written by the coeditor of the volume, Jeri W. Nieves, explores the role of nutraceuticals on bone health and includes reviews of both essential and nonessential nutrients as well as certain other dietary substances. The chapter includes discussions of calcium and vitamin D, soy compounds, dehydroepiandrosterone, antioxidants, flavonoids, carotenoids, omega-3-fatty acids, B-vitamins, magnesium, boron, strontium, silicon, phosphorus, red clover, black cohosh, and ipriflavone. The author concludes that there are few consistent beneficial findings for these substances other than for calcium and vitamin D.

The final part of this important volume, Part 7, contains seven chapters of clinical relevance that describe the effects of certain nutrition-related disorders and their effects on bone health. Chapter 35 examines the effects of eating disorders with emphasis on anorexia nervosa as this eating disorder is associated with significant weight loss and low body weight and significant loss of bone mass, density, and structure. The author describes the adverse synergistic effects of this eating disorder that usually occurs in early adolescence at the time when almost 25 % of peak bone mass is being formed in normal weight children. A key factor in the bone loss seen in anorexic girls is low estrogen levels and loss of menses. Bone density correlates inversely with the duration of amenorrhea. There is also an inverse relationship between estrogen levels and markers of bone resorption in adolescent girls with anorexia. The chapter reviews the potential nutritional and pharmacological strategies used in the treatment of anorexia.

Two chapters examine the skeletal effects of genetic diseases. Chapter 36, coauthored by Michael Holick, looks at the multifactorial adverse health effects of cystic fibrosis. We learn that cystic fibrosis

is a recessive genetic disorder that causes abnormal sodium and chloride transport resulting in lung and gastrointestinal complications, lung infections, pancreatic insufficiency, impaired digestion, and malabsorption. The low bone mass seen in cystic fibrosis patients as they age is multifactorial and is influenced by nutritional status, disease severity, glucocorticoid use, hormonal status, inflammation, gastrointestinal function, mechanical loading, and physical activity patterns. The critical issue of calcium and other bone-related nutrient absorption in the face of disease-specific metabolically related issues is reviewed. Chapter 37 reviews the autoimmune disorder, celiac disease that has both genetic and environmental components. It is characterized by innate and adaptive immune responses that are primarily triggered by the ingestion of dietary gluten, resulting in inflammation, small intestinal villous atrophy, and crypt hyperplasia. Genes that code for human leukocyte antigens (HLA) DQ2 and DQ8 are strongly associated with and confer susceptibility for celiac disease. In addition to the characteristic intestinal symptoms, celiac disease is associated with extra-intestinal complications, including those affecting skeletal health. Reduction in bone mineral density and increased risk of bone fracture, caused by malabsorption-related alteration of calcium metabolism and immune-mediated mechanisms, are frequently seen in patients with celiac disease. Reduced bone density and bone derangement are some of the most common extra-intestinal complications found in newly diagnosed celiac disease patients. The chapter summarizes the currently available information regarding the prevalence, pathogenic mechanism, and treatment of celiac disease in the context of bone health.

Chapter 38 examines the effects of both disease and treatment of HIV on the skeleton. We are reminded that HIV is a retrovirus that infects immune cells leading to progressive failure of the immune system. The importance of this unique chapter is due to the increased life expectancy of the HIV-positive population. Most HIV-infected persons in the USA will be 50 years old or older by 2015 and their risk for osteopenia and osteoporosis, and fractures increases as their life expectancy increases. The etiology of osteoporosis in HIV-infected persons is complex and may involve both HIV disease itself and antiretroviral treatment. Traditional risk factors, such as smoking, hypogonadism, and low body weight, also play a role. Bone health screening and nutritional interventions in this population are in the early stages of development.

The last three chapters present early findings and unique perspectives on the potential role of certain nutrients in either maintaining or enhancing bone health. Chapter 39 explores the new clinical findings associated with consistent exposure to low-grade inflammatory responses in populations at risk for osteoporosis. The author indicates that there are data linking dietary benefits in individuals with diseases with known inflammatory pathogenesis such as type 2 diabetes, cardiovascular disease, and cancer. With regard to bone, the limited number of intervention trials that demonstrate that calcium and vitamin D supplementation, high dairy diets, increased dietary protein, vitamin K, and omega-3 fatty acids produce modest reductions in circulating inflammatory biomarkers in people with osteoporosis, sarcopenia, or the presence of other chronic diseases is reviewed. Currently, it is not known if a reduction in inflammatory markers translates into beneficial effects on skeletal health or a reduction in fracture risk. Given the emerging clinical evidence linking low-grade systemic inflammation to osteoporosis, sarcopenia, and fractures in the elderly, the author suggests that further intervention trials are warranted. Chapter 40, co-authored by Jeri W. Nieves, examines the important drug–nutrient interactions in patients with osteoporosis who are given anti-osteoporosis drugs. We are reminded that in virtually all of the intervention trials supporting approval of the marketed anti-osteoporosis drugs, both the drug-placebo and the active cohorts were given supplemental calcium and vitamin D to assure adequate intake of these two nutrients. Although the dosages of calcium and vitamin D may not have been the same in all studies, the data reviewed indicates that no harm was seen in any study and in most, there was a benefit of supplementation. The authors review studies with estrogen, raloxifene, the bisphosphonates, denosumab, and teriparatide. The last unique chapter, which includes almost 200 relevant references and eight helpful figures, summarizes the research on the effects of space flight and long-term exposure to weightlessness in space on bone. The chapter

reviews the deleterious effects of space flight on the human body and the potential for nutritional interventions to reduce these adverse effects on bone. The specific effects of space flight on bone metabolism are enumerated. There is a discussion of the significant loss of bone during space exposure that is normalized over many months following return to Earth. The chapter includes extensive data on effects of individual nutrients as well as a discussion of the technical issues to assure incorporation of the right level of these nutrients into the space food.

The above descriptions of the 41 chapters in *Nutrition and Bone Health, 2nd Edition* attest to the depth of information provided by the well-recognized and respected editors and chapter authors. Each chapter includes complete definitions of terms with the abbreviations fully defined for the reader and consistent use of terms between chapters. Key features of the comprehensive volume includes over 200 detailed tables and informative figures, an extensive, detailed index, and more than 4,000 up-to-date references that provide the reader with excellent sources of worthwhile information. The volume also includes a dedication of the volume to Dr. Lawrence Raisz written by David W. Dempster and an insightful Foreword by Dr. Robert Lindsay.

In conclusion, *Nutrition and Bone Health, 2nd Edition*, edited by Michael F. Holick, M.D., Ph.D. and Jeri W. Nieves, Ph.D., provides health professionals in many areas of research and practice with the most up-to-date, well-referenced volume on the importance of diet and nutritional status throughout life on bone health. The volume places its emphasis on food groups, diets, and key nutrients associated with reduction of the risk of osteoporosis in overall healthy individuals and in patients with certain disease conditions that increase the risk of adverse bone effects. The volume serves the reader as the benchmark in this complex area of interrelationships between dietary intakes of numerous dietary components including calcium, vitamin D, protein, other relevant minerals, essential and nonessential nutrients, exercise, body weight, gender, race, ethnicity, and the dynamic changes in bone tissue that are continuous throughout life. Moreover, the physiological, genetic, and pathological interactions between diet and skeletal integrity are clearly delineated so that students as well as practitioners can better understand the complexities of these interactions. The editors are applauded for their efforts to develop the most authoritative and unique resource in the area of nutrition, bone health, and disease to date and this excellent text is a very welcome addition to the Nutrition and Health Series.

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About the Series Editor

Adrianne Bendich, Ph.D., F.A.S.N., F.A.C.N. has served as the Nutrition and Health Series Editor for over 15 years and has provided leadership and guidance to more than 120 volume editors that have developed the 60+ well respected and highly recommended volumes in the series.

In addition to *Nutrition and Bone Health, Second Edition*, edited by Michael F. Holick Ph.D., M.D., and Jeri W. Nieves, Ph.D., major new editions in 2012–2014 include:

1. *Nutrition and Oral Medicine, Second Edition*, edited by Dr. Riva Touger-Decker, Dr. Connie C. Mobley and Dr. Joel B. Epstein, 2014
2. *Fructose, High Fructose Corn Syrup, Sucrose and Health*, edited by Dr. James M. Rippe, 2014
3. *Nutrition in Kidney Disease, Second Edition*, edited by Dr. Laura D. Byham-Gray, Dr. Jerrilynn D. Burrowes and Dr. Glenn M. Chertow, 2014
4. *Handbook of Food Fortification and Health, volume I* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, Dr. Vinood B. Patel, 2013
5. *Handbook of Food Fortification and Health, volume II* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, Dr. Vinood B. Patel, 2013
6. *Diet Quality: An Evidence-Based Approach, volume I* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter and Dr. Vinood B. Patel, 2013
7. *Diet Quality: An Evidence-Based Approach, volume II* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter and Dr. Vinood B. Patel, 2013
8. *The Handbook of Clinical Nutrition and Stroke*, edited by Mandy L. Corrigan, M.P.H., R.D,. Arlene A. Escuro, M.S., R.D., and Donald F. Kirby, M.D., F.A.C.P., F.A.C.N., F.A.C.G., 2013

9. *Nutrition in Infancy, volume I* edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy and Dr. Sherma Zibadi, 2013
10. *Nutrition in Infancy, volume II* edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy and Dr. Sherma Zibadi, 2013
11. *Carotenoids and Human Health*, edited by Dr. Sherry A. Tanumihardjo, 2013
12. *Bioactive Dietary Factors and Plant Extracts in Dermatology*, edited by Dr. Ronald Ross Watson and Dr. Sherma Zibadi, 2013
13. *Omega 6/3 Fatty Acids*, edited by Dr. Fabien De Meester, Dr. Ronald Ross Watson and Dr. Sherma Zibadi, 2013
14. *Nutrition in Pediatric Pulmonary Disease*, edited by Dr. Robert Dumont and Dr. Youngran Chung, 2013
15. *Magnesium and Health*, edited by Dr. Ronald Ross Watson and Dr. Victor R. Preedy, 2012.
16. *Alcohol, Nutrition and Health Consequences*, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
17. *Nutritional Health, Strategies for Disease Prevention, Third Edition*, edited by Norman J. Temple, Ted Wilson, and David R. Jacobs, Jr., 2012
18. *Chocolate in Health and Nutrition*, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
19. *Iron Physiology and Pathophysiology in Humans*, edited by Dr. Gregory J. Anderson and Dr. Gordon D. McLaren, 2012

Earlier books included *Vitamin D, Second Edition*, edited by Dr. Michael Holick; *Dietary Components and Immune Function*, edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi and Dr. Victor R. Preedy; *Bioactive Compounds and Cancer*, edited by Dr. John A. Milner and Dr. Donato F. Romagnolo; *Modern Dietary Fat Intakes in Disease Promotion*, edited by Dr. Fabien De Meester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; *Iron Deficiency and Overload*, edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; *Nutrition Guide for Physicians*, edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple and Dr. Mary Struble; *Nutrition and Metabolism*, edited by Dr. Christos Mantzoros and *Fluid and Electrolytes in Pediatrics*, edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include: *Handbook of Drug-Nutrient Interactions*, edited by Dr. Joseph Boullata and Dr. Vincent Armenti; *Probiotics in Pediatric Medicine*, edited by Dr. Sonia Michail and Dr. Philip Sherman; *Handbook of Nutrition and Pregnancy*, edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch and Dr. Elliot Philipson; *Nutrition and Rheumatic Disease*, edited by Dr. Laura Coleman; *Nutrition and Kidney Disease*, edited by Dr. Laura Byham-Grey, Dr. Jerrilynn Burrowes and Dr. Glenn Chertow; *Nutrition and Health in Developing Countries*, edited by Dr. Richard Semba and Dr. Martin Bloem; *Calcium in Human Health*, edited by Dr. Robert Heaney and Dr. Connie Weaver and *Nutrition and Bone Health*, edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich is President of Consultants in Consumer Healthcare LLC, and is the editor of ten books including *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fourth Edition*, co-edited with Dr. Richard Deckelbaum (www.springer.com/series/7659). Dr. Bendich serves on the *Editorial Boards of the Journal of Nutrition in Gerontology and Geriatrics, and Antioxidants, and has served as Associate Editor for "Nutrition" the International Journal; served on the Editorial Board of the Journal of Women's Health and Gender-based Medicine, and* served on the Board of Directors of the American College of Nutrition.

Dr. Bendich was Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare and provided medical leadership for many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid-containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has co-authored over 100 major clinical research

studies in the area of preventive nutrition. She is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich received the Roche Research Award, is a *Tribute to Women and Industry* Awardee and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences. Dr. Bendich was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. In 2012, she was recognized for her contributions to the field of clinical nutrition by the American Society for Nutrition and was elected a Fellow of ASN. Dr Bendich is an Adjunct Professor at Rutgers University. She is listed in Who's Who in American Women.

About the Volume Editors

Michael F. Holick, Ph.D., M.D. is Professor of Medicine, Physiology, and Biophysics; Director of the General Clinical Research Unit, and Director of the Bone Health Care Clinic and the Heliotherapy, Light, and Skin Research Center at Boston University Medical Center. After earning a Ph.D. in biochemistry, a medical degree, and completing a research postdoctoral fellowship at the University of Wisconsin, Madison, Dr. Holick completed a residency in medicine at the Massachusetts General Hospital in Boston.

Dr. Holick has made numerous contributions to the field of the biochemistry, physiology, metabolism, and photobiology of vitamin D for human nutrition. As a graduate student at the University of Wisconsin he was the first to isolate and identify 25-hydroxyvitamin D3 in human blood and the active form of vitamin D as 1,25-dihydroxyvitamin D3. He participated in the first chemical synthesis of 1,25-dihydroxyvitamin D3 and it's analog 1alpha-hydroxyvitamin D3 that was used in the first demonstration of their utility in treating vitamin D-dependent rickets type I and hypoparathyroidism. He determined the mechanism for how vitamin D is synthesized in the skin and demonstrated the effects of aging, obesity, latitude, seasonal change, sunscreen use, skin pigmentation, and clothing on this vital cutaneous process. He introduced the concept of using 1,25-dihydroxyvitamin D3 and active analogs for the treatment of psoriasis. Dr. Holick has established global recommendations advising

sunlight exposure as an integral source of vitamin D with an app dminder.info. He chaired the Endocrine Society's Practice Guidelines Committee on Vitamin D which provided recommendations for the treatment and prevention of vitamin D deficiency for children and adults. He has also helped increase awareness in the pediatric and medical communities regarding vitamin D deficiency pandemic and its role in causing not only metabolic bone disease and osteoporosis in adults but increasing risk of children and adults developing common deadly cancers, autoimmune diseases, including type 1 diabetes and multiple sclerosis as well as heart disease.

Dr. Holick is a Diplomate of the American Board of Internal Medicine, a Fellow of the American College of Nutrition, and a member of numerous organizations, including the American Academy of Dermatology, American Society for Bone and Mineral Research, and the American Association of Physicians. He is the recipient of numerous awards and honors, including the American Skin Association's Psoriasis Research Achievement Award in 2002, the American College of Nutrition Award in 2002, the Robert H. Herman Memorial Award in Clinical Nutrition from the American Society for Clinical Nutrition in 2003, the Annual General Clinical Research Centers' Program Award for Excellence in Clinical Research in 2006, the Linus Pauling Functional Medicine Award from the Institute for Functional Medicine in 2007, Linus Pauling Prize 2009, DSM Innovation In Nutrition Award 2009, American Association of Clinical Chemist's Van Slyke Award 2010, American College of Nutrition's Communication Media Award 2011, the Delbert Fisher Research Scholar 2011, Best Doctors in America 2011–2014, and the American Society of Bone and Mineral Research Louis Avioli Award 2014. His teaching skills were recognized by being chosen to give the Boston University Lecture in 2013 and given the Educator of the Year award in the Masters of Medical Sciences Program in 2014. Dr. Holick serves on a number of national committees, including NIH and NASA and several editorial boards. He has organized and/or co-chaired several international symposia and is editor-in-chief for the *Journal for Clinical Laboratories and Laboratories Related to Blood Transfusion* and Associate editor for *Dermato-Endocrinology*. He has authored more than 500 peer-reviewed publications and written more than 250 review articles as well as numerous book chapters. He has acted as editor and/or co-editor on 13 books.

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Part I
Basics of Nutrition and Bone Biology

Chapter 1
Bone Health from an Evolutionary Perspective: Development in Early Human Populations

Dorothy A. Nelson, Sabrina C. Agarwal, and Linda L. Darga

Key Points

- Skeletal characteristics, bone health, and the risk of osteoporosis reflect our evolutionary past.
- Evolutionary mechanisms resulted in the genetic changes that helped our ancestors to adapt to diverse environments.
- Environmental and cultural factors, including diet, physical activity, work patterns, health and disease impact the skeleton.
- Anthropological techniques allow us to create models of life in past human populations providing insight into modern-day bone health.
- The major biocultural shifts during hominin evolution include the following:
  - Expansion from the tropics to a wide range of environments
  - Transition from hunting and gathering to food production
  - Change from physically active lifestyles to relative sedentism
  - Increase in life expectancy, with changes in reproductive behaviors

Keywords Hominin evolution • Bone health • Osteoporosis • Dietary calcium • Vitamin D

1.1 Introduction

The skeleton serves two primary functions: it provides biomechanical support and protection of soft tissue; and it plays a key role in mineral homeostasis. Skeletal health can be affected by a number of factors, including genetics, lifestyle, demographic characteristics, and disease. Skeletal size, strength, and structure can be affected by diet and physical activity, age, body size, ethnicity, and health status. In living persons, most of these factors can be assessed to some extent, and changes can be monitored

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in individuals over time. Techniques such as bone densitometry, assessment of biochemical markers of bone remodeling, radiography, bone biopsy, and others can be used in the assessment of skeletal status. In contrast, investigations of skeletal health in past populations are limited to various physical characteristics that happen to be preserved at a moment in time for each individual specimen or local population.

For the purposes of examining evolutionary aspects of bone health, it is fortunate that bones (and teeth) are typically preserved in the fossil record. Certain artifacts of culture may also be present in hominin(human) fossil sites, and these provide further information about adaptation. Some of the techniques used for assessing skeletal status in the living, such as bone densitometry and histomorphometry, can also be used in skeletal remains. However, it is impossible to obtain dynamic or longitudinal measurements of physiological processes, or to assess diet and physical activity accurately. Fortunately, anthropological techniques have been developed that allow us to create reasonable models of life and health in past human populations.

The stunning developments in genetic analyses have also yielded insights into the evolutionary history of our species. Not only can we assess the genetics of living populations and through next-generation analytical techniques, make comparisons among modern human populations, but breakthroughs have allowed genome sequencing of fossil populations dated to 50,000 years ago. These data have provided new understandings of the ongoing evolution of human skeletal adaptation that continues today. In this work we will examine aspects of our evolutionary past that may have affected skeletal health in human ancestors and formed the basis for observed skeletal conditions among modern human populations. In order to place bone health in evolutionary perspective, an overview of evolutionary principles and stages will be presented.

1.2 Overview of Evolution

The period during which our human ancestors evolved is miniscule in relation to the evolutionary record of all living things. The first simple organism is thought to have appeared around 3.5–4 billion years ago; evidence of the first vertebrates dates to about 525 million years ago; the first transitional tetrapod, *Tiktaalik roseae*, a transitional species between fish and land-based quadrupeds [1], to about 375 million years ago; and, finally, mammals are found in the fossil record some 200 million years ago. The animal order to which we belong, the Primates, appeared approximately 70 million years ago. If we were to fit 200 million years of mammalian evolution into a 12-h clock (Fig. 1.1), the earliest members of the Primates appear at about 7:48 [2]. The first hominids, or members of the human family, appear at 11:42; and food production develops in the final 2 or 3 s. Thus, people are relative newcomers on the earth when compared to other organisms, but the speed with which the species changed is unique among animals.

1.2.1 Evolutionary Mechanisms

Although the theory of evolution by natural selection was presented in a paper on behalf of Charles Darwin and Alfred Wallace 155 years ago and laid down in detail in Charles Darwin's book, *On the Origin of Species by Means of Natural Selection, or the Preservation of Races in the Struggle for Life* [3], in the 6th edition shortened to *The Origin of Species*, it continues to be the organizing principle for studies of life, including within our own species, albeit tweaked along the way. Since Darwin's time we have even discovered a new domain of life, single-celled micro-organisms called Archea [4]. In spite of the fact that these organisms live in habitats we might have thought incompatible with life,

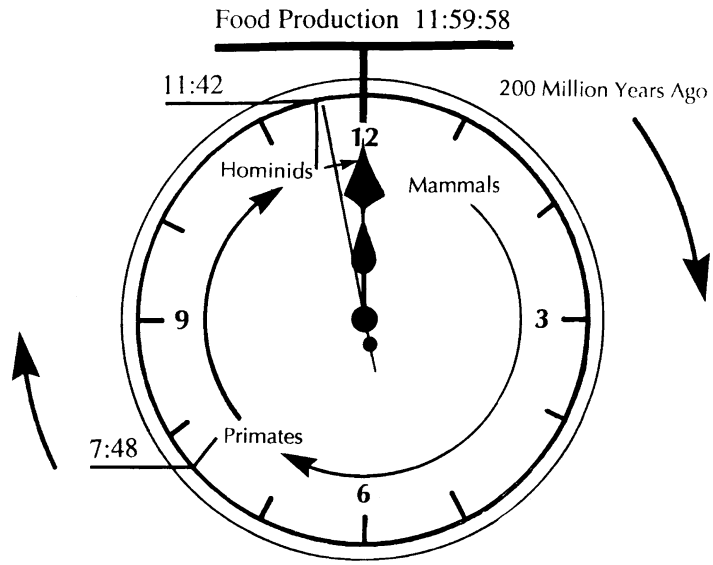

Fig. 1.1 Analog of 200 million years of evolution depicted on a 12-h clock. (Reprinted with permission from ref. [2], Fig. 1, p. 326, © Springer-Verlag.)

and they possess distinct differences from the Prokaryotes, they appear to follow an evolutionary path similar to the rest of us. Darwin's theory not only has survived the arrival of the first genetic revolution, it became wedded to population genetics in the *modern synthesis* in the 1930s and 1940s. It continues to provide the scaffolding for our own contemporary synthesis, based on the current genetic revolution. The Human Genome Project has not yet provided the personalized therapies that were hoped for, however the technological revolution that allowed sequencing of our genome has generated an explosion of understanding of not only genomics, but also of proteomics, microbiomics, epigenomics and methylomics and advances in molecular evolution, promising new insights into what being human means, as well as implications for nutrition and bone health.

The central feature of evolution is adaptation to the environment, initially our fetal environment. Adaptation is the result of the operation of natural selection, that is, the survival and reproduction of those most fit in their environment and an increase in frequency in the next generation of their genes. The most basic mechanism that alters the genetics of a population and forms the raw material for the operation of natural selection is mutation. These usually random changes in the sequence of DNA bases are typically deleterious, but may be neutral in their effects and occasionally afford an individual a step up in competing with its conspecifics for resources and survival. Natural selection then operates to increase the frequency of that allele in the population.

Another mechanism is gene flow, in which population members establish cultural and biological links to near-by or distant groups, introducing novel alleles. The human species has excelled in migrations, originating on the continent of Africa, and by about 2 million years ago, venturing into the Middle East and farther north and east to populate a new continent and eventually occupying most land surfaces on earth. After peopling uninhabited lands very different from the tropical rainforests and savannas of Africa and encountering new selective forces, human migrations have continued throughout our history. The more gene flow that occurs, the more alike two populations become, and it is gene flow that prevents speciation from occurring and maintains our single and diverse species. This is an important factor in a species such as *Homo sapiens* that is so geographically dispersed. The last evolutionary mechanism is genetic drift, the random changes in gene frequencies of populations, especially small populations, from generation to generation. Drift also occurs when members of one

population disperse to found a new population; their gene frequencies differ from the population of origin due to their being a non-representative (non-random) sample of the parent population. Genetic drift seems to be the main reason why certain genetic diseases occur at unexpectedly high frequency among some populations, such as maple syrup urine disease or Hirschsprung disease in Old Order Mennonites of southeastern Pennsylvania [5]. Despite their proximity to non-Mennonite populations, cultural factors have impeded gene flow.

Increased research attention has revealed two other factors important in human evolution. The first, epigenetics, or factors that affect gene expression that do not involve alteration of the DNA base sequence, can result from, for instance, DNA methylation and are important in turning particular genes on and off during embryological development, tissue differentiation in post-natal development and changes that result in malignant transformation of cells [6]. While increasing understanding of a basic mechanism of gene expression is exciting, it is now realized that methylation is affected by environmental factors, including dietary factors. Dietary restriction of folate, methionine and choline can alter DNA methylation and gene expression in animal studies [7]. Furthermore, changes in methylation have been shown to be passed on to the next generation in animal studies. For example, newborn rat pups were exposed to stressed, abusive mothers; this altered the methylation pattern of an important gene expressed in their brains that remained into adulthood. When those newborn pups in turn became pregnant, they passed this methylation pattern on to their pups [8]. As inheritance of environmentally modified methylation begins to be demonstrated in humans, the environment will be shown to factor into human evolution in ways beyond natural selection. Researchers have now zeroed in on the analysis of methylation patterns in the human brain at a resolution of individual DNA bases [9].

The human microbiome has also garnered the attention of scientists. There are ten times the number of organisms living in the human gastrointestinal tract, and elsewhere, than we have human cells in the body. We realize that the trillions of organisms making up the microbiome in the human gut affect our health in potentially profound ways. The implications for cultural traditions, especially in diet, nutritional status, human health and diseases, such as auto-immune diseases like asthma and colitis, are looming in importance [10]. That this microbiota has been a factor in our evolution is increasingly apparent and may at some point be tied to bone health.

1.2.2 Culture and Adaptation

In the middle of the last century, anthropologists argued that "culture" was learned symbolic behavior and the resulting material products that belonged to humans alone. Now we speak of chimpanzee cultural behavior referring to learned behavior passed down from generation to generation that is characterized by geographical variation and "traditions." Even teaching whooping cranes born in captivity to migrate as their ancestors did by getting them to follow a small, one-person airplane is referred to as "teaching them crane culture." (Morning Edition, NPR, 8/30/2013)

Despite the broad application of the concept of culture in recent decades, human culture has been defined as a society's shared and socially transmitted ideas, values, and perceptions, which make sense of life's experience, generate behavior and the material technology, goods and institutions that result. No other species' behavior, no matter how complex, can compare to human cultural adaptation. From the first tools discovered in the African Savanna [11], human material culture is not only the major means by which hominins (those in the human line of evolution) adapt but an integral part of the environment to which humans adapt. Whether it was the clothing and shelter which enabled hominins to move northward into colder climates or the use of tools to enhance the variety of foods available in a particular environment, or the alteration of their relationship to the environment, as with domestication of foodstuff gathered when it was plentiful, human adaptation has been characterized by the interaction of biology and culture and in turn, has selected for genetic changes over time in our species.

1.2.3 Bone Health and Adaptation

The subject of bone health in this chapter will be discussed in the context of transitions in human evolution. The first transition, for which there is scant fossil evidence, was the transition to bipedalism from the common quadrupedal primate ancestor to the African great apes (chimpanzees, gorillas, bonobos) and hominins. Indeed, the evolution of bipedalism is taken to be the *sine qua non* of an ancient, ancestral hominin versus an ancestral ape. We do not have a clear understanding of the selective factors giving rise to a bipedal primate in the savanna or boundary areas at the forest's edge, but the resultant species became widespread and successful. However, this transition to bipedalism set up new stresses and demands on what was originally a quadrupedal ancestor, particularly in the vertebral column and lower extremities. For example the vertebral column sustains the vertical stresses of an upright body rather than having the stress distributed horizontally as in a quadruped, with resultant lower back pain and sciatica; the use of two legs for support rather than four portended increasing hip and knee joint replacements in a longer-living biped.

On the micro-evolutionary scale, the need for specific skeletal characteristics would have continued to change as early hominins experienced bioculturally adaptive shifts. Such characteristics include the size, shape, and density of the skeleton throughout the life course. The major biocultural shifts during human evolution include the following:

1. Expansion from the tropics to a wide range of environments
2. Transition from hunting and gathering to food production
3. Change from physically active lifestyles to relative sedentism
4. Increase in life expectancy and change in reproductive behaviors

These four areas will form the focal points for discussion of bone health over the course of human evolution.

1.3 The Course of Human Evolution

It will be helpful to outline the major events in human evolution for the non-anthropologist reader (Fig. 1.2). Between 5 and 7 million years ago, one or more groups of ape-like primates began adapting to a more terrestrial bipedal niche. A skull with an ape-sized brain, dated to between 6 and 7 million years ago, was found in Chad and given the genus name *Sahelanthropus*. Some would attribute this find to the hominin clade (the evolutionary line leading to humans), but its ancestry to us is being challenged. *Orrorin tugenensis* from Kenya, based on fragmentary cranial and post-cranial bones, especially femoral fragments, and dated to 6 million years ago, has also been considered bipedal, but this too is contentious. A bit less contentious are finds attributed to *Ardipithecus kadabba* and *A. ramidus* from Ethiopia, dated between 4.5 and 6 million years ago [12]. This is very close to the time of hominin origins suggested by genetic evidence, or the molecular clock, and therefore one can expect fossils to be a mosaic of ape-like and human characteristics and open to debate [13–15]. Fossils dating from 4 million years ago and assigned to the genus *Australopithecus* are more numerous, less fragmentary and possess bipedal and dental characteristics assuring their inclusion as hominins.

This period from the origin of the hominin line to the first evidence for the genus *Homo* was characterized by much diversity. Still confined to Africa, hominins were separated into perhaps four or five genera and perhaps ten or so species, each characterized by unique skeletal and dental features. Probably some of the more robust australopithecines were primarily vegetarians, but the more gracile forms probably scavenged or caught small mammals, if observation of present-day chimpanzees is informative. In fact the remains of one species of ausralopithecine in east Africa, dated to about

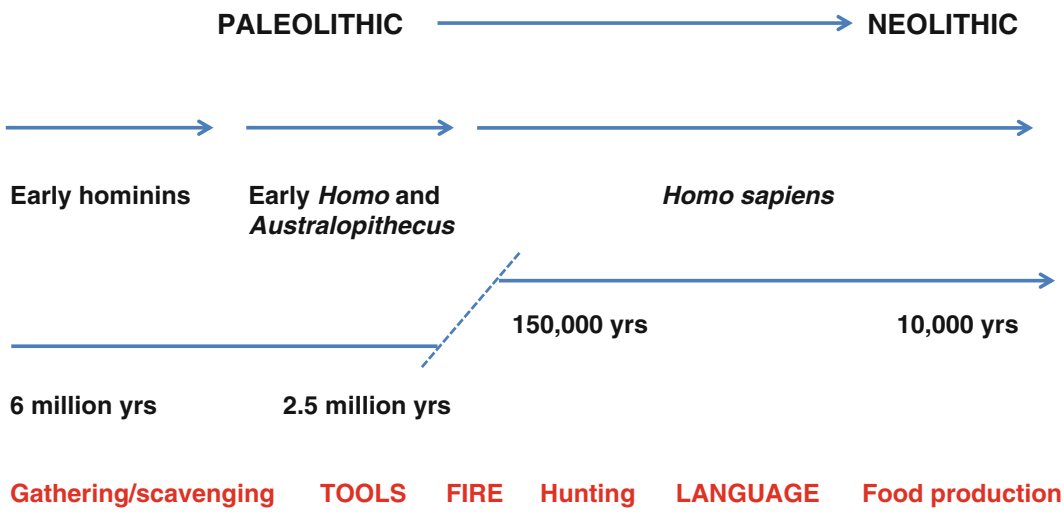

Fig. 1.2 Time line of events in human evolution over the past 6 million years (clip Art images copyright© 2010 microsoft corporation)

2.5 million years ago, is associated with butchered remains of animals [12]. Anatomically, their brains were about one-third the size of ours; their molar teeth had, at least in some species, much greater surface area; and forward-jutting faces protruded from prominent brow ridges. Where the various species' ranges overlapped, each species was probably adapted by diet and behavior to varied aspects of the environment.

About 2 million years ago, new hominins arose with noticeably smaller teeth, brains about two-thirds the size of ours, and greater stature. The origin of our genus is probably somehow associated with great climate fluctuations, and expansion and retraction of vast polar ice sheets. These ice sheets originating at each pole also resulted in environmental shifts in tropical Africa, where *Homo* was evolving; indeed the first fossils of our genus occurred at a time of variability in the fossil record of other animal species [12]. Still divided into several species and confined to Africa, the genus *Homo* is associated at many sites with some of the earliest stone tools [11]. The appearance of stone tools in the archeological record (Fig. 1.2) marks the beginning of the "Paleolithic" (Old Stone Age) period. It is likely that the early *Homo* species regularly hunted for mammals and may have scavenged the meat of large animals. As their cultural repertoire grew, by about 1.8–2 million years ago, *Homo erectus* and related species migrated north to Eastern Europe, Asia and the island of Java. By 1 million years ago, this migration arrived in Western Europe. Evidence for this spread includes not only hominin fossils but also, eventually, evidence of more advanced stone tools, living sites with shelter and the use of fire, apparently for cooking or to keep warm. Scientists debate whether *Homo erectus* or some related species made its way into Western Europe and gave rise to the Neanderthals [12].

For many decades, the origin of our species, *Homo sapiens*, has been one of the most hotly debated issues in hominin paleontology. At center stage are the Neanderthals of Western Europe. Having been characterized as brutish, thick-boned, and primitive, most authorities now [12] assign them to their own species, *Homo neanderthalensis*, and relegate them to an evolutionary dead end. Attention then turned to the origin of the genus, *Homo*, in Africa, already home to the origin of our earliest ancestors, the australopithecines. The oldest fossils attributed to our species have been found in Africa and date

to more than 150,000 years ago, accompanied by sophisticated tools. This accords with the molecular data on our species. DNA changes incorporated into the genomes have been used for decades to look at the relationship of species to each other and to date when species split to form new species and clades. The same type of data is used by molecular anthropologists to date the origin of Primates and other taxa within Primates, such as the ape-hominin split to 6–8 million years ago. These data, sampled in modern humans from around the world, have been used to estimate time to most recent common ancestor of current populations of *Homo sapiens*. Studies have repeatedly given dates for the origin of those peopling the earth today of 100,000 to <200,000 years, based on mitochondrial and y chromosomal DNA. This suggests that those populations represented by the fossil record outside of Africa, assigned to *H. erectus* and Neanderthal in Europe and Asia, have contributed little to the genomes of modern peoples. However, at least some gene flow occurred since genomic studies done on reconstituted DNA from Neanderthal bone suggest they contributed around 2 % of the DNA in non-African modern humans [16]. A newly discovered fossil group from the same time period in Siberia, the Denisovans, probably dating from somewhere between 47,000 and 100,000 years ago, also contributed to the modern gene pool [17].

According to the replacement or "out of Africa" model, based on many genetic studies, anatomically modern *Homo sapiens* arose in Africa sometime between 150,000 and 200,000 years ago and quickly (in evolutionary terms) spread throughout the Old World, replacing the Neanderthals and any other hominin species they came in contact with. In Asia and Africa, for example, *Homo sapiens* would replace archaic human populations. An alternative view, the "multiregional" model, places the Neanderthals and their contemporaries in Asia and Africa, directly in the human evolutionary line [18]. In fact, proponents of the multiregional model include the Neanderthals in the human species and consider them ancestors of modern Europeans. Critical to this approach is the understanding that gene flow would have had to be sufficient throughout the species range to prevent the diversification necessary for separate species to arise.

We do have fossil evidence now showing that by 150,000 years ago nearly-modern humans had evolved in East Africa, and more modern fossils are found subsequent to this throughout Africa and eventually the Middle East. Molecular data have accumulated that are unequivocal on these two views of modern human evolution. Studies using mtDNA, inherited through the mother's line, show that the deepest node to a most recent common ancestor is in Africa and all other modern human populations have more recent common ancestors [19, 20]. Studies seeking the most recent common ancestor of modern humans have also involved the genes on the y chromosome, inherited through the father's line, and yield somewhat younger dates of 50,000–115,000, again with the deepest split occurring in Africa and other populations separating more recently [21–23]. A recent study analyzing both types of data found similar ages from the two types of molecular data, with a common ancestor dating from a range of 100,000 to about 150,000 years ago [24]. In summary, based on molecular data, evolution of *H. sapiens* from *H. erectus*-type ancestors seems to have occurred in Africa first by about 150,000–200,000 years ago, and subsequent populations spread rapidly around the globe, essentially replacing indigenous populations.

This evolutionary period also provides evidence of big-game hunting and the tools that could be used for killing game and preparing carcasses. From a more recent period, cave art depicting large game animals, indicating their importance to the earlier cultures have been discovered, and language, which would have been helpful if not critical in developing cooperative approaches to big-game hunting must have evolved. It was apparently these innovations that led to the rapid spread and success of *H. sapiens*. The addition of meat to the diet on a regular basis, and the addition of fire to the food-processing regimen, must have dramatically altered the human diet. Dentition steadily reduced in size over evolutionary time as stone tools replaced teeth as tools, and as the texture and type of foods required less vigorous mastication. It can be inferred that bone and mineral metabolism would also have changed in response to these changes in diet and activity.

1.4 Expansion from the Tropics to a Wide Range of Environments

Reconstructions of the physical environment of our earliest African ancestors 6–7 million years ago indicate that they lived in subtropical climates, probably in a mixture of grassland/savanna and woodlands. At first, they may have scavenged sources of meat protein and procured young animals to supplement their diet of roots, tubers, seeds, fruits, and other wild plants. There are nonhuman primate examples of this adaptation among baboons and chimpanzees, both of which have been observed to hunt opportunistically but otherwise subsist mainly on a wide variety of vegetarian food. It can be inferred from this likely diet that the earliest hominins consumed more calcium than modern humans, given the almost tenfold higher calcium content in a given unit of wild plant compared with wild game [25]. The dietary calcium intake levels presumably dropped as *Homo* developed hunting tools and skills and incorporated more meat in their diets.

This decrease in calcium content of the diet most likely accelerated as our ancestors migrated into the northern climates during the ice age, or Pleistocene, about 1.8 million years ago, and relied even more on hunting as a means of subsistence [12]. If studies of more recent Arctic populations (Inuit) are relevant to these Ice Age hunters, there is some evidence that a large meat component in the diet may contribute to bone loss; however, shorter average lifespans and higher activity levels may have helped to maintain bone density [26]. One possible factor in the relationship between high meat intake and lower skeletal calcium is the resorption of skeletal calcium to buffer the effect of the acid load contained in animal proteins [27]. Additionally, calcium may be bound in the kidney by sulfates and phosphates produced by protein metabolism [28]. While high-protein diets have been suggested to reduce calcium availability, the influence of high protein intake on bone mineral and bone metabolism is controversial [29]. For example, some studies show no differences in bone mass with high-protein diets [30, 31]. However, in modern populations, a cross-cultural association has been reported between higher intakes of dietary animal protein and higher hip fractures in an analysis of data from 16 countries [32]. The advantage of increased dietary protein was perhaps balanced by adverse effects on our prehistoric ancestors' skeletons by the increased reliance on game animals.

The transition to subarctic life and reliance on big-game hunting may have been accompanied by another factor affecting calcium metabolism—decreasing exposure to ultraviolet radiation in northern latitudes, where solar radiation is weaker. Exposure to sunlight may have been reduced even further by the need to wear heavy clothing. Presumably our earliest ancestors, exposed to high levels of ultraviolet radiation, had dark skin to protect them from the adverse effects of too much sun exposure. However, dark skin would have been maladaptive in northern latitudes, where ultraviolet radiation was weaker, and the colder climate required clothing. Under these conditions, it may have been impossible to make enough vitamin D in the skin to allow optimal calcium absorption from the gut—especially when dietary calcium intake decreased. Thus, it is assumed that for members of *Homo* who lived in northern regions, loss of melanin was selected for to provide adequate vitamin D production [33]. If so it is possible that this adaptation was sufficient in populations that rarely lived past middle age, but not adequate for individuals who lived long enough to experience the well-known degenerative effects of aging on the gut and on nutrient absorption in particular. In modern populations, a high prevalence of vitamin D deficiency has been recognized in older populations [34], and this may contribute to the risk of osteoporosis.

A finding in the Neanderthal genome adds a tantalizing aspect to this discussion. Neanderthals, occupying Europe during the coldest advances of the glaciers 40,000–200,000 years ago relied on hunting large game with a relatively sophisticated tool kit [12]. Recent breakthroughs in analyzing the genome of the Neanderthals have revealed an allele for the melanocortin 1 receptor (MC1R) that regulates pigmentation in vertebrates, including humans. This allele, found in two Neanderthals' DNA, discloses a receptor with reduced function which suggests that at least some Neanderthals possessed red hair and lighter skin pigmentation and that these earlier hominins reflected modern variation in skin color [35].

1.5 Transition from Hunting and Gathering to Food Production

1.5.1 Cultural Effects

Perhaps the most dramatic transition in the prehistory of the genus *Homo* was the shift from a hunting and gathering economy to one based primarily on plant domestication. Known as the Neolithic Demographic Transition (NDT) in the Old World, about 10,000–12,000 years ago in places as widespread as the Middle East, the Indian subcontinent, and China, human populations gave up their dependence on game and collected wild plant food and adopted agriculture. Soon after, evidence of settled village life appears in the archeological record. Similar events occurred more recently in North and South America. Several studies of past populations suggest that low bone mass was not a problem in human populations until the transition from hunting–gathering to food production [36]. Factors such as a high infant and childhood mortality rate and a high incidence of injury deaths contributed to the lower life expectancy among prehistoric, technologically simple societies relying on gathering and hunting wild foods. In contrast, in early agricultural societies, infectious disease became a significant factor in limiting life expectancy. Such conditions existed partly because of larger, more sedentary populations, increased interpersonal contact, the accumulation of garbage and contaminants, and the domestication of animals.

Various indicators of bone quantity and mass have been measured in skeletal remains of past populations, including cortical thickness, cortical area, bone mineral content, and histomorphometry. Studies of archeological populations are limited by the relative imprecision with which age, sex, and other relevant characteristics can be ascribed to individual skeletons. Reconstructions of past lifeways, including dietary adaptations and physical activity levels, are hindered by our assumptions, fragmentary data, and inherent methodological errors. Bone can also be modified by its burial environment, and such biological and chemical diagenetic changes can affect the reliability of analyses. This is of particular concern with studies that rely on the use of noninvasive methods such as absorptiometry to assess bone mass [37]. Furthermore, age- and sex-related changes in bone quality and its role in bone fragility in the past have not been widely considered in archeological populations [37]. However, with these caveats in mind, it is still possible to summarize some of the current knowledge gained from studies of bone maintenance in skeletal collections.

The NDT that occurred at the time of the transition to domestication of plants and animals seemed to be a continuation of the sedentary life-style that accompanied more intensive use of resources such as marine, especially fish, resources and wild grains and an increase in population. Sedentism and soft, grain-centered diets provided the basis for shortened periods of lactation, loss of the birth control afforded by extended periods of lactation and increased fertility in women. For instance pregnancy and lactation may result in increased caries from altered salivary constituents in women: the number of caries increased in women compared to men during the transition to farming suggesting increased number of pregnancies [38]. As Cohen points out, the increase in fertility may not have been passive; increased numbers provided increased defenses at a time when conflict and aggression was probably increasing. At the same time, the transition from gathering a variety of foods to growing a few main crops with lower nutritional value resulted in a rise in nutritional deficiencies [39]. The fact that cereals are a high-density food source and easily and safely stored, does not make up for the nutritional deficiencies [39]. Shortages of vitamin C may also have affected absorption of iron. The population explosion was accompanied by a higher death rate from diseases and nutritional stress [40].

Cohen and Armelagos [41] examined overall health as demonstrated by the archeological record and found deteriorating health in 19 of 21 studies. This picture was revisited in 2011 with a reevaluation of health in transitional societies [39]. Mummert et al. found again that in 19 of 21 studies, stature decreased with reliance on cereal-based farming. Skeletal robusticity results were more varied and seemed to be related to environmental factors [39]. In addition, Steckel and Rose [42] published

further results that support this contention from studies in the Old World and additional authors corroborated deteriorating health with agriculture in the New World. Cohen [38] reviews a number of studies that document decreases in stature or sexual dimorphism, increases in infection and increases in skeletal indicators of metabolic stress, such as porotic hyperostosis and cribraorbitalia indicative of iron deficiency anemia and scurvy, enamel hypoplasia and caries with farming. Not only are cereals devoid of specific minerals and vitamins, parasites such as hookworm spread in sedentary populations [38]. Domestic crops are not only vulnerable to diseases but to loss during storage leaving a population struggling or undergoing starvation until the next harvest. Not surprisingly, studies of prehistoric populations have found a lower bone mass among transitional agriculturalists compared to gatherer-hunters [36, 43].

Studies that found a relatively low bone mass in past populations implicate such factors as chronic malnutrition associated with early agricultural adaptations, such as in Nubia (approximately 350 BCE to 1450 CE) [44–46], and in eastern and southwestern North America (from 2000 BCE to the contact period) [36]. For example, Nelson [43] reported that hunter–gatherers from 6,000 years ago in the American midwest had thicker cortices, higher bone mass (measured by single-photon absorptiometry), and better maintenance of bone in late adulthood compared with maize agriculturalists from the same region several millennia later. Ericksen [47] also suggested that nutrition was an important determinant of bone loss in her comparative analysis of age-related changes in Eskimo, Pueblo, and Arikara archeological populations. The author found radiographically measured medial-lateral cortical thinning of the humerus and femur to be most pronounced in the Pueblo sample, which relied primarily on a cereal-based diet [47]. Ericksen [48] also found differences in bone remodeling (based on density of osteons per unit area) between groups that she suggests reflect dietary differences, as well as differences in physical activity. She specifically implicates the high-protein diet of the Eskimo, and the low-protein diet of the sedentary Pueblo, in her explanation of the differences in their remodeling parameters, and a subsequent study of intracortical remodeling by Richman et al. [49] of the same skeletal material supports these findings.

Low bone mass has also been reported for some Arctic groups with an unusually heavy intake of animal protein [26]. For example, an early comparison of long bone density in U.S. blacks, U.S. whites, and Sadlermiut Inuit (AD 1500–1900), found older Sadlermiut adults to have the earliest and highest loss of bone [50, 51]. Bone core studies of various archeological Inuit skeletons, when compared to U.S. whites, also show thinner cortices, lower bone mineral content, and increased secondary osteonal remodeling suggestive of an increase in intracortical porosity and subsequent bone loss [52–54].

In summary, low bone mass has been found in some past populations from a variety of geographic regions, representing either early agriculturalists or Arctic hunters. Clearly, these are not just the ancestors of groups currently considered to have the highest risk of osteoporosis [36], suggesting a significant contribution from environmental and/or cultural factors.

1.5.2 Dietary Calcium Intake in Evolutionary Perspective

It follows from the above discussion that the sources and amounts of dietary calcium (and other relevant nutrients) changed over the time period during which our human ancestors evolved. It has been estimated that the dietary intake of calcium in Paleolithic populations was at least 1,500 mg/day [25], which is two or three times more than the typical U.S. diet affords. However, calcium intake was only one factor affecting skeletal health over the course of human evolution. The interaction of this nutrient with other dietary components, physical activity levels, exposure to solar radiation, longevity, and general health must be considered in the context of the various biocultural environments in which people lived.

It is clear that a dramatic decline in dietary calcium occurred in our recent evolutionary past with the advent first of big-game hunting and then of agriculture [2]. Cultivated foods (grains) have a much lower calcium content than uncultivated plant foods [25]. Eaton and Nelson [25] reported that, on average, cereal grains contain 29 mg of calcium per 100 g of grain, compared with nearly 133 mg/100 g in uncultivated plant sources. Furthermore, grains generally have an undesirable calcium/phosphorus ratio, and may contain phytate (which binds to calcium and reduces its availability). In the modern world, there is a wider variety of foods available, and the dietary intake of nutrients varies widely. Data from the FAO Yearbook, 1990 [55], indicate that dietary calcium intakes in the late 1980s ranged from 300 to 500 mg/day in Asia, Africa, and Latin America to 900–1,000 mg/day in some North American and European populations. This continuum does not necessarily correspond with the prevalence of osteoporosis around the world. Cooper et al. [56] estimated that in 1990, half of all hip fractures worldwide occurred in North America and Europe, although this is expected to change as life expectancy increases in the developing countries.

Explanations for the apparent paradox that higher dietary calcium intake is associated with more hip fractures include higher protein intakes and poorer vitamin D status in Western countries [27]. Thus, it is clear that calcium intake must be considered within the context of other factors. Even within a population, subgroups may have differing dietary profiles. For example, nutrient patterns by tertiles of calcium intake were studied in a group of 957 men and women, ages 50–79, residing in a community in southern California [57]. In both men and women, intakes of protein, vitamin D, magnesium, and phosphorus were significantly higher in the high-calcium tertile [57], providing a complex of nutrients that might affect the skeleton differently from the other two groups. Other lifestyle factors such as physical activity would interact with dietary habits in their effect on the skeleton. Clearly, human behavioral and dietary plasticity have allowed our species to flourish in a wide range of environments, over a wide range of calcium intakes. The exquisite adaptation of the human species to solar sources of vitamin D throughout the world with darker pigmentation near the equator and depigmentation in northern latitudes in populations going back at least as far as Neanderthals in Europe, has been thwarted by recent advocacy of sun blockers for light-complected peoples. Calcium intake, as noted for prehistoric populations undergoing the NDT, also became problematic for those who depended upon high grain diets. Also, while darker skinned people often seem to have low levels of vitamin D they may use it more efficiently than lighter skinned populations, and have increased calcium and phosphate absorption [58]. This may be why many African Americans have low observed levels based on RDA, but they do not have signs of calcium deficiency, and have higher bone density, than Euro-Americans. Kleerekoper et al. [59] also found, from a sample of almost 400 women, that African American women had greater bone mass and lower rates of bone remodeling than women of European descent.

Inuit populations demonstrate further adaptations to year-round inadequate solar radiation for making sufficient vitamin D3, given the latitudes at which they live, and low dietary calcium levels. Despite their lower vitamin D levels, it is less clear that they suffer a deficiency [58]. Another possible reason that prehistoric Inuit skeletons or current populations do not demonstrate rickets or bone disease may be due to a genetic polymorphism in the vitamin D receptor gene, at the BsmI site, with the BB haplotype designating the absence of this site and bb its presence. For instance, a population study of Euro-American women showed only 17 % were homozygous BB [60, 61] but the b allele seemed to be associated with more efficient intestinal calcium absorption and considerably less osteoporosis.

Inuit children have been encouraged to increase calcium intake, although there have been suggestions that hypercalciuria has appeared clinically [62]. Therefore Sellers et al. [62] undertook a calcium load study in ten Inuit children. After administering calcium load, hypercalciuria was significantly more frequent in these children than in a Euro-Canadian control group, and post-load and urine calcium levels were highly elevated. Eight of the Inuit children were bb and two were Bb. Thus there is the possibility that the Inuit, while inhabiting a challenging environment and diet for bone health, may

be adapted to reduced solar and dietary sources of vitamin D. Thus we see in terms of the vitamins and calcium needed, until the NDT, evolution of humans resulted in adaptations that provided adequate levels of nutrients necessary for bone health. Since the NDT, human populations seem to show more variation in nutrition levels.

1.6 Change from Physically Active Lifestyles to Relative Sedentism

1.6.1 Physical Activity in Prehistoric Times

Human paleontological and anthropological studies have shown that bone strength relative to body size has declined in recent humans compared to our earlier ancestors. Our early human ancestors had significantly different skeletal morphology as compared to modern humans that continued to adapt with changes in subsistence. For example, femoral bone strength relative to body size (measured by the polar section modulus) has shown a steady decline over the last 2 million years in the genus *Homo* likely related to reduced physical activity alongside technological advancement [63].

As discussed above, the shift from a hunting and gathering economy to one based primarily on plant domestication in the Neolithic (about 10,000–12,000 years ago) was accompanied by an increase in sedentary lifestyle. As outlined, a number of studies confirm that early agricultural groups suffered increased levels of biological stress, poorer nutrition, and elevated levels of infectious disease [64]. The shift in subsistence strategies in modern humans only intensified the reduction in bone strength. While nutritional models are most commonly used to explain low bone mass in past populations, the role of physical activity, particularly the types and intensity of physical activity, are important factors as well [65].

Evidence from the measurement of bone geometry in archaeological populations has indicated a decline in bone strength with a sedentary agricultural lifestyle [66, 67]. For example, a study of femoral cross-sectional geometry in an Amerindian sample from the Georgia Coast spanning the 4,000 years from a hunting and gathering lifestyle to agriculture production, showed a decrease in cross-sectional size with time thought to reflect the less physically demanding lifestyle with agricultural [68]. However, another study of an early agricultural population from northwestern Alabama found cross-sectional strength to be greater in both sexes as compared to hunter gatherers, interpreted as indicating a more physically demanding lifestyle in this agriculturalist group [69]. Another study of skeletal geometric properties in early agricultural populations from the southeastern U.S. Atlantic coast (Florida) has revealed similar patterns [70]. These results emphasize that workload was likely still variable in agriculturalists depending on regional and local terrain. Further, it is uncertain how these adaptations to mechanical loading observed in cross-sectional geometry may have translated into bone loss or fragility in early agriculturalists. For example, Burr et al. [71] found both cortical endosteal bone loss in an agricultural archaeological sample from the Pecos Pueblo, New Mexico, along with patterns of cortical histomorphology suggestive of an active lifestyle. The authors suggest that endosteal bone loss could have been compensated for geometrically in overall shape and in osteon dimensions, so that structural strength and fatigue properties of the tissue were maintained [71].

1.6.2 Physical Activity in Historic Times

In modern populations it is well known that physical activity can play an important role in the risk of osteoporosis, affecting both the achievement of peak bone mass in young age and the subsequent rate of bone loss and deterioration of bone quality in later life. The reduction in habitual physical activity

in modern Western populations has been suggested as a primary explanation for the increasing incidence in osteoporotic fracture [72, 73].

Previous studies of bone mineral density in historic archaeological populations, have suggested that physically active lifestyles may have played a role in reducing bone loss and fragility. For example, a study by Lees et al. [74] of femoral bone density in female archeological remains from Spitalfields, England, dated between 1729 and 1852, found no evidence of premenopausal bone loss and less severe postmenopausal loss compared to modern females, which they suggest to be the result of physical activity and possibly unidentified environmental factors. Another study of bone mineral density by Ekenman et al. [75] of medieval skeletons from Stockholm, dated between 1300 and 1530 AD, found an absence of low bone density in older age groups, and a higher diaphyseal bone density in the lower extremities as compared to modern reference values, which they also suggest could be the result of environmental factors and physical demands, such as walking and standing.

However, studies of bone loss in a British medieval skeletal population, Wharram Percy, have found differing results. Studies of cortical bone mineral density in the femur [76] and radius [77] with dual-energy X-ray absorptiometry (DXA), and bone mass in the metacarpal with radiogrammetry [78], Mays found age related bone loss in both sexes. The authors of these studies have suggested that lifestyle factors such as rigorous agricultural activity in this rural medieval population were not sufficient in preventing bone loss. However a study of trabecular microarchitecture in the Wharram Percy sample found that while loss of trabecular structure and connectivity was seen in young age, no loss in trabecular structure was seen in old age in either sex. The reasons for these different patterns of bone loss at Wharram Percy are unclear, but could reflect differences in trabecular versus cortical tissue response and skeletal site [79]. It is interesting that this archaeological sample does not show a significant number of typical fragility-related fractures, and it is possible that physical activity could have been significant enough to prevent fracture despite some bone loss [77, 80].

1.7 Increase in Life Expectancy and Change in Reproductive Behavior

The expansion of the lifespan past reproductive age is a unique aspect of the human life cycle and is uncommon among wild nonhuman primates [81]. Data from living hunting–gathering groups studied in the past century indicate that life expectancy at birth in these groups was, on average, roughly 20–40 years—much shorter than among people living in technologically advanced modern cultures [82]. However, there is some evidence that early agricultural populations had a lower mean age at death than hunter–gatherers, although this may be related to higher birth rates and not higher mortality [83]. Some estimates suggest that the average lifespan has tripled since prehistoric times [84]. Rapid increases in life expectancy at birth that began in the early twentieth century were due largely to drops in mortality among infants and children [84]. In the case of females, life expectancy is related not only to infant mortality, but also to risks associated with childbirth. Furthermore, there is no reason to believe that human *longevity* has changed over time, and there is evidence that people did indeed live into old age, at least in historical periods [85]. Jackes [85] suggests that estimates of a 10 % survival beyond age 60 would actually be conservative, highlighting the demographic data of Russell [86], which notes that a number of individuals were expected to live beyond 60 across Europe and North Africa in the first 1500 years CE, and the work of Sjovold [87], who notes a significant number of deaths between the ages of 70 and 80 in an Austrian village in the 250 years prior to 1852.

Despite lower life expectancy in the past, age-related bone loss has been documented in many archaeological populations [16, 18]. However, it should also be noted that even in modern times, fracture risk is not tied exclusively to life expectancy. Today, there is a secular trend whereby the increment in the population over the age of 80 has and will continue to rise exponentially as compared to the overall population growth [72]. However, the change in demographics does not account entirely

for the present increased incidence of several types of fragility fracture. For example, Kanis [72] notes that hip fracture incidence in Oxford, England, doubled in the 27 years since the 1950s, and similar increases have been documented in other parts of the world. Clearly, life expectancy is not the only factor involved in the increasing incidence of osteoporosis.

It is interesting that despite the findings of low bone mass in some past populations, there is little evidence of osteoporotic fractures in most of these groups [37, 79]. The low prevalence of fragility fracture in archeological samples may in part be explained as the result of mortality bias. While the low prevalence of fragility fractures in some past populations may mean that fracture was rare compared to modern populations, it could also reflect heterogeneity in the oldest age groups, whereby the oldest individuals in skeletal samples may not be developing fragility fractures because they represent an overall "healthier stock" that managed to survive into old age. This is particularly important to consider when comparing old-age individuals in the past and the present, as present-day elderly individuals have benefited from modern medicine and may not be comparable to their historical counterparts.

Perhaps a more concerning problem with using archeological skeletal samples is age-at-death estimations [32–34, 85, 88, 89]. It is increasingly evident that while some humans in the historic past did likely manage to live into old age, we cannot accurately ascribe age to skeletons older than around 55 years of age. The conservative approach in osteological studies has been to assign only broad age groups with a final open-end age group of, for example, 45 or 50+, to skeletons. However, it has been suggested that if we cannot break down our age estimation after 50 into finer groups, we may not be able to adequately study the rates of degenerative or age-related conditions [85]. While this may hold true when looking exclusively at age-related bone loss and osteoporosis, certainly the use of broad age categories is still likely adequate to discern broad changes and patterns of bone maintenance in females that are related to menopause.

From an evolutionary perspective, the increasing prevalence of osteoporosis in modern populations suggests that osteoporosis either does not impact reproductive success (i.e., it is not subject to natural selection), or that the importance of some other, related characteristic was greater than the "cost" of age-related bone loss. Longevity itself may contribute to the reproductive success of individuals or populations, perhaps through the contribution of elders in a society. For example, Hawkes et al. [90] propose that older members of a population, and grandmothers in particular, make important contributions to the survival and reproductive success of their lineal descendants past their own reproductive years. Although bone fragility is a debilitating condition that could reduce some individuals' ability to help younger generations, it could be offset by the contributions of individuals unaffected by severe bone loss. Martin [91] has suggested a light skeleton with little excess mass may be an evolutionary hallmark advantage to the bipedal human body, with a trade-off that would leave little room for substantial bone loss before risk of fracture.

It is possible that the menopause-induced loss of estrogen puts the female skeleton at risk of bone fragility only under specific conditions, particularly those found in modern populations. This includes factors such as reduced physical activity. As discussed above, physical activity levels in past populations were almost certainly higher than those of modern populations, were probably high in both sexes, and were probably maintained at a relatively high level throughout the life span. It is possible that high levels of physical activity maintained sufficient bone quantity and quality throughout much of human evolution despite menopause-related bone loss. Once this level of activity was lost in recent human history, bone fragility would have become a problem in bone health. Fertility patterns and reproductive behaviors have also changed substantially in recent human populations, considerably enough to impact female bone health. In particular, high parity and prolonged breastfeeding would have been the norm through much of human evolution that would have provided women in the past with a very different hormonal milieu and steroid exposure as compared to modern women [80]. While pregnancy and lactation are high bone turnover states due to the nutritional demands of the fetus and child, the long-term effect of pregnancy and lactation on bone loss and fragility is not clearly understood.

While longitudinal studies indicate that bone loss can occur during initial lactation, there is substantial evidence that recovery of bone occurs with extended lactation and during weaning [92–96]. Several studies have suggested that low bone mass observed in young age females in the archaeological record are evidence of physiological stress due to pregnancy and/or breastfeeding [44, 46, 97]. However, it can be argued that the loss of bone in reproductive-age women in the past was transitory, and that bone loss during reproduction would have little or no effect on long term bone fragility in women that would have survived to old age [79, 97, 98]. In fact, high parity and prolonged breastfeeding in some past populations would have offered protection against the sudden postmenopausal drop of hormones experienced by modern women [80]. It seems likely that the dramatic change in both activity and reproductive patterns in modern Western women play a role in menopause-induced bone loss.

1.8 Conclusion

In our relatively short existence on Earth, our species has undergone dramatic changes in adaptation. These include worldwide expansion into diverse environments, the development of food production, changes in physical activity, reproductive behavior, fertility and life expectancy. All of these changes likely play substantial roles in the prevalence of bone loss and osteoporosis in modern populations. In evolutionary perspective, the advantages of many of these changes for our species must have outweighed the potential disadvantages. However, skeletal health in modern populations appears to be at increasingly greater risk from modern lifestyles and environments. An understanding of our evolutionary past can hold some important lessons and provide insight into safeguarding this aspect of health as we move into the new millennium.

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Chapter 2
Gene–Diet Interactions on Bone

Serge Ferrari and David Karasik

Positive health requires a knowledge of man's primary constitution and of the powers of various foods, both those natural to them and those resulting from human skill.

Hippocrates, 480 BC

Key Points

- Nutritional and genetic factors interact to influence bone modeling and mineral homeostasis in youth and influence bone remodeling and the maintenance of bone mass.
- Candidate gene and genome-wide association studies with bone mineral density and fractures exist.
- Candidate gene studies that investigated gene–dietary interactions in osteoporosis include VDR, ESR1, and IL-6 gene with vitamin D and/or calcium intake and PPAR and lipids intake.
- Few genome-wide association studies (GWAS) to date have incorporated gene nutrient interactions into the analysis design.
- More refined phenotypes than areal bone mineral density (aBMD) are required, with a focus on cellular and molecular processes in bones in response to nutrition.
- Genome-wide interaction studies (GWIS) can contribute to better bone health by proposing individualized Recommended Dietary Allowances (RDA) for various nutrients.

Keywords Genetics • Genome-wide associations • VDR • ESR1 • IL-6 • Phenotype • Bone mineral density • Interaction • Calcium • Vitamin D

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2.1 Introduction

Osteoporosis is a complex disease, with both environmental and genetic components. Twins and parents-offspring studies have demonstrated the heritability[1] of areal bone mineral density (aBMD) and bone mineral content (BMC), hip geometry, bone turnover, microarchitecture, and eventually fractures [1]. Hence additive genetic effects explain up to 80 % of the population variance for peak bone mass [2]. The influence of these genetic factors is expressed well before puberty and will determine both the tracking of bone mass throughout growth and the susceptibility for fractures later in life [3]. In simple terms, children of parents with high bone mass are more likely to have higher bone mass themselves—and therefore lesser risk of osteoporosis later in life—in the same way as children from tall parents are more likely to be tall (heritability for height is >80 %). However there is also a clear contribution of both the internal milieu (i.e., hormones) and external factors to peak bone mass acquisition and later bone loss, including the effects of diet, such as calcium and protein intake [4–7], exercise [8], and their interaction [9]. Thus, the major genetic determination of peak bone mass does not preclude nutrients to modify the "tracking" of bone mass during growth. In fact, there are clear suggestions that nutritional and genetic factors may interact to influence bone modeling, i.e., changes in bone mass and geometry, and mineral homeostasis during the years of peak bone mass acquisition [10, 11]. Likewise, gene–environment (G*E) interactions have been found to influence bone remodeling and the maintenance of bone mass [12–14].

From the point of view of quantitative genetic theory, G*E interaction means that the genotype–phenotype relationship can be dependent on the presence of environmental factors, and vice versa. Expression of genotypes varies if the genotypes are environmentally sensitive; as an outcome, penetrance of existing alleles and new mutations varies under different environmental conditions (for instance, susceptibility to lung cancer conferred by p53 alleles will mainly be expressed in presence of smoking). Thus the environment might contribute to both phenotypic heterogeneity and mask the true contribution of genetic variants to phenotypes, by mimicking genetic heterogeneity. In our case, children from parents with high bone mass would themselves acquire a high peak bone mass, provided the alleles related to a greater bone mass acquisition will be expressed in the adequate milieu; otherwise, they may not fully achieve their genetic potential.

At the population level, the association of genes with bone mass, respectively osteoporosis risk, may therefore be confounded if exposure to the permissive, or inhibitory, factor from the environment is heterogeneous, as is often the case between different cohorts, and even within cohorts. These considerations emphasize the need to study gene–environment, and particularly gene–nutritional interactions on bone. Unfortunately, this has rarely been done, the field being dominated by genetic association studies on one side, and epidemiological and intervention dietary studies on the other, with few interactions between the two.

2.2 Candidate Gene Association Studies

The actual number of genetic variants contributing to bone health and, conversely, osteoporosis risk, is currently unknown. It has been hypothesized that the determination of bone mass involves dozens of genes with relatively small additive effects, and a few genes with rather large effects [15, 16]. Segregation studies in populations defined by a homogeneous genetic background and environment had suggested that analytical models accounting for a major gene effect could be the most appropriate

[1] Heritability (h^2, %) is defined as the proportion of the total variance for a trait across the population that is attributable to the additive effects of genes.

to describe BMD heritability [17–19]. Early population-based association studies have mostly tested the relationship between BMD and/or bone turnover markers in unrelated individuals and polymorphic candidate genes coding for bone structural molecules [20], hormones and/or their receptors implicated in calcium/phosphate and bone metabolism, cytokines involved in bone remodeling and transcription factors [21].

Nearly 20 years ago, the first association study between single-nucleotide polymorphisms (SNPs) in the vitamin D receptor gene (VDR) and aBMD was published [22], paving the way for an intensive search of the genetic markers underlying the susceptibility to bone fragility. In addition to VDR, polymorphisms in the collagen 1 alpha 1 gene (COL1A1), estrogen receptor alpha gene (ESR1), and TGF beta (TGFB1) were also amongst the first and most extensively studied [2, 13, 23–26]. A few years later, linkage of three Mendelian skeletal disorders, i.e., the autosomal recessive syndrome of juvenile onset osteoporosis-pseudoglioma (OPPG), familial high bone mass (HBM), and autosomal dominant osteopetrosis Type I (ADOI) to chromosome 11q12-13 [27–29] led to the mapping of the LDL-receptor related protein 5 (*LRP5*) [30–32]. Since the 11q12–13 locus has also been linked to femur and spine BMD in pairs of healthy Caucasian-American sisters [33], as well as with hand BMD in Russians [34], the *LRP5* became a strong candidate gene for osteoporosis. The first studies indeed suggested an association of two missense polymorphisms, i.e., that modify the amino acid sequence and signaling function of the receptor [35], namely V667M and A1330T, with aBMD [36, 37].

Since then, association of ESR1 and LRP5 polymorphisms with aBMD and fractures has been confirmed through both meta-analyses of candidate gene studies including thousands of subjects from several cohorts [38, 39], and genome-wide association studies (GWAS, see below). However, a recent meta-analysis of GWAS cohorts which reappraised 150 previous candidate genes using 36,000 SNPs confirmed only nine genes associated with these traits [40]. Besides *ESR1*, most of these nine genes clustered in either of two major bone biology pathways, namely the RANKL/OPG/RANK pathway that is indispensable for osteoclastogenesis and bone resorption, and the WNT/LRP/β-catenin pathway that plays a central role in the control of bone formation and remodeling. In contrast, association of VDR, COL1A1, TGFB1, and many other genes with bone mass was not confirmed. On another side, VDR gene polymorphisms (3′-UTR *Bsm1* genotypes) have recently been associated with falls and muscle power in two large cohorts [41]. Reasons for inconsistent results, failure to replicate, and eventually controversies in candidate genes association studies are multiple: early studies used small and therefore underpowered cohorts, leading to false positive or negative associations; genetic and phenotypic heterogeneity between cohorts, particularly concerning the definition and validation of fragility fractures; limited knowledge and availability of functional SNPs, i.e., with a direct effect on gene expression and/or protein function; and deficient modeling for G*E interactions (see below).

2.3 Genome-Wide Association (and Interaction) Studies

In the last decade, the genetic exploration of human complex diseases has become more robust. Technical advances allowing for large-scale genotyping coupled with the computational ability to analyze vast amounts of the DNA polymorphisms facilitated broad study of the genome (typically analyzing at once hundreds of thousands of “common” SNPs) for association with disease and/or phenotype [42]. Proliferation of the genome-wide association studies (GWAS) offered an unbiased approach to identify new candidate genes for human diseases. GWAS is a mapping approach that relies on linkage disequilibrium (LD) between the single-nucleotide polymorphisms and the causal variants (still largely unknown). The GWAS approach has proved productive in uncovering multiple SNPs associated with complex diseases, including osteoporotic fractures [43].

Recently, GWAS in the bone field were spread beyond the traditional aBMD, into more refined bone phenotypes, such as volumetric BMD/microarchitecture [44, 45], subpopulations such as children [46] or premenopausal women [47], or using extreme phenotypes approaches, i.e., comparing extremes of the population with high and low bone mass for instance [48]. To date, GWAS of multiple osteoporosis-related phenotypes produced ~80 candidate loci. Most of the genetic variants in these genomic regions have been intronic or intergenic, i.e., without evidence of being a functional mutation. Moreover, several osteoporosis gene variants thus identified are in genes whose protein function on bone is not necessarily well known. Hence system genetics approaches to identify the related differences in gene and/or protein expression and function may be necessary to decipher and interpret these findings [49, 50].

Another observation from current GWAS is that top-associated polymorphisms might have strong marginal effects that propelled them to the top of the associated SNP set (p value for association $< 5 \times 10e{-}8$), because their effects are not environment-dependent. Good examples in the osteoporosis field are gene variants in the RANKL/RANK/OPG pathway of bone resorption and in the Wnt/β-catenin/LRPs bone forming and remodeling pathway [43, 51]. Which genetic variants should be expected to interact with the environment, and therefore, should be taken into account, is still an unresolved question. Thus a genome-wide interaction study, which models interaction of genome-wide polymorphisms with a covariate, was rarely done. One example is that of Liu et al. [52], in which genome-wide SNP interactions with sex were studied for lumbar spine (LS) and femoral neck (FN) BMD. Despite the large meta-analytical effort (25,353 individuals from 8 Caucasian cohorts were included), no genome-wide significant evidence for gene-by-sex interaction was found to influence BMD variation in that screen, pointing out that even larger samples are required to pinpoint genome-wide interaction. Here to note, sex is a better-defined "environment" than many dietary exposures, which would need a lot of homogenization efforts between the cohorts. Nevertheless, there have been some advances in the field of gene-by-diet interactions outside of osteoporosis, such as a genome-wide gene-by-coffee environment study that identified glutamate receptor gene GRIN2A to contribute to Parkinson's disease [53]. In a stratified sample, the GRIN2A signal was present in "heavy" coffee drinkers but not in "light" coffee drinkers. Another attempt was done by the Women's Health Initiative, where G*E interaction was found within a cohort of African-American women for dietary energy intake and SNP rs9557704, with waist-to-hip circumference ratio as outcome [54]. As in the above study [53], no results exceeded the Bonferroni-corrected statistical significance threshold, again indicating a need for large powered designs to discover G*E effects in genome-wide interaction study.

2.4 Gene–Dietary Interactions in Humans

A comprehensive evaluation of gene–dietary interactions in chronic diseases ideally would combine the power of large-scale GWAS and the standardized evaluation of multiple environmental factors in the participating cohorts. However there are several difficulties that have prevented GWAS–environment interaction analyses to be successfully performed so far (with a few exceptions). Common issues are the lack of common tools used to assess environmental data in different cohorts, as well as the potentially different level of exposure between cohorts (e.g., due to geographical, cultural, religious, and socioeconomic patterns). Second, the sample size that would be required increases considerably when genome-wide interactions have to be considered. Eventually, the development of statistical tools to perform such hypothesis-free screens is lagging behind. For these reasons, most available data on gene–dietary interactions still come from candidate gene approaches, as is expanded below.

2.4.1 VDR Gene Polymorphisms and Calcium and Vitamin D Intake

Prior to the era of the human genome project and the systematic identification and annotation of reference SNPs (rs numbers), genotyping was commonly performed using DNA restriction enzymes recognizing a specific nucleotide sequence allowing them to cut, or not cut, at the polymorphic site, thus generating DNA fragments of different lengths allowing for the allele to be identified. Hence, 20 years ago, the first VDR gene polymorphisms to be used for association studies with osteoporosis traits were characterized thanks to *Bsm1*, *Apa1*, and *Taq1* enzymes [55].

Consistent with the role of $1,25OH_2D_3$ (calcitriol) in regulating intestinal calcium transport, a number of small studies have suggested that VDR polymorphisms may influence calcium absorption, parathyroid hormone (PTH) levels, and the skeletal response to calcium intake [56]. Thus, in postmenopausal women following a calcium restriction period, fractional absorption of calcium increased significantly less in women with the *Bsm1* genotype BB compared to bb (in the VDR 3′-untranslated region, UTR), despite a trend for higher calcitriol levels among the former [57]. In another study, radiocalcium absorption was higher in the bbaaTT haplotype [58]. Several other studies confirmed an association of VDR variants with intestinal calcium absorption (reviewed in [59]). In healthy young men, serum PTH levels were significantly higher in the BB genotype at baseline and remained so under either a low or a high calcium-phosphorus diet. Moreover, on the low calcium-phosphorus diet, BB subjects had significantly decreased tubular phosphate (Pi) reabsorption (TmPi) and plasma Pi levels, consistent with the biological activity of higher PTH levels [60]. These observations suggest a decreased intestinal calcium absorption and/or a lesser sensitivity to $1,25OH_2D_3$ in the intestine and parathyroid glands of subjects carrying the VDR BB genotype. Several studies also found an association between VDR polymorphisms and serum PTH levels in patients with chronic renal failure, kidney transplants, and primary hyperparathyroidism [61–65]. Another study comparing the distribution of VDR genotypes in 105 rachitic children from Nigeria with very low calcium intake (200 mg/day) and 94 healthy controls found that the VDR *Fok1* ff genotype was significantly underrepresented among cases [66], suggesting that this genotype might improve intestinal calcium absorption and thereby be protective against osteomalacia induced by dietary calcium deficiency. Similar results have recently been reported in rachitic children from Egypt and Turkey [67]. In contrast, in 72 Caucasian, African-American, and Mexican children with adequate calcium intake (above 1,000 mg/day), Ames et al. found that carriers of the VDR genotype ff had significantly decreased intestinal calcium absorption [68], which attests in favor of the interaction.

Dietary calcium intake and/or supplements have also been suggested to modulate the association of VDR polymorphisms with BMD, and conversely, VDR genotypes to modulate the effects of calcium intake on BMD. In a cohort of 144 prepubertal girls, baseline BMD at lumbar spine and femur neck was significantly lower in subjects with VDR *Bsm1* BB genotype compared to the other genotypes. BMD gain in response to calcium supplements (850 mg/day) for 1 year was increased among BB and Bb, whereas it remained apparently unaffected in bb girls, who had a trend for spontaneously higher BMD accumulation on their usual calcium diet [69]. A similar interaction between VDR *Bsm1* alleles and calcium intake on femur neck BMD was reported in a cross-sectional study of premenopausal Caucasian women [70]. A more recent study also found that the association between milk intake and bone mass accrual in 117 peri- and post-menarcheal girls was influenced by VDR promoter genotypes [71]. A significant interaction between VDR-3′ genotypes and calcium intake on BMD and BMD changes has also been shown in postmenopausal women by a number of investigators [72–75]. In a pioneering study including elderly subjects (90 % women, mean age, 73 years) with a high prevalence of osteoporosis and a low calcium intake (590 mg/day), bb subjects did apparently not loose bone at the lumbar spine over 18 months, whereas spine BMD decreased 2 % in heterozygotes and BB during this time [72]. Calcium supplements (800 mg/day) reversed bone loss in Bb subjects after 18 months, but

did not significantly alter BMD changes among the other genotypes. Another prospective study in younger postmenopausal women (mean age, 59 years) whose mean calcium intake was very low (400 mg/day) also found that lumbar spine and hip bone loss was significantly higher in BB subjects. In subjects receiving calcium supplements (500 mg/day) however, bone mass changes were similar in all genotypic groups, indicating that the response to calcium was actually greater among BB [73]. Similar to the previous two studies, a long-term follow-up (6.3 years) study in postmenopausal women (mean age 69 years) reported that among individuals with low calcium intake (below 456 mg/day), TT homozygotes for VDR *Taq 1* polymorphism (same as bb) had a significantly lower rate of bone loss at both the femoral neck and lumbar spine compared to tt (same as BB). In contrast, among those with a higher dietary calcium intake (above 705 mg/day), there were no more significant differences in BMD changes between genotypes [76]. A recent study in 578 Greek postmenopausal women confirmed that all VDR polymorphisms were associated with lumbar spine BMD in the low calcium intake group (<680 mg/day), but not in women with higher calcium intake [77] (Table 2.1). In addition, the Nurses Health Study (mean age 60 years) reported that the relative risk of hip and wrist fractures was significantly higher in BB compared to bb in a subgroup with calcium intake below 1,078 mg/day (odds ratio, 4.3), but not in the subgroup with higher calcium intake (odds = 1) [78]. On the contrary, in the Framingham Osteoporosis cohort, among men and women aged 69–90 years, BMD at the femur trochanter and ultra-distal radius (two regions rich in cancellous bone) was significantly higher in bb compared to BB in subjects whose calcium intake was greater, but not lower, than 800 mg/day [75].

Several studies suggest an interaction between vitamin D intake and/or supplements and VDR polymorphisms on BMD. The first one reported a 4 % BMD gain over 2 years with vitamin D supplements (400 IU/day) in a small group of elderly women with BB and Bb genotypes, but not bb [79]. In 230 men aged 41–76 years, the positive association between vitamin D intake and BMD at all sites was more pronounced in the subgroup with long polyA repeat polymorphisms [80]. In 221 Danish girls aged 11–12 years receiving vitamin D_3 supplements for 12 months, whole body BMD and BMC increased significantly among VDR *Fok1* FF subjects, but not among Ff or ff [81]. Similarly, in 179 Lebanese adolescent girls, VDR gene polymorphisms were associated with percent changes in bone area, BMC, and BMD at multiple skeletal sites after 1 year in the vitamin D_3 group but not in the placebo group [82]. The least increments were observed in the BB and tt genotypes. In addition, in a 3-year randomized study comparing calcitriol (0.25 μg/day) + calcium (1 g/day) to calcium alone, the addition of calcitriol further reduced vertebral fractures incidence among subjects with the VDR B allele, but not among bb [83]. Furthermore, studies from Japan indicate an interaction between VDR genotypes and BMD response to alfacalcidol [84, 85]. However, in the Study of Osteoporotic Fractures (SOF), a cohort of nearly 10,000 postmenopausal women with 10 year and more follow-up, no association between VDR polymorphisms and incident fractures was found before or after stratification by dietary calcium intake, use of calcium supplements, and use of vitamin D supplements [86].

Altogether, these studies illustrate that ignoring the interaction between VDR gene polymorphisms, calcium intake and vitamin D may mask a true association of these genotypes with BMD and osteoporosis. On another side, their rather small sample size and variable results still prevent firm conclusions to be drawn about the influence of VDR polymorphisms on the effects of calcium intake or vitamin D supplements on bone mass gain or loss.

2.4.2 Other Gene Interactions with Calcium and Vitamin D

Numerous large-scale candidate gene studies and GWAS have confirmed the association of ERα gene (ESR1) polymorphisms with BMD and fractures (see above). These studies however have not examined the possibility of an interaction with the environment, whereas some candidate gene studies have done so. In 313 late postmenopausal women with a low average calcium intake (approximately

Table 2.1 Recent studies of G*E interactions with osteoporosis-related phenotypes in humans

Gene	Tested marker(s)	Nutrient	Sample: Age, sex, ethnic composition	Phenotype	Main effect: Odds ratio[a], *P* value**	Interaction	Reference
APOE	Alleles *ε2*/*ε3*/*ε4*	Vitamin K1 intake	Scottish women, early postmenopausal ($n=2,721$)	LS BMD; FN BMD; BMD change	$P \leq 0.05$ for LS BMD and change in LS BMD	No interaction	[124]
ESR1	4 SNPs, incl. rs2077647 and rs2234693	Alcohol drinking	Japanese women (cases: 114 postmenopausal with a confirmed osteoporosis) and controls: 171 healthy (mean age of 39.0 years)	Osteoporosis risk	OR = 3.15, 95 % CI = 1.83–5.41 (Haplotype of 2 SNPs)	$P=0.03$	[125]
IL6	–634C/G	Calcium intake	Chinese girls ($n=176$, aged 9–11 years)	BMD and BMC at total body, total hip and FN	<0.05	$P<0.05$	[126]
LRP5	4 SNPs (incl. rs4988321)	Calcium intake	Greek postmenopausal women ($n=578$); stratified by calcium intake	LS & hip BMD Osteoporotic Fx	rs4988321 with LS BMD ($P=0.002$)	$P=0.016$	[127]
MTHFR	rs1801133 (C677T)	Riboflavin intake	Dutch men and women ($n=5,035$, age 55+ years old)	Fracture	n.s.	$P=0.0002$	[108]
MTHFR	rs1801133 (C677T)	Maternal folate and B6 intake	U.K. Caucasian children (9.9 years old, $n=5,816$)	BMD: total body less head region ($N=5,816$), lumbar spine ($N=3,196$)	$P<0.001$ (spine BMD)	$P=0.1$ (spine BMD)	[109]
PPARG	13 SNPs (incl. rs2028760, rs1801282, rs1805192)	Dietary fat intake	US Caucasian men ($n=867$, 62.2 ± 9.1 years) and women ($n=925$, 60.5 ± 9.1 years)	FN, TR, and LS BMD	$P<0.05$ (mostly in men for FN)	$P<0.01$ (mostly in men for FN)	[115]

(continued)

Table 2.1 (continued)

Gene	Tested marker(s)	Nutrient	Sample: Age, sex, ethnic composition	Phenotype	Main effect: Odds ratio[a], *P* value**	Interaction	Reference
PPARG	10 SNPs (incl. rs12497191, rs4135263, rs1151999, and rs1152003)	Polyunsaturated fatty acids (PUFA) intake	Two Danish cohorts: a case-control ($n=809$) and perimenopausal ($n=1,716$) women allocated to hormone therapy or not at baseline and followed for 10 years	Vertebral Fx; LS and FN BMD	VertFx risk (OR = 1.48–1.76, $P=0.005$–0.04 for rs12497191, rs4135263, and rs1151999) increased BMD ($P \leq 0.02$ for rs1151999)	$P<0.03$ (with rs1151999 and BMD)	[114]
VDR	BsmI, TaqI, and Cdx-2	Calcium intake; low threshold = 680 mg/day	Greek postmenopausal women ($n=578$)	LS and hip BMD Osteoporotic Fx	n.s.	In low calcium intake group, all variants were associated with LS BMD ($P<0.05$)	[77]

Abbreviations: *BMC* bone mineral content, *BMD* bone mineral density, *CI* confidence interval, *FN* femoral neck, *Fx* fracture, *LS* lumbar spine, *PUFA* polyunsaturated fatty acid, *TR* trochanter

**Statistical significance: when there is more than one phenotype or SNP, lowest *P* value is provided. *n.s.* nonsignificant

[a]Odds ratio [OR] and 95 % confidence interval [CI]

600 mg/day), including 142 women with a history of osteoporotic fractures, no significant association was found between ESR1 polymorphisms alone or in combination with VDR polymorphisms on BMD, nor on biochemical markers of bone and mineral metabolism [87]. Furthermore, in 484 Japanese women aged 24–80 years old, ESR1 polymorphisms did not seem to influence the relationship between calcium intake and BMD changes over up to 5 years [88]. In contrast, in early postmenopausal women enrolled in a 5-year prospective study of hormone replacement therapy (HRT) or placebo in addition to calcium and vitamin D, there were no significant differences in BMD among ESR1 genotypes at baseline, but significant differences in lumbar spine BMD changes between *PvuII* intronic genotypes PP (−6.4 %) and pp (−2.9 %) in the absence of HRT, suggesting an interaction between calcium-vitamin D supplements and ESR1 on bone loss [89]. However, these differences were no more apparent in women receiving HRT. In a small prospective study of calcium supplementation in Chinese girls segregated by ESR1 *PvuII* genotypes, the 1 year percentage change in 1/3 radius BMD was greater among PP than other genotypes [90].

IL-6 gene codes for an inflammatory cytokine that is implicated in the activation of osteoclasts and inhibition of bone formation by osteoblasts. IL-6 expression in bone is triggered by estrogen deficiency and parathyroid hormone, and is thereby implicated in postmenopausal bone loss [91, 92]. A common −174G>C polymorphism (frequency of the C allele is ~0.4 among Caucasians) and a rare −573G>C polymorphism (frequency of the C allele is ~0.06 among Caucasians) in the IL-6 promoter region have been shown to influence the level of IL-6 gene transcription in vitro [93, 94]. Accordingly, serum levels of IL-6 are approx. 50 % lower in −174CC compared to −174GG [93], whereas both the −174C and −573G alleles are associated with significantly lower levels of C-reactive protein (CRP) and C-terminal cross-links of Type 1 collagen (CTx)—a marker of bone resorption—in postmenopausal women [94, 95]. Moreover, BMD at various skeletal sites decreased more in older vs. younger postmenopausal women with the −174G allele than in those without it (−9 % to −10 % in GG and GC vs. −5 to −6.1 % in CC) [95]. Another group specifically studied the rate of decline in hip BMD with the *IL-6* 174GC polymorphism [96]. Compared to women with the *GG* phenotype, women having the *CC* genotype had slower rates of bone loss in the total hip and femoral neck in ~3.5 years of follow-up and 33 % lower risk of wrist fractures over an average of 10.8 years [96]. Two studies also reported an association of IL-6 polymorphisms with peak bone mass, one in young males [97] and the other in premenopausal females [98]. However, the latter study did not find a lower rate of bone resorption or bone loss associated with the −174CC genotype in 234 postmenopausal women (mean age, 64 years).

The interaction between IL-6 promoter polymorphisms and factors known to affect bone turnover, namely years since menopause, estrogen status, physical activity, smoking, dietary calcium, vitamin D, and alcohol intake, was examined in the Offspring Cohort of the Framingham Heart Study [99]. This cohort comprised 1,574 unrelated men and women (mean age 60 years) with bone mineral density measurements at the hip. In models that considered only the main effects of IL-6 polymorphisms, no significant association with BMD was observed in either women or men. In contrast, interactions were found between IL-6 −174 genotypes and years since menopause, estrogen status, dietary calcium, and vitamin D intake in women. Thus, BMD was significantly lower with genotype −174 GG compared to CC, and intermediate with GC, in women above 15 years past menopause, in those without estrogens or with calcium intake below 940 mg/day. In estrogen-deficient women with poor calcium intake, hip BMD differences between IL-6 −174 genotypes CC and GG were as high as 16 %. In contrast, no such interactions were observed in men. These data therefore suggest that sex, age, HRT, and dietary calcium all influence the association between IL-6 alleles and bone mass. They also perfectly illustrate the fact that ignoring G*E interactions may prevent true associations from being detected, thereby potentially explaining the absence of signals at the IL-6 locus in osteoporosis GWAS (see above) [40].

2.4.3 MTHFR and Folate

The enzyme methylenetetrahydrofolate reductase (MTHFR) synthesizes 5-methyltetrahydrofolate, which is the main circulating form of folate and the methyl donor for the conversion of homocysteine (Hcy) into methionine. Hcy interferes with collagen cross-linking and elevated plasma Hcy is associated with a two- to fourfold increased risk of hip fracture [100, 101]. Several observational studies also suggest that low blood concentrations of folate and vitamin B_{12} may be associated with decreased BMD and an increased risk of fracture [102]. In turn, polymorphisms in MTHFR may be important determinants of Hcy levels and osteoporosis risk, and may be further determined by interactions between MTHFR and dietary folate, vitamins B12 and B6 (Table 2.1).

A recent meta-analysis of 20 studies including more than 20,000 cases and controls has confirmed a 23 % increase in fracture risk in both gender with the MTHFR 677TT genotype [103]. The effect was predominant on vertebral fractures and in subjects younger than 60 years. A modest association with BMD at all sites was also found. A low marker density (<100,000 SNPs) GWAS in the Framingham cohort also identified the MTHFR locus, among others, to be associated with BMD [104]. In this cohort, an interaction between MTHFR genotypes and plasma folate levels on hip BMD was also reported [105]. A significant interaction among quartile of energy-adjusted riboflavin (B2) intake, MTHFR 'TT' genotype, and femur neck BMD has also been found in another cohort [106]. In a cohort of 1,223 elderly women, the MTHFR C677T polymorphism was not directly associated with plasma Hcy levels, but an interaction was present with folate and riboflavin intakes, such that those with a TT genotype and low dietary folate or low dietary riboflavin intake had higher homocysteine concentrations [107]. In this study, the 5-year hip BMD loss was significantly greater in the highest tertile of Hcy, but showed no association with MTHFR polymorphisms nor an interaction between those and folate intake. In the Rotterdam study of ~5,000 individuals aged more than 55 years, MTHFR C677T alleles were not associated with fracture risk overall, but in the low riboflavin intake group, TT had a 22 % increase in Hcy levels and a twofold increase in fracture rate [108]. Eventually, in a large cohort of 10-year-old boys and girls (ALSPAC), the association between MTHFR genotype and spine BMD was attenuated particularly in girls by high maternal dietary intakes of vitamin B6 (pyridoxine) and folate during pregnancy but not by child's dietary intakes at 7 years of age [109].

2.4.4 PPAR Gamma and Lipids Intake

PPAR-γ is a key transcription factor to regulate the fate of bone marrow mesenchymal stem cells (MSCs) into adipocytes rather than osteoblasts, and is also playing a role in osteoclastogenesis [110, 111]. Increased expression as well as activation (for instance with glitazones) of PPAR-γ has been shown to induce bone loss and fragility. Ogawa and colleagues first reported that a silent C>T transition located in exon 6 of the PPAR-γ gene was associated with total body BMD, but not bone turnover markers, in 404 healthy postmenopausal Japanese women [112]. In 2005, Rhee and colleagues attempted to replicate findings for this same polymorphism in 263 healthy Korean women ages 37–73, but were unable to demonstrate any differences in BMD of the spine or hip across genotype groups [113]. They did however report a significantly higher serum osteoprotegerin level among the major allele homozygotes compared to the combined heterozygotes plus minor allele homozygotes. A recent study in two Danish cohorts including men and postmenopausal women with and without HRT found a significant association of several PPAR-γ polymorphisms with vertebral fractures, although curiously the risk was higher among heterozygotes only [114] (Table 2.1). An association with BMD was also seen, but was different in subjects with high or low body weight and among the two cohorts. In

one of the cohorts, an interaction between a distinct PPAR-γ polymorphism and polyunsaturated fatty acids (PUFA) intake on BMD was suggested.

In a subset of unrelated men and women from the Framingham Offspring Cohort, 13 SNPs in PPAR-γ were examined for sex-specific interaction with percent of energy intake from total fat on aBMD of the femoral neck (Table 2.1). Significant interactions were found between several SNPs and fat intake in both men and women [115]. Similar results were observed for aBMD of the trochanter and the lumbar spine. For example, compared to men on a low-fat diet, men on a high-fat diet had lower BMD of the femoral neck when homozygous for the C allele for a given SNP, but a higher BMD of the femoral neck when homozygous for the A allele at this same SNP.

2.5 Conclusions

Genetically speaking, humans today live in a nutritional environment that markedly differs from that for which our genetic constitution was selected [116]. Thus, gene polymorphisms which appeared a few tens of thousands of years ago in the human genome as an adaptation to a changing environment (Hunter-Gatherer, then Agricultural), but have become *mis*-adapted to our current nutritional and lifestyle habits, may in turn contribute to a number of common disorders including osteoporosis [117], but also diabetes, hypertension, and other "diseases of civilization," After 20 years of research in the field of osteoporosis genetics, our knowledge about the gene variants underlying susceptibility to the disease remains trivial. Indeed, the additive effects of currently identified genetic variations on aBMD remain less than 6 % of the total population variance for this trait, which is far from the 60–80 % heritability predicted by family studies (see Introduction). Where is then the missing heritability for osteoporosis? It probably lies in expressed polymorphisms (eSNPs), i.e., SNPs associated with differential mRNA expression levels [118]; low frequency (<<1 %) alleles, which by definition have been segregated out of the population because they carried a survival disadvantage for the human species (hence a larger effect size of the allele would be expected compared to a more common allele); in genetic variants located in noncoding, but conserved, potentially regulatory regions of our genome [119]; copy-number variations, and in epigenetic modifications of DNA that influence gene expression patterns.[2] Continuous progresses in technological developments have started to allow the analysis of such genetic variations and it is therefore expected that more of the additive genetic effects on bone mass and fracture risk will be uncovered in the next few years. Nevertheless, the study of G*E interactions remains crucial, as illustrated by recent studies of gene–dietary interactions in osteoporosis (Table 2.1).

Despite this evidence, few GWAS to date have incorporated G*E interactions into the analysis structure [121, 122] and this is primarily due to the challenges associated with such an approach. The statistical power is usually low, and the analytical methodology is underdeveloped or cumbersome. One of the major limitations of such studies is the relative imprecision of dietary intake estimates by any questionnaire, with poor reproducibility within individuals and large variation within and between populations and across time. Biomarkers stemming out of proteomic, metabolomic, and nutrigenomic analyses are being developed, which could provide more reliable insights into gene–dietary interactions [123].

Animal models can also potentially help to understand the gene–diet interactions mechanistically, as shown for dietary fat interactions with PPAR-γ [115]. Also, more refined phenotypes than aBMD are required, with a focus on cellular and molecular processes in bones in response to nutrition. In the future, advances in the osteoporosis genetics field may allow for individualized Recommended Dietary Allowances (RDA) for various nutrients, including calcium and folate, and for targeted interventions aiming at improving lifestyle factors for better bone health.

[2] Genome-wide methylation arrays are now available (see reference [120]).

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Chapter 3
Bone Physiology: Bone Cells, Modeling, and Remodeling

David W. Dempster and Lawrence G. Raisz

Key Points

- The skeleton undergoes constant change through the processes of modeling and remodeling.
- Modeling is defined as change in the size or shape of skeletal elements that occurs when formation and resorption are independent e.g., during growth or in an adaptive response to a change in mechanical loading.
- Remodeling is defined as resorption and formation that occur essentially at the same site and in a programmed manner in which formation is temporally and spatially coupled to resorption.
- Modeling and remodeling are achieved by the concerted action of osteoclasts and osteoblasts, either working independently (modeling) or in sequence (remodeling). Both processes are regulated by osteocytes.
- Osteocytes also act as mechanosensors, contribute to calcium homeostasis and regulate serum phosphate by secretion of fibroblast growth factor 23.
- Bone resorption and formation are regulated a host of endocrine and paracrine factors, including, but not limited to, parathyroid hormone, vitamin D, insulin-like growth factor, receptor activator of nuclear factor kappa B, and sclerostin.
- Good nutrition (calcium, magnesium, vitamin D, protein, etc.) is essential for skeletal well-being during growth, adulthood and, particularly, in older age.

Keywords Bone remodeling • Modeling • Resorption • Formation • Osteoclast • Osteoblast • Osteocyte

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3.1 Introduction

There has been a remarkable expansion of the understanding of growth and remodeling of the skeleton based on advances in cell and molecular biology and genetics, as well as identification of the many local and systemic factors that regulate bone cell function. Despite this, there remain many unanswered questions concerning the regulation of these processes. This chapter focuses on the interplay of local and systemic factors in bone remodeling, particularly those that are most likely to be affected by changes in nutritional state. For a recent review of this topic, see Ref. [1].

3.2 Skeletal Development

The skeleton is initially formed by a series of programmed mesenchymal condensations followed by formation of a cartilaginous template for all of the major skeletal structures. The conversion of the cartilaginous template to bone involves either endochondral bone formation in which the cartilage is initially calcified or membranous bone formation in which the bone cells arise adjacent to, but separate from, the cartilage anlage, which does not calcify but simply is resorbed [2].

Many genes have been identified that act early in embryonic development to determine the specific form of skeletal structures. Remarkably, there is a single transcriptional pathway that is critical for the conversion of the cartilaginous to a bony skeleton. The transcription factors RUNX-2/Cbfa-1 and a downstream factor, Osterix, are apparently necessary and sufficient for mesenchymal precursors to differentiate into osteoblastic cells [3, 4]. The knockout of either of these factors results in a failure to form a bony skeleton, whereas in humans heterozygous for RUNX-2 there is a specific pattern of deformities termed cleido-cranial dysplasia [5]. The shape of a skeleton is determined genetically, but the size is determined by local growth factors, particularly insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) and their receptors and binding proteins. Thus, knockouts of IGF-1 or IGF-2 result in decreased skeletal size with an extreme decrease occurring when both genes are deleted [6, 7]. Growth hormone (GH)-dependent IGF secretion is important in early postnatal growth, but appears to be less important in prenatal growth when local IGF production, independent of GH, predominates. Maternal imprinting of the IGF-2 scavenger receptor limits skeletal growth, while paternal imprinting of IGF-2 enhances growth [8, 9].

Other factors may have both stimulatory and inhibitory effect on skeletal size. For example, fibroblast growth factor (FGF) can activate a specific receptor, FGF-3, to produce cessation of cartilage growth [10]. Activating mutations of this receptor result in dwarfism. Cartilage growth and differentiation is under complex local regulation by parathyroid hormone-related peptide (PTHRP) and genes involved in local signaling such as Indian hedgehog (Ihh) [11]. Insulin also acts on the IGF receptors and increased glucose-stimulated insulin production in fetuses of mothers with poorly controlled diabetes may be responsible for their increased birth weight [12].

3.3 Modeling and Remodeling

The skeleton undergoes constant change through the processes of modeling and remodeling. Modeling is defined as change in the size or shape of skeletal elements that occurs when formation and resorption are independent—for example, the enlargement of long bones by periosteal apposition and endosteal resorption. Remodeling is defined as resorption and formation that occur essentially at the same site. Remodeling may either be in balance, that is, the amount of new bone formed at the site is equal

to the amount previously resorbed, or there may be remodeling imbalance with either gain or loss of bone because the amount of new formed is greater or less than the amount resorbed. Both modeling and remodeling are subject to systemic and local regulation. Trabecular bone remodeling is probably the predominant mechanism for fulfilling the homeostatic role of the calcium-regulating hormones, whereas cortical bone remodeling is probably of particular importance in the response to loading, the repair of microdamage, and maintenance of cell viability. Although modeling is greatest during skeletal growth, it continues throughout life, largely in response to mechanical forces. For example, increased strain due to weakening of the skeleton by endosteal resorption can produce new periosteal apposition even late in life. Recently, modeling has been shown to occur in response to anabolic agents used in the treatment of osteoporosis [13, 14].

3.4 The Bone Remodeling Cycle

The remodeling cycle involves a series of linked cellular events that has been turned the bone multicellular unit (BMU) [15]. Bone remodeling occurs on trabecular surfaces in the form of shallow irregular Howship's lacunae, whereas in cortical bone it occurs as a cylinder to form the Haversian canal, which is then replaced to form an osteon. There are four phases: activation, resorption, reversal, and formation (Fig. 3.1). Histological studies suggest that the BMU is compartmentalized, that is, there are lining cells that separate the BMU from the rest of the marrow space [16, 17]. This allows for a substantially different local milieu for BMU, with levels of local factors and ions that differ from

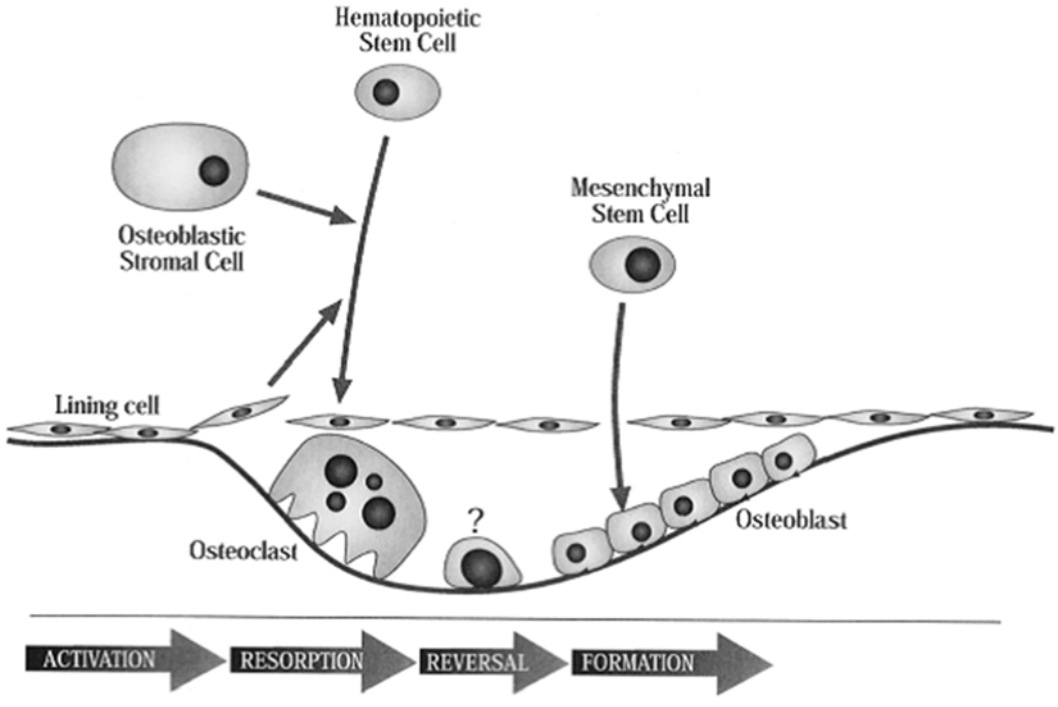

Fig. 3.1 Bone remodeling. The current concept of trabecular bone remodeling is illustrated here. Either osteoblastic stromal cells or lining cells may activate the resorption process. The bone remodeling units may be compartmentalized. The mechanism of entry of osteoclasts is not clear; in Haversian remodeling, the osteoclasts are presumable carried in by vessels. Although a single layer of osteoblasts is illustrated, multiple layers are required to refill the remodeling space

those in the general extracellular fluid. Premature loss of the remodeling compartment during the remodeling cycle has been implicated in the reduced bone formation that accompanies glucocorticoid excess [17].

3.4.1 Activation

Our understanding of the activation of bone resorption has advanced greatly in recent years with the discovery of a ligand–receptor system that explains the old observation that stimulators of bone resorption act largely on cells of the osteoblastic lineage and that this indirectly results in activation of osteoclasts [18] (Figs. 3.2 and 3.3). Stimulation of resorption results in the production of receptor activator of NFκB ligand (RANKL) on stromal cells, osteoblastic cells, and T-cells. RANKL then interacts with a receptor, RANK, on precursor cells of the hematopoietic lineage to initiate differentiation of these cells to form multinucleated osteoclasts and to maintain their resorbing activity. This system is held in balance by the production of a decoy receptor, osteoprotegerin (OPG), which is also produced by osteoblasts and which may be downregulated by stimulators of resorption. While this system is clearly important in initiating and regulating osteoclast activity, additional steps may be necessary that are not as well understood. In addition to delineating the BMU compartment, the lining cells may be involved in removal of a protein layer covering bone

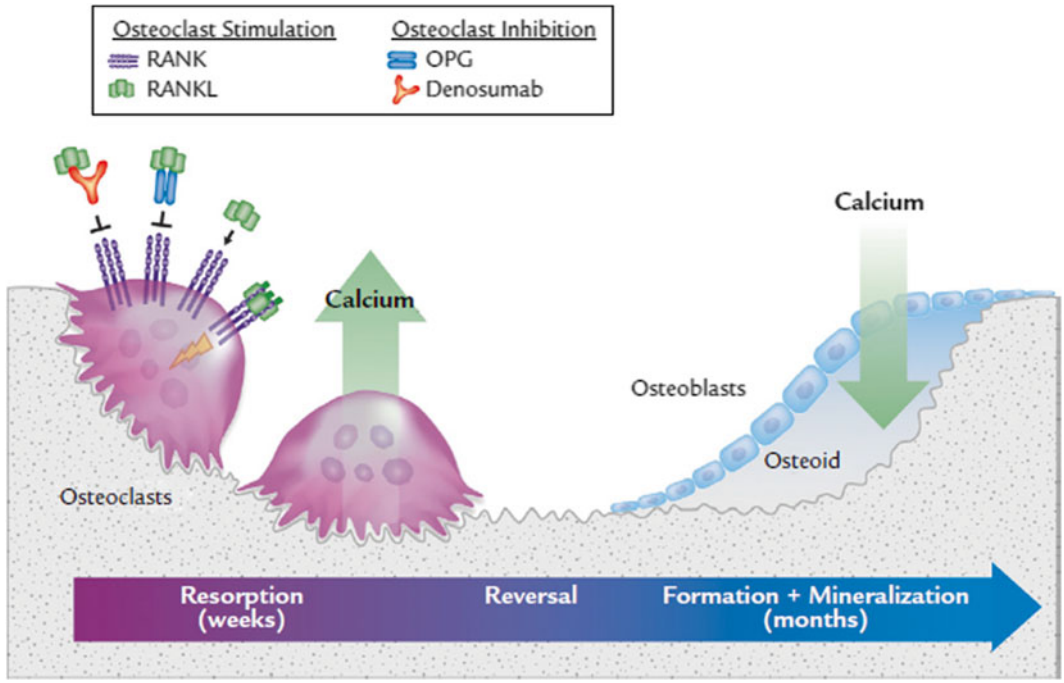

Fig. 3.2 A basic multicellular unit (BMU) engaged in bone remodeling. Osteoclasts initiate the remodeling cycle when receptor activator of nuclear factor kappa B ligand (RANKL) binds to its receptor (RANK) on the surface of osteoclasts and their precursors. This binding is prevented naturally by osteoprotegerin (OPG) and pharmacologically by the drug denosumab, thereby inhibiting osteoclast formation, activity, and survival. Osteoclastic resorption releases calcium and phosphate, which are replaced more gradually during the bone formation phase of the cycle. In this manner, the bone cells can participate in calcium homeostasis without long-term harm to the structural integrity of the skeleton. (Reproduced with permission from Ref. [18].)

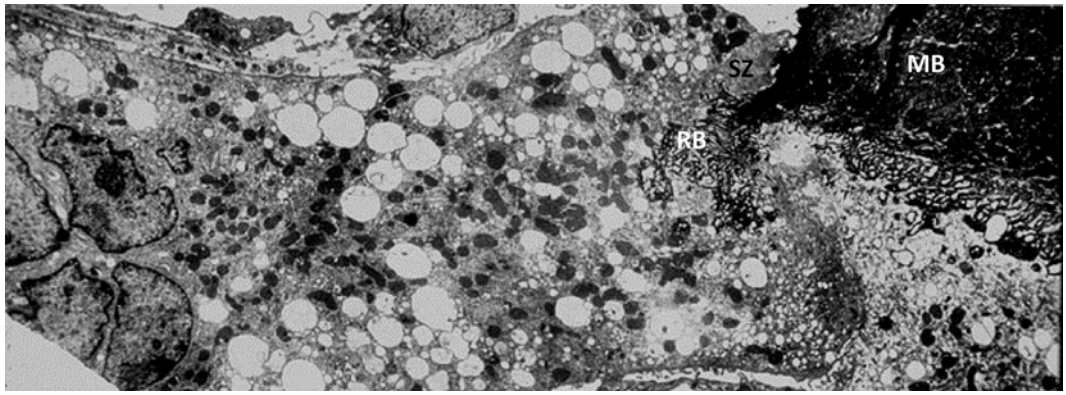

Fig. 3.3 Transmission electron micrograph of a rat osteoclast resorbing mineralized bone (MB). The cell is highly polarized with profiles of four nuclei towards the *left* and the ruffled border (RB) and sealing zone attached to the bone at the *right*. Note the numerous mitochondria and vacuoles in the cytoplasm

that is necessary for osteoclast access [19]. As the lining cells form a compartment, then the osteoclast precursors need to traverse the lining cell membrane, presumably through penetrating vessels, and this may also be a site for regulation.

3.4.2 Resorption

The size, duration, and depth of the resorption phase are presumably controlled at least in part by RANKL, which maintains osteoclast viability. However, control of the movement of osteoclasts along the bone surface or in Haversian canals cannels is less well understood. Moreover, we do not know the precise mechanism by which osteoclast resorption is stopped. High concentrations of calcium in the ruffled border area may result in separation and inactivation of osteoclasts [20]. Transforming growth factor-β (TGFβ) released from the bone matrix may also cause osteoclast apoptosis, but TGFβ may also increase osteoclastic activity [21, 22]. Calcitonin rapidly stops osteoclastic activity partly by blocking cell attachment, but this is probably an emergency brake on osteoclasts to protect against hypercalcemia rather than a general mechanism for stopping osteoclast activity [23, 24]. There is evidence that osteoclasts can undergo fission to produce mononuclear cells that are capable of resorption (Fig. 3.4). It has been estimated that as much as 2/3 of the resorption cavity may be excavated by such mononuclear osteoclasts [25, 26].

3.4.3 Reversal

The process of reversal is the least well understood phase of the remodeling cycle. Mononuclear cells are present on the bone surface that may complete the removal of matrix, deposit the mucopolysaccharide-rich material, the so-called cement line, between old and new bone, and possibly produce factors that attract and activate osteoblast precursors. It is not clear whether these mononuclear cells are derived from the mesenchymal or hematopoietic lineage cells or how they are regulated. Studies of the reversal phase [19, 27] suggest that bone lining cells rather than cells of the monocyte–macrophage lineage are present and that they digest protruding collagen fibrils and then deposit a thin osteopontin-rich cement line that is necessary before osteoblastic bone formation can begin.

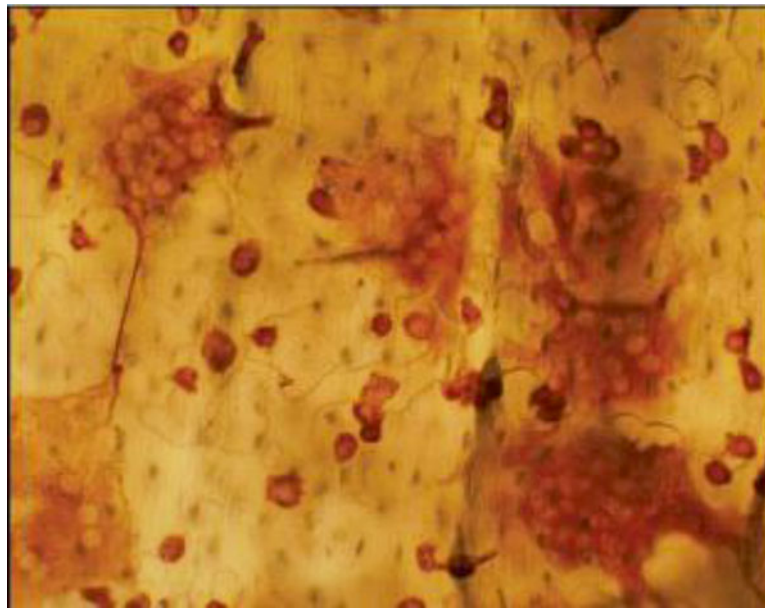

Fig. 3.4 Multinucleated and mononucleated human osteoclasts in culture on a bovine bone slice. The cells are stained for the calcitonin receptor. (Reproduced with permission from Ref. [25].)

3.4.4 Formation

The formation phase involves the replication, migration, and differentiation of mesenchymal cells to form osteoblasts (Fig. 3.5) and may also involve activation of previously dormant lining cells. The formation phase lasts for several months, compared to the few weeks of the resorption and reversal phases. Many factors are known to affect the magnitude of bone formation as discussed below, but the specific signals that lead to initiation and cessation of formation and that result in positive, equal, or negative balance between resorption and formation under different circumstances are still not well understood. It is clear, however, that the osteocyte is a key player in regulating bone formation by production of the potent osteoblast inhibitor, sclerostin [28].

3.5 The Osteoclast Lineage

The original concept that osteoclasts were derived from an alternative differentiation pathway from cells of the monocyte–macrophage lineage required some revision [29–31]. Studies demonstrated that pre-B-cells (B220-positive) can differentiate into osteoclasts if their normal pathway into mature B-cells is blocked. B220-positive plus cells may also differentiate to macrophages. Presumably all of these cells are derived initially from multipotent hematopoietic stem cells. Osteoclasts differentiate initially as mononuclear cells that express tartrate resistant acid phosphatase (TRAP) and may even express calcitonin receptors even before the critical process of fusion occurs. The large multinucleated osteoclast is a highly differentiated cell that is able to resorb bone because it adheres to the mineralized surface through a sealing zone and develops within that zone a ruffled bordered area in which the secretion of hydrogen ions and proteolytic enzymes can remove mineral and degrade matrix. The highly convoluted cell surface can form vacuoles, which contain the breakdown products that are

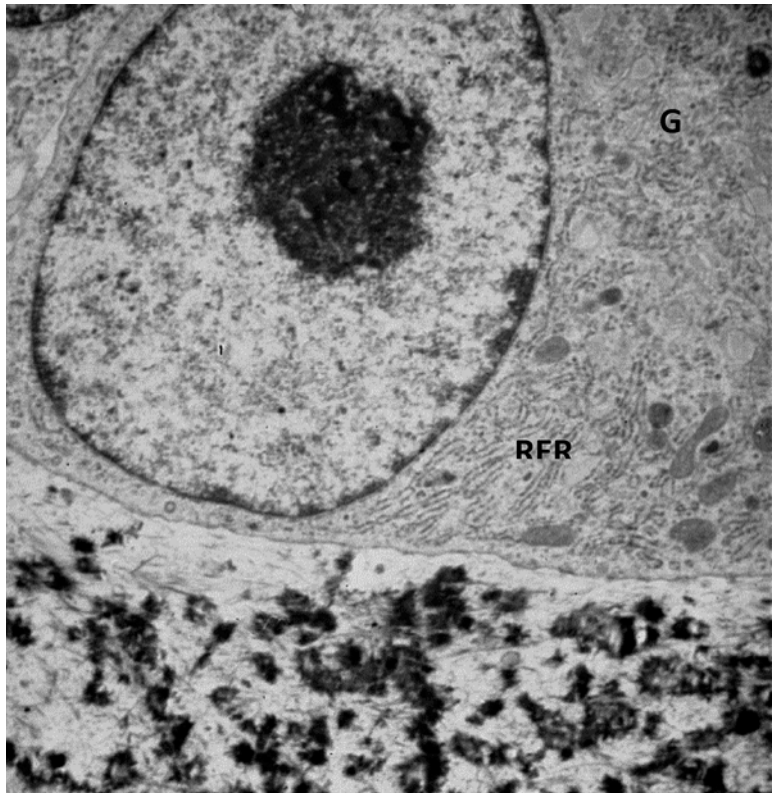

Fig. 3.5 Transmission electron micrograph of a rat osteoblast. Note the well developed Golgi apparatus (G) and plentiful rough endoplasmic reticulum (RER), consistent with a cell engaged in the synthesis and secretion of proteins. Secretory vesicles can be seen fusing with the plasma membrane. Collagen fibrils are present in the extracellular space and the dark clusters are crystals of hydroxyapatite being deposited in the matrix during the early stages of mineralization

transported thought the cell and release these products to the extracellular fluid. The osteoclast has a number of specialized features that permit it to function, including integrins for binding to bone, carbonic anhydrase and proton pumps to facilitate hydrogen ion secretion, lysosomal enzymes, particularly cathepsin K, to break down matrix, and ion-transport systems to permit the transport of calcium, phosphate, and excess bicarbonate to the extracellular fluid. Osteoclasts are terminally differentiated, relatively short-lived cells in which the nuclei undergo apoptosis. It is not clear whether all of the nuclei undergo apoptosis simultaneously or whether there are nuclei with different life spans within a single multinucleated cell. See Ref. [32] for a recent, comprehensive review of this topic.

3.6 Osteoblasts and Osteocytes

The differentiation of mesenchymal cells to fully functional osteoblasts that can deposit bone matrix and promote mineralization is a complex, multistep process. Freidenstein [33] coined the terms inducible and determined osteoprogenitor cells (IOPCs and DOPCs) to indicate that there were stromal that were pluripotential and could be induced to form osteoblasts, while other cells further along the differentiation pathway were irreversibly differentiated. This concept was revised because cells that have many of the characteristics of DOPCs can be diverted in culture to other phenotypes such as

Table 3.1 The multifunctional osteocyte

• Mechanosensation and detection of microdamage
• Regulation of phosphate homeostasis by secretion of Fibroblast Growth Factor-23 (FGF-23)
• Inhibition of bone formation by secretion of sclerostin
• Bone resorption ("osteocytic osteolysis") by expression of carbonic anhydrase

adipocytes or cartilage cells [34]. Thus it seems more likely that the process of differentiation is a continuum with many different stages. Moreover, the differentiated osteoblasts probably do not represent a single phenotype. There appear to be variations in the expression of genes such as osteocalcin among osteoblasts. Moreover, the morphology and rate of collagen synthesis of osteoblasts may vary from very high in the largest columnar cells to low in more flattened osteoblasts. Osteoblasts not only produce a collagenous matrix but regulate its posttranslational modification and mineralization through the production of noncollagen proteins. While osteogenic precursor cells were once thought to be fixed in marrow stroma and in the periosteum, recent data suggest that at least some osteoblast precursor cells may be found in the peripheral circulation [35, 36]. Once an osteoblast has completed its task of matrix formation, it can undergo several fates. Some osteoblasts become buried as osteocytes, while others form lining cells. The regulation of these alternative pathways undoubtedly has considerable impact on the amount of newly formed bone and its structure.

The osteoblasts, lining cells, and osteocytes in each BMU form a syncytium in which each cell is connected to each other by multiple cytoplasmic processes. This syncytium probably fulfills many functions in bone. One clearly important function is to detect and transmit the skeletal response to mechanical forces. This impact loading of the skeletal is amplified by fluid shear stress around the canalicular processes, and the osteocyte cell bodies produces signals involving change in ion flux, nitric oxide production, and prostaglandin production, which can initiate modeling and remodeling responses [37, 38]. Microdamage as a consequence of repetitive loading may also provide a signal for these responses. However, mechanosensation is but one function that the osteocyte performs. Recent studies indicate that the osteocyte participates in remarkable array of processes (Table 3.1) [39, 40].

3.7 Other Cell Types

Angiogenesis plays a critical role in bone modeling and remodeling [41]. Resting and proliferative cartilage are normally resistant to vascular invasion, but hypertrophic cartilage, particularly after calcification of the matrix, appears to induce vascular invasion. This brings chondroclasts that resorb the calcified cartilage and stromal cells that initiate the bone formation in the primary spongiosa. Haversian remodeling also depends on the entry of new blood vessels into bone. Epidermal growth factor (EGF) produced either by endothelial cells or by cells of the osteoblastic lineage plays a critical role in this process. Endothelial cells may also produce cytokines and other regulatory factors such as nitric oxide and prostaglandin, which influence bone cell function.

Interactions between marrow and bone are critical in bone modeling and remodeling. Not only is the marrow the source of stromal cells and osteoclast precursors, other marrow elements may play an important regulatory role. For example, T-cells are an important source of both membrane-associated and soluble RANKL [41–43]. This is likely to be of particular importance in inflammatory bone loss and malignancy, but may also be important in physiological remodeling. Increased hematopoiesis is associated with expansion of the marrow cavity at the expense of bone in disorders such as thalassemia [44]. There is an ill-defined association between mast cells and bone loss. Patients with mastocytosis develop osteoporosis associated with increased interleukin (IL)-6 production [45], and increased mast cell numbers have been described in idiopathic osteoporosis as well. Finally,

there appears to be a reciprocal relationship between adipocytes and osteoblasts in bone [34]. There is an increase in adipocyte numbers in the marrow with aging, which may be exaggerated in osteoporotic patients.

3.8 Systemic Hormones Regulating Bone Remodeling

Metabolic functions of the skeleton are largely controlled by two calcium-regulating hormones, PTH and 1,25-$(OH)_2$D. Calcitonin appears to play a lesser role—indeed, calcium homeostasis is not impaired in patients with either calcitonin excess such as those with medullary carcinoma of the thyroid or with calcitonin deficiency after total thyroidectomy. Whereas the primary function of PTH is to maintain serum ionized calcium concentration, the level of PTH also establishes the overall rate of bone turnover. Thus, increased PTH levels in hyperparathyroidism result in increased bone remodeling. After an initial period of bone loss, this process often appears to be in balance [46].

Early studies show that both PTH and PTHRP are critical for early skeletal development [47]. Stimulation of bone formation by PTH and also by PTHRP is probably critical in establishing optimal skeletal mass and structure. Once the skeleton is fully formed, PTH deficiency, that is, hypoparathyroidism, is associated with low bone turnover and high bone mass.

The relative dominance of the catabolic or anabolic effects of PTH appears to depend on both the level and duration of increased hormone levels. In rats that are given the same dose of PTH intermittently or continuously, the former administration results in increased bone formation with no increase in bone resorption, while the latter results in increased bone resorption with no increase in bone formation [48]. A plausible explanation is that the ability of PTH to increase RANKL and decrease OPG levels depends on more prolonged stimulation of osteoblasts, while only a transient stimulation is sufficient to induce growth responses, probably related to increased production of local growth factors including IGF and possibly TGFβ, IL-6 fibroblast growth factor (FGF), and prostaglandin E_2 (PGE_2). PTH effects also differ for different types of bone. In hyperparathyroidism, loss of cortical bone usually predominates while trabecular bone is preserved at least in the iliac crest, although there may be a subset, particularly of postmenopausal women, in whom PTH excess increases spinal trabecular bone loss. There is evidence for an increased risk of vertebral fracture in primary hyperparathyroidism [49] and recent studies using high resolution peripheral quantitative computed tomography (HRpQCT) suggest loss of bone from both cortical and trabecular compartments in the radius and tibia [50, 51].

PTH excess may play a role in the pathogenesis of osteoporosis. Patients with calcium and vitamin D deficiency will show an increase in PTH levels, and this will increase bone resorption. Interestingly, the age-related increase in PTH levels may be related not only to calcium and vitamin D deficiency but also to estrogen deficiency, since women given estrogen replacement do not show this increase.

The most important role of the vitamin D hormone system is to maintain the intestinal absorption of calcium and phosphate and supply these ions for bone mineralization. Indeed, skeletal growth and development can be relatively normal in the absence of 1,25-$(OH)_2$D or its receptor if calcium and phosphate supplies are adequate. In humans and experimental animals lacking the vitamin D receptor, but given sufficient amounts of calcium and phosphate, the major phenotypic abnormality is alopecia [52]. 1,25-$(OH)_2$D can also directly stimulate bone resorption at high concentration [53]. This effect may be particularly important in conditions of marked calcium and phosphate deficiency when 1,25-$(OH)_2$D synthesis is maximal. Resorption takes these essential ions from the skeleton to provide adequate supplies for soft tissues.

There is considerable debate about the role of 1,25-$(OH)_2$D in osteoblast function. An anabolic effect of this hormone and particularly of certain analogs has been described, but nothing comparable to effect of PTH has been demonstrated. Transgenic mice that overexpress the vitamin D receptor in osteoblasts exhibit enhanced bone formation and reduced resorption [53] Vitamin D receptors have

been found in many other cells types, particularly hematopoietic cells. Abnormalities of vitamin D metabolism may also affect immune responses [54]. Vitamin D may play a role in neuromuscular function, and impaired balance and muscular strength are associated with vitamin D depletion. This may be in part related to changes in calcium and phosphate availability to nerve and muscle.

3.9 Growth-Regulating Hormones

The major hormones that regulate tissue growth and metabolism all have a major influence on skeletal growth and remodeling, including the growth hormone–insulin-like growth factor (GH–IGF) system, thyroid hormones, and adrenal glucocorticoids. Recently discovered nutritional regulators, leptin and ghrelin, may also affect the skeleton, although current data are limited and conflicting.

The GH–IGF system determines body size, including the size of the skeleton before epiphyseal closure, and regulates the distribution of body fat, lean body mass, and bone modeling and remodeling after epiphyseal closure. This system can be considered as both a systemic and local regulator of bone metabolism. GH can stimulate IGF production not only in the liver but also in other target organs, including bone. Thus, skeletal growth is not markedly impaired by targeted deletion of IGF-1 production in the liver [55]. However, deleting liver IGF-1 and its binding protein does impair growth [56]. The GH–IGF system stimulates both resorption and formation. In human studies, GH treatment has resulted in transient decreases in bone mass due to a more rapid increase in resorption with a subsequent gradual and progressive increase in bone formation resulting in an overall increase in bone mass after 2–3 years of treatment [57]. The function of this system is markedly influenced by a number of binding proteins [58]. Among these, IGFBP2–4 all appear to have inhibitory effects on IGF action, whereas IGFBP5 appears to be stimulatory and may even have direct anabolic effects. The main circulating binding protein, IGFBP3, can inhibit the effects of IGF-1 in vitro, but prolongs its half-life in the circulation and enhances the in vivo anabolic response to IGF-1.

IGF-2 plays a critical role in skeletal development, but its role in the adult skeleton is less clear. Both IGF-1 and IGF-2 are subject to nutritional regulation, and a decrease in these factors associated with protein–calorie malnutrition probably mediates the associated impairment of growth and loss of bone mass.

Thyroid hormone has an effect similar to PTH on bone. In hyperthyroidism, bone resorption and formation are both increased while PTH levels are decreased [59]. However, thyroid hormone does not stimulate vitamin D activation, and calcium absorption may be decreased in hyperthyroid patients. Conversely, in hypothyroid patients, bone turnover is decreased and PTH levels may be increased. Thyroid hormone excess, either as primary hyperthyroidism or due to excessive thyroid hormone therapy, can contribute to bone loss. Both a history of hyperthyroidism and hypothyroidism [60] are associated with an increased risk for fractures. Hyperthyroidism may accelerate growth in children, while hypothyroidism clearly diminishes growth. The latter may be the result of an impairment of the GH–IGF axis.

Adrenal glucocorticoids have complex effects on bone cell function [61, 62]. Glucocorticoids enhance bone cell differentiation. Thus, decreased glucocorticoid production or increased inactivation of glucocorticoids may impair fetal skeletal growth and development. In children, glucocorticoid excess can markedly impair skeletal growth. This may not require high concentrations of glucocorticoid, but depends more on the fact that diurnal rhythm of glucocorticoid secretion is lost so that there is no period in the afternoon and night of extremely low secretion. This diurnal rhythm is essential for skeletal growth. During the period of low glucocorticoid secretion, the GH–IGF-1 axis is activated and there appears to be an increase in bone formation as indicated by a nocturnal rise in the bone formation marker, osteocalcin. Osteocalcin is particularly sensitive to glucocorticoids. There is a glucocorticoid response element (GRE) in the osteocalcin promoter whereby glucocorticoid receptor binding can inhibit transcription [63]. Glucocorticoid responses can be controlled not only by the

circulating levels but also by the level of glucocorticoid receptors in the cell and by the rate at which glucocorticoids are inactivated by the enzyme 11β dehydrogenase [64].

The biphasic effects of glucocorticoids can be demonstrated in organ culture. There is an initial increase in collagen synthesis, probably related to an increase in the cell responsiveness to IGF-1, followed by a prolonged decrease associated with decreased IGF-1 production. Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis and results largely from the direct inhibition of bone formation by glucocorticoids [65]. However, there may also be an increase in bone resorption, probably as an indirect result of decreased sex hormone production and decreased calcium absorption and secondary hyperparathyroidism. PTH levels are not consistently increased in patients with glucocorticoid excess [66].

Two recently identified hormones that are critical nutritional regulators may also play a role in bone. Leptin is a product of adipose tissues that decreases appetite. There are animal models of obesity with either leptin resistance or leptin deficiency. In these models hypogonadism may occur, yet despite this bone mass is increased. This led to the hypothesis that leptin might inhibit bone formation. Animal studies suggested that this was a indirect central effect and that there was a central nervous system hormone, possibly a β-adrenergic agonist that stimulates bone formation, which is downregulated by leptin [67]. However, studies of the peripheral effect of leptin suggest that it is a direct stimulator of bone formation an inhibitor of bone resorption [68, 69]. These paradoxical central and peripheral pathways have not yet been fully resolved.

Ghrelin was originally identified as a hormone produced by the stomach that stimulates appetite [70]. However, it is now recognized as a growth hormone (GH) secretagogue. Because GH secretagogue receptors are expressed by osteoblasts, ghrelin may play a role in metabolism, but a recent systematic review and meta-analysis [71] failed to find a convincing association between ghrelin and bone mineral density or fractures.

3.10 Sex Hormones

Sex hormones play a critical role in the regulation of skeletal development and bone remodeling [72, 73]. Estrogen deficiency is probably the single most important factor in the pathogenesis of osteoporosis in both men and women. Estrogen acts on the skeleton not only through its direct effects on cartilage and bone cells but also through its effects on other hematopoietic cell lineages and possibly also on endothelial cells in bone [74–78]. A large number of pathways by which estrogen might act have been identified, but their relative importance in skeletal physiology is still not established. Analysis of estrogen action is complicated by the fact that there are two estrogen receptors ERα and ERβ, and that there may also be estrogen effects on the cell membrane that are independent of either receptor [79]. Studies of men with estrogen deficiency, due either to loss of ERα or to a defect in aromatase the enzyme that converts androgen to estrogen, show a similar phenotype. They have failure of epiphyseal closure, increased bone turnover, and low BMD. Current studies also suggest that activation of ERα is the most important pathway for skeletal affects [80, 81]. Estrogen may inhibit resorption by altering the production of cytokines in marrow cells, increasing OPG production or inducing osteoclast apoptosis, possibly by increasing TGFβ [23, 82–84]. High concentrations of estrogen can clearly stimulate bone formation in animal models and may enhance the bone formation response to mechanical forces [81, 85]. In estrogen deficiency, bone formation is increased, but the increase is clearly inadequate to compensate for the increase in bone resorption. Hence there is a relative defect in bone formation. The orphan receptor, estrogen receptor-related receptor α, has recently been identified, which may modulate estrogen responses [86].

The effects of estrogen on bone may involve different pathways and a different dose–response relation from its effects on classic target organs such as the uterus and breast. Estrogen agonists/antagonists, formerly termed selective estrogen receptor modulators (SERMs), which do not stimulate the breast or uterus, can still inhibit bone resorption and prevent bone loss after ovariectomy [87]. Bone

turnover can be decreased by low doses of estrogen, and fracture risk is increased in women with extremely low serum estradiol concentrations [88, 89]. Indeed, the estrogens produced by aromatase in fat tissue may be sufficient to protect the skeleton in postmenopausal women, hence the association of low body weight, low bone mass, and increased fracture risk in this population. Estrogens have multiple effects on hematopoietic cells. In animal models, estrogen deficiency is associated with an increased number of B-cells in the marrow, and B-cell precursors may be a source of osteoclasts [31]. Both estradiol and raloxifene can affect β-lymphopoiesis [90]. Estrogen can modulate activation and cytokine production of T- and B-cells as well as cells of the monocyte–macrophage lineage.

Our understanding of the role of testosterone in bone metabolism is complicated by the fact that testosterone can be converted to estrogen by many tissues, probably including bone [91]. Studies using dihydrotestosterone, which cannot be aromatized, suggests that androgens can both stimulate bone formation and inhibit bone resorption [92, 93]. Whether these effects are mediated only through the androgen receptor or by binding to the estrogen receptor as well is not established. Androgens are probably responsible for the stimulation of bone growth that occurs at puberty and can probably stimulate bone formation in adults as well. However, studies in humans suggest that estrogen is far more important than androgen in inhibiting bone resorption [94]. Androgen effects on muscle may indirectly produce skeletal responses by altering mechanical forces exerted on bone [95]. The role of progesterone in bone is controversial. A number of authors have suggested that progesterone may stimulate bone formation. However, markers of bone formation were not increased by administration of progestins to postmenopausal women, either untreated or on estrogen therapy [96–98]. Other hormones of the pituitary/reproductive system have also been implicated in bone remodeling. In particular, inhibin may suppress bone remodeling [99]. Inhibin deficiency could play a role in the increased bone remodeling and bone loss that occurs in the perimenopause, at a time when estrogen levels are not generally decreased but inhibin production declines as indicated by a rising follicle-stimulating hormone level.

3.11 Local Regulatory Factors

The recognition that there are a large number of local factors that regulate bone remodeling has been one of the major advances in bone biology in recent decades [100, 101]. An adequate review of this topic is beyond the scope of this chapter, not only because of the number of factors but because of the complexity of their actions and interactions. Cytokines, such as IL-1, and tumor necrosis factor-α (TNFα), growth factors, such IGF-1 and the TGFβ and the related bone morphogenetic protein (BMP) family, small molecules such as prostaglandins, leukotrienes, and nitric oxide, and neuropeptides have all been found to affect bone formation and resorption and have been implicated in meta-physiological and pathological skeletal responses.

The concept that cytokines play a role in bone began with an observation made 30 years ago that supernatants of human mononuclear cells cultured with an activator, such as an agglutinin or an antigen to which the patient had cell-mediated immunity, could cause bone resorption. This was initially called osteoclast activating factor. Subsequent studies indicated that a major component was IL-1 but TNFα and other cytokines were shown to have quite similar activities. These cytokines can also affect bone formation, although both inhibitory and stimulatory responses have been reported. Other cytokines, such as IL-6 and IL-11, can stimulate both bone resorption and formation. A number of cytokines can inhibit bone resorption, including IL-4, IL-13, IL-18, and interferon (IFN)-γ or IFN-β. Recently it has been found that IFN-β expression was increased in cells of the osteoclastic lineage treated with RANKL [102]. This appears to be a second balancing system for preventing excessive osteoclastogenesis, analogous to the RANKL-OPG system. Studies with cytokine inhibitors and knockouts of the cytokines or their receptors have confirmed these effects in murine models [103]. The relative importance of these cytokines in human bone remodeling and particularly in the

pathogenesis of osteoporosis has not been established. It is possible that a deficiency of inhibitory cytokines as well as an excess of those that stimulate resorption could play a pathogenetic role in bone loss.

Prostaglandins, particularly prostaglandin E_2 (PGE_2), are potent multifunctional regulators of bone metabolism [101]. The predominant response to PGE_2 is stimulation of bone resorption and formation, although there are also inhibitory responses under certain conditions. Stimulation of bone turnover by PGE_2 is likely to be involved in inflammatory bone loss and appears to play an important role in the response to mechanical forces. The systemic and local factors that stimulate bone resorption increase the production of PGE_2 via the inducible enzyme cyclooxygenase-2 (COX-2). As a result, bone resorptive responses both in vivo and in vitro may be blunted in animals which cannot form prostaglandins because COX-2 is inhibited or deleted or because specific receptors for PGE_2, particularly the EP2 and EP4 receptors, are inactivated [104–107]. PTH, 1,25-$(OH)_2$D, thyroid hormone, IL-1 and TNFα, BMP-2 and TGFβ [108, 109] can all induce COX-2. Osteoclastogenesis in response to these factors is diminished in bone marrow cultures from knockout mice. However, these factors also have the capacity to stimulate bone resorption by prostaglandin-independent pathways. PGE_2 can itself stimulate COX-2 [110]. This auto-amplification pathway is probably important in the skeletal response to mechanical forces.

A number of other small molecules have been implicated in bone remodeling. Nitric oxide (NO) can act as both an inhibitor of bone resorption and a stimulator of bone formation and NO donors are being explored as a potential treatment for osteoporosis [111, 112]. Leukotrienes, neuropeptides, and nucleotides have also been shown to act on bone [113–116].

Bone cells produce and/or respond to many different growth factors [117]. As discussed above, IGF-1 is a potent stimulator of bone formation, which also increases bone resorption. IGF-1 is not only produced by bone cells but is probably stored in bone matrix together with binding proteins and may be released when these binding proteins are degraded [118]. The large family of TGFβ-related proteins including several of the BMPs may also be stored in the matrix. There is a specific release pattern for TGFβ involving release from a binding protein and activation of the precursor molecule [119]. This may occur during bone remodeling and has been implicated in the inhibition of resorption and the initiation of formation during the reversal phase of the bone remodeling cycle. Other growth factors, such as vascular endothelial growth factor (VEGF), EGF platelet-derived growth factor (PDGF), and FGF, are also likely to be local regulators of bone formation and possibly also bone resorption [120–123]. There may be an amplification system for these factors involving PGE_2. VEGF can stimulate prostaglandin production in endothelial cells, while EGF, PDGF, and FGF can stimulate prostaglandin production in bone. PGE_2 has been shown to stimulate the production of the VEGF and FGF in bone cells [124].

Another important local regulatory pathway was been discovered through the genetic analysis of unusual families that have high bone density and of a rare genetic form of bone loss, the osteoporosis–pseudoglioma syndrome [125–127]. High bone density is associated with an activating mutation of low-density lipoprotein receptor related-5 (LRP-5), while deletion of this receptor results in the osteoporosis–pseudoglioma syndrome. This receptor is associated with the Wnt signaling pathway, which is critical for craniofacial development. Neither the specific ligand that activates the LRP-5 receptor nor the mechanisms by which it affects bone mass have yet been identified.

3.12 Regulation by Calcium, Phosphorus, and Other Ions

Calcium is not only essential for regulation of neuromuscular activity and cellular function throughout the body, but it also may play a specific role as a local regulator in bone. High concentrations of calcium are likely to develop in the ruffled border area, where the hydrogen ion concentration is high

and mineral is being dissolved. These high concentrations of calcium have been shown to inhibit osteoclast function, probably largely by causing a loss of cell adhesion. This effect may also require an increase in intracellular calcium ion concentration. There is controversy concerning the mechanism by which osteoclasts sense calcium. There may be a specific calcium receptor, but this appears to differ from the calcium-sensing receptor of the parathyroid glands. High local concentrations of calcium may also affect osteoblast function [128].

Although extracellular ionized calcium concentration is tightly regulated, the extracellular concentration of phosphate shows wide variation, not only during different stages of skeletal growth and maturation in humans but also among different mammalian species. In general, higher phosphate concentrations are associated with more rapid skeletal growth and mineralization; for example, serum phosphate concentrations are elevated during the rapid growth phases of early infancy and puberty. In organ culture, increasing phosphate concentration has been shown to enhance both matrix formation and mineralization [129]. There is also a reciprocal relation between calcium and phosphate concentrations, particularly because of the effect of parathyroid hormone to decrease renal tubular absorption of phosphate and lower serum levels while increasing serum calcium concentration. Phosphate has complex effects on osteoclastic bone resorption. Phosphate depletion is associated with increased serum calcium and increased resorption rates in organ culture, which may be in part due to a change in the physical chemical gradient for removal of mineral [130]. Defects in osteoclast phosphate transport, on the other hand, are associated with impaired osteoclastic bone resorption [131].

Magnesium can affect bone remodeling both directly and indirectly. In severe magnesium deficiency, parathyroid hormone secretion is impaired, bone resorption is decreased, and hypocalcemia can develop [132]. The low magnesium levels may also directly affect both osteoblast and osteoclast function [133]. The skeleton is a reservoir not only for calcium and phosphate but also for sodium and for hydroxyl ions. The skeleton forms a second line defense after the kidney in acid–base balance, providing both phosphate and hydroxyl ions to buffer hydrogen ion excess. Severe chronic acidosis can cause bone loss. It has been postulated that the typical Western high-protein diet represents a acid load that may cause mineral loss [134].

Fluoride is a potent stimulator of osteoblasts and can cause increased bone formation [135, 136]. Unlike the anabolic effect of PTH, the response to fluoride can produce bone that is structurally abnormal, resulting in increased fragility rather than increased strength. Fluoride also is incorporated into the hydroxyapatite mineral, producing an alteration in crystal structure. In humans, low doses of fluoride may increase bone strength and decrease fracture risk, but the use of fluoride for the treatment of osteoporosis has been largely discontinued because of the relatively narrow range between therapeutic and adverse effects.

Strontium is incorporated into the bone mineral and may have an anabolic effect on osteoblasts. Strontium ranelate is an effective agent to increase bone mass and lower fracture risk in osteoporotic patients [137].

3.13 Conclusion

The major goal of this chapter has been to provide the reader with help in asking appropriate questions concerning possible pathways that might influence bone resorption and formation and that might in turn be influenced by nutrition. While this field is evolving extremely rapidly and many new regulatory factors and new interactions are likely to be discovered, the regulatory mechanisms outlined here are important to understand not only because they are affected by nutrition but also because they may be the key to understanding interactions between nutrition and genetics.

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Chapter 4
Estrogens, Progestins, SERMs, and Osteoporosis

Robert Lindsay

Key Points

- Loss of ovarian function among mature women increases bone remodeling by increasing the frequency of remodeling site activation and enhancing the avidity of osteoclasts.
- The ubiquitous occurrence of menopause and the bone loss that follows renders osteoporosis and its attendant fracture risk a major health problem for the female population.
- Estimates suggest that the average woman has about a 50 % chance of a fracture in her remaining years.
- Replacement estrogen reduces that risk by essentially reducing remodeling toward premenopausal levels.
- Estrogens have potent effects in many organ systems, not all of which are beneficial.
- Estrogens and hormone therapy (estrogen plus a progestin to protect the uterus) remain important agents for treatment of menopausal symptoms, with the recommendation that the lowest effective dose be given and that treatment be given for the shortest time possible.
- As long as estrogen is given skeletal protection is to be expected, but when estrogens are stopped rapid bone loss ensues leading to the need for therapy in at risk women.
- When estrogens are used for osteoporosis prevention, care must be taken to ensure adequate calcium and vitamin D.

Keywords Estrogen • Remodeling • Osteoclast • Menopause • Hormones • Women's Health Initiative • Progestin

4.1 Introduction

The increased frequency of fractures among the elderly has been known for centuries. Indeed the fact, that in most cultures, fractures consistent with osteoporosis occur more commonly among aging female of the species was described by Bruns [1] who first demonstrated this for proximal femur fractures in 1882. It is now more than 70 years ago that Fuller Albright [2] noted that vertebral

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fractures occurred more commonly among women who had had their ovaries removed prior to the average age of menopause. Albright went on to show that estrogen intervention reversed the negative calcium balance in women presenting with osteoporosis, and suggested (mistakenly as it turned out) that estrogens were anabolic [3]. Henneman and Wallach [4], following Albright's patients noted that those on estrogen did not lose height whereas in women not on estrogen there was a higher frequency of height loss (for detailed discussion see [5, 6]. Thus the link between estrogens and skeletal homeostasis became established.

It is, however, only comparatively recently that data have been generated that establish beyond all doubt that estrogen intervention not only prevents bone loss after menopause but also reduces the risk of fractures of wrist, hip, and spine [7–11]. The most compelling evidence comes from the Women's Health Initiative, a study that has become somewhat infamous over the past 10 plus years since the data were first released [12, 13]. While discussions still continue about heart disease [14, 15], breast cancer [16], and Alzheimer's disease, the data on fracture prevention are quite secure and confirmatory of all we understood beforehand [17]. However, since fractures generally occur in older individuals lifestyle and nutrition might be expected to play a role in the development of weak bones that are then at risk of fracture [18, 19]. Since estrogen plays such an important role in skeletal homeostasis it seems relevant to add a short chapter to this volume not only reviewing hormonal effects but trying to evaluate what we know about the interaction of hormones with the other factors impacting upon skeletal metabolism throughout life.

4.2 Pathophysiology

Irrespective of the mechanism, in all situations of estrogen deprivation there is an increase in skeletal remodeling that in many, but not necessarily all, women, results in a loss of bone mass and disruption of skeletal architecture that increases fracture [20, 21]. It is the process of bone loss that results in disrupted architecture, and leads to increased risk of fracture. Since the skeleton is subject to multiple influences (lifestyle, diet, estrogen status) it is not unreasonable to assume each will impact in some way upon the effects of the others [18, 19].

It is the process of bone loss that leads to the architectural abnormalities that are the hallmark of the osteoporotic skeleton, and the increased fracture risk. It is also worth stating that those who start with low bone mass usually have small bones (a genetic influence) and small bones are more likely to fracture on any given trauma than large bones.

Recker has shown that in many individuals the increased remodeling that occurs across menopause can persist into old age [22]. If the increased remodeling were due simply to increased recruitment of new remodeling sites then a new steady state should be reached with stable bone mass. In many individuals that is not the case. Bone mass (measured as BMD by DXA) continues to decline at a variable rate, implying a negative balance within each remodeling site, that may be modulated by a variety of insults. The interaction of these is incompletely understood. Although individually these features are well known and understood there is still considerable heterogeneity in the rate and duration of bone loss. Within the skeleton, bone loss is dependent on surface and thus is higher in skeletal sites with a high proportion of cancellous bone, which in turn is more likely to be influenced by exogenous factors including diet and lifestyle.

The mechanisms by which estrogen might affect the skeleton have been the subject of considerable investigation. Perhaps the best documented are the effects on the RANK-ligand pathway. Estrogens appear to modulate the balance between the osteoblast derived factors RANK itself and the decoy receptor osteoprotegerin. Thus, in states of reduced estrogen supply the balance is tipped in favor of RANK-L resulting in increased osteoclast recruitment and activity, and in estrogen replete states the

balance is more toward the decoy receptor that then reduces the impact of RANK-L and inhibits osteoclast recruitment. In addition many other pathways and cytokines have also been implicated including the interleukins (1, 6, and 11 in particular), TNF-alpha, lymphotoxin, M-CSF, and GM-CSF. It also seems that the WNT signaling pathway is involved. Estrogens also stimulate the secretion of TGF-beta which inhibits bone resorption and stimulates formation. Finally, estrogens are pivotal in controlling the formation of BMPs particularly BMP-6 in human osteoblast cell lines. Given the array of effects and the fact that many of these pathways are likely to be influenced by diet and lifestyle to a variable degree, it is perhaps not surprising that unraveling the interactions has proven difficult, if not impossible.

Although we pay most attention to menopause as a stimulus to bone loss, it is evident that bone loss occurs in all situations in which estrogen supply is diminished. In young women interruption of the hypothalamic-pituitary-ovarian axis also causes bone loss and is often compounded by other insults to the skeleton. The most significant clinical problem is that of anorexia nervosa where the nutritional deprivation magnifies the bone loss related to estrogen deficiency, and serves as a model for the potential interactions [23]. Bone loss also follows the use of Depo-Provera as a birth control method, that reduces estrogen supply [24]. Clinically one of the more difficult situations follows the treatment of breast cancer among premenopausal women. Here the loss of ovarian function together with the effects of chemotherapy can produce severe bone loss that cannot be ameliorated by estrogen intervention for obvious reasons.

4.2.1 Estrogen Intervention

The original observational studies of Albright have been confirmed by multiple clinical trials. Albright demonstrated improved calcium balance after estrogen therapy and as noted incorrectly suggested that estrogens through their effect on calcium balance could be anabolic to the skeleton [3]. Henneman and Wallach following up on the patients Albright treated with estrogen showed that those women maintained their height, while untreated women had sporadic height loss using this as a surrogate for vertebral fractures [4].

The first controlled clinical trials of estrogen were published in the 1970s. These showed, using noninvasive methods of assessing bone mass (X-ray and single photon absorptiometry) that estrogen prevented bone mass loss in both ovariectomized women as well as those who had passed a natural menopause [7, 9, 25]. Many similar studies have been published since that time, with more sophisticated imaging (QCT and DXA) than was possible in the original studies [26, 27]. As long as estrogens are supplied, bone remodeling remains within premenopausal range and bone resorption and new bone formation are approximately in balance, although with some degree of variability.

The largest of the clinical trials using the surrogate marker of BMD is the Women's Health Initiative (WHI) a study that has become somewhat controversial since its original publication over 10 years ago. However, WHI confirmed the earlier studies, showing that estrogen intervention helped preserve bone mass and, more importantly went on to show reduction in fractures (33 % reduction in vertebral fractures and a 27 % reduction in non-vertebral fractures for conjugated estrogen alone and a relative hazard for hip fracture of 0.67 in the conjugated estrogen progestin arm). These data were confirmed by the million women study in the UK, a prospective but observational study [12].

The most studied of the estrogens is conjugated equine estrogen, but meta-analysis indicate a similar effect for other active estrogens including estradiol [28]. Dose relationships suggest that oral doses as low as 0.3 mg conjugated estrogens per day, and transdermal delivery of 25 μg/day are protective to the skeleton [29, 30]. A large meta-analysis in 2002 found 57 studies for prevention and treatment of osteoporosis using estrogen, with or without a progestin [31]. In the 55 included in the analysis,

estrogen by any route of administration, with or without a progestin resulted in net increases in BMD of about 5 % in the spine and 2.5 % at the hip, when compared with placebo. In the touted Women's health Initiative (WHI) which evaluated 0.625 mg conjugated estrogen, BMD in a subgroup (1,024 E+P; 938 E alone) increased by 3.7 % at year 3 in the E+P group and 1.8 % at year 6 in the hip. In the spine BMD increased by 3.3 % at year 3 and by 7.5 % at year 6, with a 5.1 % gain in BMD at year 6 in the estrogen alone arm [12, 13]. These are not comparable results, however, since all participants in the E alone arm had had hysterectomy performed prior to menopause with ovariectomy. Although most of those studies evaluate prevention of bone loss, estrogens are also effective treatment for patient with the established disease.

4.2.2 Route of Administration

Estrogens may be delivered orally, transdermally, intravaginally, or parenterally. The majority of the fracture data from clinical trails are based on oral therapy. However, there are good data that indicate that transdermal estrogens also reduce bone turnover and preserve BMD [30, 32, 33]. High doses of intravaginal estrogen appear similarly beneficial. The data tend to suggest that an adequate estrogen dose supplied by any route will effectively control skeletal remodeling and prevent osteoporosis. Whether the same holds true for those who present with the established disease remains unclear.

4.2.3 Discontinuation of Treatment

The effects of estrogen on bone dissipate rapidly when treatment is discontinued with increased rates of bone loss and a concomitant increase in the risk of fracture. Thus within a few years BMD in prior estrogen users is no different from those who have had no estrogen exposure. Fracture risk also increases and again within a few years reverts to baseline risk. Indeed some data suggest that the risk of fractures may eventually exceed that seen without intervention.

4.2.4 Interactions

The negative effects of estrogen deprivation after the menopause are sufficiently dominant that, at least in the first few years they cannot be reversed by modifications in lifestyle or nutrition. However, these studies do not adequately address whether attenuation of the bone loss can be obtained, by modifying calcium intake or even vitamin D. Such data cannot easily be obtained from clinical trials since supplements of calcium and often vitamin D are background treatment in studies designed to demonstrate the efficacy of a pharmacologic agent. The severe effects of both nutritional deprivation and estrogen loss as seen in anorexia, nervosa tend to suggest an interaction, but since the nutritional insult is so severe it is difficult to draw conclusions about interactions in a more physiological setting. To answer this question Nieves et al. conducted a systematic review of all estrogen studies, since several had been performed prior to the routine administration of supplements in clinical studies [34]. The data are quite compelling indicating that the beneficial effects of estrogen were dependent on the level of calcium intake throughout the study. Thus where calcium supplements were provided the increments seen in BMD were significantly better than those observed in studies that evaluated estrogen alone (± progestin). No fracture data are available to confirm the BMD effects.

4.2.5 Estrogen Dose

Early studies were performed using synthetic estrogen or what can be considered to be a "full" dose of conjugated equine estrogens. However, more recent data have suggested that lower doses of estrogen, perhaps 0.3 mg CEE can produce a positive effect on BMD [29, 31, 35]. Data from the Study of Osteoporotic Fractures also suggest that modest increments in estrogen (in that case endogenous) can produce beneficial effects on the skeleton [36].

4.2.6 Other Effects of Estrogen Intervention

In addition to the effects of ET/HT on bone and fractures WHI also evaluated multiple outcomes associated with oral administration of conjugated estrogen. The study reported an increased risk of breast cancer, cardiovascular disease, and possible increased risk of dementia [37]. Subsequent analyses have raised the possibility that the benefit risk ratio for ET may be different than for HT. In addition therapy provided close to menopause provides effective treatment for menopausal symptoms and early treatment may mitigate some of the concerns (as may reduced dose or non-oral administration).

4.3 Conclusion

Estrogens remain first line treatment for severe menopausal symptoms. Non-estrogenic agents (e.g., SSRI's) can be used for milder symptoms. Estrogens when used should be given in the lowest effective dose, and for the shortest time to achieve benefit. When estrogens are considered for the prevention of osteoporosis, alternatives should be reviewed, before initiating treatment. For those with an intact uterus a progestin should be added, or the use of estrogen plus a SERM (bazodoxifene) considered. Treatment should be reviewed on a regular basis. BMD measurements or the use of biochemical markers of bone turnover can be used to determine effectiveness. Calcium intake should be set at 1,000–1,500 mg/day, preferably from diet, although supplements may be used to bring total intake to that level. Vitamin D should be checked and supplements used to bring serum 25(OH)D above 30 ng/ml. Treatment should be reviewed at least annually and transition to non-hormonal medications as soon as feasible.

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Chapter 5
Bone Biomechanics and the Determinants of Skeletal Fragility

Lamya Karim and Mary L. Bouxsein

Key Points

- In the USA alone, there are over 1.5 million fractures each year, including 280,000 hip fractures and 500,000 vertebral fractures.
- The number of fractures is projected to double or triple in the next 30–50 years.
- Low BMD is among the strongest risk factors for fracture, but there are limitations of BMD.
- Skeletal fragility and skeletal loading are also important.
- Standard engineering concepts can be used to evaluate structural failures and considering the various components that influence whole bone strength.
- Whole bone strength is determined by the amount of bone, the spatial distribution of the bone mass, and the intrinsic properties of the materials that make up bone.
- Key dietary factors may influence the determinants of bone strength.

Keywords Biomechanics • Skeletal fragility • Bone strength • Bone mineral density • Microarchitecture • Bone matrix properties • Vitamin D • IGF-1 • Bone biomechanics • Fractures • Osteoporosis • Structural properties • Bone geometry • Bone microarchitecture • Nutrition

5.1 Introduction

Fractures are among the most dramatic and devastating sequelae of aging of the human skeleton. In the USA alone, there are over 1.5 million fractures each year, including 280,000 hip fractures and 500,000 vertebral fractures. Of greater importance, however, is the fact that based on current demographic trends predicting a "graying" of the population worldwide, the number of fractures is projected to double or triple in the next 30–50 years. This marked increase in the number of fractures represents a great societal burden, as caring for individuals with fractures will have a large impact on health care costs. Accordingly, interventions to reduce fracture incidence are needed. Although several drug therapies are efficacious for reducing fracture risk, they do not eliminate fractures entirely.

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Moreover, access to and compliance with therapies is relatively poor, and thus, fractures remain a large and growing public health concern [1].

Strategies to reduce fracture risk must be based on a sound understanding of the cellular, molecular, and biomechanical mechanisms that underlie the increased risk of fractures with aging. In the past, the predominant view was that age-related fractures were simply an unavoidable consequence of bone loss due to estrogen deficiency. Whereas low BMD is among the strongest risk factors for fracture, a number of clinical studies have demonstrated the limitations of BMD measurements in assessing fracture risk and monitoring the response to therapy. These observations have brought renewed attention to the broader array of factors that influence fracture risk, including those that are directly related to skeletal fragility as well as those related to skeletal loading. In support of this view, an NIH consensus conference defined osteoporosis as "a skeletal disorder characterized by compromised bone strength leading to an increased risk of fracture" [2]. This definition underscores the role of bone strength, and implies that understanding bone strength is key to understanding fracture risk.

This chapter reviews the etiology of age-related fractures from a biomechanics viewpoint, by introducing a standard engineering concept used to evaluate structural failures and considering the various components that influence whole bone strength, with discussion of how key dietary factors may influence the determinants of bone strength.

5.2 Biomechanics of Age-Related Fractures

From a mechanical perspective, fractures represent a structural failure of the bone whereby the forces applied to the bone exceed its load-bearing capacity (Fig. 5.1). The forces applied to the bone will depend on the specific activity, and will vary with the rate and direction of the applied loads. For example, the loads applied to the proximal femur during a fall will depend on the height of the fall, the direction of the fall, the impact surface, the extent of soft-tissue overlying the hip, and the ability of the individual to protect oneself from the fall impact by extending a hand.

The load-bearing capacity of a bone (or "whole bone strength") depends on the amount of bone (i.e., size), the spatial distribution of the bone mass (i.e., shape), and the intrinsic properties of the materials that comprise the bone [3, 4]. Moreover, the mechanical behavior of a bone will depend on

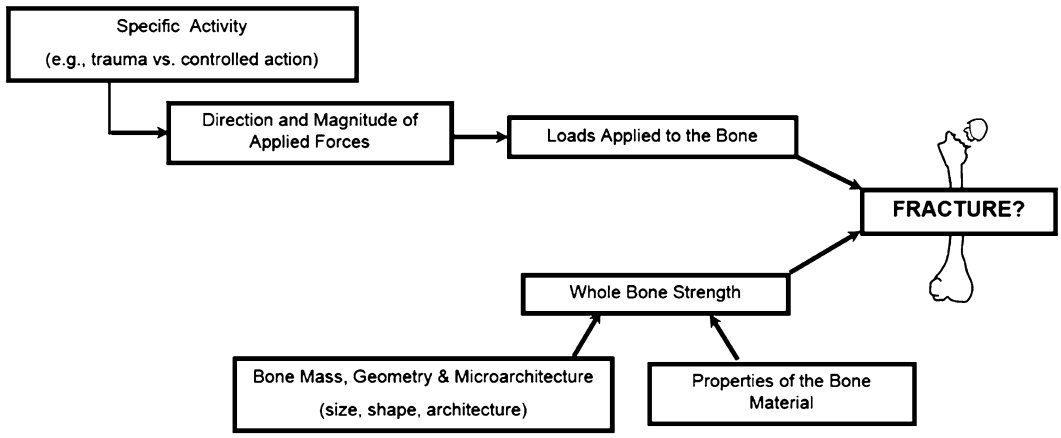

Fig. 5.1 Etiology of age-related fractures. This diagram illustrates the concept that fractures occur when the forces applied to a bone exceed its strength. Various factors influence both the loads applied to the bone, as well as whole bone strength

the direction and magnitude of the loads applied to it. For example, the proximal femur withstands much higher loads when tested in a single-leg stance configuration than it does when tested in a configuration designed to simulate a sideways fall [5]. Further discussion of the determinants of whole bone strength is presented in the next section.

The importance of the interaction between skeletal loading and bone strength has been demonstrated in several clinical studies [6, 7]. For instance, the Study of Osteoporotic Fractures examined elderly women who fell and suffered a hip fracture ($n=130$), women who fell and suffered a wrist fracture ($n=294$), and women who fell and did not have a fracture ($n=467$) [6]. They reported that among women who fell on or near their hip, those who fell sideways or straight down were at increased risk for hip fracture (odds ratio = 4.3), whereas those who fell backward were less likely to suffer a hip fracture (odds ratio = 0.2). Those who fell forward were more likely to suffer a wrist fracture. In another study, Greenspan and colleagues [7] compared community-dwelling elderly individuals who fell and suffered a hip fracture ($n=72$) to those who fell and did not fracture ($n=77$). They found that low hip BMD, low body mass index, and characteristics related to the fall itself were *independent* risk factors for hip fracture. Similarly, in women, both low BMD and low trochanteric soft tissue thickness contribute independently to hip fracture risk [8]. Taken together, these studies confirm an important interaction between bone strength (as reflected by BMD), skeletal loading (as assessed by fall traits and/or fall-related cushioning), and fracture risk.

Considering this broad paradigm of fracture etiology, interventions to reduce fractures may be targeted at either reducing the loads applied to the bone and/or at increasing whole bone strength. Examples of strategies to reduce the loads applied to the skeleton include fall prevention programs, trochanteric padding (e.g., "hip pads"), and counseling individuals in proper lifting techniques to minimize loading on the spine.

5.3 Determinants of Whole Bone Strength

The ability of a bone to resist fracture (or "whole bone strength") depends on the amount of bone (i.e., mass), the spatial distribution of the bone mass (i.e., shape and microarchitecture), and the intrinsic properties of the materials that comprise the bone (Fig. 5.2). Bone remodeling, specifically the *balance* between formation and resorption, is the biologic process that mediates changes in the traits that

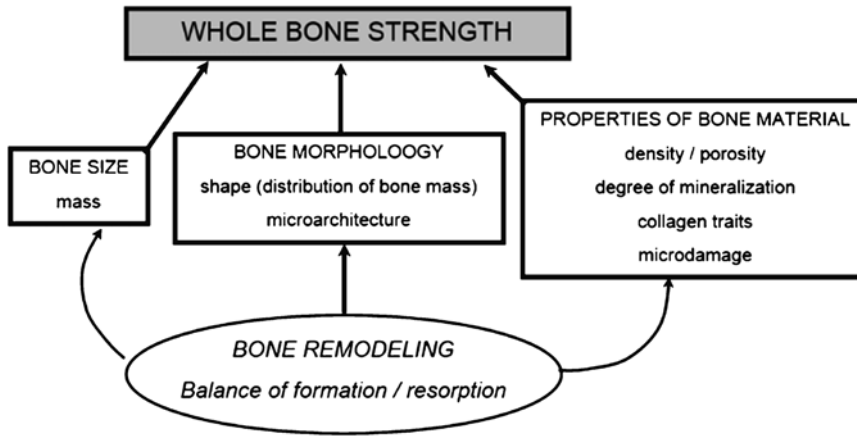

Fig. 5.2 Determinants of whole bone strength. The key determinants of whole bone strength include the bone mass, the bone geometry and microarchitecture, and the properties of the bone matrix

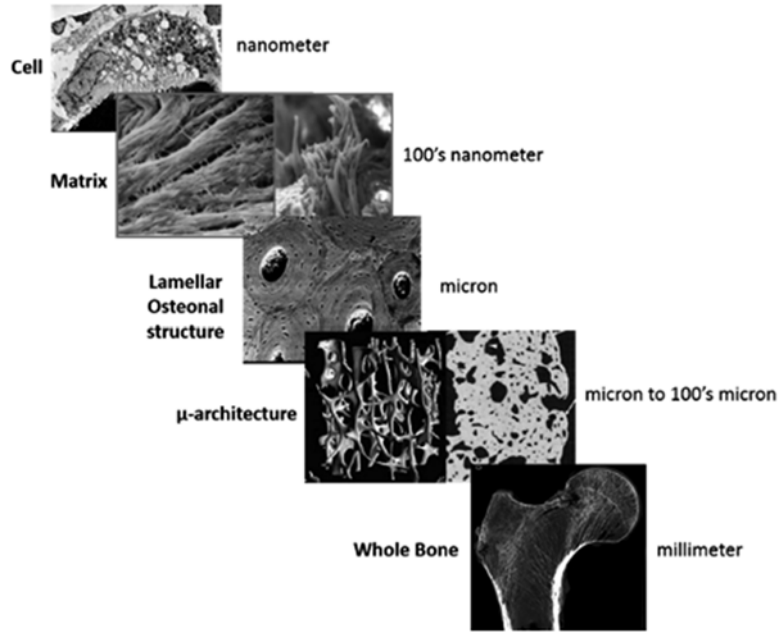

Fig. 5.3 Hierarchical nature of factors contributing to bone strength. Whereas fractures occur at the whole bone level, the bone biomechanical properties are influenced by alterations at many different length-scales: (1) activity at the cellular level, (2) the composition of the bone matrix, (3) the osteonal and lamellar structural, (4) the cortical and trabecular microstructure; and (5) the bone mass, geometry, and size

influence bone strength. Thus, conditions, diseases and drugs that impact bone formation and/or resorption will influence bone's resistance to fracture.

In considering these determinants of bone strength, one must keep in mind several important concepts. First, unlike most engineering materials, bone is continually adapting to changes in its mechanical and hormonal environment, and is capable of self-renewal and repair. Thus, in response to increased mechanical loading, bone may adapt by altering its size, shape, and/or matrix properties. This type of adaptation is readily seen by the greater size of the bones in the dominant versus non-dominant arm of tennis players [9]. In addition, favorable changes in bone geometry may occur in response to deleterious changes in bone matrix properties. For example, in a mouse model of osteogenesis imperfecta, a defect in the collagen that leads to increased bone fragility can be compensated for by a favorable change in bone geometry to preserve whole bone strength [10].

A second important concept concerns the hierarchical nature of the factors that influence whole bone strength (Fig. 5.3). Thus, properties at the cellular, matrix, microarchitectural, and macroarchitectural levels may all impact bone mechanical properties [11]. Importantly, the various factors are interrelated, and therefore one cannot expect that changes in a single property will be solely predictive of changes in bone mechanical behavior. The challenge is to identify which changes have the greatest impact on whole bone biomechanical properties, and therefore fracture risk.

5.3.1 Material Versus Structural Properties of Bone

In any discussion of bone biomechanical properties, it is important to distinguish between the *material* and *structural* properties of bone. During any activity, a complex distribution of forces (or loads) is applied to the skeleton. With the imposition of these forces, bones undergo deformations. This

relationship between the forces applied to the bone and the resulting deformations characterize the *structural behavior, or structural properties,* of the whole bone. Thus, structural properties are influenced by the size and shape of the bone, as well as the properties of the bone tissue. In contrast to the structural behavior, the *material behavior, or material properties*, of bone tissue is independent of the specimen geometry. Thus, the material properties reflect the intrinsic biomechanical characteristics of cortical and trabecular bone. In evaluating the effect of disease or therapies on bone properties, biomechanical engineers evaluate the effects on both structural and material behavior of bone. Although the biomechanical properties of the whole bone are functionally the most important outcome, assessing bone *material* properties may be critical for understanding the mechanisms that underlie changes in whole bone properties.

There is a vast literature describing the factors that influence the intrinsic (i.e., material) properties of trabecular and cortical bone, including the response not only to slow, monotonic loading but also to high rate loading and cyclic loading. The material properties of *trabecular bone* are influenced by many factors; however, the strongest determinants are apparent density (or bone volume fraction) and the microstructural arrangement of the trabecular network. Sampled over a wide range of densities, the stiffness and strength of trabecular bone are related to density in a nonlinear fashion, such that the change in strength is disproportionate (i.e., greater) than the change in density [12–15]. For example, a 25 % decrease in density, approximately equivalent to 15 years of age-related bone loss, would be predicted to cause approximately a 50 % decrease in the stiffness and strength of trabecular bone. However, given the heterogeneous nature of trabecular bone, it is clear that density alone does not explain all of the variation in trabecular bone mechanical properties. Both empirical observations and theoretical analyses indicate that trabecular microarchitecture plays an important role (see below).

The primary determinants of the biomechanical properties of *cortical bone* include porosity and the mineralization density of the bone matrix (or tissue mineral density). Indeed, over 80 % of the variation in cortical bone stiffness and strength is explained by a power-law relationship with mineralization and porosity as explanatory variables [16–19]. Other properties that influence cortical bone mechanical behavior include, but are not limited to, its histologic structure (primary, lamellar, versus osteonal bone), the collagen content and orientation of collagen fibers, extent and type of collagen cross-linking, the number and composition of cement lines, and the presence of fatigue-induced microdamage.

A few of the factors that influence both the structural and material behavior of bone will be briefly presented in the sections that follow. However, readers interested in a more in depth discussion of bone biomechanics are referred to several excellent reviews on this topic [20, 21].

5.3.2 Role of Bone Geometry

With regard to whole bone biomechanical behavior, the overall size of the bone (i.e., mass) as well as its shape (i.e., distribution of mass) play important roles [22]. Consistently, laboratory testing of the strength of human cadaveric vertebra, distal radii and proximal femora has shown, not surprisingly, that larger bones are stronger than smaller bones [23–27]. Moreover, clinical observations support the importance of bone size. For example, decreased cross-sectional area of the radius is a risk factor for wrist fracture among both young girls [28] and postmenopausal women [29]. Smaller bone size also predicts fractures in older men [30]. In addition, women with smaller vertebral bodies have an increased risk of vertebral fracture [31–33], and men with smaller femoral neck size have increased risk of hip fracture [34].

The loads applied to the skeleton generally are a combination of compression or tension forces with bending or torsional moments. The resistance to bending and torsional loading is particularly important, as the highest stresses in the appendicular skeletal are due to these loading modes.

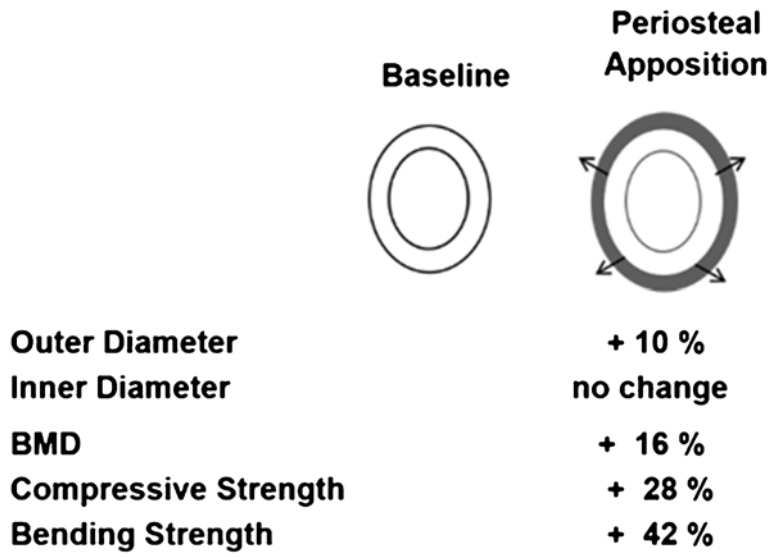

Fig. 5.4 Influence of bone geometry on BMD, and compressive and bending strength. This figure illustrates the positive impact of increased periosteal diameter on long bone biomechanical properties, showing that a small increase in bone's outer dimensions will improve bending and torsional strength more than would be measured by the BMD change

The most efficient design for resisting bending and torsional loads involves distributing the bone material far from the neutral axis of bending or torsion (generally this axis is near the center of the bone). The distribution of mass about the neutral bending axis is quantitatively described by a geometric property termed the area *moment of inertia*. Importantly, the area moment of inertia of a solid circular bar is proportional to its diameter to the fourth power (i.e., moment of inertia $\propto$ diameter^4). Thus, small increases in the external diameter of a long bone can markedly improve its resistance to bending and torsional loading (Fig. 5.4).

It is interesting to consider how age-related changes in bone geometry attempt to preserve whole bone strength. Considerable evidence indicates that age-related declines in the material properties of bone tissue are accompanied by a redistribution of cortical and trabecular bone. Specifically, in the appendicular skeleton these changes involve endosteal resorption along with periosteal apposition, leading to an age-related increase in the diameter of long bones but a decrease in cortical thickness [35–38]. This increase in outer diameter helps to maintain the resistance to bending and torsional loads. For many years, it has been suggested that men undergo this pattern of favorable geometric adaptation to a greater extent than women and therefore may be one reason for lower fracture rates in elderly men than women [35, 36, 39–41]. However, cross-sectional data from 3D-quantitative computed tomography (QCT) imaging challenge this paradigm, demonstrating that both men and women undergo favorable geometric changes with aging [42, 43].

5.3.3 Role of Bone Microarchitecture

5.3.3.1 Trabecular Microarchitecture

Although bone density is among the strongest predictors of the mechanical behavior of trabecular bone, both empirical observations and theoretical analyses show that aspects of the trabecular microarchitecture influence trabecular bone strength as well [14, 15, 44, 45]. Trabecular architecture can be

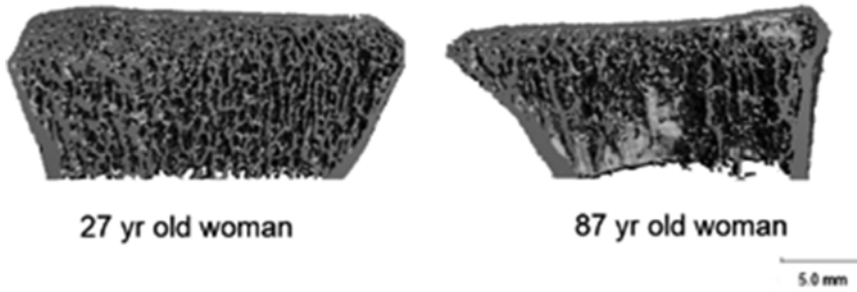

Fig. 5.5 In vivo assessment of trabecular microarchitecture. These HR-pQCT images of the distal radius in a 27-year-old woman (*left*) and a 87-year-old woman (*right*) show the profound skeletal deterioration associated with aging, and demonstrate the ability of in vivo measurements to assess these changes

described by the shape of the basic structural elements and their orientation. The trabecular structure is generally characterized by the number of trabeculae in a given volume, their average thickness, the average distance between adjacent trabeculae, and the degree to which trabeculae are connected to each other. In addition, recent work utilizes image processing algorithms to characterize individual trabecular elements, counting the number of plate- and rod-like elements, as well as their spatial orientation (e.g., vertical, oblique, transverse) [46, 47]. For many years, assessment of trabecular microarchitecture was possible only by two-dimensional histomorphometry of iliac biopsies. However, newer imaging modalities such as micro-computed tomography (μCT) and magnetic resonance imaging allow for three-dimensional assessment of trabecular structure on excised bone specimens [48–50]; while high resolution peripheral quantitative computed tomography (HR-pQCT) allows assessment of bone microstructure in vivo at the distal radius and tibia [51–54] (Fig. 5.5).

Laboratory studies have demonstrated moderate to strong correlations between trabecular bone architecture and biomechanical properties of trabecular bone [55–59]. Generally, however, trabecular bone microarchitecture traits are strongly correlated with trabecular bone volume [48, 55, 56]. Therefore, discerning the independent effects of specific architectural features on bone mechanical properties has proven challenging. Nonetheless, including indices of trabecular architecture enhances prediction of the biomechanical properties of human trabecular bone [60]. To further address this issue, analytical studies have been used to investigate how specific changes in microarchitecture influence trabecular bone mechanical behavior [61–63]. For example, Silva and Gibson employed an analytical model of vertebral trabecular bone, and compared the predicted loss in mechanical properties due to equal loss of bone mass either by trabecular thinning or by removal of trabecular elements [61]. They found that for the same decline in bone mass, loss of trabecular elements was two to five times more deleterious to bone strength than thinning of the trabecular struts, implying that maintaining connectivity of the trabecular network is critical.

Another potential mechanism whereby trabecular bone properties decline with increased bone resorptive activity is the hypothesis that the presence of resorption cavities themselves serve as a site of local weakness where cracks in the trabeculae may initiate [64]. van der Linden and colleagues evaluated this possibility using an analytical model of vertebral trabecular bone, wherein they induced a 20 % decline in bone mass either by thinning the entire trabecular structure or by randomly introducing resorption cavities [62]. They made two important observations: (1) the reduction in bone strength was greater when bone loss occurred by introduction of resorption cavities than by trabecular thinning, and (2) in both cases the predicted decline in vertebral trabecular bone strength was larger (30 % for trabecular thinning and 50 % for introduction of resorption cavities) than the decline in bone mass. Altogether these findings confirm the deleterious impact of excessive bone resorption on trabecular

bone strength, and provide a partial explanation for why small changes in bone mass can have marked effects on vertebral fracture risk.

Decades ago, Parfitt discussed the potential deleterious effect of increased bone resorption on trabecular microarchitecture and strength [64]. The importance of trabecular bone microarchitecture has since been supported by clinical studies showing altered trabecular microarchitecture in iliac bone biopsies in subjects with fragility fractures compared to age-matched controls with no fractures [65–68]. More recent data from in vivo imaging of the appendicular skeleton shows that men and both premenopausal and postmenopausal women with history of fracture have deteriorated bone microarchitecture, even when adjusting for possible differences in BMD [54, 69–71]. In some studies, fracture cases and controls had similar BMD, but the individuals with fracture had worse microarchitecture [51, 54]. Furthermore, recent studies have shown that increased skeletal fragility in type 2 diabetes may be due in part to increased cortical porosity [72, 73], and that alterations in bone microarchitecture may contribute to race- and ethnic-related differences in fracture rates [53, 74, 75]. Altogether these clinical observations point to an important role of trabecular architecture in fragility fractures.

5.3.4 Role of Bone Matrix Properties

In addition to macroarchitecture and microarchitecture, features of the bone matrix itself influence bone mechanical properties. Thus, characteristics that affect bone mechanical properties include the composition of the matrix, include (but are not limited to) the relative ratio of inorganic (i.e., mineral) to organic (i.e., water, collagen, and non-collagenous proteins) components; the degree of matrix mineralization; mineral crystal size and maturation; the extent and nature of collagen cross-links; and the amount and nature of matrix microdamage [76].

5.3.4.1 Matrix Mineralization

It is well established that the degree of matrix mineralization, or ash content, strongly influences cortical bone mechanical properties [16, 77, 78]. Thus, the elastic modulus and strength of cortical bone are positively related to the degree of matrix mineralization. However, the ability of cortical bone to absorb energy may either increase (if the bone is relatively undermineralized to begin with) or decrease (if the bone is already fully mineralized) with increasing mineral content [79].

Bone tissue mineralization is shows minimal variation with age and across skeletal sites [80, 81]. This is in stark contrast to the marked variation in bone structure that is seen with aging and across skeletal sites [48, 82]. Drug therapies that decrease bone turnover will eventually increase the degree of matrix mineralization by prolonging the period of secondary mineralization [83, 84]. In contrast, agents that increase bone turnover may lead to a transient decrease in the degree of matrix mineralization as new remodeling units are initiated and new bone laid down. Thus, iliac crest biopsies from postmenopausal women treated with anti-resorptive therapy (calcium and vitamin D, raloxifene, risedronate, and alendronate) show an increase in the degree of mineralization that mirrors the suppression of bone turnover [85–88], whereas iliac crest biopsies from men treated with teriparatide show a slight decrease in the degree of mineralization [89]. These effects on matrix mineralization may contribute to the anti-fracture efficacy of these agents, with increased mineralization expected to lead to increased bone stiffness. This would be expected to be a favorable change in those who may have a slightly undermineralized bone matrix due to a longstanding elevated rate of bone

turnover due to estrogen deficiency and/or secondary hyperparathyroidism due to vitamin D deficiency. Indeed, long term treatment with alendronate "normalizes" the distribution of tissue mineral density in iliac crest biopsies [90].

5.3.4.2 Collagen Characteristics

Bone is a composite material with two primary constituents, mineral and collagen. Although collagen has long taken a back seat to mineral with regards to skeletal fragility, mounting evidence indicates an important role for age- and disease-related changes in collagen content and structure [91]. The majority of evidence suggests that in normal bone, the mineral provides stiffness and strength, whereas collagen affords bone its ductility and ability to absorb energy before fracturing [92]. Posttranslational modifications of collagen, including increased amounts of non-enzymatic glycation have been shown to influence bone mechanical properties [91, 93]. Specifically, increased posttranslational modifications has been shown to reduce mechanical strength and/or toughness in avian bone [94], diabetic rat bone [95], and in human cancellous bone [96]. However, their contribution to age-related skeletal fragility remains undefined.

5.3.4.3 Non-collagenous Proteins

In addition to modifications of the collagen network, non-collagenous proteins such as osteopontin and osteocalcin in the bone matrix can also affect bone material properties. Osteopontin is important for mineralization [97] and plays a role in bone resorption by anchoring osteoclasts to the mineral matrix of the bone surface [97, 98]. Osteocalcin stimulates mineral maturation and inhibits bone formation [99]. It recruits osteoclast precursors to bone resorption sites and helps with their differentiation into mature osteoclasts. These proteins have been recently considered to act as the glue that holds mineralized collagen fibers together [100]. Under an applied force this non-fibrillar component stretches, energy dissipates by the breaking of sacrificial bonds between adjacent collagen fibrils, and harmful crack formation is prevented [101]. Thus, alterations to the matrix composition of both collagenous and non-collagenous proteins may alter bone biomechanical properties.

5.3.4.4 Microdamage

Throughout life, physiologic loading of the skeleton produces fatigue damage in bone. Although the optimal methods to quantify microdamage in bone are under debate, numerous studies show that the accumulation of damage weakens bone (reviewed by Burr [102]). Moreover, it appears that microdamage initiates activation of remodeling, presumably to repair the damaged tissue [103, 104]. This intriguing observation suggests that one role of bone remodeling is to repair fatigue-induced microdamage in bone. It has then been hypothesized that excessive suppression of bone turnover may reduce the capacity of bone to repair microdamage, and eventually lead to reduced mechanical properties. While canine studies have shown that bisphosphonate treatment leads to increased microdamage accumulation [105–108], there is limited evidence that this accumulation directly leads to negative effects on bone mechanical properties [109, 110]. Thus, there is ongoing debate regarding the optimal level of bone turnover to prevent architectural deterioration while preserving the ability of bone to maintain calcium homeostasis, respond to altered mechanical loading and to repair microdamage. Further, the role of microdamage in age-related fragility fractures has yet to be established [110].

5.4 Effect of Altered Nutrition on Bone Biomechanical Properties

5.4.1 Influence of Vitamin D Deficiency on Bone Biomechanics

One of the main roles of vitamin D is to promote calcium and phosphate absorption, to allow for normal bone mineralization. In children, vitamin D deficiency leads to rickets, characterized by bowing of the long bones due to inadequate mineralization of growth plate cartilage and bone matrix. In adults, vitamin D deficiency leads to osteomalacia and/or secondary hyperparathyroidism. Importantly, individuals with wrist and hip fractures show increased prevalence of vitamin D deficiency [111, 112]. Yet, until recently, the means by which low levels of vitamin D influence bone strength were poorly understood.

A recent study by Busse and colleagues provided novel insights into the mechanisms that underlie increased skeletal fragility in vitamin D deficiency. In particular, they tested the hypothesis that increased fracture risk with vitamin D deficiency is not solely due to inhibited bone mineralization, but rather that there are multiple pathologic changes that contribute to reduced bone strength [113]. These investigators examined iliac bone biopsies from normal and vitamin D deficient individuals, and performed histologic analyses along with detailed biomechanical testing and high resolution imaging to determine the ability of the bone material to resist crack formation and growth. As anticipated, bones from vitamin D deficient subjects had increased thickness of the unmineralized osteoid layer covering the bone surface, with decreased cortical and trabecular bone thicknesses. Analysis of the external cortex revealed higher porosity and widening of Haversian canals, consistent with defective mineralization. However, interestingly, in those with vitamin D deficiency the bone itself had a higher degree of mineralization and was less resistant to microcrack formation and growth than bone from subjects with normal vitamin D. The authors hypothesized that the thickened osteoid layer inhibits osteoclasts from accessing the underlying bone, and hence the bone becomes "older" and more brittle, resulting in a bone material with reduced fracture toughness. This innovative study of human bone examined at multiple length-scales with several complementary techniques has provided improved understanding into the multiple mechanisms whereby vitamin D deficiency reduces bone strength.

5.4.2 Influence of Undernutrition and Overnutrition on Bone Biomechanics

Undernutrition, whether due to reduced food availability or voluntary caloric restriction, has profound negative effects on bone strength. Low caloric intake reduces IGF-1 levels and may contribute to reduced bone size, lower BMD, and poor bone microarchitecture. As discussed above, basic biomechanical principles dictate that whole bone strength is directly related to bone size, and thus, these principles explain the observations of smaller bone size in individuals with wrist [29], hip [34], and vertebral fractures [32]. In support of the prominent role of IGF-1 on skeletal health, genetically altered mice with low IGF-1 levels exhibit reduced bone size, bone mineral density, and cortical thickness compared to controls [114]. Caloric restriction in mice leads to profound negative effects on bone mass, bone microarchitecture, and bone strength [115], whereas anorexia in young women is associated with increased fracture risk, reduced bone mass, deficient bone microarchitecture, and lower estimated bone strength [116–118].

Overnutrition, including high-fat or high-carbohydrate diets, can also have deleterious effects on bone strength. Whereas increased body weight was thought to be protective against fractures, emerging evidence suggests that obesity is a risk factor for fracture at some, but not all, skeletal sites [119, 120]. Moreover, individuals with type II diabetes, who are generally overweight, have an increased

risk of fracture despite having normal to high BMD [121]. The biomechanical mechanisms underlying increased fracture risk in cases of overnutrition are incompletely understood. Certainly, overweight individuals may be at increased risk of falls, and when they do fall, they will apply higher forces to the skeleton than normal weight individuals. Independent of these effects, there is some evidence that obese individuals have bone geometry and microarchitecture insufficient for their greater body weight [122–125].

5.5 Conclusion

In conclusion, this chapter reviews several concepts related to the biomechanical aspects of skeletal fragility. First, fractures occur when the loads applied to bone exceed its strength. Therefore, strategies to reduce fractures should consider interventions aimed at reducing the loads applied to bone as well as to maintaining or increasing bone strength. Second, whole bone strength is determined by the amount of bone (i.e., size or mass), the spatial distribution of the bone mass (i.e., shape or architecture), and the intrinsic properties of the materials that comprise the bone. Areal bone mineral density measurements by DXA reflect some of the components of bone strength, including bone mass, the degree of mineralization and to some extent bone size. It is because of this that BMD measurements are moderately to strongly correlated with the strength of human cadaveric vertebrae, radii and femurs. However, BMD measurements do not reflect other components of bone strength, including the 3D distribution of bone mass, trabecular and cortical microarchitecture, and the intrinsic properties of the bone matrix. Alternative methods for noninvasive assessment of bone geometry, microarchitecture, and strength are currently being used in clinical research studies, and hold promise for more sensitive and specific assessment of fracture risk [126–128] (Fig. 5.6).

Nutrition, diseases, and therapeutic interventions influence not only BMD but also the various components of bone strength that were discussed in this chapter. Thus, assessment of fracture risk and treatment efficacy in the individual patient must consider the repertoire of factors that may be influencing bone strength.

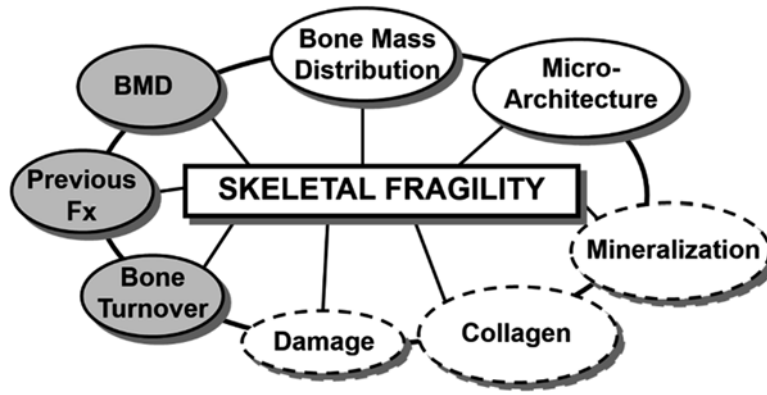

Fig. 5.6 Assessment of skeletal fragility: today and tomorrow. The *boxes* shaded on the *left* indicate tools that the clinician has in hand today to assess skeletal fragility. In comparison, the importance of assessing bone mass distribution and microarchitecture is currently being evaluated in clinical studies (*solid lines*). Assessment of bone mineralization, collagen characteristics, and microdamage is currently not capable by noninvasive assessments (*dotted lines*), though these factors likely influence bone strength and skeletal fragility

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Chapter 6
Clinical and Research Applications of Bone Mineral Density Examinations

Leon Lenchik, Scott Wuertzer, and Thomas C. Register

Key Points

- Bone densitometric techniques allow quantitative measurement of BMD and are commonly divided into central and peripheral.
- Central methods allow measurement of BMD in the spine and proximal femur and include dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT).
- Peripheral methods allow measurement of BMD in the phalanges, forearm, tibia, or calcaneus and include peripheral dual X-ray absorptiometry (pDXA) and peripheral quantitative computed tomography (pQCT).
- Although it does not measure BMD, quantitative ultrasound (QUS) is often included with peripheral methods.
- The most important principle of monitoring using DXA is that the same bone (or ROI) must be measured and analyzed the same way.

Keywords Bone densitometry • Bone Mineral Density (BMD) • Dual X-ray Absorptiometry (DXA) • Quantitative Computed Tomography (QCT) • Peripheral Dual X-ray Absorptiometry (pDXA) • Peripheral Quantitative Computed Tomography (pQCT) • Quantitative Ultrasound (QUS) • Techniques for measuring bone mineral density • Fractures • Osteoporosis • Fracture risk assessment • Diagnosis of osteoporosis

6.1 Introduction

For over a quarter of a century, various types of examinations measuring bone mineral density (BMD) yielded essential information about bone health and fracture risk and have made a significant impact on osteoporosis research as well as on patient management. Yet care must be exercised when interpreting the results of these, "densitometric," examinations, as pitfalls are common and may be overlooked.

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6.2 Techniques for Measuring BMD

Conventional radiographs are used routinely for the diagnosis of fractures and to help with fracture management. Prior to fracture, the assessment of bone mineralization on conventional radiographs is subjective, characterized by poor inter- and intra-observer reproducibility as well as low correlation with quantitative measurement of BMD [1–4]. In the absence of fracture, the ability of conventional radiographs to assess bone health and fracture risk is limited; thus, the use of bone densitometry has become routine.

Bone densitometric techniques allow quantitative measurement of BMD and are commonly divided into central and peripheral. Central methods allow measurement of BMD in the spine and proximal femur and include dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT). Peripheral methods allow measurement of BMD in the phalanges, forearm, tibia, or calcaneus and include peripheral dual X-ray absorptiometry (pDXA) and peripheral quantitative computed tomography (pQCT). Although it does not measure BMD, quantitative ultrasound (QUS) is often included with peripheral methods.

The technical characteristics of densitometric devices are typically summarized in terms of precision (i.e., reproducibility based on multiple measurements) and trueness (i.e., comparison of measured value with "true" mineral content). When used properly, bone densitometry devices have very low precision errors [5–10]. In particular, the coefficient of variation of posterioanterior (PA) spine measurement with DXA approaches 0.5 % [7, 8].

6.2.1 DXA

Although usually applied to the lumbar vertebra and proximal femur, DXA allows measurement of BMD of virtually any skeletal region. DXA scanners consist of an X-ray source and associated X-ray collimators and detectors. On central DXA devices, the X-ray source is usually located below the scanner table and is coupled via a C-arm to X-ray detectors located above the table. During scan acquisition, the scanner C-arm moves but does not contact the patient.

The physical basis for DXA measurements may be summarized as follows: X-ray photons are differentially attenuated by the patient based in part on their energy and on the density of the tissue through which they pass [11]. Dual-energy X-rays are required to determine how much of the attenuation of X-ray photons is attributable to bone rather than soft tissue. Manufacturers of DXA equipment use different approaches for producing and detecting dual-energy X-rays. One approach uses K-edge filtering to split the polyenergetic X-ray beam into high-energy and low-energy components. These systems require the use of energy-discriminating detectors and an external calibration phantom. The other approach uses voltage switching between high and low kVp during alternate half-cycles of the main power supply. These systems require current-integrating detectors and an internal calibration wheel. DXA scanners also differ according to the size and the orientation of the X-ray beam. Older scanners use a "pencil beam," with a collimated X-ray beam and a single detector moving in tandem. Most scanners in use today are "fan beam," with an array of X-rays and detectors, allowing for shorter scan acquisition times and higher image resolution.

There are several limitations of DXA for measuring BMD [5]. Perhaps the most important is that DXA provides an areal measurement of BMD (g/cm^2) rather than the volumetric measurement (mg/cm3) provided by QCT. Areal measurements do not take into account bone thickness and are influenced by body size and bone size. Young men typically have higher areal BMD (aBMD) than young women, who have smaller skeletons. Various studies [12–21] have addressed this issue by adjusting areal BMD for bone size either by (1) dividing aBMD by height, height squared, or square root of

height, (2) dividing aBMD by square root of bone area to calculate bone mineral apparent density (BMAD), (3) dividing aBMD by vertebral body width, (4) estimating vertebral volume from PA and lateral spine DXA scans. Unfortunately, in clinical practice, there is no simple way to account for this limitation because the DXA software does not perform a volumetric adjustment. Determination of BMD in individual bone compartments is also limited by the two-dimensional nature of DXA, which integrates cortical and trabecular BMD in the path of the X-ray beam [5, 11]. In contrast, QCT allows differentiation of trabecular and cortical BMD [5, 6].

Despite these limitations, central DXA is generally considered the "gold standard" for the clinical measurement of BMD. This is justified by the fact that this technique has been the most widely studied and requires only a low level of radiation exposure (<10 microSv) [22, 23]. Because DXA has been used in most epidemiological studies, it is well known how aBMD relates to fracture risk [24–35]. DXA has also been used in most pharmaceutical trials for selection and monitoring of subject populations [36–47]. Perhaps the main reason why DXA is considered the clinical gold standard relates to the widespread and growing consensus on its use for the diagnosis of osteoporosis, the assessment of fracture risk, and in therapeutic guidelines [48–56]. For these reasons as well as the low cost and broad availability, it is likely that central DXA will continue to be the most widely used approach to clinical BMD measurement.

As research applications of BMD measurements continue to increase, QCT offers some important advantages over DXA.

6.2.2 QCT

Although usually applied to the lumbar spine, QCT allows measurement of BMD in the proximal femur, forearm, tibia, and thoracic spine, and could be applied to any bone in the body. Measurements are obtained using any clinical CT scanner with a dedicated software package and calibration phantom.

The physical basis for QCT measurements may be summarized as follows [5, 6]: Computed tomography (CT) uses X-rays to generate an image based on total linear X-ray absorption coefficient. CT uses the absorption coefficients to generate an attenuation numbers in Hounsfield Units (HU), calibrated so that water = 0 HU. The QCT software converts the HU of the bone mineral phantom, to obtain a linear regression correction curve from which the patient's BMD is calculated [5, 6].

Initial work with QCT used two-dimensional, single-slice acquisitions through the mid-portion of the lumbar vertebrae. Although valuable for research, it had low acceptance among clinicians due to insufficient precision and high radiation dose [57, 58]. Widespread availability of spiral multidetector CT scanners has revolutionized diagnostic imaging and with it the QCT field. Modern QCT measurements are performed with faster data acquisition (<10 s), improved precision (CV = 1.3–1.7 %), and lower radiation doses (1.5–3 mSv) [59].

Measurement of BMD using QCT offers many advantages over DXA [6, 59]: (1) QCT allows for volumetric BMD measurement, less influenced by the patient's height and weight, (2) QCT allows separate measurement of trabecular BMD and cortical BMD, (3) QCT measurement of trabecular BMD is less influenced by degenerative disease, (4) QCT allows quantitative assessment of bone size, bone volume, and other morphometric parameters from which information on bone strength can be derived.

There are several limitations of QCT for measuring BMD [6, 59]: (1) The radiation dose is much higher than DXA, (2) There is insufficient standardization on regions of interest with regards to size, placement, and analysis.

Despite these limitations, there is increasing clinical acceptance of QCT. The International Society for Clinical Densitometry (ISCD) and the American College of Radiology (ACR) have recently published practice guidelines for the clinical use of QCT [59, 60].

The use of QCT in research has been growing even more rapidly [61–73]. The use of volumetric BMD for finite element analysis is especially promising [69–73].

6.2.3 *Peripheral Devices*

Peripheral devices include peripheral DXA, peripheral QCT, and quantitative ultrasound (QUS). These provide greater portability, ease of use, and lower radiation than central devices.

The physical basis of central DXA and peripheral DXA devices is the same [5, 74]. The main differences among peripheral DXA devices are the type of skeletal sites that are measured (i.e., phalanges, forearm, or calcaneus) and the X-ray beam geometry that is used (i.e., pencil beam or cone beam).

The physical basis of central QCT and peripheral QCT devices is the same [6, 59]. Peripheral QCT devices are dedicated scanners used to measure the forearm or the tibia. They offer the same advantages over DXA as central QCT, including the ability to differentiate trabecular from cortical BMD [6, 59]. Due to the lower radiation dose compared to standard CT, there is increasing use of pQCT in children and adolescents.

Although QUS devices do not measure BMD, they are usually included in discussion of peripheral methods for assessment of bone [5]. The main advantage of QUS over DXA and QCT is lack of ionizing radiation. There is much greater technologic diversity for QUS devices than for any densitometric method. Most QUS devices measure the speed of sound (SOS) and/or the broadband ultrasound attenuation (BUA). SOS is expressed in meters per sec (m/s). BUA is expressed in decibels per megahertz (dB/MHz). SOS and BUA values are lower in patients with osteoporosis when compared to normal controls. Differences among QUS devices are related to measurement site (i.e., phalanges, calcaneus, or multiple sites), sound transmission (i.e., trabecular transverse, cortical transverse, or cortical axial), transducer coupling (i.e., water- or gel-based), data acquisition (i.e., fixed single-point or variable imaging), definitions of velocity and attenuation, and calibration methods [75].

Although the clinical use of peripheral devices has been declining, practice guidelines developed by the ISCD are available [59, 74, 75].

There is continuing interest in peripheral devices in the bone research community [76–78]. The use of QUS in the pediatric population is especially promising because it provides an opportunity to detect skeletal disease without exposure to ionizing radiation [76–78].

6.3 Rationale for Bone Densitometry

6.3.1 *Fracture Trials*

Bone densitometry is a powerful clinical tool due to its ability to predict the risk of fracture. Since the critical measures of the impact of osteoporosis are fractures and their outcomes, the ability to identify individuals at high risk for fractures is paramount.

Many cross-sectional and longitudinal studies have shown that BMD measured by DXA is highly associated with osteoporotic fractures [24–35]. In general, for each standard deviation decrease in BMD, the risk of osteoporotic fracture doubles [79] (Fig. 6.1). DXA measurements at the hip predict hip fracture better than measurements at other skeletal sites (relative risk 1.9–3.8) [79].

Compared to DXA, QCT measured BMD for prediction of fractures has been less well studied. In postmenopausal women, QCT has been shown to predict spine fractures as effectively as DXA [59]. Hip fracture prediction using QCT is less well established [59]. The combination of QCT measured BMD and CT measured geometric parameters may improve prediction of fracture [72].

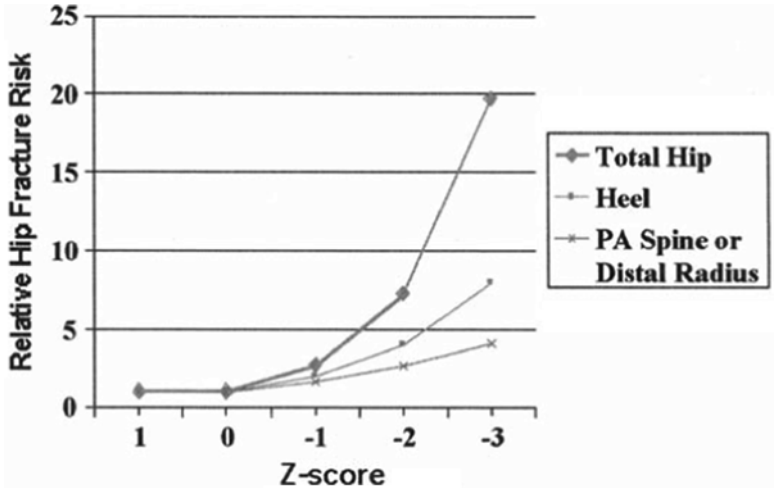

Fig. 6.1 Relative risk of hip fracture compared to individuals of the same age and with an average BMD obtained from measurements of BMD at various skeletal sites using DXA. In the Study of Osteoporotic Fractures the relative risk of hip fracture per 1 SD decrease in BMD was 2.7 (CI 2.0–3.6) when BMD was measured at the proximal femur, 2.0 (CI 1.5–2.7) when BMD was measured at the heel, and 1.6 (CI 1.2–2.2) when BMD was measured at the PA spine or distal radius [17]. Unlike the case of predicting hip fracture risk, in which hip BMD measurement appears superior to other methods, in predicting any osteoporotic fracture, all BMD measurements (i.e., central and peripheral) had comparable relative risk. *PA* posterior–anterior

Peripheral devices have also been shown to predict fracture risk [74–86]. Calcaneal and forearm BMD measurements using pDXA have been shown to predict vertebral and overall fractures [74]. Forearm BMD measurements using pQCT have been shown to predict hip fractures [59]. Calcaneal measurements using QUS have been shown to predict vertebral, hip, and overall fractures [75, 84, 85].

6.3.2 Biomechanical Studies

It is generally accepted that osteoporotic fractures result from diminished bone strength, which is in turn determined by both BMD and "bone quality." Biomechanical studies have shown that BMD is a powerful predictor of bone strength. Material properties of trabecular bone specimens vary according to BMD and anatomic site. It has been estimated that 60–90 % of the variability in elastic modulus is explained by apparent density [87–91]. There is high inverse association between femoral BMD and failure load. Correlations between vertebral BMD and failure load are higher for DXA ($r = 0.80$–0.94) than QCT ($r = 0.30$–0.66), reflecting the contributions of both the size of the bone (which influences areal BMD) as well as the cortical component of bone to biomechanical strength [92–94].

6.4 Clinical Use of BMD Measurements

In clinical practice, BMD measurements are usually obtained using central DXA. Practice guidelines for osteoporosis from various professional organizations usually include DXA [50–54]. The BMD measurements are typically used to help with the diagnosis of osteoporosis, assessment of fracture risk, selection of patients for pharmacological therapy, and monitoring of disease progression or therapeutic efficacy [95–98].

6.4.1 Diagnosis of Osteoporosis

Osteoporosis is commonly diagnosed by presence of a fragility fracture of the spine or proximal femur. Prior to fracture, measurement of BMD allows diagnosis of osteoporosis, which is analogous to diagnosis of hypertension prior to the occurrence of stroke.

6.4.1.1 Diagnosis of Osteoporosis Using DXA

With DXA, the diagnosis of osteoporosis is made according to the T-score, a standardized score unique to bone densitometry. T-scores are calculated by subtracting the mean BMD of a young-normal reference population from the subject's measured BMD and dividing by the standard deviation of a young-normal reference population. T-score based diagnosis relies on the World Health Organization (WHO) criteria [99]: Osteoporosis (T-score less than or equal to –2.5), osteopenia or low bone mass (T-score between −1.0 and −2.5), and normal (T-scores equal to or above −1.0). According to the ISCD, this diagnostic approach should be used only in postmenopausal women and men age 50 years and older [55].

The reason that T-scores rather than BMD values are used to diagnose osteoporosis is explained in part by the fact that manufacturers of DXA scanners use different approaches to BMD measurement. Each manufacturer uses a different approach for producing and detecting the dual-energy X-rays, for calibrating the scanners, and in some cases for defining the regions of interest where the BMD is measured. Thus, the same BMD value cannot be used for diagnosis of osteoporosis using different devices.

By definition, the T-score is dependent on the peak bone mass of young normal adults. But the peak bone mass as well as the change in BMD with aging and disease vary between the sexes and among ethnic groups [100].

The use of DXA to diagnose osteoporosis in men has received special scrutiny [13, 33–35, 54–56, 101–108]. DXA measured areal BMD is approximately 10 % higher in young men than in young women [13]. This is primarily related to the fact that men have higher body weight and larger bones than women, and aBMD measurement by DXA is influenced by body weight as well as bone size [13]. All DXA manufacturers calculate T-scores and Z-scores in men based on the male reference data, hence at a given aBMD value the T-scores in men and women are different. Recently, several professional organizations including the ISCD have proposed an alternative approach for the diagnosis of osteoporosis in men [56]. The new approach would use female reference data to calculate T-scores in men [105, 106]. Proponents of the new approach point to studies that show men fracturing at the same BMD as women [34, 102]. Others argue for retaining the current approach to T-score calculation in men, based on studies that show men fracturing at higher BMD values than women [103, 104].

The use of DXA to diagnose osteoporosis in non-Caucasians also poses some challenges [101, 109]. In general, aBMD in young adult men and women is higher in African-Americans than in Caucasians or Hispanics. The approach to diagnosis in non-Caucasians is complicated by the fact that some DXA manufacturers adjust their T-scores for race, whereas others do not (i.e., Hologic adjusts in women and men, Norland adjusts in women but not men, GE-Lunar does not adjust) [101]. Because there are many races and ethnicities (and admixing of races is common) and the relationship between BMD and fracture risk in non-Caucasians is less established, the best approach to diagnosis in these individuals is unknown. Currently, the ISCD recommends using the Caucasian reference data for non-Caucasians [55].

In premenopausal women, men younger than 50, and children, the WHO criteria do not apply. In these groups, Z-scores provide an important measure of skeletal health [55]. The Z-score is calculated similarly to the T-score except that an age-matched reference population is used. In premenopausal women, men younger than 50, and children with Z-scores below -2, the ISCD recommends avoiding terms such as osteopenia and osteoporosis. Instead, the ISCD suggests terminology such as, "bone mass below the expected range for age" [109].

6.4.1.2 Diagnosis of Osteoporosis Using Other Technologies

It is widely accepted that the WHO diagnostic thresholds are appropriate only for DXA measurements at particular skeletal sites (e.g., PA spine, hip, and forearm) [55]. The diagnostic approaches to osteoporosis using QCT, pQCT, pDXA, and QUS have been controversial [110].

The use of WHO criteria for QCT is not recommended because it would result in over diagnosis of osteoporosis [6, 59]. To provide an alternative method for diagnosing osteoporosis using QCT, specific diagnostic thresholds based on spinal trabecular BMD have been proposed [6, 60]. The thresholds have been endorsed by the American College of Radiology [60].

The use of WHO criteria for peripheral devices is not recommended [110]. T-scores obtained on peripheral devices are not comparable to those obtained with central DXA because these techniques use different physical principles, measure different skeletal sites with different rates and patterns of bone loss, and use different normative databases [110]. Ideally, unique diagnostic thresholds for each technique would be established based on risk profiles for future osteoporotic fractures. At present, the ISCD does not recommend using peripheral devices for diagnosis [55].

6.4.1.3 Other Limitations of T-score Based Diagnosis

Diagnostic criteria based on T-scores have several other important limitations. The use of any threshold for diagnosing osteoporosis may be misleading, since the relationship between decreasing BMD and increasing risk of fracture is continuous rather than threshold-based and there is substantial overlap in BMD among fracture and non-fracture patients. Thus, it is impossible to define a threshold BMD value for a population below which everyone will fracture or above which no one will fracture. Despite that limitation, the T-score based diagnostic threshold has been essential for clinical practice, especially in the United States, where the risk of a disease is generally not interchangeable with the diagnosis of a disease. For example, various threshold-based diagnostic approaches are used for hypertension, hypercholesterolemia, and type 2 diabetes, where similar continuous relationships between measured and outcome variables exist.

Another problem with the T-score based approach to diagnosis relates to the fact that T-scores are dependent on an "appropriate" reference database (i.e., young adult reference means and standard deviations). However, there is poor agreement among reference databases of different manufacturers as well as between reference data from various study populations [111–114]. Different manufacturers may use different inclusion and exclusion criteria when gathering normative data. Furthermore, a single manufacturer may use different reference populations at different skeletal sites and regions of interest. If the standard deviations are different, the resultant T-scores are different, even when the mean BMD values for two normative populations are the same [113]. For these reasons, the same patient measured on different devices is likely to have different T-scores.

6.4.2 Fracture Risk Assessment

For many years epidemiological trials have shown that for each standard deviation decrease in BMD there is approximately a twofold increase in risk of fracture [79]. Yet, in clinical practice, the use of BMD to calculate fracture risk has posed some challenges.

6.4.2.1 Fracture Risk Assessment Using DXA

With central DXA, various approaches to risk reporting have been advocated, including absolute risk, relative, risk, short term risk, and long term risk. At the same time increasing evidence suggests that non-BMD risk factors contribute substantially to overall fracture risk [32].

Recently introduced into clinical practice, the World Health Organization fracture risk assessment tool FRAX® tool has helped standardize the clinical approach to fracture risk assessment using DXA [115, 116]. The tool estimates 10-year risk for major osteoporotic fracture (clinical vertebra, proximal femur, distal forearm, and proximal humerus) and hip fracture. The risk estimation is based on non-BMD risk factors (age, gender, weight, height, previous fracture, parental hip fracture, current smoking, use of glucocorticoids, rheumatoid arthritis, secondary osteoporosis, alcohol intake) and DXA measured BMD of the femoral neck. Although many non-BMD factors have been recognized as increasing the risk for fracture, not all are used in FRAX. FRAX incorporates only those risk factors that are easily measurable, common, and have been shown in large trials to predict fracture risk, independent of BMD. Importantly, some common risk factors such as high bone turnover do not have sufficient data to be included. Other risk factors such as frailty and high frequency of falls are not easily measured and are not included for that reason.

Like other fracture risk models, FRAX has limitations [117–121]. In particular, it is difficult to assign numerical fracture risk to many types of patients (i.e., younger women, patients with secondary osteoporosis, and patients on therapy), where the relationship between BMD and fracture risk is not known.

6.4.2.2 Fracture Risk Assessment Using Other Technologies

There is increasing evidence that BMD measured by technologies other than DXA are able to predict the risk of osteoporotic fracture. The ISCD has provided some guidance for the clinical assessment of fracture risk using these technologies [55, 59, 74, 75]: Spinal trabecular BMD measurement by QCT can be used to predict spine, but not hip, fractures in postmenopausal women; Distal radius BMD measured by pQCT can be used to predict hip, but not spine, fractures in postmenopausal women; Heel QUS measurement can be used to predict hip, vertebral, and global fractures in postmenopausal women and hip and nonvertebral fractures in men over age 65; BMD measurement with pDXA can be used to predict vertebral and global fractures in postmenopausal women. Applying these recommendations to clinical practice remains challenging. Ideally, a tool similar to FRAX would be developed for various BMD measurement technologies. Since this is unlikely, clinicians must use their own judgment on how incorporate these techniques into their practice.

6.4.3 Selection of Patients for Therapy

Clinical decisions about which patients should be offered pharmacologic therapy for osteoporosis are often based, at least in part, on measurement of BMD.

6.4.3.1 Selection of Patients for Therapy Using DXA

Utilization of DXA-based BMD thresholds to help select patients for pharmacologic therapy has been part of various widely used professional practice guidelines [50–54]. Such an approach is appropriate because evidence for fracture reduction exists mainly in subjects enrolled in pharmaceutical trials based on the presence of low BMD and/or the presence of a vertebral fracture. Enrollments based on BMD typically employed DXA for measurement. Furthermore, treatment efficacy for various pharmaceutical agents was determined using fracture and BMD as endpoints. The BMD monitoring also typically utilized DXA for measurement.

6.4.3.2 Selection of Patients for Therapy Using Other Technologies

There are no current guidelines for using non-DXA technologies to help select patients for therapy. Part of the problem is that most experts are against using these technologies for the diagnosis of osteoporosis. In the absence of diagnosis, treatment thresholds would need to be based on fracture risk. If a fracture assessment tool becomes available that employs QCT, pQCT, pDXA, or QUS, these technologies may play a more prominent role in therapeutic decisions.

6.4.4 Monitoring of Therapy

Monitoring of therapy using BMD measurements is possible as long as the devices used have low precision errors and the skeletal sites being monitored respond well to therapy [49]. To determine if interval change is statistically significant, the precision of the device must be known and the study must be technically valid. Least significant change is calculated from the precision error and used when monitoring patients [49]. Because of excellent precision and greatest responses to therapy, measurement of the spine BMD with DXA or QCT is preferable to other densitometric methods and measurement sites.

6.4.4.1 Monitoring of Therapy Using DXA

When monitoring patients using DXA the following considerations for site selection apply: PA spine is preferred because it has the lowest precision error and because it is most responsive to therapy [122]. If PA spine cannot be used, total hip may provide a viable alternative.

Monitoring time interval depends on the precision error and the expected change in BMD with therapy. Some therapies are associated with large increases in BMD, while others may have modest or no significant BMD changes [36–47]. Patient factors (i.e., glucocorticoid therapy, hyperparathyroidism, weight loss, etc.) also influence expected changes in BMD. However, in most cases, clinicians monitor therapy not to identify patients who gain BMD, but to identify those who lose BMD while on therapy. For that purpose, a 1–2-year follow-up interval is generally appropriate. In contrast, higher risk patients, such as those receiving glucocorticoids, may be followed as early as 6–12 months [49].

6.4.4.2 Monitoring of Therapy Using QCT

QCT is valuable for monitoring changes in BMD, in part because it can measure the more metabolically active trabecular bone compartment. According to the ISCD, "trabecular BMD of the lumbar spine measured by QCT can be used to monitor age-, disease-, and treatment related BMD changes" [59].

6.4.4.3 Monitoring of Therapy Using Peripheral Technologies

Monitoring bone status using peripheral technologies is not recommended [59, 74, 75].

6.5 Approach to Clinical DXA Interpretation

6.5.1 Site Selection

Typically, two skeletal sites are measured with DXA, the lumbar spine and proximal femur [55, 123]. The rationale for measuring both spine and hip is as follows: approximately 20–30 % of patients have significant spine–hip discordance, in which T-scores at one site are of a different diagnostic category than the other site [124, 125]. It is desirable to find the site with the lower BMD. Another reason for measuring both relates to fracture prediction, spine BMD is a better determinant predictor of spine fractures, whereas hip BMD is a better determinant predictor of hip fractures. Finally, in patients with severe degenerative disease of the spine, hip BMD offers more accurate monitoring. Various causes of spine–hip discordance have been described, including differences in achievement of peak bone mass and the rate of bone loss at different skeletal sites. For example, in perimenopausal women, rate of bone loss is greater in cancellous bone (e.g., spine) than cortical bone (e.g., hip). Body weight, obesity, exercise, and other parameters may also differentially influence BMD in these sites.

In patients in whom the spine and/or the proximal femur scans are invalid, a forearm scan may be obtained. For example, patients with spine instrumentation, fractures, severe degenerative disease, or scoliosis may benefit from a forearm scan [55]. Patients with bilateral hip replacements (or other instrumentation), severe hip arthritis, or patients who exceed the weight limit of the scanner table are candidates. Finally, forearm scan is useful in patients with hyperparathyroidism, since the mid-radius region of interest (also known as 1/3 or 33 % radius) measures primarily cortical bone which is particularly susceptible to loss due to hyperparathyroidism [55].

6.5.2 Scan Acquisition and Analysis

It is important to weigh the patient and measure his or her height, because these parameters may influence the selection of appropriate scan mode and in some cases influence the Z-score. Changes in weight and height loss should be recorded, as they may influence DXA results. Importantly, height loss could indicate the presence of a vertebral fracture and require additional evaluation with spine X-rays.

DXA examinations must be obtained using the manufacturer's recommendations for patient positioning, scan protocols, and scan analysis. The patient should change into a hospital gown (or equivalent) to remove potential artifacts related to street clothing.

The lumbar spine scans are acquired with the subject's body aligned with the scanner table and legs elevated using the standard positioning block. The scan should extend from the mid portion of L5 to the mid portion of T12 vertebra. BMD of lumbar vertebrae 1–4 are measured. The proximal femur scans are acquired with the subject's leg internally rotated using the standard positioning device. BMD of the femoral neck region, total hip region, and trochanteric region are measured. The forearm scans are acquired with the subject sitting in a chair adjacent to the scanner table and his or her nondominant forearm placed in the standard positioning device. BMD of the mid radius region, ultra distal radius region, and total forearm region are measured. Total body scans are acquired with the subject supine and aligned with the scanner table.

6.5.3 DXA Results

Presentation of DXA results varies according to the manufacturer and software version and is usually configurable by the DXA operator. Common features include a summary of patient demographics, an image of the skeletal site scanned, a plot of patient age versus BMD, and numerical results. For various regions of interest, the numerical results include the BMD values (g/cm^2), T-scores, and Z-scores. Other data, including BMC, area, %BMD, vertebral height, etc. may be provided.

When interpreting DXA examinations it is important to check whether correct patient demographics were entered into the DXA computer. Incorrect age, gender, and race may influence T-scores and/or Z-scores.

The next step is to evaluate the DXA image for proper patient positioning, scan analysis, and artifacts. On properly positioned PA spine scans, the spine appears straight (aligned with the long axis of scanner), both iliac crests are visible, and the scan starts in the middle of L5 and ends in the middle of T12 (Fig. 6.2). On properly positioned hip scans, the femoral shaft is straight (aligned with the long axis of scanner), the hip is internally rotated, and the scan includes the ischium and the greater trochanter (Fig. 6.3). The lesser trochanter is a posterior structure, and its size is the best indication of the degree of rotation of the proximal femur during a DXA study. BMD values are affected by the degree of rotation of the proximal femur and the position of the femoral neck region of interest, and internal or external rotation will cause an increase in the measured BMD. On properly positioned forearm scans, the forearm is straight and the distal ends of the radius and ulna are visible. On properly positioned total body scans, the patient is centered and the entire body is within scan limits.

It is important to rescan the patient when positioning errors occur. For example, if PA spine images show the spine is tilted or not centered, or that one or both iliac crests or T12 or L5 are missing, the patient should be rescanned. On the image of the hip scan, if the femoral shaft is angled (adducted or abducted) or the leg is not properly rotated (too much lesser trochanter is visible), the patient should be rescanned. On images of forearm scans, if the forearm is not centered, radius and ulna are angled, or the distal cortex of radius and ulna are not visible, the patient should be rescanned.

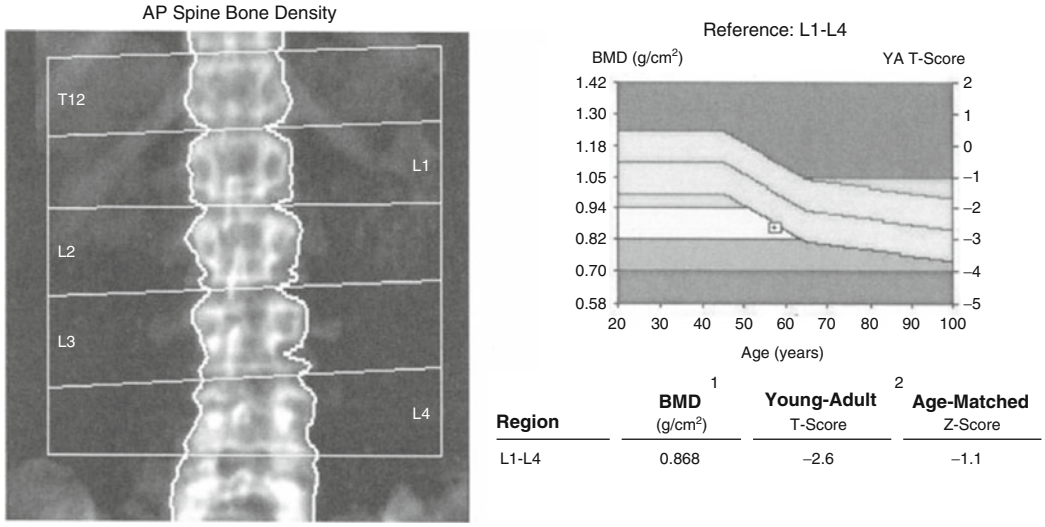

Fig. 6.2 DXA scan of the PA spine in a 56-year-old Caucasian woman not receiving any pharmacological therapy. Note proper patient positioning and scan analysis. The T-score is within the WHO range for osteoporosis

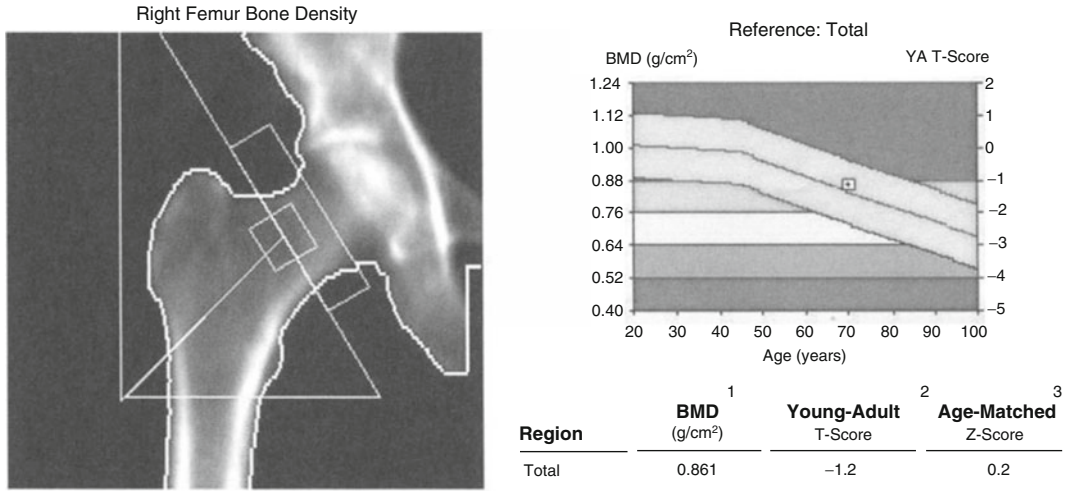

Fig. 6.3 DXA scan of the right proximal femur in a 69-year-old Caucasian woman on estrogen replacement therapy. Note proper patient positioning and scan analysis. The T-score is within the WHO range for osteopenia

DXA images should also show appropriate scan analysis (e.g., region of interest size and location). On the spine image, the vertebral bodies should be numbered correctly (Fig. 6.2). This is especially true in patients with four or six lumbar vertebrae, in whom numbering should begin at the level of the iliac crest—typically, corresponding to the L4–5 disk space. On the hip image, the femoral neck region must not include the greater trochanter or the ischium (Fig. 6.3). Femoral neck region of interest placement is manufacturer-specific (GE-Lunar measures the midportion of the femoral neck, Hologic measures the base of the femoral neck). For this reason, it is important to follow the manufacturer specific recommendations. On forearm scans, the distal radius region should not include the articular surface of the distal radius.

It is important to be able to recognize artifacts on DXA images. Common artifacts include degenerative disease and fractures (Fig. 6.4). Degenerative disease typically manifests as disk space narrowing, subchondral sclerosis, osteophytes, and facet osteoarthritis. Spinal degenerative disease often increases BMD [126, 127]. Severe osteoarthritis of the hip may increase BMD in the femoral neck or total hip (because of buttressing of medial femoral neck), whereas BMD in the trochanteric region is relatively unaffected [128]. When degenerative disease of the spine is limited to several vertebrae, those vertebrae should be excluded from the region of interest used for diagnosis or for monitoring. When disease is diffusely distributed, a forearm scan should be added. In general, vertebral fractures increase BMD. Vertebral compression fractures often appear as having decreased height compared to adjacent vertebrae. In some cases, the PA spine DXA image will not show a known fracture. In such cases, discrepant BMD values for individual vertebrae may indicate the presence of a compression fracture. Comparison to a VFA DXA image or lumbar spine X-ray is necessary to identify such fractures definitively. Fractures must be excluded from the region of interest used for scan analysis to prevent overestimation of BMD. Nonvertebral fractures may also increase BMD [129]. For this reason, avoid measures of the hip or forearm which have previous fracture.

Other artifacts include postoperative changes (i.e., laminectomy defects, spinal instrumentation and/or fusion), vascular calcifications, gastrointestinal contrast, calcium tablets, gallstones, renal stones, pancreatic calcifications, and various metallic devices. External artifacts, including buttons, zippers, bra clips, wallets, and jewelry, should be removed prior to scanning.

Patients should not be scanned if there has been recent gastrointestinal contrast, because contrast in overlying tissues invalidates BMD result; the patient is uncooperative and cannot remain still

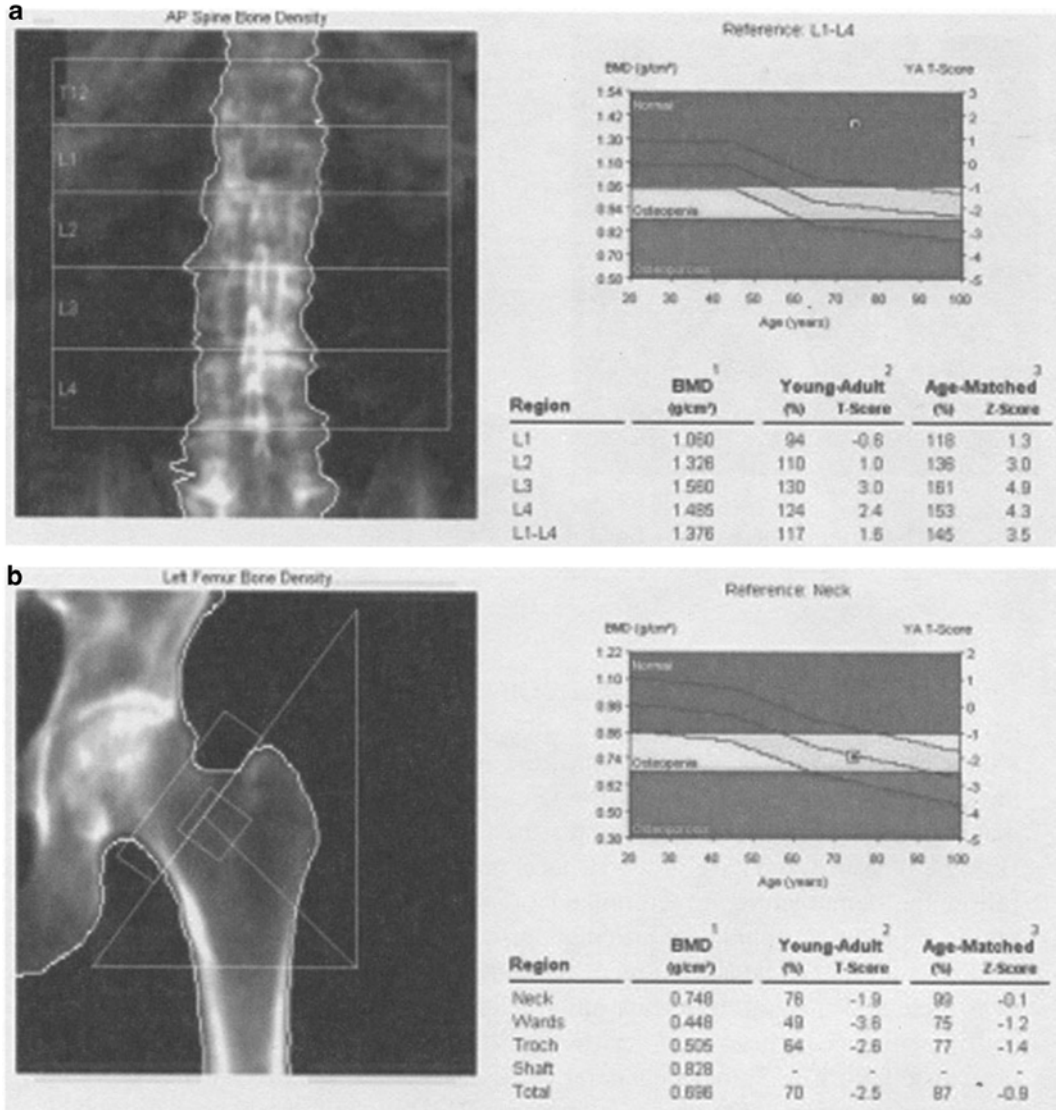

Fig. 6.4 (**a**) DXA scan of the PA spine in a 74-year-old Caucasian woman not receiving therapy. Note asymmetric sclerosis and osteophytes involving multiple vertebra indicating degenerative disease. Corresponding X-ray showed a fracture of L1 vertebral body. Although the T-score is within the WHO range for normal, the BMD is falsely elevated by degenerative disease and fracture. (**b**) DXA scan of the left proximal femur in the same woman shows the T-score in the total hip and trochanter within the WHO range for osteoporosis. This case illustrates a common occurrence in elderly patients, in which the spine BMD is in the normal range due to the presence of artifacts and yet the hip BMD is in the osteoporotic range

throughout the examination (motion artifacts invalidate BMD result); the patient is obese (DXA scanner tables have a weight limit of 250–450 lb).

The final step is to evaluate the numerical results. DXA results are expressed in absolute BMD units (g/cm^2), T-scores, and Z-scores. The absolute BMD is used to longitudinally monitor disease progression or response to therapy. T-score is used to make a diagnosis of osteoporosis in postmenopausal women and men over age 50. Z-score is used for diagnosis in premenopausal women, younger men, and children.

6.5.4 Diagnosis

In making the diagnosis of osteoporosis the following approach is used [55, 123]: Upon ensuring proper positioning and analysis, and if necessary, exclusion of artifacts, the lower of the T-scores of the PA spine and hip is used. In the spine, using the weighted average of L1-L4 is preferred (Fig. 6.2). Vertebra affected by focal structural abnormalities (i.e., fracture focal degenerative disease, or surgery) should be excluded from analysis. There should be an incremental increase in BMD from L1 to L4. Individual vertebral T-scores should be within 1 SD. When these two conditions are not met, the DXA image should be scrutinized to detect an artifact that should be excluded from analysis. Occasionally, a radiograph is needed for clarification.

In the hip, using the lowest T-score of the total hip (total femur) and femoral neck is recommended (Fig. 6.3). Ward's region and trochanteric region should not be used to diagnose osteoporosis. It is important to note that discrepancies between sites may indicate a true difference in BMD, but they may also be falsely elevated due to degenerative joint disease or fractures, or falsely decreased due to osteolytic bone tumors or postoperative changes

In making the diagnosis of osteoporosis using DXA, it is essential to recognize that low BMD does not explain its etiology. In particular, low BMD in postmenopausal women does not always imply postmenopausal osteoporosis due to estrogen deficiency but can also be due to secondary causes of osteoporosis. Furthermore, a single low BMD result may evolve in different ways, resulting, for example, from a low peak BMD followed by normal rate of loss, or a normal peak BMD with accelerated rate of loss.

6.5.5 Monitoring

When monitoring patients with DXA it is important that follow-up scans demonstrate consistent patient positioning and scan analysis [49]. The most important principle of monitoring using DXA is that the same bone (or ROI) must be measured and analyzed the same way. DXA images on the two comparison studies should be inspected to make sure that the ROI is the same size and position. When monitoring patients longitudinally, BMD values rather than T-scores should be compared, since the T-scores depend on a normative database that may change with software upgrades.

6.6 BMD Measurement in Research

6.6.1 Importance

Traditional and emerging methods for measurement of BMD and bone quality are important for both clinical and basic science research applications. In clinical trials and epidemiologic studies, skeletal imaging and BMD evaluation facilitates a greater understanding of the underlying determinants responsible for bone structure and strength. BMD is also an important translational tool linking basic science studies to clinical applications. Studies using bone density measurements have led to the identification of novel biomolecular pathways and/or molecules for targeting to improve bone health. With patient therapies, BMD represents an important phenotype which can be readily monitored noninvasively to assess responses to treatment in the research environment.

6.6.2 Applications

6.6.2.1 Epidemiology and Basic Science: Identification of Determinants of BMD

Skeletal biologic processes (and BMD) are dependent upon genetic and environmental influences and there is increasing interest in understanding the potential influences of a variety of conditions and diseases, including disorders of glucose metabolism (i.e., diabetes, insulin resistance, and metabolic syndrome), obesity and weight loss, sarcopenia, aging, amenorrhea, menopause, androgen deficiency, and other endocrine disorders, on the skeleton. Comprehensive studies combined with increasing use of systems biology approaches to understand biomolecular pathways involved in these relationships should provide new mechanistic insights into these complex systems.

Epidemiologic approaches as well as clinical studies will continue to be critically important for investigating determinants of BMD. These studies have been useful in identifying novel genes (such as LRP5) associated with specific skeletal BMD phenotypes [130, 131]. Further identification of genetic determinants underlying variability in BMD is an important ongoing area of investigation, and recent studies of GWAS datasets have revealed a variety of genes and genomic regions associated with BMD [132–134]. Determination of interrelationships and inter-tissue regulation between BMD/bone metabolism and other phenotypes, such as diabetes, atherosclerosis, and vascular calcification, represent areas of active investigation [62–64, 135–138]. Many of these epidemiologic studies are cross-sectional in nature, measuring multiple phenotypes across a specific population at a given point in time. Longitudinal epidemiologic studies using repeated measures of BMD and other phenotypes provide opportunities to evaluate relationships between changes in phenotypes relative to time and environmental influences. Longitudinal investigations into the importance of diet, endogenous and exogenous hormones, lifestyle parameters, behavior and stress, weight loss and gain, occupations, etc. are all being investigated and should provide insights into strategies for preservation of skeletal health in our aging population. Diet and nutritional contributors to BMD under recent or current investigation include dietary isoflavones, vitamins (D, K, etc.), proportions of macronutrients, specific protein constituents (animal vs. vegetable sources), carbohydrates (simple vs. complex, fructose), fats, minerals, etc. Dietary acid load and its relationship to skeletal health is also an area of interest.

6.6.2.2 Clinical Research Studies

Randomized, double blind, placebo controlled trials which monitor BMD over time provide the most effective means of evaluating efficacy of various treatment approaches. Recent and current experimental trials include evaluations of the effects of dietary constituents and supplements, exercise and weight loss regimens, and new and emerging drug therapies. Although BMD is often the primary outcome being assessed in randomized trials, the ultimate validation of the efficacy of a treatment is a reduction in the risk of fracture. Trials with fracture outcomes require large numbers of participants and longer experimental periods in order to generate reliable and statistically significant results, and are often impractical.

6.6.2.3 Preclinical Research Studies

Preclinical research studies in animal models allow evaluation of many of the above factors in a controlled environment which is not possible in human studies, and have the potential to provide important information into the relative efficacy of various treatments on the skeleton. DXA, QCT, microCT are all useful for monitoring the skeletal effects and relevant underlying mechanisms of emerging

investigational drugs, treatments, and dietary regiments in studies which can be tightly regulated and monitored in animal models. Nonhuman primate models have been particularly useful for predicting human BMD responses to drug as well as dietary regimens [139, 140].

6.6.3 Future Applications

Ongoing research to extend skeletal imaging outcomes beyond BMD to include bone geometry and microarchitecture have the potential to significantly improve the evaluation of bone quality and strength in the future [141]. This extended bone phenotyping is already providing new information regarding fracture risk and new insights into the mechanisms underlying responses to treatment. While these approaches are currently limited to research and some clinical trial applications, they may have more widespread clinical application in the future.

6.7 Conclusion

Despite growing interest in experimental techniques, such as quantitative magnetic resonance imaging, examinations measuring bone mineral density are vital to osteoporosis research as well as to patient management. When interpreted properly, the results of these examinations provide essential information about bone health and fracture risk.

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Chapter 7
Nutritional Epidemiology: Nutritional Assessment and Analysis

John J.B. Anderson and Katherine L. Tucker

Key Points

- Dietary assessment can determine the amount of foods, nutrients, energy, and other dietary components consumed.
- Methods include the 24-h recall, diet record, food frequency questionnaire, and others.
- Cross-sectional and longitudinal studies of the skeleton have used assessment methods to determine if any benefits accrue from defined diets or from single or multiple nutrient supplements
- Randomized controlled trials for one or more years designed to test nutrients besides calcium are needed for advancing our knowledge of skeletal effects and recommending dietary intakes of nutrients across the life cycle.

Keywords Food frequency • 24 h recall • Nutritional epidemiology • Diet assessment • Calcium • Phosphorus • Vitamin D

7.1 Introduction

This overview of nutritional assessment is a revised version of the chapter (Chap. 7) in the first edition [1]. Better understandings of diet–bone linkages help in both the promotion of bone health and the prevention of osteopenia, osteoporosis, and skeletal fractures in late life. Many nutritional factors contribute to skeletal development during the first two decades of life, to the maintenance of the adult skeleton, and to attempts to bolster bone mass and bone density in late life. Many nutrients are of importance for adults and the elderly, notably calcium, phosphorus, and vitamin D, but also protein, magnesium, vitamins C and K, carotenoids, and others. On the other hand, although phosphorus and vitamin A are both essential for bone status, too much of these nutrients may have negative consequences, and care to avoid excess intakes in the context of the US diet is advised. Several trace

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minerals found in bone likely exist by their omnipresence in the earth's crust including silicon, an under-researched, but important mineral for bone status [2] and an increasing number of phytonutrients, such as carotenoids, are understood to protect bone with aging.

Prior reports of nutrient intakes of older adults show that major changes in nutrient consumption begin at approximately 70 years of age, when older men and women start to have marked reductions in both macronutrient and micronutrient intakes from foods [3–5]. Since micronutrient requirements do not generally decrease, and in some cases increase, with aging, the selection of micronutrient-dense foods remains of major importance throughout adulthood.

Several chapters in this book provide information on other risk factors for bone health, including nutritional variables and lifestyle factors. The use of data on dietary intakes of calcium, for example, permits statistical analyses that uncover associations or linkages between assessments of dietary variables and measurements of bone mass (bone mineral content, BMC) or density (bone mineral density, BMD) by dual energy X-radiography (DXA). DXA is the main device used today for assessing adult bone status. Epidemiological and biostatistical methods, using both linear and nonlinear techniques, generate statistical meaning of any diet–bone associations, but not of the mechanistic aspects of the linkages that require biological information.

This chapter covers methods used in nutritional assessment with selected references to published reports that utilize the types of nutritional assessments that relate dietary nutrients to bone status.

7.2 Dietary Intake Assessment

Capturing the intake of nutrients across a 24-h period or longer is much more difficult than it may appear [6]. Validity of dietary data varies by method and by nutrient, depending on time period to be assessed. Common dietary methodologies include the diet record, where individuals record detail on everything they eat or drink, the 24-h recall (24HR), where respondents are asked to report everything they ate or drank the day before with detail on preparations and recipes, and the food frequency questionnaire (FFQ), where individuals report their usual pattern of intake over the past year for different types of foods, with or without additional information on portion sizes and preparation. For epidemiologic analyses relating dietary intake to bone status or fracture outcome, we are usually interested in usual long-term intake, which may be estimated with multiple individual days or with one or more food frequency questionnaires. Because diet records require educated and compliant volunteers, they are less useful in large population-based epidemiologic studies. The respondent burden is high, and poor completion rates and variable completion quality limit their validity. Further, dietary records have been shown to underestimate and misrepresent usual intake, as individuals tend to consume less when focused on recording their intake. For these reasons, we discuss only the 24-h recall (24HR) and food frequency questionnaire (FFQ) methods below.

7.2.1 24-Hour Recall

The 24HR is widely used in large surveys, including the National Health and Nutrition Examination Survey (NHANES) in the USA. An interviewer asks each participant to recall everything ingested as a food or beverage, or taken as a supplement, during the previous 24 h. Although incomplete information is always a concern with this method because of memory lapses or lack of knowledge of specific recipes of dishes consumed, recent advances in data entry, such as the USDA automated multiple pass system, along with food models, booklets or other portion size aids [7, 8], have greatly improved

completeness and validity [9]. Because the recall is open-ended, it allows for diverse foods and preparations and is, therefore, an excellent choice for multiethnic groups or new populations, for whom specific questionnaires have not been developed. Recalls are usually interviewer-administered, with direct input into a computer system, either in person or over the telephone [10, 11]. New programs, such as the National Cancer Institute (NCI) computer-assisted 24HR, (ASA24), allow compliant individuals enter their own data [12].

Data from 24HR provide valid group and subgroup nutrient intake means and, as such, is an excellent tool for population surveillance. For use in relation to individual outcomes such as bone mineral density or incidence of fracture, however, the 24HR has important limitations. Primarily, the day-to-day variation in individual intake limits the 24HR as a measure of usual intake [13, 14]. To the extent that intraindividual variation exceeds interindividual variation in the population under study, large numbers of individuals may be grossly misclassified relative to their usual intake, resulting in attenuation of or complete inability to see associations with outcome variables. For example, if someone who rarely eats meat happens to be interviewed the day after going out for an annual steak dinner, their recorded intake will not even closely represent their usual intake. At the population level, this variation can be assumed to be random error. Therefore, the group mean will be reasonably valid, but misclassification of individuals will weaken the ability to detect true associations. This variation differs by nutrient [14, 15], depending on whether they are concentrated in infrequently consumed foods (like vitamin A in liver), or if part of a regular daily pattern (like milk intake with meals). As an example, using data from Finland, 7–14 days were considered adequate to classify most nutrients, but nutrients with high variability may require 21 days or more [16].

Most large studies cannot afford to collect multiple days of intake to stabilize the within person estimates toward their usual intake. To the extent that the day-to-day variability is truly random, statistical corrections may be used when at least 2 days of intake are available to estimate intraindividual or interindividual variation. This ratio can then be used to better estimate the true association in linear models with continuous outcome variables [13, 17]. Because the effect of this random error is always toward the null, this means that most observed associations will underestimate the truth. The application of a correction for this can then provide evidence for an association that may not be seen directly.

In addition to this correction for random error, the National Cancer Institute (NCI) designed a method to improve nutrient intake estimates from recalls, by adding a propensity (frequency) questionnaire to record intake of episodically consumed foods (like liver). This approach will help to avoid the extreme misclassification in cases where low nutrient intakes are captured on the 2 days of recorded recall, but where an individual reports relatively frequent consumption of an important source of that nutrient on other days. An example would be low retinol intakes on two random days that did not include weekly consumption of liver (a high retinol food). To consider this information, the NCI developed a two step model to (1) estimate the probability of intake from 2 recalls, and (2) fit a model with the transformed recall data, adjusted for episodically consumed foods from the FFQ [18]. Although this approach improves estimates, it still does not allow precise assignment of nutrient intake to individuals. A further limitation of corrections for intraindividual/interindividual variation is that the within-to-between variability is not constant across populations, so that adjustments for the full sample may bias estimates of subgroups [19].

Addition concerns for validity in the 24HR include differential underreporting [20] by certain subgroups in the population, including obese individuals [21–23], smokers [21], women [22], and restrained eaters [24, 25]. Although some statisticians have experimented with adjustment for this nonrandom error in reporting behavior, it will vary by population and is unlikely to be easily corrected. To the extent that the underreporting represents portion sizes rather than exclusion of specific food groups, adjustment for total energy intake will help to improve ranking in nutrient intake distributions relative to total intake or requirement, thereby improving ability to detect associations with outcomes.

7.2.2 Food Frequency Questionnaire (FFQ)

Because of the need for repeat measures and rather complex post-measurement statistical adjustment with the 24HR, the food frequency questionnaire (FFQ) is the main tool used in large epidemiologic studies. Unlike 24HR, FFQ capture usual intake over a period of time, usually the past year, in a single administration. The FFQ consists of a food list, where like foods are grouped together to minimize length. Respondents are then asked to note the frequency of consumption of each food type, for example, "red meat (beef, pork, and lamb) and meat dishes". Responses include a range of options such as: rarely or never; less than once/month; two to three times/month; one to two times/week; three to six times/week; once/day; twice/day; three or more times/day. Portion size options may be provided or, in the Willett FFQ, may be assumed from other population-based data. Nutrient intake is calculated by multiplying the frequency by the portion size to obtain an amount, and then calculated from the weighted nutrient content for key foods within the food line item. For red meat in the US population, this may include a heavy weighting for ground beef, lower weightings for steak or beef stew, pork chops or roast, and lower still for lamb, which together add to 100 % to form a composite food with appropriate nutrient content.

As is evident from this example, FFQ will contain measurement error due to limitations in the food list, individual food weighting assumptions, and un-captured variation in portion size. Despite these limitations in individual specificity, however, FFQ have been shown to rank intakes well after total energy intake adjustment [15], particularly when developed for and validated with a specific population [26, 27]. What is important to note is that the assumptions included in the food list and specific food weightings within each line item are based on the most frequently consumed foods and recipes in either national data or another data set. Therefore, when a subgroup varies considerably in their dietary pattern, the use of FFQ developed for the majority US population will misclassify them. In fact, the most commonly used FFQ have shown poor results in minority populations. For example, validity coefficients for energy intake in the Block FFQ were 0.44 for non-Hispanic white women, but only 0.14 for Hispanic women [28].

For this reason, it is important that the FFQ selected for use has been calibrated for use and validated in each group with different dietary patterns targeted for study analyses.

7.3 Dietary Recall vs. Food Frequency Questionnaire: Focus on Micronutrients

Given the limitations discussed above, the choice of 24HR vs. FFQ ultimately depends on the goals of the specific study. 24HR estimate both macro and micronutrients more precisely than do FFQ in the short term, while FFQ obtain data on usual intake over a longer period of time, but lack the precision of individual portion sizes and recipes. Energy-adjustment usually improves the accuracy of micronutrient intake ranking in populations for which the questionnaire has been designed and validated, but captures less accurate quantitative estimates of actual micronutrient intakes. The extent of misclassification from a few recalls will depend on the intraindividual variation of intake in the population under study. To illustrate this, we will discuss a few specific micronutrients of importance to bone health: calcium, phosphorus and vitamin D.

7.3.1 Calcium Intake Assessment

Assessment of total calcium intake requires quantification of the amounts of calcium naturally in foods, of calcium used in foods as a fortificant, and lastly as supplemental calcium. A recent estimate of total

calcium intake by adults, using NHANES data from 2003 to 2006 showed that intakes ranged from a mean of 728 mg/day in men 81 year of age and older to 968 mg/day in men 31–40 year, and from 581 mg/day in women 81 year and older to 730 mg/day in those 31–40 year [29]. These data suggest that most adults do not meet the Recommended Allowances of 1,000 mg/day for ages 19–50 year (both genders), 1,000 mg/day for males between 50 and 70 year, and 1,200 for females beyond age 50, of the Institute of Medicine [30]. The same analysis by Mangano et al. [29], noted that from 33 % of younger to 56 % of older men, and from 42 % of younger to 69 % of older women take supplements containing some amount of calcium—adding an average of 74 (younger men) to 393 (older women) mg/day to their intakes. Importantly, however, non-supplement users tended to also have lower dietary intake than supplement users, and thus constitute a large group with clearly low calcium exposure.

The means reported above are for specific age and sex groups, based on 24HR data in the national survey. Estimates for individuals in the NHANES recalls, whether a single day in earlier surveys, or the average of 2 days in more recent years, will be limited and subject to misclassification from intra-individual day to day variation. For calcium, the coefficients of variation within (CVw) and between (CVb) individuals, calculated from the NHANES 2007–2008 data, were 52 %:37 %: for adult men and 46 %:38 % for adult women [15]. To the extent that within-person variation exceeds the variance between-individuals (as it does here), more days will be needed to obtain a stable estimate for individual usual intake. With only a few days, individuals are likely to be misclassified in the distribution, making it difficult to find significant associations with outcome measures, such as bone mineral density. Still, the practice of consuming milk and dairy products, the major sources of calcium, tends to be reasonably regular and the ratios described above (1.2–1.4) are not extreme relative to many other nutrients. High intraindividual and interindividual variability in micronutrient intakes is another reason why most epidemiologic studies use the FFQ, rather than the 24 HR, and adjust for total energy intake to standardize relative to likely individual requirement.

Regardless of the method for data collection, the frequent use of calcium supplements by a large subset of the population will distort the distribution for total calcium intake. For this reason, it is common practice to add supplement use in statistical models separately from linear measures of dietary intake. This may be done either as a yes/no variable or in categories of intake levels (0 = no supplement use; 1 = some-250 mg (the amount that may be in a multivitamin-mineral supplement; and 2 = >250 mg (suggesting specific calcium supplement use). Another approach is to calculate total calcium intake by summing diet and supplement intakes, but then to perform analyses in quartile or quintile categories (understanding that the highest categories will be due largely to supplement use).

7.3.2 Phosphorus Intake Assessment

In contrast to calcium, phosphorus is found in practically all foods and, increasingly, is added to processed foods to improve flavor or texture and to increase shelf life [31, 32]. Public health concern has surfaced because of the excess amounts of phosphorus consumed in the USA and the resulting lower dietary calcium to phosphorus ratio (Ca:P). The addition of numerous phosphorus compounds to our food supply is becoming an important public health concern [33, 34]. Although adequate phosphorus intake is necessary for healthy bone formation, excess phosphorus may pose significant risk. Most of the research on the damaging effects of high phosphorus exposure has been conducted with kidney patients, where it is known that high serum phosphate is associated with elevated risk for heart disease and mortality [35–37]. However, it is now believed that high phosphorus exposure may contribute to cardiovascular disease (CVD) in the general population. It has been shown to stimulate fibroblast growth factor-23, secreted by osteocytes, and parathyroid hormone, secreted by the parathyroid gland, both of which have been associated with elevated CVD risk [38–41]. High levels of added

phosphorous compounds may also contribute to low bone mineral density. For example, regular cola consumption has been linked with lower bone mass in adult women [42].

Research in this area remains limited in part, because it is difficult to accurately assess the usual long-term exposure to added phosphorus compounds in individuals. Food composition tables are currently incomplete for phosphate additives, and vary considerably in actual content even within category. For example, poultry, which is widely consumed, may be sold without additives, or it may be basted in a plastic shrink-wrap package, with phosphate compounds in the liquid. This information is not currently collected in most dietary methods. Even without this information, however, using the known food composition of phosphorus currently in the database, US adults obtain considerably more that the RDA of 700 mg per day for men and women [43]. Data from the NHANES, 2005–2006 show that many adults exceed twice that amount (ranging from a mean of 1,270 mg for men aged 71 year and older, to almost 1,730 mg in men 31–50 year; and from 985 in women aged 71 year and older, to about 1,200 in women aged 31–50 year [44]. Even more concerning is that we know that these intakes are underestimates. Several studies compared estimated intake from the national nutrient database with direct chemical analysis of food and found that phosphorus intake was likely underestimated by 25–30 % [9, 45–47]. In addition, it is important to note that the phosphorus from additive salts is highly absorbed and bioavailable relative to that found in foods.

Given the rather large error in our current estimates of actual phosphorus exposure, it is also difficult to estimate the actual day-to-day variability in intake. However, as measured in the NHANES 2007–2008, the CVw /CVB for phosphorus intake were reported as 37 %/30 % in adult men and 37 %/29 % in adult women, similar to those for calcium [15]. This suggests that the intraperson variation exceeds between-person variation, but not to an extreme degree, so that rankings would be possible with several days of recall or, as is usually done, with a single good quality FFQ measure. To the extent that individuals tend to consume processed foods vs. not on a regular basis, the actual values for phosphorus intake may have an extended distribution and the true intra/inter person variation may be lower than currently estimated.

For all these reasons, it is important to continue to improve the status of the national nutrient databank to keep up with rapid changes in the food supply. In addition, future analyses with phosphorus intake may benefit from methods similar to those used with supplements or other nutrients added to the food supply vs. naturally in the food matrix (such as natural folate vs added folic acid), to account for these differences in bioavailability [48]. Further long-term investigations are needed to understand the potentially adverse effects of high serum PTH under high phosphorus intake conditions or when the phosphorus to calcium intake ratios continue to exceed 2:1, as is already true for most US adults.

7.3.3 Vitamin D Intake Assessment

Vitamin D has recently gained enormous attention due to increasing understanding of its importance to numerous systems for maintaining health. This vitamin is unique in that it has historically largely been obtained endogenously, by activation of 7-dehydrocholestrol in the skin by sunlight to provitamin D3, which is then converted to cholecalciferol. This process works well only with regular direct sun exposure and in northern latitudes, clear seasonal variation in vitamin D status is apparent, with drops in the winter and spring in the Northeastern United States [49]. Surprisingly, however, many recent surveys have shown low or deficient vitamin D status in populations even in lower latitude sunny areas [50, 51]. Concern for skin cancer, increasing use of sunscreen, and availability of air-conditioned cars and buildings has led to lower sun exposure throughout the world. Because of this, more attention has been given to vitamin D intake from both food and supplements. However, vitamin D is in very few foods. In the USA, most of our vitamin D intake comes from fortified milk, fatty fish, eggs, some fortified yogurts, and fortified breakfast cereals. Still, dietary intake of vitamin

D, as measured by FFQ, does associate significantly with serum concentration, as has been shown in the Framingham Heart Study [52]. Because vitamin D is found in high concentration in limited foods, like fatty fish, within person day to day variation will be much larger than between-person variation. Therefore, a few 24HR are unlikely to rank usual intake well and use of an FFQ is advisable.

The most recent Institute of Medicine (IOM) Panel set the RDA for vitamin D at 600 IU (15 mg)/day for most individuals, and at 800 IU (20 mg)/day for those 71 year and older. They further recommended that serum 25(OH)-vitamin D concentration should be above 20 ng/mL (50 nmol/L), although many researchers argue that concentrations much greater than this may offer better protection against chronic disease [53]. Because of the attention to this nutrient, increasing numbers of individuals are taking supplements either the year round, or during the winter months and the self-dosing ranges greatly. Therefore, as described above for calcium, it is advisable to create a three-level categorical variable, to indicate no use, relatively low use, or high dose supplement use per day. Some studies of vitamin D include questions about sunlight exposure, such as walking or sitting outside, and whether they travel to the south during winter months. The best assessment of vitamin D status, however, is the measurement of serum 25(OH)-vitamin D.

7.3.4 Intake Assessments of Other Micronutrients

Three of the more critical micronutrients that affect bone status have been highlighted above, but the active investigation of nutrient–bone linkages in recent decades has demonstrated that many micronutrients and macronutrients are important, including protein, vitamins C and K, magnesium, and carotenoids and other phytonutrients [54–59]. For this reason, a full dietary assessment is optimal in order to obtain information on the full complement of nutrients and foods, rather than to use a brief screener. The use of brief screeners for calcium and vitamin D have been popular, but have been shown to have many limitations, including limitations in the food list that lead to biased intake assumptions, lack of ability to include lower amounts of calcium intake from commonly consumed foods, like bread, that can add up to important amounts, lack of ability to adjust for total energy to improve ranking estimates and importantly, and lack of ability to consider the role of other nutrients, either as additional important dietary components in preserving bone mineral density or in fracture risk reduction, or as potentially confounding variables in the analysis.

One important issue with dietary variables is collinearity of nutrients in common foods. To some extent we have this with dairy products, which are the major sources not only of calcium, but also of vitamin D (fortified) and to a large extent, phosphorus. In addition, they contain protein, potassium and other nutrients that likely work together to improve the effect of calcium on the bone. Therefore, testing adjusted regression models with a full array of micronutrients is recommended before declaring a significant effect of a specific nutrient on the outcome. For this reason, more studies are examining whole foods and dietary patterns as well as single nutrients [60].

7.4 Examples of Bone Studies Using Nutritional Assessments

Relationships between diet, nutritional status and bone status or fracture risk have been studied in many different ways. Population based epidemiologic approaches include cross-sectional studies, to examine associations at a single point in time and prospective cohort studies, to improve estimates of likely causality, by measuring the dietary exposure prior to either change in bone status or to future fracture. Because there may be considerable variation across studies due to socio-economic, cultural and genetic differences in distributions of risk or to issues of study design, no single study is definitive. Therefore, after many studies are done, summary reviews help to assess the cumulative strength

of observed associations, using methods such as systematic review or meta-analysis. Below are just a few examples of these.

7.4.1 Cross-Sectional Studies

The strength of epidemiologic results depends on study design as well as the validity of the measures. Many studies, including the NHANES, are cross-sectional surveys that assess correlations between nutritional intake measures and outcomes, like BMD, at the same point in time. As an example, an analysis of NHANES 2005–2006 data did not show a significant association between concurrent calcium intake and BMD of the hip or lumbar spine in adults aged 50 and older. In this case, calcium

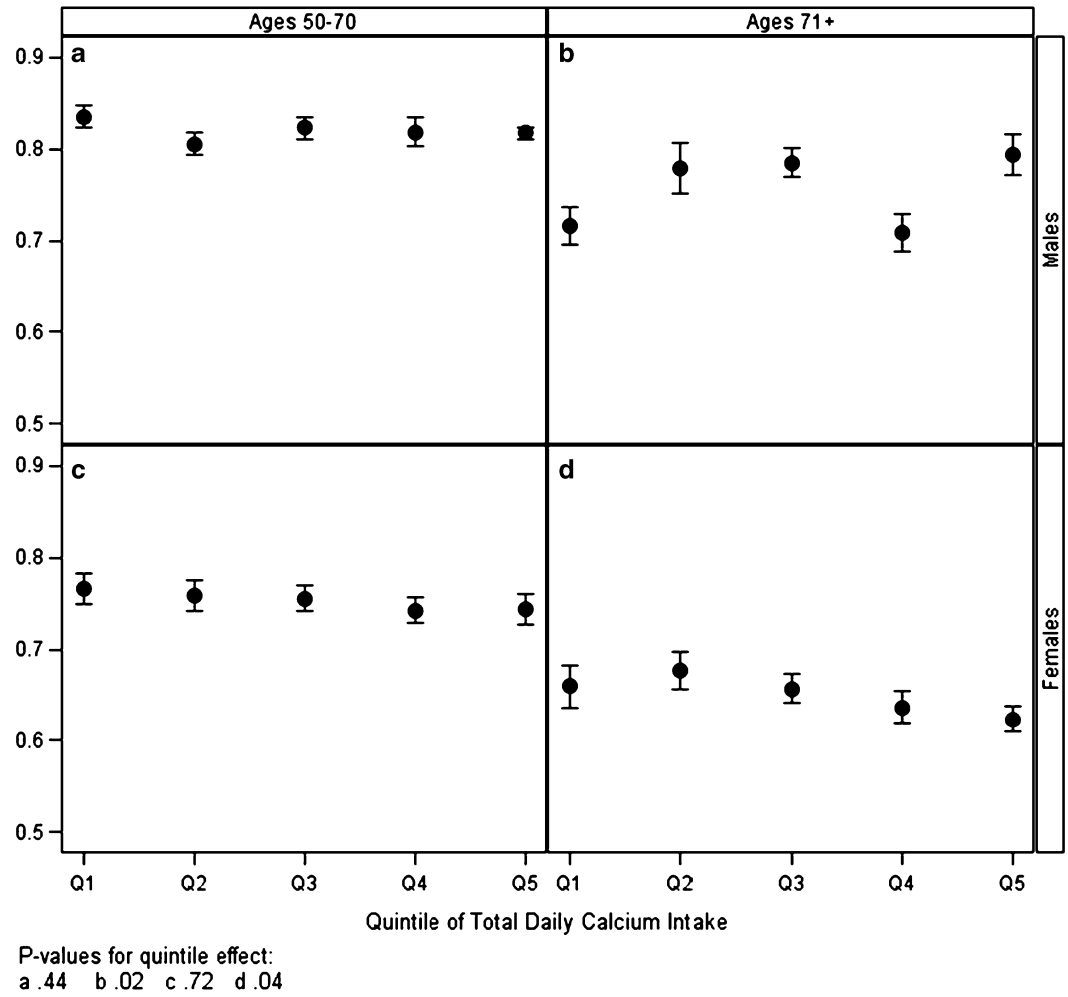

Fig. 7.1 Calcium intake and bone mineral density of the hip (proximal femur) adjusted for body mass index in a sample of older US men and women. (Permission to reproduce this figure is given by the Journal of Clinical Endocrinology and Metabolism: Anderson, J.J.B., Roggenkamp, K.J., and Suchindran, C.M. Calcium intakes and femoral and lumbar bone density of elderly U.S. men and women: National Health and Nutrition Examination Survey 2005–2006 analysis. J Clin Endocrinol Metab 97: 4531–4539, 2012.)

intake was analyzed as quintile categories and the only adjustment in the analysis was for BMI [61]. Data for the hip are shown in Fig. 7.1. As noted above, with this model, most individuals in the highest categories will be calcium supplement users. In other words, the data presented in this report from a representative national survey of older US adults confirms earlier understandings that suggest that calcium loading, i.e., high dose provided by a single supplement, has little or no effect on femoral or lumbar bone density in older adults. Cross sectional studies provide good evidence of association, but on their own, do not imply causation. Because both the calcium intake and the BMD are measured at the same period of time, the possibility always remains of confounding by other variables, such as age, medication use, or physical activity (among others), or by reverse causality, where individuals with a poor outcome have changed their dietary intake in response to the problem, rather than developing the problem due to long term low intake of the nutrient.

7.4.2 Prospective Studies

Stronger evidence is obtained with prospective studies. Dietary intake is measured at a baseline time point and either loss in bone over time, or incident fracture is assessed. In this case, the exposure is measured before the outcome so there is stronger likelihood that the intake may be contributing to the outcome. One example is a study [62] of prospective data from the Swedish Mammography Cohort that were used to assess fracture rates in older women over a 19-year follow-up period. Calcium intake was measured by FFQ at baseline and women were divided into quintiles of intake. In this study, the association of fracture with dietary calcium was nonlinear, with higher risk of any fracture in the lowest intake quintile (<751 mg/day) relative to the third quintile (882–996 mg/day) (Hazard Ratio = 1.18 (95 % confidence interval 1.12–1.25); of first hip fracture (HR = 1.29 (1.17–1.43) and development of osteoporosis (HR = 1.47 (1.09–2.00)). However, higher intake of calcium above the third quintile did not reduce the risk of fractures of any type, or of osteoporosis, and the highest intake quintile (>1,137 mg/day) was actually associated with greater risk of hip fracture, hazard ratio 1.19 (1.06 to 1.32). The authors suggest that moderate intake of calcium combined with adequate intake of other micronutrients is likely to be sufficient to meet the structural and functional demands of the skeleton, while high levels of intake may increase the rate of hip fractures. However, they caution that high levels are likely result from supplement use, which may be more common in individuals who perceive themselves to be at high risk.

7.4.3 Systematic Reviews and Meta-Analyses

The history of nutritional sciences has been to focus on the single nutrients that may contribute to reduced risk of disease, and this has been true for bone health as well. The most often studied of these are calcium and vitamin D. When multiple observational cross-sectional and prospective studies show congruent protective associations between a nutrient and an outcome, such as fracture risk, randomized clinical trials have been implemented with supplements vs. placebo to determine with more confidence whether or not the result is causal and therefore may be used in clinical practice. Trials themselves have limitations and multiple trials in differing populations are needed to show effectiveness. Once the body of literature has advanced to include sufficient numbers of trials, it is helpful to evaluate the sum total of results to get a better idea of what the evidence suggests in its totality.

An early review, that examined the relationship between calcium supplementation and bone, was based on 15 trials, but with a small number of participants n = 1,806) [63]. The authors concluded that calcium had small, though weak, benefit to bone, but no fracture reduction was demonstrated.

The small size of this analysis limited its statistical strength for drawing conclusions. A more recent review of both prospective studies and meta-analysis of clinical trials is based on larger numbers of older adults in prospective cohort studies [64]. However, the conclusion was essentially the same—that high calcium consumption or use of calcium supplements was not significantly related to risk of hip fracture in either men or women. Neither the pooled results from prospective cohort studies nor those from randomized controlled trials supported an association between calcium intake or supplementation and fracture risk in women or men.

7.5 Discussion

Nutritional assessments of calcium have been especially helpful in bone studies of older adults and the elderly, but the few investigations examining phosphorus and vitamin D intakes have been less insightful, largely because of methodological issue, such as inadequate food compositions tables for foods fortified with phosphate additives and of vitamin D skin production in those exposed to sunlight (UVB) in critical times of the year. Despite relatively large errors of measurement in micronutrient assessments, consistency of micronutrient–bone linkages across studies, especially meta-analyses, suggest that ball-park estimates of intakes have provided reasonable data to support current thought about the importance of micronutrients for the promotion of health and the reduction of osteoporosis and fractures, especially of the hip (Table 7.1).

Calcium Intakes: Early thinking was that calcium supplements would increase measurements of bone mineral content and density and several studies did report skeletal benefits of calcium supplements. One early report, however, clearly did not [65]. Calcium assessments over the last 10 years or so have generated important understandings of calcium requirements during the later decades of life, long after peak bone development in the second decade of life and consolidation by the end of the third decade. Older adults, both male and female, need smaller amounts of calcium each day in order to maintain bone mass or density [61, 62] and to prevent hip fractures [62, 64]. The maintenance of

Table 7.1 Calcium, vitamin D, and phosphorus: effects on bone health and food sources

Nutrient	RDA and UL for most adults	Too little	Sources to encourage	Too much	Sources to avoid
Calcium	RDA: 1,000–1,200 mg/day UL: 2,000 mg/day	Low BMD	Milk Yogurt Sardines or fish with bones Tofu Fortified soy milk	Brittle bones	Excess supplements
Vitamin D	RDA: 600 IU/day UL: 4,000 IU/day	Low BMD	Fatty fish Fortified milk, yogurt or breakfast cereal Supplements as needed	Brittle bones	Excess supplements
Phosphorus	RDA: 700 mg/day UL: 4,000 mg/day	Poor bone development	Milk Yogurt Tofu Beans Nuts Lean meats and fish	Brittle bones	Cola Processed or basted meats Commercial baked products or salad dressing Processed cheese

BMD also applies to both women who were omnivorous or lacto-ovo-vegetarian [66]. The cited reports used food frequency questionnaires or repeat recall measures.

A major finding emerged from the recent publications using the appropriate assessment tools: routine calcium intakes that maintained bone mass or density typically did not reach recommended intake amounts (RDAs) in large percentages of study participants. After a minimal daily intake of calcium, i.e., about 600 mg per day, was achieved, hip fracture rates were prevented; intakes higher than the RDAs had no additional benefits in terms of BMD or fracture prevention, except for the highest quintile in the prospective study of older Swedish women which had an increase in fracture rate [62]. In the USA at least, the high intakes of calcium in NHANES populations and other national surveys have resulted from calcium supplement use [61, 67]. Most studies published over a decade or more ago supported the benefit of calcium supplements for older adults in increasing bone mass and density, but, since the first meta-analysis [63, 68], the preponderance of studies have not shown such robust skeletal benefits, if any [61, 62, 64]. Calcium supplements are no longer recommended by the US Preventive Services Task Force [69].

Phosphorus Intakes: The major concern about phosphorus is excessive intake from additives [34]. Deficient intake is rarely a problem and it typically results because of poor protein nutrition and starvation (marasmus) or semi-starvation status. Phosphate salt additives are not available for processed foods in food composition tables. So, assessing phosphorus amounts in the diet is basically not possible; only the phosphate content of unprocessed (or raw) foods can be totaled. Even without food additive phosphates, typical estimated phosphorus intakes are twice as great as calcium intakes, on average. Therefore, a calcium-phosphorus ratio of 0.5 certainly contributes to an acute intake of PTH following meals. The long-term effects of such diets on bone mass and density has not been investigated.

Vitamin D Intakes: Vitamin D consumption from foods can be reasonable assessed using food composition tables, but the unknown quantity of vitamin D skin production during the months with UVB exposure has been difficult to obtain by skin film badges or other methods. So, overall vitamin D status remains a mystery. The best tool so far to assess vitamin D status is the serum 25-hydroxyvitamin D concentration with the three arbitrary classes of deficiency, insufficiency, and sufficiency [70]. Sufficiency is arbitrarily classified as having serum 25-hydroxyvitamin D concentrations greater than 30 ng/mL (or 75 nmol/L). Insufficiency is within the range from 21 to 29 ng/mL and deficiency is a measurement less than 20 ng/mL. In his previous review [53, 70] he only used two classes: deficiency (<20 ng/mL) and sufficiency (20 ng/mL and greater). Using these definitions, large percentages of adults have been shown to be deficient or insufficient in surveys of populations of the USA and other western nations. The meaning of such widespread low serum concentrations has been difficult to establish without evident clinical signs of osteomalacia. Theoretically, any concentration of serum 25-hydroxyvitamin D above zero should provide sufficient substrate for renal production of serum 1,25-dihydroxyvitamin D, the hormonal form that enhances both intestinal calcium absorption and osteoblastic bone formation.

Since the utility of dietary assessment of vitamin D is extremely limited, vitamin D status is now primarily based on serum 25-hydroxyvitamin D measurements. Presumably an adequate status for bone health is a serum concentration of 20 ng/mL or greater.

Intakes of Other Nutrients: The evidence for the importance of intakes of other nutrients such as protein, vitamins C, K, certain B vitamins, magnesium, potassium and carotenoids and other nutrients is much more recent and remains active. Together, however, the evidence is coalescing toward the importance of a high quality nutrient-rich diet for the protection of bone status and prevention of fracture, rather than the use of calcium supplements, as has been widely promoted in the past.

7.6 Conclusions

Nutritional assessments have been essential for the analysis of the association between the usual intake of a nutrient, such as calcium, and bone parameters, such as BMD, during the later stages of life. Most studies have examined postmenopausal women because of the greater incidence of osteoporosis and hip fractures in this gender, but men if they live long enough will suffer from osteoporosis and hip fractures as well. Calcium intake has relatively little impact at superannuated ages as long as consumption exceeds approximately 600 mg a day, according to findings of the prospective study of Swedish women [62]. In western nations phosphorus intakes are substantially increased by food additives, and high total P intakes that are almost twice as large as calcium may have adverse skeletal effects. Vitamin D intakes contribute to vitamin D status, as assessed chemically by measuring serum 25-hydroxyvitamin D, but sun exposure to UVB apparently has a greater benefit that vitamin D intake from foods, including D-fortified foods. Individuals with a serum 25-hydroxyvitamin D deficiency clearly need to improve their intake to support calcium metabolism and balance, but it remains unclear whether insufficient individuals need vitamin D supplements. Nutritional assessments of calcium have been very helpful to researchers, but so far the same cannot be said for nutritional assessments of phosphorus and vitamin D.

Assessing dietary intake accurately for use in epidemiologic studies poses significant challenges. Despite this, many advances have been made. Whereas early work concentrated almost exclusively on calcium, we now know that many nutrients contribute to optimal bone health and fracture prevention. Unfortunately, calcium supplements alone have not proven to be the panacea originally expected. Rather, adequate intake of many different nutrients appears to be necessary, within an overall healthy dietary pattern, to maintain optimal bone health. Future work will continue to explore the optimal combinations of foods and an expanding range of nutrients and phytochemicals that may optimize healthy aging, including maintenance of bone status.

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Chapter 8
Dietary Pattern Analysis in Nutritional Science Research: A Review of Current Evidence Relating Dietary Patterns to Indices of Bone Health and Fracture Risk

Adrian D. Wood and Helen M. Macdonald

Key Points

- The dietary pattern approach to nutritional science research may help to overcome some of the limitations associated with single nutrient studies.
- This chapter outlines how to generate dietary patterns with commonly available statistical software and then examine their association with bone mineral density (measured by dual energy X-ray absorptiometry).
- A summary of the current evidence relating dietary patterns to indices of bone health is provided.
- Few studies have assessed dietary pattern associations with incident fracture.
- Nutrient dense dietary patterns (as seen with high intakes of plant based foods, lean protein or oily fish, for example) have been consistently positively associated with BMD.
- It is difficult to make specific public health recommendations regarding dietary patterns or food groups given the heterogeneity of the different dietary pattern approaches and the study populations used in different studies.
- There is a requirement for consistency in future dietary pattern research with thorough consideration of potential confounding factors.

Keywords Dietary patterns • Bone health • Bone mineral density • Falls • Fracture • Postmenopausal women

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8.1 Introduction

Nutritional research in relation to bone health has historically focused on calcium and vitamin D with adequate intakes of these nutrients required for the prevention and cure of rickets in children and osteomalacia in adults [1]. In recent years, the research focus has widened to include other nutrients, particularly those associated with fruit and vegetable consumption. Bioactive phytochemicals (e.g. antioxidant carotenoids), minerals (calcium, magnesium, potassium, silicon, and boron), vitamins (C, K, and folate), or the buffering of excess dietary acidity from a fruit and vegetable rich diet all have their advocates. Although epidemiologic investigations of diet–disease relationships cannot prove causality, they can be employed to predict disease outcomes in the longer term. However, nutrient associations with bone mineral density (BMD) or fracture incidence may be confounded due to colinearity between nutrients from common food sources and the accuracy of food composition databases. Further, it is unrealistic to formulate dietary advice based on data from single nutrient studies.

A holistic approach in which combinations of foods that represent what people actually eat (rather than a list of nutrients) may be more helpful in determining the role of diet in bone health. The dietary pattern approach has increasingly been recognised for its potential to provide more meaningful information on whether diet can affect disease risk [2]. For example, dietary patterns have been employed to examine prospectively their association with cardiovascular disease (CVD) risk [3], type II diabetes mellitus [4], and colorectal cancer [5] in large scale epidemiologic studies.

Dietary patterns can be generated using a priori knowledge or by statistical manipulation of dietary data (different approaches to dietary pattern generation and analysis are summarised in Table 8.1). In a priori analyses the investigator may utilise dietary guidelines or score dietary intakes against existing dietary pattern information such as with the Mediterranean Diet Score [6]. Empirical analyses employ statistical techniques such as principal components analysis (PCA) which aims to explain the maximum amount of variation in dietary intake data with the minimum number of independent components (dietary patterns), or cluster analysis which groups individuals according to the types of foods that they commonly consume. Newer statistical techniques include reduced rank regression which aims to explain the maximum amount of variation in study outcomes (e.g. BMD) with the minimum number of dietary pattern variables. Partial least squares (PLS) analysis is another approach which aims to find the relation between dietary variables and outcome measures.

Although dietary patterns can be generated empirically, they still contain a subjective element, with food item groupings (not always done) and the selection and description of dietary patterns for further analysis being predominantly investigator driven (other factors such as population demographics may also contribute). Dietary patterns tend to explain only a small amount of the overall variation in surrogate bone health outcomes. Nevertheless, diet remains an important area on which to focus research as it supports other approaches to maintaining bone mass and reducing falls and may help to

Table 8.1 Summary of different methods employed for dietary pattern generation and analysis

Method	Summary description
Mediterranean diet score	Aims to describe adherence to the Mediterranean diet by utilising a 10-point dietary scale which incorporates components of the traditional Mediterranean diet
Principal components analysis	Aims to explain the maximum amount of variation in dietary data with the minimum number of independent components (dietary patterns)
Cluster analysis	Cases or individuals are clustered according to commonly consumed food items
Reduced rank regression	Aims to explain the maximum amount of variation in study outcome measurement(s) with the minimum number of dietary pattern variables
Partial least squares analysis	Identifies the relation between foods or food groups and study outcome measurements (e.g. bone turnover markers or BMD)

steer future research by highlighting potentially important interventions for testing. In this chapter we provide a detailed overview of how to generate dietary patterns using commonly available statistical software and we examine the relationship between BMD and empirically derived dietary patterns in a well-characterised cohort of postmenopausal women. Finally, we review and summarise the current evidence relating dietary patterns to indices of bone health and fracture risk.

8.2 Dietary Pattern Analysis in Nutritional Research

Details of the different methodologies which can be employed in dietary pattern analysis and the advantages and disadvantages of their approach have been reviewed previously [7]. In this section, using dietary data from a large cohort of Scottish postmenopausal women as an example, we provide a step-by-step outline of how to generate dietary patterns using PCA. PCA is the data reduction technique employed in the majority of dietary pattern studies on surrogate bone health markers published to date. We then show data on the relationship between bone mineral density (BMD) and our empirically derived dietary patterns and discuss important methodological considerations of dietary pattern generation, analysis, and interpretation.

8.2.1 Dietary Assessment

For dietary pattern analysis it is recommended that dietary intake be determined with an appropriate dietary assessment tool which has been validated for the population under study. Our semi quantitative FFQ was designed to assess habitual dietary intake in a North of Scotland population [8]. It measures usual intake of 98 foods in the present and past (up to 12 years and at age 20–30) organised into 20 sections. It has been validated using 7-day weighed records [9]. Nine pre-specified frequency responses are possible, ranging from rarely or never eaten to eaten every day. The weight of each food item consumed is obtained by multiplying the frequency of intake with its assigned portion size. The question on fruit intake is open ended allowing respondents a greater opportunity to specify what they ate. Dietary intakes from the open ended questions were calculated by multiplying the number of portions per week by the portion size. Daily intakes of macro and micronutrients from the diet were calculated using the UK Composition of Foods version 5 [10].

8.2.2 Data Collection

Participants from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort attended study visits during 2007–2011 at the Clinical Research Facility, University of Aberdeen, UK. Weight was measured on a balance scale (Seca, Hamburg, Germany) and height was measured with a stadiometer (Holtain Ltd., Crymych, UK). Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) at the hip (total) and lumbar spine (L1-L4) (Lunar iDXA, GE Medical Systems Inc, Madison WI). Daily phantom measurements were performed. A total of 1,679 postmenopausal women completed dietary questionnaires, the majority of whom also had DXA scans. In vivo precision was 0.54 % for lumbar spine BMD and 0.56 % for mean total hip BMD (left hip only: 0.68 %, right hip only 0.75 %) assessed by repeat scans in 60 volunteers.

8.2.3 Food Groupings and Energy Adjustment

The grouping of food items for dietary pattern analysis is sometimes conducted prior to statistical data reduction, to help with later interpretation. Grouping choices may vary from study to study depending on the dietary habits of the population under investigation, although food items similar in composition and nutrient profile are typically grouped together. An example of investigator driven food groupings is shown in Table 8.2. If grouping of foods is done, it may be a useful check to conduct analysis on ungrouped foods to confirm that both approaches generate similar dietary patterns (although the output is generally more difficult to interpret). In epidemiologic studies examining nutrient associations

Table 8.2 An example of investigator driven food groupings employed for analysis of dietary patterns

Food group	Food items included in the group
Red meat	Beef, lamb, pork, ham, bacon
White meat	Chicken, turkey, and other poultry
Processed meat	Tinned meat, pork and beef sausages, haggis, offal, and all processed meat dishes
White fish	All types of white fish
Oily fish	Kippers, herring, salmon, tuna and tinned fish
Other fish	Fish pie and other fish dishes
Eggs and egg dishes	Eggs, eggs in baked dishes
Yoghurt and cream	Full fat and low fat yoghurts, all types of cream
Cheese	Full fat and low fat hard and soft cheeses
Potatoes	All potato products including French fries
Vegetables	Green, root, salad, peppers, onions, tomatoes
Fruit	All types of fresh fruit
Milk	All types of milk including dried, condensed, and soya
Bread	All types of bread or rolls, butteries
Pulses	Baked beans, kidney beans, lentils, peas and sweet corn
Rice and pasta	Wholemeal and normal pasta, all types of rice
Cereal	Porridge and all breakfast cereals
Biscuits	Sweet and savoury biscuits, oatcakes
Cakes	Sponge, fruit, and plain cakes, pastries, pancakes, and scones
Desserts	Milk, sponge, and fruit based desserts, custard, ice cream
Tinned and dried fruit	All tinned and dried fruit
Chocolate and candy	All types of confectionary
Soups	Homemade, tinned, and dehydrated packet soups
Potato chips and nuts	Nuts, peanut butter, potato chips, and tortilla chips
Milk based sauces	All types of milk based sauces
Condiments	Bottled sauces, salad cream, mayonnaise
Sweet spreads	Jam, honey, marmalade
Fats and oils	Lard, dripping, vegetable oils, butters, and margarines
Coffee	Ground and instant, decaffeinated and caffeinated
Tea	All teas
Sugar in hot drinks	Sugar in hot drinks
Fruit and veg juice	Fruit and vegetable juices
Non-diet fizzy drinks	Non-diet carbonated drinks and colas
Diet fizzy drinks	Diet carbonated drinks and colas
Beer	Low alcohol and normal beer
Liquor	All spirits
Wine	All types of wine

with health or disease outcomes, energy intake is considered as a confounding covariate and dietary variables tend to be energy adjusted. As the field of nutritional science has evolved to include an increasing number of studies relating dietary patterns to disease risk or functional outcomes, investigators have as a general rule, continued to adjust for energy, usually by regressing the food or food group onto total energy intake [11]. In practice however, we have consistently observed no difference between dietary patterns depending on whether dietary data were energy adjusted or not (unpublished data), although components generally become inverted.

8.2.4 Statistical Analysis and Interpretation of the Dietary Patterns

Data reduction techniques for the generation of dietary patterns are a feature of many commonly available statistical software packages. We utilised the statistical software programme SPSS for Windows (version 20.0, SPSS Inc., Chicago, IL), for the present analysis. Intakes for each of the grouped food items in grams per week were calculated prior to creating dietary patterns. Non-normally distributed intakes of grouped foods were transformed prior to analysis, and intakes were adjusted for total energy intake using the residuals method. PCA was run to derive dietary patterns and factor loadings for each of our grouped food variables. Dietary pattern scores were saved as variables and a scree plot and factor score table were generated. Components (dietary patterns) are independent of each other. The eigenvalue of a component is the percentage of variance in the dietary data explained by that component, while the factor loadings of a component are the contributions made by each food group variable. We retained only those components with an eigenvalue >1, as those with eigenvalues <1 do not explain sufficient amounts of the overall variation to be included. In our analysis, food groups with an absolute factor loading score of $>\pm 0.3$ were considered to contribute to a dietary pattern so as to better identify the main contributors for each component. Data out with these parameters are not removed from the analysis; rather they are suppressed from view to simplify interpretation of the factor score table of the generated components. The factor score for each component is computed by summing the observed intakes of the food items weighted by the factor loading, giving every participant a score for each identified dietary pattern. PCA aims to explain the maximum amount of variation in the diet with the minimum number of variables. The scree plot (a graph of variance explained plotted against the number of factors that are generated) can be examined to help determine whether this data reduction technique has achieved its aim. Figure 8.1 shows the scree plot for energy adjusted dietary intakes from our analysis.

The steep slope of the line at the beginning of Fig. 8.1 indicates that the first few components (dietary patterns) explain the majority of the variance in dietary intake data. As the number of components increases, the slope of the line becomes shallower demonstrating that the additional components do not contribute substantially to the overall variance in the data. Output tables from the PCA analysis show factor loading scores (which can be positive or negative) for each food group in a given dietary pattern. These values indicate that a person eats more of a food with a positive loading and less of a food with a negative loading. The names of the dietary patterns generated from the PCA can be conveniently labelled according to the dominant food types in the respective patterns. We would caution against lending too much weight to descriptor labels as they are subjective and advise instead on utilising factor loading data where it is available for more accurate interpretation of a given dietary pattern.

We examined associations between dietary component scores (generated from PCA) and BMD measurements by calculating Pearson correlation coefficients. Multiple linear regression was employed to further investigate the relationship between dietary pattern factor scores and BMD where appropriate. Age, weight, height, physical activity level, and national deprivation category were entered as independent predictor variables in a stepwise manner.

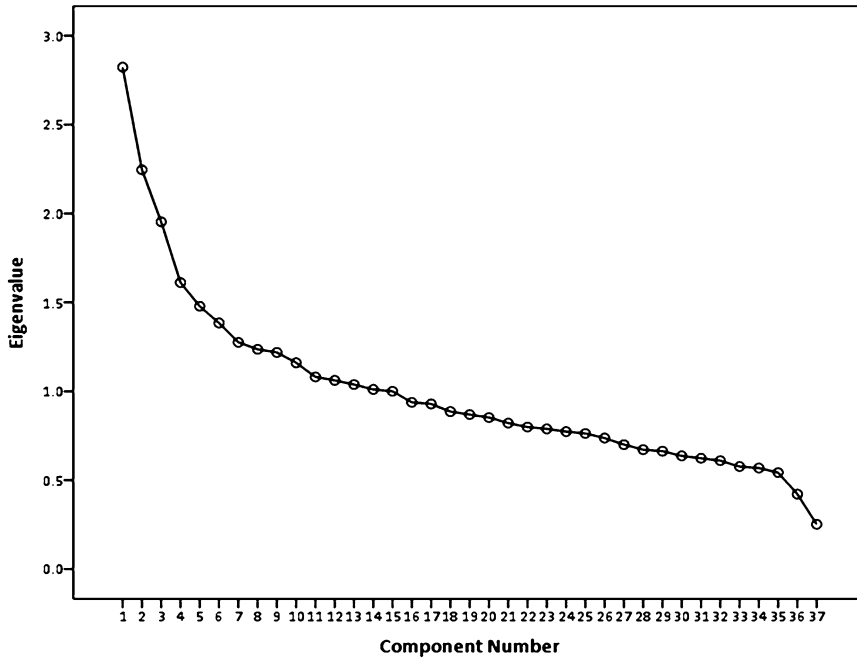

Fig. 8.1 Scree plot generated using PCA

8.3 An Investigation of the Relationship Between Dietary Intake and Bone Mineral Density in Our Study Cohort

Study participants were postmenopausal women aged 66 (2.1) years (mean (SD)). Approximately two thirds of participants in our cohort (68 %) were either overweight or obese (BMI $\geq$ 25 kg/m^2). Three components generated from our analysis explained 19 % of the total variance in dietary intake data among our population. Component one, consistent with a "prudent" dietary pattern, was characterised by high positive loading scores for fruit, vegetables, lean protein, and oily fish. Component two, consistent with a "processed foods" dietary pattern, was characterised by high intakes of red and processed meats, cakes, and desserts. The third principal component was characterised by positive loading scores for red, white, and processed meats, potatoes, and fats or oils.

Our data showed that the "prudent" dietary pattern was positively associated with BMD at the hip and lumbar spine (Pearson $r = .05$ and .04 respectively; $P < .05$). The third dietary component was also positively associated with hip and lumbar spine BMD (Pearson $r = .12$ and .07 respectively; $P < .01$). These associations remained significant for hip BMD only, after adjustment for age, weight, physical activity level, and national deprivation category. Further examination by multiple linear regression showed that weight was the strongest independent predictor of BMD (23.3 %). Dietary components 1 and 3 explained 0.2 and 0.3 % respectively of the overall variation in hip BMD (Table 8.3) showing that our dietary patterns explained a relatively small percentage of the overall variation in BMD (in comparison to previous data from our cohort [12] although similar to the amount of variation explained by smoking status and physical activity level).

Table 8.3 Multiple linear regression analyses to identify independent predictors of mean hip BMD in the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort

Component 1 "prudent"				
Independent variable	Variation explained (%)	Unstandardised β	95 % CI	*P*
Weight	23.3	.005	.005, .006	<.001
Height	.3	−.001	−.002, .000	.006
Dietary component 1	.2	.007	.001, .012	.03
Total	23.8			
Component 3				
Weight	23.3	.005	.005, .006	<.001
Dietary component 3	.4	.007	.002, .013	.01
Height	.2	−.001	−.002, .000	.03
Total	23.9			

8.4 Current Evidence Relating Dietary Patterns to Indices of Bone Health and Fracture Risk

Over the last decade, several observational studies have explored associations between dietary patterns and surrogate markers of bone health [12–21]. The main findings of these investigations are summarised in Table 8.4. Overall, nutrient dense dietary patterns, characterised generally by high intakes of plant-based foods, as well as lean protein or oily fish, have been consistently positively associated with surrogate markers of bone health [12, 16–21]. These associations transcend marked variations in study size, design, setting, and cohort characteristics.

In recent years, a number of studies have progressed beyond assessing surrogate biomarkers and examined dietary patterns in relation to osteoporosis related fracture risk either in prospective [23, 24, 26, 27] or retrospective cohort [22] or case control studies [22, 28]. Overall, the data are inconsistent. Details of these studies and of their main findings are summarised in Table 8.5.

8.5 Discussion

We have provided a step-by-step overview of how to generate dietary patterns by PCA for use in observational studies of diet–disease relationships. Our data are consistent with other published findings suggesting that dietary patterns rich in fruits, vegetables, or oily fish may positively affect bone health. It remains unclear which plant based food components confer such potentially protective effects, although there exist many potential candidates. Beneficial effects of oily fish may relate to vitamin D, particularly at northerly (high) latitudes. Alternatively, high oily fish consumption may reflect an eating pattern characterised by low intakes of potentially bone deleterious saturated fatty acids.

The positive association between dietary component 3 and hip BMD may relate to the high positive loading score for dietary protein in this dietary pattern through adequately supporting bone remodelling (although the protein–bone relationship remains controversial). High dietary protein has conventionally been thought to negatively affect bone via increased potential renal acid load associated with elevated urinary calcium excretion [29]. A recent study conducted in the UK showing an increase in femoral neck BMD for women ($n=238$, mean age (SD) = 22.8 (1.7) years) across tertiles of a "nuts and meat" dietary pattern generated by PCA supports our observations. Our findings are also consistent with data from the meta-analysis of Darling et al. [30] showing that all protein supplementation has a small positive effect on lumbar spine BMD. However, results from a pooled analysis of cohort

Table 8.4 Summary of studies assessing the association of dietary patterns with indices of bone health[a]

Studies assessing surrogate markers of bone health							
Study, year (reference)	Study design (follow-up)	Participants (country)	Women (%)	Dietary pattern analysis	Primary outcome(s)	Factors adjusted for in analysis	Main findings
Karamati et al., 2012 [13]	Cross sectional	160 aged 50–85 years (Iran)	100	Principal components analysis	Left femoral neck and lumbar spine (L1–L4) BMD	Age, BMI, physical activity, parity, smoking, education, fragility fracture history, history of HRT, supplement intake, antiresorptive drug use, age at menarche, relative accuracy of energy reporting	Odds ratio for risk of having BMD at lumbar spine below the median for high fat (OR = 2.29, 95 % CI: 1.05, 4.96; $P = .04$) and nutrient poor (OR = 2.83, 95 % CI: 1.31, 6.09; $P < .01$) dietary patterns
Whittle et al., 2012 [14]	Cross sectional	251 men, mean (SD) age: 22.4 (1.6) years; 238 women, mean (SD) age: 22.8 (1.7) years (UK)	48.7	Principal components analysis	Lumbar spine (L2–L4), and femoral neck BMD	Age, BMI, smoking, physical activity, father's social class, mean energy intake	Women in top vs. bottom quintile for "nuts and meat" dietary pattern had greater femoral neck BMD by 0.074 g/cm^2 ($P = .049$)
Fairweather-Tait et al., 2011 [15]	Cross sectional	2,464 mean (SD) age: 56.3 (11.9) years (UK)	100	Principal components analysis	Total hip, femoral neck, and lumbar spine BMD	Age, age squared, BMI, smoking, physical activity	Traditional English dietary pattern score negatively associated with hip neck BMD (β = −0.055, 95 % CI: −0.090, −0.020; $P = .01$) Energy adjusted alcohol intake positively associated with lumbar spine BMD (β = 0.050, 95 % CI: 0.017, 0.083; $P = .014$)
McNaughton et al., 2011 [16]	Cross sectional	527 aged 18–65 years (Australia)	100	Factor analysis	Lumbar spine (L2–L4), total hip, and forearm BMD	Age, height, energy intake, smoking, sport, walking, education, calcium intake	Mediterranean type dietary pattern score positively associated with BMD at hip (β = 0.0022, 95 % CI: 0.0001, 0.0044; $P = .042$) and spine (β = 0.0037, 95 % CI: 0.018, 0.0056; $P < .0001$)

Hardcastle et al., 2011 [12]	Cross sectional	3,236 aged 50–59 years (UK)	100	Principal components analysis	Femoral neck and lumbar spine (L2–L4) BMD, urinary fPYD/Cr and fDPD/Cr (nmol/mmol), serum P1NP	Weight, height, current smoking, physical activity level, age, social deprivation category, HRT use, menopausal status	Processed food dietary pattern score negatively associated with BMD at femoral neck (β = −0.009, 95 % CI: −0.013, −0.004; $P < .001$) and lumbar spine (β = −0.008, 95 % CI: −0.013, −0.003; $P < .002$) Snack food dietary pattern score negatively associated with BMD at femoral neck (β = −0.007, 95 % CI: −0.012, −0.003; $P = .001$) and lumbar spine (β = −0.009, 95 % CI: −0.013, −0.004; $P < .001$)
Sigiura et al., 2011 [17]	Cross sectional	293 mean (SD) age: 60.2 (6.2) years (Japan)	100	Principal components analysis	Radial BMD	Age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, total energy intake, intakes of calcium, magnesium, potassium, vitamin D	Women in top vs. bottom tertile for "β-cryptoxanthin" dietary pattern score had significantly lower odds ratio for risk of low radial BMD (OR = 0.30, 95 % CI: 0.11, 0.77; $P = .017$)
Langsetmo et al., 2010 [18]	Prospective cohort	1,928 men mean (SD) age: 58.8 (13.5) years; 4,611 women mean (SD) age: 61.2 (12.2) years (Canada multicentre)	70.5	Factor analysis	Lumbar spine (L1–L4), femoral neck, trochanter, Ward's triangle, and total hip BMD	Age, BMI, height, centre, education, smoking, alcohol consumption, activity, sedentary time, milk consumption, supplements (vitamin D and calcium), antiresorptives, corticosteroids, recent (<5 years) menopause, oophorectomy	Each SD increase in energy dense dietary pattern score associated with BMD decreases for men >50y and postmenopausal women respectively of 0.009 g/cm^2 (95 % CI: 0.002, 0.016) and 0.004 g/cm^2 (95 % CI: 0.000, 0.008)

(continued)

Table 8.4 (continued)

Studies assessing surrogate markers of bone health							
Kontogianni et al., 2009 [19]	Cross sectional	220 mean (SD) age: 48 (12) years (Greece)	100	Principal components analysis	Lumbar spine (L2–L4) BMD and total body BMC	BMI, smoking status, physical activity level, low energy reporting	Score of dietary pattern characterised by high consumption of fish and olive oil and low intake of red meat was positively associated with lumbar spine BMD ($\beta = 0.185$; $P = 0.02$) and total body BMC ($\beta = 0.140$; $P = 0.05$)
Okubo et al., 2006 [20]	Cross sectional	291 mean (SD) age: 46.4 (3.7) years (Japan)	100	Factor analysis	Forearm BMD	Age, BMI, grasping power, current smoking, fracture history, HRT use, age at menarche, parity, calcium and multivitamin supplements	Women in top vs. bottom quintile for healthy dietary pattern score had significantly greater BMD ($P < .05$)
Tucker et al., 2002 [21]	Cross sectional	345 men mean (SD) age: 75.1 (4.9) years; 562 women mean (SD) age: 75.3 (4.8) years (USA)	62	Cluster analysis	Proximal right femur BMD (femoral neck, trochanter, and Ward's area)	BMI, height, age, energy intake, physical activity score, smoking, vitamin D and calcium supplement use, season For women, further adjustment for oestrogen use	For men, BMD in the fruit, vegetable, and cereal rich dietary cluster was significantly greater at all three proximal femur sites than two or more of the other dietary cluster groups ($P = .05$). Mean BMD in the candy dietary cluster was significantly lower than in the fruit, vegetable, and cereal cluster group for the Ward's area and femoral neck sites ($P < .001$) Women in the candy dietary cluster group had lower BMD than all but one other group (sweet baked products) at the radius ($P < .01$)

[a]Updated from Wood AD, Macdonald HM (2013) Interactions of dietary patterns, systemic inflammation, and bone health. In: Burckhardt P, Dawsonhughes B, Weaver C, editors. Nutritional Influences on Bone Health: Springer [2]

Table 8.5 Summary of studies assessing the association of dietary patterns with fracture incidence

Studies assessing fractures							
Study, Year (reference)	Study design (follow up)	Participants (country)	Women (%)	Dietary pattern analysis	Primary outcome (s)	Factors adjusted for in analysis	Main findings
Zeng et al., 2013 [22]	Case control	Cases: 581 mean (SD) age:71 (6.7) years Controls: 581 Age matched (China)	Data not given	Principal components analysis	Hip fracture	BMI, education, household income, house orientation, smoking, alcohol drinking, tea drinking, physical activity, daily energy intake, family history of fractures, calcium supplement use, multivitamin use For women, further adjustment for years since menopause, oral contraceptive use, and oestrogen use	Odds ratio of hip fracture risk for healthy (T3 vs. T1: OR = 0.42, 95 % CI: 0.24–0.73; $P = .004$), prudent (T3 vs. T1: OR = 0.51, 95 % CI: 0.28–0.90; $P = .03$), or high fat (T3 vs. T1: OR = 1.78, 95 % CI: 1.12–2.83; $P = .015$) dietary patterns
Samieri et al., 2013 [23]	Prospective cohort (8 years)	1,482 Aged >65 year (France)	62.9	Principal components analysis	Hip, wrist, and vertebral fractures	Age, gender, total energy intake, educational level, marital status, BMI, self-reported osteoporosis, osteoporosis treatment, calcium and/or vitamin D supplementation	Hazard ratio of wrist and any site fracture risk respectively for nutrient dense dietary pattern (HR = 0.82, 95 % CI: 0.67, 1.00; $P = .05$, HR = 0.87, 95 % CI: 0.76, 0.99; $P = .04$) Hazard ratio of hip fracture risk for south-western French dietary pattern (HR = 0.78, 95 % CI: 0.61, 0.99; $P = .04$)

(continued)

Table 8.5 (continued)

Studies assessing fractures							
Benetou et al., 2012 [24]	Prospective cohort (9 years)	188,795 Mean (SD) age: 48.6 (10.8) years (Europe multicentre)	70.1	Modified Mediterranean diet score	Hip fracture	Age, sex, education, smoking status, BMI, height, physical activity, total energy intake, history of CVD, cancer, fracture For women, further adjustment for menopausal status, HRT use	Hazard ratio of hip fracture risk with increased adherence to modified Mediterranean diet score by 1 point (HR = 0.93, 95 % CI: 0.89, 0.98; $P = .004$)
Langsetmo et al., 2011 [25]	Retrospective cohort (10 years)	3,539 women Mean (SD) age: 67.6 (8.6) years 1,649 men Mean (SD) age: 64.6 (10.0) years (Canada multicentre)	68.2	Factor analysis	Low trauma fractures	BMI, BMD, falls, prior fracture, co morbidities, smoking, milk consumption, supplements (vitamin D and calcium) For women, further adjustment for diagnosis of osteoporosis, antiresorptive use, education, alcohol use, physical activity, and sedentary hours	Hazard ratio of low trauma fracture risk per 1 SD of nutrient dense dietary pattern in men (HR = 0.83, 95 % CI; 0.64, 1.08; NS) and women (HR = 0.86, 95 % CI; 0.76, 0.98; $P = .05$)
Monma et al., 2010 [26]	Prospective cohort (4 years)	1,178 Aged >70 years (Japan)	55.8	Factor analysis	Fall related fractures	Age, gender, BMI, energy intake, experience of falls in previous 6 months	Hazard ratio of fall related fracture risk for vegetable (T3 vs. T1: HR = 2.67, 95 % CI: 1.03, 6.90; $P = .025$) and meat (T2 vs. T1: HR = 0.36, 95 % CI: 0.13, 0.94; $P = 0.056$) dietary patterns

McTiernan et al., 2009 [27]	Dietary modification intervention (8.1 years)	48,853 postmenopausal women Aged 50–79 years (United States multicentre)	100	Adherence to low fat, increased fruit, vegetable, and grain diet	Osteoporosis related fractures, falls, and BMD	Age, ethnicity, education, BMI, physical activity, alcohol intake, smoking, income, total energy intake, percentage of energy from fat, family history of fracture, social support, optimisation, life events, hostility, negative emotion construct, use of multivitamins, Hormone Therapy trial randomization arm, number of fruit, vegetable, and grain servings per day	Hazard ratio of hip fracture according to dietary modification treatment (HR = 1.12, 95 % CI: 0.94, 1.34; $P = .21$) The intervention group had a lower rate of reporting ≥2 falls than did the comparison group (HR = 0.92, 95 % CI: 0.89, 0.96; $P < .01$)
Xu et al., 2009 [28]	Case control	Cases: 209 postmenopausal women aged 50–70 years Controls: 209 age matched subjects (China)	100		Forearm fracture		

studies showed no association of protein intake with relative risk of hip fracture suggesting that the small benefit to BMD may not affect long term fracture incidence [30].

In the majority of published dietary pattern studies assessing bone health, it is unclear whether observed associations may be weighted by particular types of food items within grouped food variables. We propose that the dietary pattern approach could be progressed to incorporate reduced rank regression and statistical methods such as PLS analysis as the field evolves. For example, the PLS method can be employed to highlight which foods contribute the most to a given dietary pattern, and to examine specific foods within groups and their association with study outcomes. Reduced rank regression aims to generate dietary components that explain the maximum amount of variation in a set of study outcomes. The potential advantages of this approach have been reviewed elsewhere (with a focus on studies of CVD and stroke risk) [31]. This method has been employed with success in a 4-year longitudinal study of children ($n=308$ age = 3.8–4.8 years at baseline) assessing fat and bone mass [32]. A dietary pattern characterised by a high intake of dark-green vegetables, deep-yellow vegetables, and processed meats was related to low fat mass and high bone mass (this pattern explained 3.3–5.2 % and 3.9–5.8 % of the variation in fat mass and bone mass respectively over the 4 years of the study).

There are of course limitations to the dietary pattern approach, particularly with association studies assessing surrogate biomarkers. Dietary patterns may be expected to differ geographically due to the consumption of different types of foods; care should therefore be taken when comparing the results of studies conducted across different countries. There are many lifestyle covariates which may influence BMD, most notably, bodyweight, physical activity level, smoking status, use of calcium and other dietary supplements, menopausal status, and use of hormone replacement therapy. Although many studies adjust for such factors during statistical analysis, residual confounding may be unavoidable due to measurement error or missing or uncollected data. In addition, individuals "at risk" of a disease (e.g. family history or previous fracture) may have modified their diet, but still have an elevated risk. Finally, association studies cannot determine causality, and there may be mechanisms involved which bypass surrogate bone health markers but affect fracture incidence.

Dietary pattern data in relation to falls-related fracture risk is sparse, although such studies are predominantly prospective in nature. Despite sharing a common primary end point, these investigations differ in terms of participant population, study design, methodology employed for dietary pattern generation, and assessment of study covariates. There was one dietary modification intervention trial (48,853 postmenopausal women aged 50–79 years) which was conducted across multiple centres in the USA assessing the effect of the Women's Health Initiative Dietary Modification adherence low fat, and increased fruit, vegetable, and grain intervention on falls and fall related fracture. After a mean 8.1 years of follow-up, the intervention group (an intensive behavioural modification programme of 18 group sessions in the first year and quarterly maintenance thereafter) had a lower rate of reporting two or more falls than did the comparison group, although there was no difference in fracture risk [27]. This study was originally designed as a trial on hormone replacement therapy. In a large scale study of 188,795 men and women (mean age of 48.6 ± 10.8 year followed up over 9 years) across multiple European centres, increased adherence to a Mediterranean diet was associated with a reduction in hip fracture risk [24]. The Mediterranean Diet Score was originally developed for use in a Greek population. A frequently encountered limitation of the approach is in the difficulty of categorising or scoring commonly consumed food items (particularly composite dishes) which may be reflective of prudent dietary choices in non Mediterranean populations. Two further prospective studies show contrasting findings. Data from a Japanese cohort (1,178 men and women aged >70 years) showed that a vegetable rich dietary component (generated by factor analysis) was associated with an increased risk of fall related fractures [26], whereas in a similarly sized study conducted in France (1,482 men and women aged >65 years), nutrient dense and south-western French dietary patterns were respectively associated with reduced any site and hip fracture risk [23]. The Japanese study may be limited by its small number of incident fracture cases ($n=28$) and as the traditional Japanese diet is quite different from a Westernised one, participants with very high loading scores for the vegetable factor may also have had lower intakes of potentially beneficial dietary components such as protein.

8.6 Conclusion

Overall, although a healthy dietary pattern consistently appears to protect bone health, it is difficult to make precise recommendations either in support of or to avoid specific dietary patterns or food groups to help maintain bone mass or reduce fracture risk given the heterogeneity of current data. It is clear that care needs to be taken in interpreting the data on dietary patterns, with particular consideration given to the study design and how patterns were generated. The effect sizes are small as seen for many chronic diseases, with many factors making a contribution to overall risk. It may be appropriate to adopt a population specific approach for future research to account for differences in availability of traditional food items across countries. Analyses should be consistent and potential confounding and limitations need to be thoroughly considered. Such an approach could help to clarify whether dietary patterns predict fracture risk, and may aid with formulation of public health advice for individuals with low bone mass or at risk of osteoporosis who are not at an age where treatment is appropriate or in the generation of hypotheses for testing in future research.

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Chapter 9
Nutrition and Oral Bone Status

Elizabeth Krall Kaye

Key Points

- Alveolar bone loss is a key diagnostic feature of periodontal disease.
- Higher intakes of nutrients that modulate the inflammatory response and food sources of whole grains and dietary fiber are inversely associated with risk of alveolar bone loss.
- Prevalence of periodontal disease is lower among persons with healthy eating patterns and ideal body weight.
- Prospective studies and clinical trials are needed to clarify these associations and determine optimal intake levels for the prevention of periodontal disease.

Keywords Periodontal diseases • Alveolar bone loss • Diet • Nutrition • Obesity

9.1 Introduction

Loss of tooth-supporting alveolar bone is one aspect of periodontal disease, a chronic inflammatory condition that affects nearly half of the adult US population [1]. The most common forms of periodontal diseases, gingivitis and adult periodontitis are initiated by bacteria found in plaque that forms a biofilm on the tooth surface. Periodontitis is a significant cause of tooth loss [2] and has also been associated with higher risk of various chronic systemic diseases such as heart disease and diabetes [3, 4]. Treatments for periodontitis include deep cleaning, medications to control inflammation, and surgery [5]—all of which entail considerable cost to dental patients and insurers. Prevention of periodontal diseases could have far-reaching medical and social benefits. Measures to prevent periodontal diseases focus primarily on individual behaviors to control bacteria levels and inflammation such as brushing, flossing, regular dental checkups, and abstinence from smoking [5]. Less attention has been given to the role of good nutrition. Nutritional factors related to inflammation and bone integrity may have roles in preventing oral bone loss, but their effectiveness is uncertain.

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9.2 Structure of Teeth and Oral Bone, and Periodontal Diseases

The tooth is a calcified structure that is functionally divided into the enamel-covered crown which protrudes above the gum line and aids in the mastication of food, and the cementum-covered root which is embedded in a socket in the alveolar process of the maxilla or mandible. The demarcation between the crown and root is referred to as the cemento-enamel junction (CEJ). Cementum is a bone-like substance comprised of about 45 % hydroxyapatite that attaches to alveolar bone by the collagenous fibers of the periodontal ligament. Alveolar bone proper is a layer of cortical bone that immediately lines the tooth socket, while supporting alveolar bone of the mandibular and maxillary processes is made up of both cortical and trabecular bone. Cementum has limited capability to remodel and is not resorbed under normal conditions. In contrast, alveolar bone, like bone tissue in other parts of the body, continuously undergoes cycles of resorption and formation.

In the mandible, the alveolar crest is the uppermost border of the alveolar bone proper (in the maxilla, it is the lowermost) and this landmark defines the extent of alveolar bone loss, one of the key measures of periodontal disease (Fig. 9.1). Under healthy conditions, the alveolar crest lies about 1–2 mm from the CEJ. In periodontitis, there is vertical loss of bone. Severity of alveolar bone loss is typically expressed as the height of alveolar bone (root tip to alveolar crest) in millimeters or as a percentage of total distance from the tooth root to the CEJ.

The alveolar processes are covered by mucosal gingival (gum) tissue that normally serves as a barrier between the mouth and bone. Gingivitis is defined as inflammation of the gums without involvement of the underlying tissues and presents as bleeding when a probe is inserted between the gum and the root of the tooth. If gingivitis is left untreated, it may advance to periodontitis in which periodontal ligament and alveolar bone are broken down by proteolytic enzymes, pro-inflammatory factors, and reactive oxygen species generated by the host immune response [6]. As bone height erodes, the gingiva recedes, the periodontal ligament detaches from the tooth root, and a pocket develops between the root and gum in which bacteria can accumulate and further aggravate periodontal tissues.

Bleeding on probing, attachment loss, and pocket depth are clinical measures of gingivitis and periodontal disease widely used in prevalence and research studies. Each of the measures of periodontal disease has strengths and limitations. Unlike the measurement of alveolar bone loss, attachment

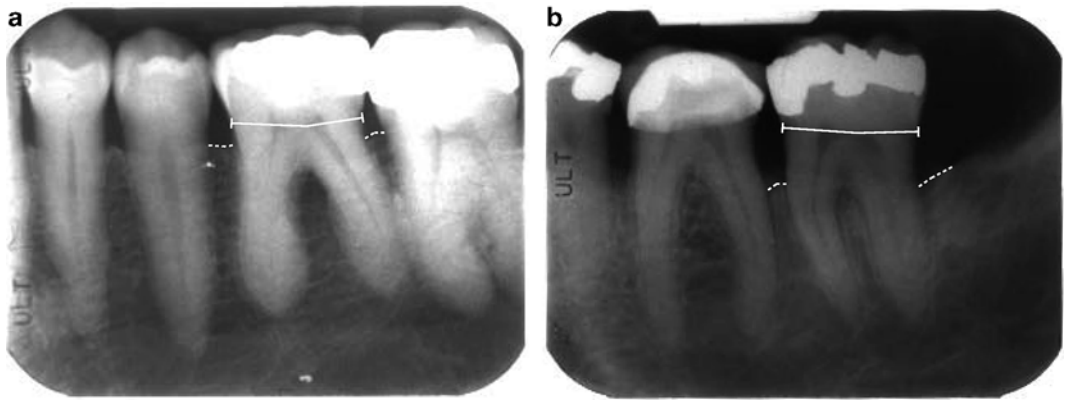

Fig. 9.1 Dental radiographs showing a molar tooth with little to no alveolar bone loss (Panel **a**) and a tooth with more than 20 % alveolar bone loss (Panel **b**). *Solid white lines* indicate approximate locations of the cemento-enamel junctions (CEJ). In the healthy periodontium, the crest of alveolar bone is slightly apical to (toward the tooth root) the CEJ. *Dotted white contours* indicate actual crest of the alveolar bone

loss and probing depth do not require dental radiographs, and so are comparatively less burdensome and less expensive to obtain. However, they are indirect gauges of oral bone loss because they reflect damage to both bone and mucosal tissues and are reversible to some extent with treatment.

Periodontal disease is one major reason for tooth loss in middle-aged and elderly persons; the other is caries [3]. Other factors that contribute to periodontal disease and tooth loss include poor dental hygiene, lack of access to dental care, genetics, and systemic diseases such as diabetes [4]. Osteoporosis has also been associated with increased periodontal disease and tooth loss but, as with heart disease, it is not clear if the relationship is causal [7].

Age is a significant risk indicator for periodontal disease and tooth loss. In the National Health and Nutrition Examination Survey (NHANES) conducted in 2009–2010, attachment loss and probing depth were measured at six sites per tooth. Severity of periodontitis was defined on the basis of number of sites or teeth with increasing levels of attachment loss and/or probing depth [1]. According to NHANES, 8.7 % of all US adults over the age of 29 had mild periodontitis, 30.0 % had moderate disease, and 8.5 % had severe disease. The population aged 65 years and older was much more likely than 30–34-year-olds to be affected by moderate (53 % vs. 13 %, respectively) and severe periodontitis (11 % vs. 2 %, respectively).

The prevalence of tooth loss and edentulism (total tooth loss) also increase with age. Among persons age 65 years and older surveyed in 1999–2004, the average number of teeth retained was 19 (28 teeth are considered full dentition) and 27 % of this age group was edentulous [8]. In contrast, the average number of teeth present among all 20–64 year-olds was 25, and only 4 % were edentulous [8].

9.3 Nutrition and Periodontal Diseases

Nutritional factors may influence the progression of periodontal disease via mediation of the inflammatory response or by direct effects on the integrity and metabolism of oral bone and soft tissues.

Although plaque bacteria are necessary for the initiation of periodontal disease, much of the tissue damage is the result of a hyperinflammatory host response. In response to infection, the local cells and leukocytes recruited to the site of infection release proteolytic enzymes, prostaglandins, and cytokines such as tumor necrosis factor and interleukins [6, 9]. These factors destroy the bacterial pathogens but at the same time damage periodontal tissue. Ideally, the tissues are repaired upon resolution of the infection, but if the pro-inflammatory state is prolonged, the potential for progression of tissue damage remains. In addition to the disease process at the local level, it is believed a state of chronic, low-grade systemic inflammation also interacts with periodontal disease in a bidirectional manner; systemic pro-inflammatory factors may contribute to and exacerbate the breakdown of periodontal tissues, while chronic periodontitis also increases the overall level of systemic inflammation.

Many nutritional factors influence the inflammatory response. Diets with excess calories or high levels of refined carbohydrates, saturated fats, or trans fatty acids stimulate inflammation through the generation of reactive oxygen species and oxidative stress [6]. A state of chronic low-grade inflammation exists in obesity, given that adipose tissue is a significant source of pro-inflammatory cytokines, and in the metabolic syndrome, a cluster of three or more risk factors for cardiovascular disease of which abdominal obesity and glucose intolerance are components. In contrast, healthy dietary patterns are associated with lower systemic levels of pro-inflammatory markers such as TNF-a, IL-6, CRP and adhesion molecules [10]. Specific components of the diet that correlate with lower levels of circulating inflammatory markers include increased intakes of whole grains, dietary fiber, fruits and vegetables, polyphenols, omega-3 fatty acids, vitamin D and antioxidant vitamins, and moderate alcohol use [6, 10]. Many of these nutrients have been examined in relation to clinical periodontal disease in humans, but fewer data are available for alveolar bone loss.

9.3.1 Nutrition and Alveolar Bone Loss

Animal models of experimentally induced periodontitis have demonstrated that loss of alveolar bone is increased in obesity [11] and after consumption of a high-fat [12] or high phosphorus/low calcium diet [13], and can be mitigated by administration of omega-3 fatty acid [14, 15], milk proteins [16], or cocoa flavonoids [17]. The effect of ethanol is unclear [18]. Several of these observations have been borne out in human investigations, although the number of epidemiologic studies is small and diversity of the populations is limited. Table 9.1 provides details of the study populations and results.

9.3.1.1 Obesity and Metabolic Syndrome

The Department of Veterans Affairs Dental Longitudinal Study (DLS) has collected dental radiographs for measurement of alveolar bone loss (ABL) in a cohort of over 1,000 men since the late 1960s [19]. In these men, body mass index was significantly associated with risk of moderate to severe ABL incidence [20]. Men who were obese (body mass index ≥30 kg/m^2) at study baseline were 60 % more likely to experience moderate to severe ABL over several decades of follow-up compared to normal-weight men. Among normal weight and overweight men, there was a trend for increased ABL if waist circumference-to-height ratio was greater than 50 % [20]. In a cross-sectional study of metabolic syndrome and periodontal disease among middle-aged men and women, participants with moderate to severe periodontal disease based on ABL were about 2½ times more likely to have metabolic syndrome than those minimal or no bone loss [21].

In the absence of dental radiographs, the all-male Health Professionals Follow-Up Study (HPFS) assessed periodontal bone loss with a validated questionnaire item, "Have you been professionally diagnosed with periodontal disease with bone loss?" [22]. Obese men in this cohort had a 30 % higher hazard rate of periodontal disease incidence relative to normal weight men, and there was a modest increased risk (9 %) among overweight men as well [23]. Waist-to-hip ratio, an indicator of abdominal adiposity was positively correlated with the hazard of new periodontal disease [23].

9.3.1.2 Whole Grains and Dietary Fiber

The VA DLS began administering food frequency questionnaires in the mid-1980s and associations of ABL progression with several individual nutrients have been reported. Daily intake of foods that are good to excellent sources of dietary fiber (at least 10 % of the Reference Daily Intake per serving) was associated with reduced risk of alveolar bone loss progression [24]. On average, the men consumed three servings of high-fiber foods per day. Each one serving increment of all high-fiber foods was associated with an 8 % lower risk of ABL progression. The association was stronger for the fruit (14 % reduction in risk) and vegetable (18 % reduction) food groups [24].

Using food frequency questionnaire data from the HPFS, Merchant et al. [25] estimated the risk of periodontal disease incidence in men according to levels of various sources of whole grains and dietary fiber. They reported that the risk of developing periodontal disease was highest among men who consumed fewer than 0.6 servings/day of whole grains, and risk decreased with increasing whole grain consumption. Men who consumed three to four servings per day (one serving was equivalent to ¾ cup of whole grain cereal or one slice of whole wheat bread) were 23 % less likely to develop periodontitis than men who consumed zero to one servings of whole grains per day. No significant associations were found between periodontal disease risk and total fiber, fruit fiber, or vegetable fiber.

Table 9.1 Studies of nutritional status and oral bone loss

Study	Nutritional status measures	Study design and population	Alveolar bone loss measure	Primary results
Gorman et al., 2012 [20]	Baseline obesity status[a] Baseline waist circumference-to-height ratio (<50 % vs. ≥50 %)	Prospective design, up to 29 year follow-up 1,038 white males participating in DLS Mean age 48 at baseline	Incidence of moderate/severe ABL (≥40 % ABL) on two or more teeth	Higher risk of ABL in obese men relative to normal weight men (Hazard ratio, HR = 1.6, CI: 1.07–2.38)
Nesbitt et al., 2010 [21]	Diagnosis of metabolic syndrome	Cross-sectional design 112 men (mean age 57) and 78 women (mean age 60.0)	Alveolar bone loss (none/slight vs. moderate/severe)	Greater odds of metabolic syndrome in participants with moderate/severe ABL than those with none/slight bone loss (OR = 2.61, CI: 1.1–6.1)
Jimenez et al., 2012 [23]	Obesity status[b] Quintiles of waist circumference (WC) and Waist–Hip Ratio (WHR)	Prospective design, up to 20 year follow-up. 36,910 males participating in HPFS Mean age 52–54 years at baseline	Self-reported incident periodontal disease with bone loss	Higher risk of periodontal disease in obese men relative to normal weight men (HR = 1.30; CI : 1.17–1.45); in highest quintile of WC (HR = 1.27, CI: 1.11–1.46) and WHR (HR = 1.34, CI: 1.17–1.54) relative to lowest respective quintiles [c, d]
Schwartz et al., 2012 [24]	Dietary fiber: Servings/day of high-fiber foods, grains, fruits, and vegetables in past year. Total grams fiber/day	Prospective design, up to 24 year follow-up 625 white males participating in DLS Mean age 48 at baseline	Incidence of moderate to severe ABL (from no ABL to ≥40 % ABL) on any tooth, or tooth loss	In men age ≥65, ABL risk 24 % lower per each serving of high fiber foods (HR=0.76, CI: 0.60–0.95) or high fiber fruits (HR=0.86, CI: 0.78, 0.95). No associations among men age <65
Merchant et al., 2006 [25]	Whole grains: Quintiles of whole-grain, refined grain, total fiber, and fiber from cereals, fruit, and vegetables in past year	Prospective design, up to 24 year follow-up. 34,160 males participating in HPFS Mean age 52–55 years at baseline	Self-reported incident periodontal disease with bone loss	Periodontitis risk declined 6 % per each 1.0 g/day increase in whole-grains (RR = 0.94, CI: 0.90–0.97). Risk of disease 23 % less in highest vs. lowest whole-grain quintiles (RR = 0.77, CI: 0.66–0.89). No association with refined grain or fiber intakes
Alshouibi et al., 2013 [26]	Vitamin D: total vitamin D intake (IU/day) in past year. Categorized into <400, ≥400 to <800, and ≥800 IU/day	Repeated measures cross-sectional design over 24 years 562 white males participating in DLS Mean age 62–63 at baseline	ABL ≥40 % on three or more teeth	Lower odds of ABL in vitamin D intake ≥800 IU/day category relative to <400 IU/day category (OR = 0.54, CI: 0.30–0.96)
Garcia et al., 2011 [27]	Vitamin D (≥400 IU/day) plus Calcium supplement (≥1,000 mg/day) users vs. non-users	Prospective observational study, 1 year follow-up 29 women ≥5 years postmenopausal, 22 men aged 50–80 years All had moderate-to-severe periodontal disease and received maintenance therapy during follow-up	Change in alveolar crest height and alveolar bone density	Patients who used supplements had 17 % more alveolar crest height than non-users at baseline and 6 months, 11 % more by end of study; not statistically significant Alveolar bone density higher in supplement users at 6 and 12 months relative to non-users ($p = 0.07$)

(continued)

Table 9.1 (continued)

Study	Nutritional status measures	Study design and population	Alveolar bone loss measure	Primary results
Millen et al., 2012 [28]	Vitamin D: plasma 25(OH)D	Cross sectional design 920 postmenopausal women Age range 50–79 years	Worst site and whole-mouth average alveolar crest height (mm)	No difference in worst site ABL or whole-mouth mean ABL among plasma 25(OH)D quintiles. E.g. whole-mouth mean ABL in extreme quintiles: Q1 (5.98–40.73 nmol/L): 2.4 mm Q5 (77.55–193.56 mnol/L): 2.4 mm
Pitiphat et al., 2003 [29]	Alcohol: frequency of beer (1 bottle or can), wine (4-oz glass), and liquor (1 drink or shot) in past year	Prospective design, up to 12 year follow-up 39,461 males participating in HPFS Mean age 53–55 years at baseline	Self-reported incident periodontal disease with bone loss	Higher risks (RR) of periodontitis among men reporting usual alcohol intake at all levels compared with non-drinkers: >0 to <5 g/d: (1.24, CI: 1.09–1.42) 5 to <15 g/d: (1.18 (1.04–1.35)) 15 to <30 g/d: (1.18 (1.01–1.38)) >30 g/d: (1.27 (1.08–1.49))
Tezal et al., 2001 [30]	Alcohol: drinks per week of beer, wine, and liquor. Categorized into <5 vs. ≥5 drinks/week, and <10 vs. ≥10 drinks/week	Cross sectional design 1,371 men and women Age range 25–74 years	ABL (mm), and categorized into healthy (0.4 to <2 mm), low (2 to <3 mm), moderate (3 to <4 mm) or severe (≥4 mm)	No significant association of ABL category with ≥5 or ≥10 drinks/week. Mean ± SD ABL comparable in drinks/week categories: <5 drinks/week: 2.58 ± 1.35 mm ≥5 drinks/week: 2.86 ± 1.60 mm <10 drinks/week: 2.60 ± 1.39 mm ≥10 drinks/week: 2.85 ± 1.43 mm
Jansson et al., 2008 [31]	Alcohol: centiliters pure alcohol per day	Prospective design, 20 year follow-up 315 men and women Mean age 55 years at baseline	Longitudinal bone loss (individual's mean difference in ABL from baseline to follow-up)	No significant association of alcohol with longitudinal ABL. E.g. change in %ABL in highest and lowest categories: 0–1 cl/d: 10.2 ± 8.20 % >5 cl/d: 9.36 ± 8.12 %

Abbreviations: *ABL* alveolar bone loss, *DLS* VA dental longitudinal study, *HPFS* health professionals follow-up study, *FFQ* food frequency questionnaire, *HR* hazard ratio, *OR* odds ratio, *CI* 95 % confidence interval, *RR* relative risk, *Cl/d* centiliters per day, *G/d* grams/day, *25(OH)D* 25-hydroxyvitamin D

[a,b] Obesity status: underweight (<18.5 kg/m^2) [b]; normal weight (18.5-24.9 kg/m^2) [a,b]; overweight (25-29.9 kg/m^2) [a,b]; obese (≥30 kg/m^2) [a,b]

[c] WC < 34.5 (lowest quintile) vs. ≥40 in (highest quintile)

[d] WHR <0.90 (lowest quintile) vs. ≥0.99 (highest quintile)

9.3.1.3 Vitamin D

In the DLS, vitamin D intake level of 800 IU per day or more was associated with approximately a 50 % reduction in the odds of moderate-to-severe ABL compared to intakes less than 400 IU/day [26].

A group of 51 postmenopausal patients with moderate-to-severe periodontal disease, some of whom regularly took vitamin D and calcium supplements, was followed for a 1-year period [27]. Change in alveolar crest height was height similar in supplement users and nonusers. Alveolar bone density was higher in supplement users at 6 and 12 months relative to nonusers, but the difference was not statistically significant [27]. Millen et al. [28] found no association between plasma 25-OH and alveolar crest height in postmenopausal women.

9.3.1.4 Alcohol

It is unclear if alcohol intake is related to alveolar bone loss. Using the self-reported periodontal bone loss status in the HPFS, alcohol intake was associated with 18–27 % higher risk of periodontal disease even at very low intake levels (0.1–4.9 g/day) [29]. However, radiographic studies have not found such a relationship. Tezal et al. [30] studied 1,371 subjects between the ages of 25 and 74 years and categorized their alcohol consumption into <5 or ≥5 drinks per week. One standard drink contains 14 g of ethanol. Participants with moderate or severe ABL were more likely to be in the higher consumption category but the difference was not statistically significant. Similar results were found when the consumption groups were cut off at <10 or ≥10 drinks per week [30]. Jansson et al. [31] also found no significant association between alcohol intake and ABL among 513 individuals in a longitudinal study. Even among subjects with the highest alcohol consumption (5 cl, or approximately 8 g, pure alcohol per day or more), the extent of alveolar bone loss was similar to that in nondrinkers.

9.3.2 Nutrition and Clinical Periodontal Disease Measures

Attachment loss and probing pocket depth are more commonly used measures of periodontitis in surveys and large studies. Results of studies using these clinical measures of disease generally support findings from studies of ABL and nutrition. The majority of the studies have been cross-sectional in design, so it is not possible to determine the directionality of the associations.

Obese persons have about 35 % higher odds of clinical periodontal disease than normal weight individuals and there is a trend of higher periodontitis prevalence with increasing body mass index [32, 33] and worsening periodontitis with weight gain [34]. Clinical measures are consistently worse in persons with metabolic syndrome relative to healthy people [33, 35] or persons with only 1 or 2 risk factors [36].

Specific nutrients and dietary components that have been associated with lower incidence or prevalence of periodontitis are vitamin C [37, 38], omega-3 fatty acids [39, 40], calcium [41], folate [42], carotenoids [43], vitamin D [44], dairy products [45, 46] and green tea [47]. Alcohol dependence is linked to severe periodontitis [48] but the association with low and moderate alcohol intakes is uncertain. An analysis of NHANES III data found significantly increased odds of attachment loss with intakes as low as five drinks per week [49], but another study, in contrast, reported an inverse relationship to attachment loss and gingival bleeding [50].

These nutrients may also be surrogate markers for healthy eating patterns and health-promoting lifestyle behaviors, in which multiple nutrients interact to reduce inflammation and repair tissues, and in which there is a relative lack of nutrients that initiate oxidative stress. Higher scores on a healthy

eating index were associated with less clinical attachment loss, as was greater physical activity [51]. Higher dark green and yellow vegetable intake was inversely related to incidence of attachment loss [52]. In an analysis of NHANES III data (1988–1994), Al-Zahrani et al. [53] found lower prevalence of periodontal disease among participants who engaged in at least one health-enhancing behavior (high quality diet, normal weight maintenance, or engaging in exercise). Low scores on the USDA Healthy Eating Index were related to more calculus deposits on the teeth [54], which are the substrate for oral bacterial growth.

9.4 Conclusion

Periodontal bone loss is influenced by multiple local and systemic factors. There is abundant evidence to suggest nutritional status may influence the susceptibility to develop periodontitis and the course of progression by maintaining the integrity of bone and collagen tissues and by modulating the inflammatory response. However, based on the existing evidence, it is not clear what nutritional advice beyond maintaining a healthy balanced diet can be given to dental patients in order to prevent or slow oral bone loss. More prospective studies and clinical trials are needed to determine the optimal nutrient intakes for preventing oral bone loss.

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Chapter 10
Nutrition Counseling for Skeletal Health

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Key Points

- Nutrition plays an important role in skeletal health throughout the life cycle.
- Evidence-based nutrition recommendations that can be used to promote skeletal health.
- The total diet or overall pattern of food consumed is the most important focus of healthy eating A healthful dietary pattern is associated with prevention of chronic diseases as well promoting skeletal health.
- The Surgeon General's report on bone health and osteoporosis recommendations include consuming recommended amounts of calcium and vitamin D, maintaining a healthful body weight, and being physically active, along with minimizing the risk of falls.
- Meeting calcium recommendations and weight bearing physical activity build strong bones, optimizes bone mass, and may reduce the risk of osteoporosis later in life.
- Nutrition counseling using the Nutrition Care Process is an effective structure for tailoring evidenced-based recommendations to an individual's unique needs.

Keywords Yacoub • Karmally • Skeletal health • Nutrition counseling

10.1 Introduction

It is estimated that more than 10 million Americans over the age of 50 have osteoporosis, including 7.8 million women and 2.3 million men. Another 33.6 million over the age of 50 have low bone mass and thus are at risk for osteoporosis [53]. The direct costs of osteoporosis in 2001 were between $11.6 and $17.1 billion. Osteoporosis not only imposes direct costs on society but indirect costs as well, including the costs of morbidity and premature mortality. Moreover, as older Americans remain in the workforce, osteoporosis results in loss of productivity.

While calcium and vitamin D are two of the most important nutrients involved in skeletal health, having an overall balanced diet including a variety of the food groups will help optimize the nutrient requirements for health. Adequate intake of dietary calcium, an abundant mineral, making up more than 99 % of bones and teeth, is especially important during childhood to increase bone mass.

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Table 10.1 Institute of medicine dietary reference intake for calcium and vitamin D

Calcium				Vitamin D		
Life stage group	Estimated average requirement (mg/day)	Recommended dietary allowance (mg/day)	Upper level intake (mg/day)	Estimated average requirement (IU/day)	Recommended dietary allowance (IU/day)	Upper level intake (IU/day)
Infants 0–6 months	*	*	1,000	**	**	1,000
Infants 6–12 months	*	*	1,500	**	**	1,500
1–3 years old	500	700	2,500	400	600	2,500
4–8 years old	800	1,000	2,500	400	600	3,000
9–13 years old	1,100	1,300	3,000	400	600	4,000
14–18 years old	1,100	1,300	3,000	400	600	
19–30 years old	800	1,000	2,500	400	600	4,000
31–50 years old	800	1,000	2,500	400	600	4,000
51–70 year old males	800	1,000	2,000	400	600	4,000
51–70 year old females	1,000	1,200	2,000	400	600	4,000
>70 years old	1,000	1,200	2,000	400	800	4,000
14–18 years old, pregnant/lactating	1,100	1,300	3,000	400	600	4,000
19–50 years old, pregnant/lactating	800	1,000	2,500	400	600	4,000

*For infants, Adequate Intake is 200 mg/day for 0–6 months of age and 260 mg/day for 6–12 months of age
**For infants, Adequate Intake is 400 IU/day for 0–6 months of age and 400 IU/day for 6–12 months of age
Institute of Medicine. Dietary Reference Intake for Calcium and Vitamin D. Washington, DC: National Academies Press, 2010. http://www.iom.edu/~/media/Files/Report%20Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin%20D%20and%20Calcium%202010%20Report%20Brief.pdf [1]

Recommendations for calcium are based on the optimal amount to promote bone density and prevent bone loss which can lead to fractures, osteoporosis, and osteomalacia. The Recommended Dietary Allowance (RDA) for calcium, shown in Table 10.1, ranges from 1,000–1,200 mg/day depending on age and gender [1]. The National Health and Nutrition Examination Survey (NHANES) found that most Americans fall below those recommendations and dietary calcium intake has been shown to decline in older age [2].

Vitamin D, which increases calcium absorption in the intestinal tract, does not occur naturally in many foods. Therefore, foods fortified with vitamin D, such as dairy products, milk substitutes, and cereal, are the main dietary sources for several people. See Table 10.2 for a detailed list of dietary sources of Vitamin D [3]. Other major sources of vitamin D are sun exposure and supplements [2]. Adequate intake of vitamin D is essential for bone development, especially in infants and children, to prevent rickets and osteomalacia and osteoporosis later in life [4]. Vitamin D status is measured by serum 25-hydroxyvitamin D which has a half-life of about 3 weeks. There is currently no optimal level of serum 25(OH)D, which is dependent on various factors including life stage and whether skeletal or nonskeletal effects are being measured [5].

The U.S. Preventive Services Task Force (USPSTF) conducted a systematic review evaluating the evidence regarding the benefit of supplementation with vitamin D and calcium. They concluded there is not enough evidence to support supplementation with calcium and vitamin D to prevent fractures in men or premenopausal women. The USPSTF also concluded that there is not enough evidence to support the benefit of supplementation with more than 400 IU of vitamin D3 and more than 1,000 mg of calcium to prevent fractures in non-institutionalized postmenopausal women. They also advise against daily supplementation with 400 IU or less of vitamin D3 and 1,000 mg or less of calcium in preventing fractures for non-institutionalized postmenopausal women; however, supplementation with

Table 10.2 Dietary sources of vitamin D

Serving size	Dietary source	Vitamin D (IU)
3 oz	Trout, cooked	645
3 oz	Salmon, sockeye, cooked	447
3 oz	Halibut, Atlantic and Pacific, cooked	196
3 oz	Sardines, canned in oil with bone	164
1 cup	Yogurt, nonfat, fruit variety, fortified	127
1 cup	Skim milk, with added vitamins A and D	115
1 cup	Soymilk, fortified	114
¾ cup	Cereals ready-to-eat, general mills, whole grain total	100
1 cup	Cereals ready-to-eat, Kellogg's Raisin Brain	95
3 oz	Tuna, white, canned in water	68
1 large	Egg, whole, cooked	41
1 cup	Mushrooms, shiitake, cooked	41

US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl [3]

vitamin D may be beneficial for preventing falls in community-dwelling adults 65 years or older. A potential harmful side effect of supplementation is an increased risk of renal stones [6]. The Agency for Healthcare Research and Quality examined 165 primary articles and 11 systematic reviews, which included over 200 primary articles, to evaluate the health outcomes of calcium and vitamin D. One systematic review showed a small improvement in BMD in the spine and other areas for postmenopausal women supplemented with calcium and vitamin D [7]. Another systematic review which included 19 studies in postmenopausal women and older men showed a correlation between vitamin D supplements and BMD. The dosage given was between 400 IU and 1,400 IU per day. The effect of vitamin D alone on BMD could not be determined due to the possible effect of other nutrients used such as calcium and vitamin K. In one randomized control trial (RCT), there was improvement in bone turnover markers, but not BMD with supplementation of 200 IU of vitamin D [8].

Magnesium is another abundant mineral associated with a higher BMD; however, according to NHANES, the majority of adults do not meet the RDA of 420 mg/day and 320 mg/day for adult males and females, respectively. The role of magnesium alone in preventing bone loss is unclear as many dietary sources of magnesium contain other nutrients associated with bone health such as calcium and phosphorus. Some limited studies have shown increased BMD with magnesium supplementation, but the evidence is inconclusive and cannot be generalized to a healthy population [51]. Good dietary sources of magnesium include almonds, cashews, peanuts, kidney beans, black-eyed peas, lentils, and milk. See Table 10.3 for exact values and portions [3].

Phosphorus or phosphate (as present in biological systems) is another important mineral for maintaining bone health. The majority of phosphorus (85 %) is found in bone and teeth. Dietary sources of phosphate include protein-rich foods such as beans, meat, fish, eggs, and dairy, as well as cereal grains and peanuts. See Table 10.4 for exact values and portions [3]. A phosphate deficiency can lead to rickets and osteomalacia [9].

Vitamin C is not only necessary for forming collagen, but it is also important for bone mineralization and osteoblast activity [10]. Some studies done on postmenopausal women have shown increased BMD with vitamin C supplements, but there is no strong evidence in its role for preventing osteoporosis [11]. Good dietary sources of vitamin C include fruit, particularly citrus as well as peppers, and some green vegetables such as broccoli, kohlrabi, and kale.

Fluoride is an element that supports the health of bone and teeth and about 99 % of its presence in the body is in hard tissues. Through mineralization, it contributes to healthy bones and teeth. The Academy of Nutrition and Dietetics advises dietitians to encourage the use of fluoride for all age

Table 10.3 Dietary sources of magnesium

Serving size	Dietary source	Magnesium (mg)
1 cup	Spinach, cooked	157
1 oz	Pumpkin seeds	156
1 cup	Soybeans, cooked	148
1 cup	Black beans, cooked	120
1 oz	Brazil nuts	107
1 cup	Oat bran, cooked	88
1 cup	Brown rice, cooked	84
1 cup	Kidney beans, cooked	80
1 cup	Chickpeas, cooked	79
1 oz	Almonds	76
1 cup	Lentils, cooked	71

US Department of Agriculture, Agricultural Research Service.2012 USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl [3]

Table 10.4 Dietary sources of phosphorus

Serving size	Dietary source	Phosphorus (mg)
1 cup	Oat bran, raw	690
1 cup	Ricotta cheese, part skim	450
1 cup	Bulgur, dry	420
3 oz	Sardines, canned with bone	417
8 oz	Yogurt, low fat	327
1 cup	Soybeans, green, cooked	284
1 cup	Navy beans, cooked	262
1 cup	Kidney beans, cooked	251
1 cup	Pinto beans, cooked	251
1 cup	Skim milk	247

US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl [3]

groups for oral health, bone health, and overall health [12]. While some research shows that fluoride can stimulate osteoblast to form bone when given in large enough doses, the current levels in the water are not enough to have an effect on osteoblast activity or fracture prevention. Besides water, other sources of fluoride include foods and beverages made with fluoridated water and fluoridated topical gels, foams, and rinses.

10.2 Fruits and Vegetables and Bone Mass

Fruits and vegetables are good sources of vitamins, minerals, and antioxidants which are involved in bone building and the maintenance of bone ([2], [51]). Some studies have shown that dietary acid negatively effects bone health [52]. In order to balance the body's acid load, it relies on skeletal reserves to produce bicarbonate and maintain acid–base balance. Minerals such as potassium and magnesium found in fruits and vegetables offset the acid production from the various components of the diet by producing bicarbonate and creating an alkaline environment. Western diets pose a risk

for fractures due to the high dietary acid load secondary to low consumption of fruits and vegetables and higher consumption of grains and animal based proteins (meat, fish, and eggs) which form acid when metabolized [13].

10.3 Protein and Bone Mass

While dietary protein does contribute to the body's acidic load, studies have yielded mixed results on its effect on bone health; however, protein is necessary for preserving skeletal muscle which has a positive correlation to bone health [2]. Adequate protein intake can help reduce the loss of skeletal muscle mass, which is especially important in the elderly, who are at greater risk for falls and fractures. The loss of muscle strength and mass during aging leads to a condition known as sarcopenia. Patients with sarcopenia who suffered hip fractures are almost twice more likely to develop osteoporosis than patients with hip fractures and normal muscle mass [50].

Fatty fish such as salmon, mackerel, herring, sardines, and albacore tuna are high quality and lean proteins rich in omega-3 fatty acids and many important vitamins such as A and D and minerals including selenium, calcium, iodine, and zinc. Many studies have established its positive role in diseases such as cancer, heart disease, and diabetes. [47] found that in premenopausal women in Spain, it can also have a positive effect on BMD in the phalanges, measured with bone ultrasound. In 65 premenopausal women, significant differences were found in those who consumed 0–2 servings of fish per week versus 5–7 servings per week. The latter group had a higher intake of vitamin D and zinc and higher bone mass in the phalanges. While these are important nutrients involved in bone mass, results from previous studies have shown that omega-3 fatty acids is a contributor to bone health [47].

10.4 Omega-3 Fatty Acids and Bone Density

Polyunsaturated fatty acids (PUFA) status, which is measured by omega-3 and omega-6 PUFAs in red blood cells (RBC), may have an effect on hip fracture risk. In a case–control study, Orchard et al. studied postmenopausal women in the Women's Health Initiative (WHI). In 324 matched pairs of women with hip fractures from the Bone Mineral Density cohort and from the WHI Observational Study, alpha linolenic acid (ALA) in RBC, eicosapentaenoic acid (EPA), and total omega-3 PUFAs, but not docosahexaenoic acid (DHA) was associated with lower hip fractures. In the highest RBC omega-6/omega-3 ratio, hip fracture nearly doubled. In addition, the positive effect of RBC omega-3 on lowering hip fracture risk was associated with ALA and EPA, not DHA [14].

Omega-3 PUFA, particularly, EPA and DHA are found in fatty fish while ALA can be found in vegetable oils including soybean, rapeseed, flaxseed, chia seeds, and walnuts. Some green vegetables also contain small amounts of ALA such as kale, spinach, and purslane. Sources of omega-6 PUFAs are found primarily in vegetable oils including corn, safflower, sesame, soybean, pumpkin, wheat germ, and walnuts. Consuming more omega-3 and decreasing the ratio of omega-6 to omega-3 fatty acids are associated with higher BMD [15].

The 2010 Dietary Guidelines for Americans [16] recommend an intake of 0.7–1.6 g of ALA or 0.6–1.2 % of energy intake and 0.25 g EPA + DHA per day; however, the National Health and Nutrition Examination Survey (NHANES) 2009–2010 show that Americans consume less than 2 g of omega-3 per day and 0.12 g of EPA + DHA [16, 17]. The American Heart Association recommends consuming two servings of 3.5 oz cooked fatty fish per week to boost intake of EPA and DHA to 0.5 g per day for healthy adults [18].

10.5 Caffeine Consumption and Bone Density

Caffeine has been shown to increase calcium excretion and decrease intestinal absorption which may result in decreased bone density. With each cup of coffee, there is a 5 mg loss of calcium. Studies have yielded mixed results with some showing that high caffeine intake may have a negative impact on BMD only when calcium intake is also low [19]. One meta-analysis of ten prospective studies showed an association with increased risk of fracture with each cup of coffee, particularly in women [20]. Conversely, a recent long-term study in a Swedish Mammography Cohort of 61,433 women found that while high coffee consumption (four or more cups per day) versus low consumption (less than one cup per day) was related to a slight decrease in bone density, it did not increase fracture risk [21]. While results are inconsistent, consuming adequate calcium should be encouraged, particularly in populations that consume a lot of caffeine either through coffee, tea, or soda beverages.

10.6 Dietary Patterns and Bone Density

Dietary patterns similar to the Mediterranean diet such as a high intake of plant-based foods, fish, and olive oil with a low intake of red meat is associated with higher BMD [22]. The Framingham study found that diets that were rich in fruits, vegetables, milk, and cereals had significantly are associated with higher bone density than diets primarily consisting of salty foods, pizza, soda, meat, bread, and potatoes [23]. Another study measuring the different dietary patterns of Australian women in relation to BMD found that dietary patterns similar to a Western type diet (high in fat, processed meats, calorically dense, and nutrient empty) had a negative effect on bone density. Conversely, dietary patterns similar to a Mediterranean diet which is rich in legumes, seafood, seeds and nuts, wine, rice, and vegetables had a positive effect on BMD [24].

10.7 Populations at Risk for Bone Related Diseases

In addition to those consuming a Western diet, Asian populations are also at risk for bone related diseases. Asian diets are typically low in dairy and fall short on their daily calcium intake. Consumption of dark vegetables may be their main sources of calcium. Vegetarians and vegans are also at risk for low bone density due to their lower intake of protein and calcium [2].

Lactose intolerant individuals often avoid milk and dairy products and consequently are at risk for low bone mineral density. Many individuals avoid milk not only due to intolerance but due to perceived intolerance or because of an aversion. Compared to the rest of the US population, and based on self-reports, African-Americans had a higher perceived lactose intolerance and consequently consumed less calcium, vitamin D, and other bone building nutrients found in dairy [25, 26].

There are varying degrees of lactose intolerance or sensitivity. Even in the more severe cases, individuals can usually still tolerate small amounts of dairy products, but many avoid them all together [26]. To improve tolerance, milk can be consumed with meals and other dairy products can be incorporated slowly into the diet in smaller quantities. Dairy foods that are better tolerated include lactose free products, hard cheeses, which are lower in lactose, and yogurt, which has live cultures that help break down lactose [25].

10.8 Role of Exercise in Bone Health

In addition to maintaining a balanced diet with adequate nutrients, exercise is a beneficial component of skeletal health. Exercise that has been shown to improve BMD includes modest impact activity and resistance training. Resistance training can help maintain muscle mass and tone which reduces the risks of falls and fractures [11].

10.9 Obesity and Bone Health

While there has been evidence that excess body weight can increase risk of bone-related diseases, a recent study has shown that specifically bone marrow fat in obese individuals is associated with lower BMD. Bone and fat cells share a common stem cell that forms osteoblasts, adipocytes, and marrow fat, and so bone marrow fat may serve as a marker of bone health. In a study of young obese men and women, Bredella et al. [27] data found that higher serum triglycerides, intrahepatic lipids (IHL), intramyocellular lipids (IMCL), and serum lipids were associated with higher bone marrow fat, whereas higher HDL cholesterol was associated with lower bone marrow fat. In addition to weight reduction counseling, educating obese individuals on a cardiovascular protective diet to lower serum lipids as well as reducing intake of fat and sugar may have the added benefit of reducing their risk of developing osteoporosis.

10.10 Soy and Bone Health

Soy products contain isoflavones, a type of phytoestrogen which binds to estrogen receptors and acts as selective estrogen receptor modulators (SERMS). Some studies show it may play a positive role in menopausal bone health. However, due to the lack of sufficient human trials and the differences in soy derivatives used in each trial and variability of soy products on the market, there is no conclusive evidence to support the use of soy products in preventing bone loss [2]. For many people who do not consume dairy, fortified soy products are still a practical option for alternative sources of calcium and vitamin D.

10.11 Nutrition Counseling

10.11.1 Assessment of the Patient in Nutrition Counseling

Nutrition counseling, using the Nutrition Care Process is an effective structure for tailoring evidenced-based recommendations to an individual's unique needs (Nutrition Care Process SNAPshots, [28]. The first step is to assess the individual based on various factors including their readiness to change (using Prochaska's Stages of Change model), [29] their level of food and nutrition knowledge, and their typical eating patterns including dietary restrictions, intolerances or allergies, and cultural or religious eating habits.

The stages of change model, also called the Transtheoretical Model is a valuable tool in motivational interviewing to assess an individual's readiness to change which will guide the focus of nutrition counseling sessions. There are five stages in the model categorized as: precontemplation (no intention of change), contemplation (thinking about change), preparation (intention to change within the foreseeable future), action (actively making changes), and maintenance (defined as engaging in a behavior for at least 6 months) [29].

A counseling style adopted by health professionals originally used to treat addictions is motivational interviewing (MI). This process is a way of communicating that includes reflective listening and involves the client in decision making while using change talk. Through motivational interviewing and asking open-ended questions, the nutrition professional can determine which stage the patient/client is in, design an intervention that fits within that particular stage, and ideally move them forward to the next stage of change. This non-judgmental, client-driven, and goal-centered approach opens the lines of communications to facilitate positive behavior change [30].

Before immediately making diet recommendations for nutrients involved in bone health, it is necessary to first assess the client's baseline food and nutrition knowledge. This can be determined by asking them which foods are high in calcium and vitamin D, to name the requirements for those nutrients, or a short questionnaire asking about nutrition and bone health.

Obtaining a thorough diet history is important and should not only focus on calcium and vitamin D consumption, but the overall diet should be assessed. This can be done by asking open-ended questions about their typical intake, any food restrictions, food preferences, food aversions, and/or intolerances, and cultural food practices.

Patients' values and health beliefs and lifestyle practices can be explored by asking questions such as:

- What are your concerns about lowering risk for bone disease?
- What are your family activities to promote exercise?
- Tell me about your favorite traditional foods so that they can be included in your eating pattern.
- Who decides the menu for your family?
- Who does the grocery shopping and food preparation?
- Do family members eat most meals together?
- Can you describe your current eating pattern?
- What portion of your dinner plate has rice, bread, vegetables, and animal protein?

Open questions help move the change process along. The patient/client should be given an opportunity to express commitment to specific changes in behavior. These should be asked in a respectful manner. A 24 h recall and a short, validated calcium and vitamin D questionnaire [31] can also be administered to assess the adequacy of those nutrients in their diet. If data such as bone mineral density (BMD), intestinal calcium absorption, concentration of parathyroid hormone (PTH), serum vitamin D levels, and T and Z scores, are available, these should also be reviewed during the initial assessment. These serve as objective signs and symptoms to reflect the patient/client's baseline bone health.

10.12 Diagnosis

After assessing the patient/client, diagnosing them with a nutrition problem is the next step. Understanding the *problem*, *etiology*, and *signs/symptoms* (PES), will help narrow down the specific nutrition problem that can be modified through nutrition intervention.

For example, if the patient/client has low bone mineral density (BMD), as measured by a DXA scan (Dual X-ray Absorptiometry), and their intake of calcium is low due to lactose intolerance, the PES statement could be "inadequate intake of calcium related to low intake of dairy secondary to

lactose intolerance as evidenced by low T and Z scores." In addition to identifying the problem, the PES statement concisely identifies the barrier to healthy eating which can give direction and focus to the counseling session. There may be several risk factors and problems contributing to low bone mineral density which can be captured by more than one PES statement. It is important to note that the problem should be solved through nutrition intervention. In the previous example, lactose intolerance is a contributing factor, but not the primary problem because it is not something that can be changed with nutrition counseling.

10.13 Intervention

If lactose intolerance is preventing the patient/client from consuming calcium-rich foods from dairy, one intervention is to educate the patient/client on increasing the intake of calcium-rich foods from other sources such as vegetables and calcium fortified foods or lactose-free dairy products. While this might be helpful information, it does not necessarily mean they will change their behavior. Nutrition counseling goes beyond merely providing the patient/client with information. By utilizing various behavior change strategies, the intervention is more likely to be effective and meet the patient/client's individual needs.

The approach that the registered dietitian will take depends on the patient/client's stage of change. Motivational interviewing can be helpful in resolving uncertainty about making behavioral changes by actively listening, reflecting, asking open-ended questions, and maintaining a nonjudgmental and supportive environment [32].

Once the barrier to making changes is identified, the nutrition counselor can guide the patient/client to help them come up with their own solutions. The patient/client solution is more likely to be effective in meeting their needs. If the nutrition counselor gives educational information and offers solutions, it is important to follow it up with asking the patient/client what they think about the solution and if it helps to overcome the barrier.

When the client moves into the action phase of the transtheoretical model, goal setting is an important next step. It is an important part of the Nutrition Care Process and motivational interviewing. While it is important for the client to come up with their own goals, they may need help in setting realistic expectations and small, achievable goals. Goals can be both short-term and long-term. Helping the client set specific and measurable goals will simplify monitoring and evaluating progress [32].

If a client does not eat enough foods with calcium, one goal could be to increase the intake of low-fat dairy to three servings per day by having one serving at each meal. If the client is lactose-intolerant and prefers not to include lactose-free milk, a similar goal could be to increase the servings of calcium-rich plant foods to five to seven servings per day. Other sources of calcium, shown in Table 10.5, include yogurt as well as calcium-fortified tofu and soy beverages [3].

When making recommendations on ways to increase calcium and vitamin D intake, it is important to consider the whole diet and the bioavailability of those nutrients. Certain nutrients/food components consumed with calcium may either increase or decrease its absorption. While vitamin D enhances calcium absorption, phytate, which is found in plant foods, such as whole grains, legumes, and nuts, binds to calcium and reduces its solubility when present in a certain proportion to calcium. Fiber is another nutrient that can have an effect on calcium absorption. Studies done on supplementation with locust bean gum and high esterified pectin, both soluble fibers, showed a reduction in calcium's availability; however, supplementation with inulin increased calcium absorption [33].

Oxalate, which is found in plant foods like kale and spinach, can also affect calcium bioavailability when present in a certain proportion to calcium. Oxalate binds to calcium and forms an insoluble compound, thus reducing calcium bioavailability. The greater the amount of oxalate present, less calcium is absorbed [34].

Table 10.5 Dietary sources of calcium

Serving Size	Dietary Source	Calcium (mg)
1 cup	Ricotta cheese, part skim	669
8 oz	Fat-free yogurt	452
0.5 cup	Tofu, raw, prepared with calcium sulfate	434
1 cup	Collard greens, cooked from frozen	357
1 cup	Orange juice, fortified	348
3 oz	Sardine with bone	325
1 cup	Soymilk, fortified with calcium and vitamins A and D	299
1 cup	Milk, nonfat, with added vitamins A and D	299
1 oz	American cheese, fortified with vitamin D	296
1 cup	Soybeans, green, cooked	261
1 cup	Turnip greens, cooked from frozen	249
1 cup	Spinach, cooked	245
1 cup	Kale, cooked from frozen	179
1 cup	Low-fat cottage cheese	138
1 cup	Okra, cooked from frozen	136

US Department of Agriculture, Agricultural Research Service. 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page. http://www.ars.usda.gov/ba/bhnrc/ndl [3]

The 2010 Dietary Guidelines Advisory Committee (DGAC) evaluated the nutritional adequacy of nondairy foods such as fortified soy or rice drinks, tofu, leafy greens, bony fish, and fortified orange juice to meet calcium needs. In a study measuring the nutritional impact of removing or adding dairy foods to the American diet, Fulgoni III et al. used two models, MyPyramid and NHANES to replace calcium in the diet with nondairy foods. To equal the amount of calcium in a serving of dairy, it would require 1.1 servings of fortified soy drink, 0.6 servings of fortified orange juice, 1.2 servings of bony fish, or 2.2 servings of leafy greens. They found that while it was possible to meet calcium requirements through these nondairy alternatives, it created shortfalls of other nutrients such as magnesium, phosphorus, potassium, and protein. Also, given Americans' current dietary patterns, consuming these nondairy foods is not always a practical substitute for calcium [35].

These are important considerations when counseling individuals who are vegan, lactose-intolerant, or from cultural backgrounds who do not consume dairy. When replacing dairy foods with nondairy calcium substitutes, they should be incorporated into the diet in a healthful and practical way while also meeting overall dietary recommendations.

10.14 Monitoring and Evaluating

After intervention, monitoring and evaluating outcomes are the next steps in the Nutrition Care Process to determine if goals are being met. By comparing current nutritionally relevant data to previous findings during the assessment, the patient/client's progress can be measured. There are four different categories in the Nutrition Care Process of monitoring and evaluating outcomes.

- Food/Nutrition-Related History. This involves food and nutrient intake, food and nutrient administration, medication, complementary medication use, knowledge/beliefs, food supplies availability, physical activity, and nutrition quality of life. The various subcategories used within each outcome will depend on the nutrition diagnosis. In relation to bone health, a comparison of knowledge about foods for optimizing bone health can be made by asking similar questions as the initial

assessment. The patient/client can be asked to name foods important for bone building or take a posttest about nutrition and bone health. Another method for monitoring and evaluating outcomes is collecting and analyzing 24 h recalls or three day food diaries at various time points and comparing them to each other and to nutritional guidelines [28].

- Anthropometric Measurement is the second category to monitor outcomes which includes height, weight, body mass index (BMI). If the patient/client's weight is a part of their nutrition assessment or a sign/symptom related to their diagnosis, continuing to obtain these measurements at each follow-up would be a part of their monitoring and evaluating process [28].
- Biochemical data, medical tests, and procedure include laboratory data and tests which will vary depending on the disease and outcomes that will be measured [28]. Upon follow up, biochemical markers of bone health should be compared to the initial assessment and to reference standards to evaluate progress.
- Nutrition-Focused Physical Findings refers to physical appearance, muscle and fat wasting, swallow function, appetite, and affect [28]. These findings will also vary depending on the nutrition diagnosis and may not all apply to certain health conditions.

10.15 Parenteral Nutrition and Metabolic Bone Disease

Patients who have intestinal failure may require parenteral nutrition (PN) and are at an increased risk of vitamin D deficiency. Vitamin D deficiency results from fat malabsorption that occurs in gastrointestinal diseases such as short bowel syndrome or gastrointestinal motility disorder as well as from decreased oral intake of vitamin D. A complication related to long-term parenteral nutrition is metabolic bone disease (MBD) that may be a result of multiple factors including "malabsorption of calcium and phosphorus, medications, high protein intake, metabolic acidosis, aluminum contamination, fluoride exposure, and vitamin K deficiency" [36].

A recent review found that metabolic bone disease was reported in 41–46 % of patients on home PN. Most metabolic bone disease cases from PN are a result of mishandling of calcium, phosphorus, vitamins D and K in addition to underlying gastrointestinal malabsorption such as Crohn's disease. Providing enough calcium and phosphorus in the PN solution is necessary for bone health [37].

Negative calcium balance can occur from inadequate calcium in the PN solution coupled with increased urinary calcium excretion. Due to the risk of calcium-phosphate precipitation, it is a challenge to provide enough of these nutrients in a parenteral solution. This is of particular concern in neonates because of their high calcium and phosphorus requirements and low fluid needs. The risk of precipitation increases when calcium and phosphorus concentrations are high, amino acid concentration is low, environmental temperature and pH is increased, as well as increased hang time of PN [38].

Managing patients on long-term PN involves routinely checking for signs of MBD such as loss of height or bone pain and comparing DXA measurements and T scores from baseline and following up with repeated measurements. Routine tests for serum vitamin D levels and PTH are also beneficial in assessing the risk for MBD as well as determining if nutrient intake needs to be adjusted. While providing enough calcium and phosphorus in a PN solution is necessary to prevent MBD, giving an excess of phosphorus can result in bone loss due to hyperparathyroidism [38].

Protein is another factor that can affect bone density and needs to be taken into consideration. Patients newly starting on PN may require a higher load of amino acids in their PN solutions for wound healing and to restore protein loss, but once it is restored, the concentration should be reduced. To reduce the risk of excessive urinary calcium excretion and chronic metabolic acidosis, no more than 1.5 g/Kg/day of protein is recommended in a PN solution [38].

Preterm and very low birth infants are also an increased risk of metabolic bone disease as the last trimester is when they accumulate most bone-building nutrients. Timing of calcium and phosphorus

administration may also play a role in bone strength in premature infants. Administering calcium and phosphorus early on at higher doses in a parenteral solution can prevent loss of bone strength in premature newborns [39].

10.16 Maternal Nutrition and Fetal Bone Development

Maternal intake of bone building nutrients, particularly calcium and vitamin D, can influence fetal bone development. Calcium is actively transported to the fetus via the placenta at [40, 41] beginning week 12 of gestation and it peaks at week 36. During the second and third trimester, the absorption of calcium by the mother increases which is dependent on calcium intake [40].

For adequate fetal bone metabolism, it is recommended for a pregnant woman to consume at least 1,000 mg per day. There is not enough evidence to support supplementation of calcium in women who are meeting their needs. However, positive outcomes of calcium supplementation have been shown in women with low intake of calcium (<500 mg/day) for a prolonged period [40]. Maternal calcium needs can be met by eating and drinking three to four servings of low-fat dairy a day which includes milk, yogurt, cheese, and products prepared with these foods. Fortified cereals and soy milk, tofu, and green vegetables such as kale, bok choy, and Brussels sprouts can also help meet calcium needs.

Vitamin D, which aids in the absorption of calcium is especially important for neonates to prevent calcium deficiency and rickets. Since breast milk is not a good source of vitamin D, maintaining adequate vitamin D status during pregnancy is especially important for the future health of the child. There is debate and mixed results on whether the current recommended intake of 600 IU per day for pregnant women is sufficient to prevent deficiency [42]. Since good sources of vitamin D such as fish are also high in mercury, alternative sources should be given when counseling a pregnant client. Vitamin D can be found in fortified cereals, fortified dairy, and in small amounts in eggs and mushrooms.

Besides calcium and vitamin D, the overall quality of a maternal diet should be considered in regards to offspring bone health. The Generation R Study in Rotterdam, Netherlands, measured maternal dietary intake in the first trimester and its effect on childhood bone mass content (BMC). A higher intake of protein, vitamin B-12, calcium, and phosphorus in the first trimester was correlated with a higher BMC in childhood, whereas maternal carbohydrate intake was associated with lower childhood BMC [55].

10.17 Implementing DGAC 2010 for Skeletal Health

When educating patients/clients on skeletal health, the DGAC 2010 should be incorporated into counseling to promote a healthful eating pattern and balanced diet. In addition to focusing on bone building nutrients, meeting overall nutrient needs is vital to prevent deficiencies as well as to maintain a healthy body weight by increasing physical activity and monitoring caloric intake. The DGAC advises to reduce consumption of certain foods which contribute to chronic diseases and weight gain. These include foods with high levels of sodium, saturated fat, trans fat, cholesterol, solid fats, added sugar, and refined grains and alcohol [16].

Sodium intake should be limited to less than 2,300 mg (mg) for the average person and less than 1,500 mg for individuals who are 51 years and older and African Americans of any age or individuals with hypertension, diabetes, or chronic kidney disease. Eating fresh foods and limiting processed and canned foods can help reduce sodium intake [16].

Saturated fat should comprise of less than 10 % of calories and solid fats like butter and margarine should be replaced with monounsaturated fats like olive and canola oil. In addition to reducing

saturated fat, dietary cholesterol should be limited to 300 mg or less per day by reducing intake of egg yolks, full fat dairy products, and meats. Alcohol could be consumed in moderation which is equivalent to one drink per day for women and two drinks per day for men, if permitted by the physician [16].

Nutrients that are of concern and need to be increased in the American diet include potassium, dietary fiber, calcium, and vitamin D. To meet calcium needs, the DGAC 2010 guidelines recommend 3 cups of low-fat dairy per day for children, adolescents, and adults aged 9–18 years, 2.5 cups per day for children aged 4–8 years and 2 cups per day for children aged 2–3 years. A study done on young female athletes found that an increase in low-fat dairy products resulted in a reduced incidence rate of stress fractures as well as improved hip BMD [43].

Increasing fruit and vegetable intake to at least 2.5 cups per day, especially dark green and red and orange will provide a good source of fiber and nutrients that support skeletal health. The adequate intake (AI) for dietary fiber is 25 g per day for women and 38 g per day for men. Dietary fiber can also be met by replacing at least half of refined grains with whole grains and by consuming more beans and peas which are good sources of protein, potassium, folate, and they also contain iron and zinc [16]. Fiber is not only beneficial for cardiovascular and digestive health, but preliminary studies show that prebiotic fiber, particularly fructans may also support skeletal health by increasing calcium absorption. This is thought to occur due to prebiotics' effect on lowering intestinal pH secondary to the production of short chained fatty acids (SCFA), which improves calcium solubility and absorption [44].

A double-blind randomized study assigned adolescents aged 9–13 years to drink a carbohydrate supplement with 8 g per day of either a prebiotic inulin-type fructan (ITF) or maltodextrin for the control group. Both supplements were mixed with calcium-fortified orange juice. The participants' baseline calcium absorption was measured followed by a repeat measurement after drinking the carbohydrate supplements for 8 weeks. Calcium absorption test was repeated at 1 year from baseline and bone mineralization was also measured. Calcium absorption was significantly higher at 8 weeks and at 1 year compared to the control group. Bone mineralization was measured by whole-body bone mineral content (BMC) and bone mineral density (BMD) and both factors increased in the fructan supplement group [45].

DGAC also recommends choosing a variety of protein rich foods including seafood, beans and peas, nuts, and lean meats, poultry, and eggs. Eating seafood twice a week (or 8 oz per week) in place of poultry or meat is also recommended to meet the goal of 1,750 mg of EPA + DHA [16].

10.18 Conclusion

When counseling patients/clients on skeletal health, the nutrition care process is a helpful tool to provide individualized care and tailored recommendations. By isolating the nutrition problem, identifying barriers, and actively involving the patient/client in reaching their goals, it improves their chances of success. After intervention, monitoring and evaluating outcomes can be achieved by comparing current measures of bone health, including biomarkers, knowledge, and intake, with previous findings.

While nutrients involved in skeletal health include calcium, vitamin D, magnesium, phosphorus, fluoride, and vitamin C, following a healthful dietary pattern is important. This can be achieved by implementing recommendations set in place by DGAC, the RDAs and DRIs. Maintaining a healthful body weight and lean muscle mass and engaging in regular physical activity will also support skeletal health.

Some populations may be at risk for bone related diseases including vegans, those who do not consume dairy either due to lactose intolerance or cultural reasons, and Western diets that fall short on many

bone building nutrients. Patients/clients who do not consume lactose should be advised on alternative sources of calcium and vitamin D including lactose-free products and fortified cereals and soy products. Recommendations should be culturally sensitive and include foods that the patient/client likes.

Careful consideration should be given to pregnant women, preterm infants, and patients with intestinal failure, especially ones requiring parenteral nutrition due to their higher needs of bone building nutrients. For healthy bone development, adequate nutrition should begin during pregnancy and maintained through adulthood.

The USDA MyPlate is a useful tool to balance intake of food groups. This consists of filling up half of the plate with non-starchy vegetables (e.g., broccoli, kale, spinach, and green beans), a quarter of the plate with starch (preferably whole grains such as barley, brown rice, and quinoa), and the other quarter with lean protein (chicken, turkey, lean beef, and fish). A healthful drink includes skim or low-fat milk with a serving of fruit. Incorporating these dietary instructions as part of an overall healthful lifestyle can enhance bone health.

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Part II
Nutrition and Bone: Effects of Life Stages and Race

Chapter 11
Nutrition in Pregnancy and Lactation

Bonny L. Specker

Key Points

- Pregnancy and lactation are periods of significant change in calcium and bone metabolism for the mother.
- Physiological changes that occur insure that there is an adequate calcium supply for fetal growth, milk production, and maternal bone recovery.
- During pregnancy, low maternal calcium intake is associated with low neonatal BMC and maternal vitamin D deficiency influences fetal bone development and neonatal calcium homeostasis.
- Whether maternal vitamin D status during pregnancy influences infant growth trajectories or bone accrual later in childhood is not known.
- Due to potential adverse effects of high maternal vitamin D concentrations on the offspring, it is important that all current and future supplementation trials investigate the influence of not just low serum 25-OHD concentrations, but also high concentrations, on these outcomes.

Keywords Fetal programming • Weaning • Bone density • Bone accrual • Vitamin D deficiency

11.1 Introduction

Significant changes in maternal calcium metabolism occur during pregnancy and lactation. These changes provide a sufficient calcium supply to the fetus for skeletal growth and to the newborn infant in the form of adequate maternal milk production. This chapter summarizes the literature on the effects of human pregnancy, lactation, and weaning on calcium metabolism and maternal bone health; the role of maternal dietary calcium and vitamin D on maternal calcium metabolism and bone health; the epidemiological evidence relating parity and lactation to osteoporosis and fracture risk; and the role of maternal calcium and vitamin D intake on calcium homeostasis, growth, and bone development of the offspring.

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11.2 Calcium and Bone Metabolism During Pregnancy, Lactation, and Weaning

Significant calcium demands are placed upon the mother during pregnancy and lactation. A term infant contains approximately 25–30 g of calcium, the majority of which is accumulated during the last trimester [1]. It is estimated that fetal calcium accretion increases from about 50 mg/day at 20 weeks of gestation to a maximum of about 330 mg/day at 35 weeks gestation [2]. Calcium demands upon the mother continue to be high during lactation when approximately 250 mg/day of calcium is lost to breast milk [3]. Important physiological adaptations occur during pregnancy and lactation to meet these increased demands. Figures 11.1, 11.2, and 11.3 summarize the physiological adaptation that occurs during pregnancy, lactation, and weaning in well-nourished women.

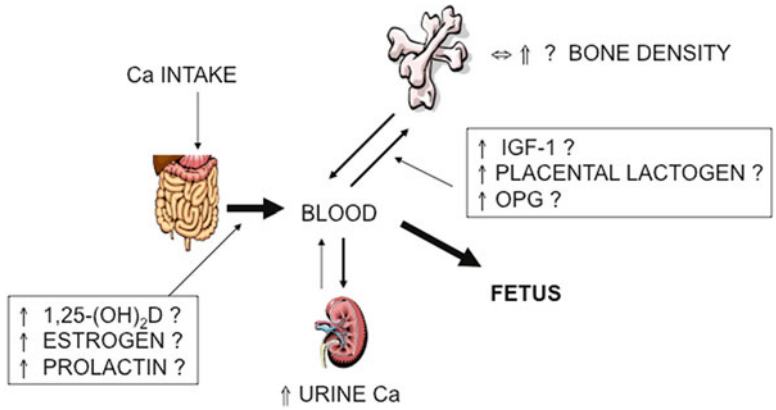

Fig. 11.1 Schematic illustration of the role of intestinal calcium absorption, renal calcium excretion, and bone turnover in providing calcium to the fetus. The size of the *arrows* indicates the relative magnitude of the flux of calcium

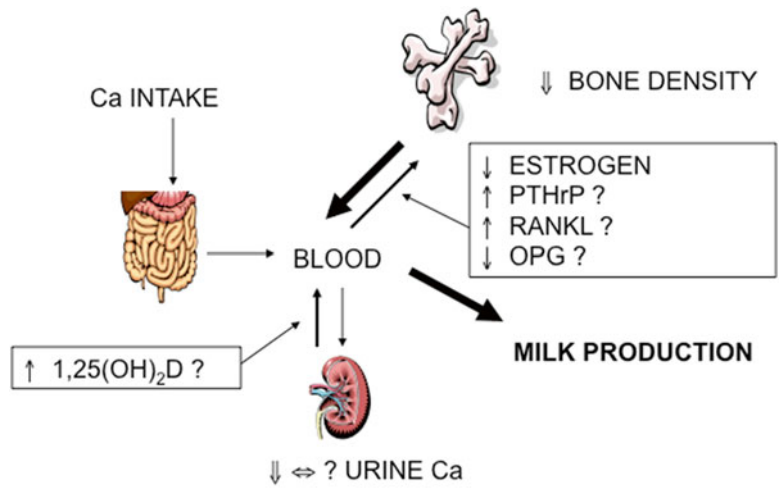

Fig. 11.2 Schematic illustration of the role of intestinal calcium absorption, renal calcium excretion, and bone turnover in providing calcium for milk production. The size of the *arrows* indicates the relative magnitude of the flux of calcium

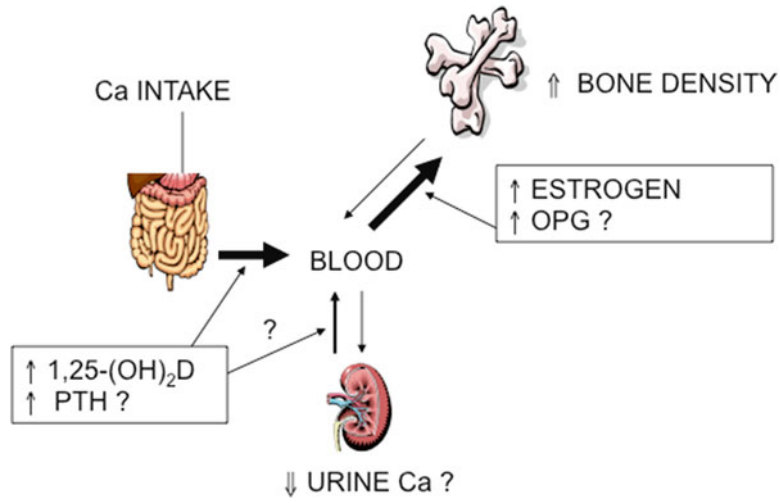

Fig. 11.3 Schematic illustration of the role of intestinal calcium absorption, renal calcium excretion, and bone turnover in providing the mother with sufficient calcium during weaning. The size of the *arrows* indicates the relative magnitude of the flux of calcium

11.2.1 Pregnancy

11.2.1.1 Calcium and Bone Metabolism

In addition to increased food consumption, there are three possible calcium sources that may supply the mother with the necessary calcium to support fetal growth: increased intestinal calcium absorption from the diet, increased renal calcium conservation, and increased bone calcium mobilization (Fig. 11.1). Under normal circumstances, the active metabolite of vitamin D, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), increases the efficiency of intestinal calcium absorption, decreases renal calcium excretion, and in conjunction with parathyroid hormone (PTH), mobilizes calcium from bone. Increased fractional calcium absorption appears to be an important mechanism for obtaining extra calcium during pregnancy [4]. Fractional calcium absorption, which is typically about 35 % in the nonpregnant state, increases to approximately 60 % during the third trimester [5, 6]. This would represent an additional 300 mg/day of calcium that is obtained solely from increased intestinal absorption among women consuming 1,200 mg calcium/day. Calcium absorption is positively associated with serum 1,25-$(OH)_2$D concentrations in late human gestation [5, 6].

During lactation, low calcium intake leads to increased serum concentrations of 1,25-$(OH)_2$D [7], and this adaptation also may occur among pregnant adolescents and adult women with low calcium intake [8]. A recent kinetic study by O'Brien and coworkers measured serum 1,25-$(OH)_2$D concentrations and bone calcium deposition (VO+) in 46 adolescent and adult women during early and late pregnancy and in the postpartum period [8]. They found higher serum 1,25-$(OH)_2$D concentrations were associated with decreased rates of bone calcium deposition during late pregnancy, which was more evident in adolescents and adult women with low calcium intakes.

The events that lead to increased serum concentrations of 1,25-$(OH)_2$D during pregnancy are not clear. PTH, which is usually considered the stimulus for the increased renal hydroxylation of 25-hydroxyvitamin D (25-OHD) to 1,25-$(OH)_2$D, has not been shown to be high during pregnancy in women consuming adequate amounts of calcium (≈1,000 mg/day) [5, 6] or low amounts of calcium (≈500 mg/day) [9]. It has been speculated that the 1,25-$(OH)_2$D present in the maternal circulation

may be of placental origin [10]. Other suggested mechanisms for the increase in calcium absorption involve estrogen, placental lactogen, or prolactin concentrations as controlling factors, all of which are known to increase during pregnancy [5, 11, 12]. Renal calcium conservation does not occur during pregnancy and renal calcium excretion increases even when calcium intakes remain relatively constant [5, 6]. The increase in renal calcium excretion is likely a result of both increased absorptive load of calcium along with an increase in glomerular filtration rate that occurs during pregnancy [5, 6].

Bone is continuously broken down and formed. The sequence of events involved in bone turnover includes activation of osteoclast precursors, followed by osteoclastic bone resorption and then osteoblastic bone formation. Markers of bone turnover are increased in late gestation [5, 6, 9]. Both markers of bone formation (bone-specific alkaline phosphatase and procollagen 1 carboxypeptides) and bone resorption (tartrate-resistant acid phosphatase, deoxypyridinoline) are elevated above nonpregnant concentrations. Although studies consistently find an increase in serum concentrations of markers of bone turnover, few have reported changes in bone density (see below). Increased concentrations of insulin-like growth factor (IGF-1) and placental lactogen have been suggested as possible mechanisms behind increased bone turnover during pregnancy [13, 14]. It also has been suggested that the increase in bone turnover markers may be explained in part by the increased turnover of soft tissue collagen of the uterus and skin, or possibly may be of placental or fetal origin [15]. Receptor activator of nuclear factor-κ B ligand (RANKL) is important in osteoclast differentiation and also in development of alveolar structures of the mammary gland during pregnancy [16]. The cytokine osteoprotegerin (OPG) acts as a decoy receptor for RANKL and prevents its function, thereby decreasing bone resorption. Studies by both Uemura et al. [17] and Naylor et al. [18] found maternal serum OPG concentrations to steadily increase with gestation. The authors speculated that higher OPG concentrations, possibly of placental origin, might play a role in the control of bone metabolism during pregnancy.

11.2.1.2 Changes in Bone Mineral

Studies of bone changes during pregnancy are difficult to conduct due to radiation exposure. One study measured bone density of the distal radius *during* pregnancy using single photon absorptiometry (SPA) [6], while other longitudinal studies have measured bone density before and after pregnancy using methods that are more sensitive for detecting small bone changes (dual energy X-ray absorptiometry (DXA) and quantitative computed tomography (QCT)). Ultrasound measurements throughout pregnancy also have been used to investigate bone changes. It is important to note that the timing of the final postpartum measurement is critical due to rapid bone changes that occur following delivery in lactating women. Unfortunately, many of the studies that investigated the effect of pregnancy on maternal BMD changes measured bone at approximately 6 weeks following delivery, a time when lactation-induced bone loss may have already occurred [19].

Studies by Cross et al. [5] and Ritchie et al. [6] did not find significant bone changes during pregnancy. However, Naylor and coworkers found a significant decrease in spine BMD [13]. These contradictory findings may be partly explained by differences in sample sizes and the type of bone measured. All three studies had small sample sizes. Cross and coworkers measured only the ultradistal (predominantly trabecular bone) and one-third distal radius (predominantly cortical bone) in nine women using SPA and found no change *during* pregnancy. Ritchie and coworkers obtained QCT and DXA measurements before and after pregnancy (1–2 weeks postpartum) and found no significant changes in spine (predominantly trabecular) or total body BMD among 14 women. However, Naylor and coworkers found a significant decrease in spine BMD measurements obtained from a total body DXA scan in a study of 16 women who were measured prior to pregnancy and within 2 weeks of delivery [13]. If a change in BMD were to occur, then it likely would be at a trabecular bone site, such as the spine, since trabecular bone is more sensitive to hormonal changes. Spine BMD measurements

from a total body scan have greater variation than QCT or spine regional DXA scans (coefficient of variation 3.6 % for spine BMD from a total body scan vs. <1 % for spine BMD from a regional scan or QCT). Changes in fat distribution before and after pregnancy theoretically may affect DXA BMD measurements of the spine, although this has been reported to be more of a problem with lateral measurements than anterior-posterior spine BMD measurements [20]. Whether changes in fat distribution affect spine measurements from a whole body scan is not known but may have influenced the findings of Naylor and coworkers.

Sowers et al. used bone ultrasound measurements to determine whether there were differences between adolescents and adults in bone changes during pregnancy [21]. Adolescent mothers had greater decreases in speed of sound (SOS, speed of signal transmission through the heel), broadband ultrasound attenuation (BUA, degree of attenuation of the high-frequency sound waves), and quantitative ultrasound index (combination of SOS and BUA into a single measure) than adult mothers. However, measurements were made at approximately 16 weeks gestation and at 6 weeks postpartum. Significant bone changes could have occurred between birth and 6 weeks postpartum if the mothers were lactating. No information was provided on whether or not any of these women were breast-feeding and whether breast-feeding rates differed between the two groups (adolescent vs. adult). In addition, no dietary information was provided and it is not clear whether this may have affected the results. Bezarra and coworkers found that pregnant adolescents consuming a low calcium diet had greater serum PTH concentrations and lower urinary calcium excretion than pregnant adults consuming a similarly low calcium intake [9]. However, serum deoxypyridinoline and bone-specific alkaline phosphatase concentrations were not different between adolescent and adult mothers and the authors suggested that pregnant adolescents protect their bones during pregnancy, possibly by decreasing urinary calcium excretion.

11.2.2 Lactation

11.2.2.1 Calcium and Bone Metabolism

Evidence does not indicate that the adaptation in maternal calcium metabolism that is seen in pregnancy occurs during lactation, a time when the mother has to adapt to meet the calcium needs of milk production (Fig. 11.2). Numerous studies have shown that intestinal calcium absorption during lactation is not influenced by the maternal calcium intake and is increased in the postpartum period in both lactating and non-lactating women [6, 22–24]. However, findings on renal calcium excretion are less consistent. Some studies report a decrease in calcium excretion compared to either pre-pregnancy concentrations or a non-lactating postpartum control group [6, 22, 25], while others report no effect [5, 26]. Since urinary calcium excretion is influenced by dietary calcium intake, it is difficult to determine whether differences in excretion are due to differences in calcium intake or renal handling of calcium. A calcium kinetic study in seven women measured both while lactating and not lactating, found lower rates of urinary calcium excretion during lactation that was independent of calcium intake [22]. Although some studies have found serum 1,25-$(OH)_2$D to be higher during lactation [7, 27, 28], other studies find no association [5]. If 1,25-$(OH)_2$D is elevated during lactation the mechanism is not clear since most studies find that serum intact PTH concentrations are low [29, 30] or do not differ with lactation [5, 25].

Biochemical markers of bone resorption and formation are elevated during lactation [25, 29, 31, 32], and a kinetic study found that bone resorption exceeds bone deposition during early lactation [22]. The increases in serum concentrations of bone turnover markers are not influenced by either dietary calcium or physical activity [32], and many individuals have suggested that the estrogen deficiency that occurs with lactation may be responsible for increased bone turnover. Other factors that are

associated with estrogen also may be important. PTH-related peptide (PTHrP), which has similar biological actions as PTH, is synthesized in mammary tissue and secreted into milk. Although its role in lactation is not clear, PTHrP concentrations have been reported to be inversely associated with estradiol concentrations and positively associated with the degree of bone loss during lactation [33]. However, not all studies have observed a relationship between lactation-induced bone loss and PTHrP [34]. PTHrP and prolactin also enhance production of mRNA for RANKL [16], and serum OPG concentrations are positively associated with serum estradiol concentrations [35]. High PTHrP and low estrogen levels during lactation theoretically would lead to high RANKL and low OPG resulting in increased bone resorption. The study by Uemura and coworkers reported a significant drop in serum OPG concentrations immediately following delivery to one month postpartum [17], a time when bone resorption increases significantly.

11.2.2.2 Changes in Bone Mineral

Significant bone loss during lactation, especially at axial bone sites, has been reported in numerous prospective studies [3, 25, 29, 36–40]. Bone loss occurs early in lactation and begins to recover once menses resumes [39, 41–43]. The amount of bone loss appears to be related to the length of amenorrhea, but ranges on average from 3 to 5 %. This amount of loss is considerable given that women typically lose 1 to 3 % per year during the postmenopausal period.

Several trials of calcium supplementation during lactation have found that the lactation-induced bone loss, breast milk calcium concentrations, and intestinal calcium absorption are not modified by the mother's calcium intake [31, 43–46]. However, one study in adolescent mothers, using older SPA methodology, found that increased calcium intake prevented lactation-induced bone loss [47]. In this study, one group of adolescent mothers received routine dietary counseling (control group) while the other group received counseling to increase calcium intake to 1,600 mg/day. However, the study was not randomized and the group with the higher calcium intake also had significantly higher intakes of calories, protein, vitamin D, and phosphorus. The control group had a significant decrease in BMC (10 % loss) while the experimental group did not (3 % loss in BMC). It is not stated whether the change in BMC differed between the two groups. Several concerns have been raised concerning the results of this study, including a higher rate of bone loss than seen in other studies and two of the women had bone measurements greater than four standard deviations above normal at baseline [48, 49]. It is possible, however, that adolescent mothers may respond to lactation differently than adult mothers while consuming a low calcium diet. Bezerra and coworkers found that among women with low calcium intakes (<500 mg/day) urinary deoxypyridinoline concentrations were higher among non-lactating nonpregnant (NLNP) adolescents compared to NLNP adult women. Urinary deoxypyridinoline concentrations, a marker of bone resorption, were higher in both adolescent and adult lactating mothers compared to NLNP, but there was no difference between the adolescent and adult mothers. Serum PTH and bone-specific alkaline phosphatase concentrations were higher, and urinary calcium excretion lower, in lactating adolescent vs. adult mothers, findings similar to what was observed in NLNP. These results indicate that adolescent mothers are more sensitive to low calcium intakes during lactation than adult mothers.

A large randomized, double-blind study of calcium supplementation among women with habitually low calcium intake (<800 mg/day) found that calcium intake did not modify bone changes observed during lactation [45]. Although calcium supplementation (1 g/day) attenuated the bone loss, the effect was similar among both lactating and non-lactating women (Fig. 11.4). There was no difference in breast milk calcium concentrations between mothers receiving calcium supplements and those receiving placebo. A randomized calcium supplementation trial among Gambian women who typically consume very low levels of calcium found no effect of calcium intake on breast milk calcium

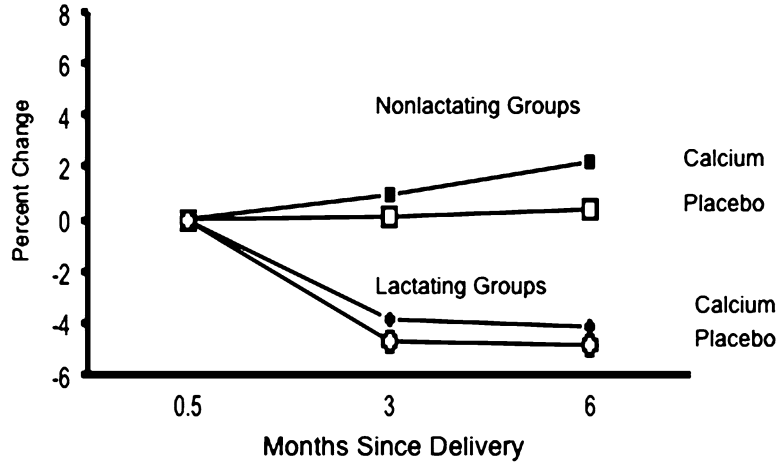

Fig. 11.4 Effects of calcium supplementation and lactation on the mean (+SEM) percent change in the bone mineral density of the lumbar spine during the first 6 months postpartum. Values are adjusted for baseline bone mineral density, height, weight, change in weight, dietary intake of calcium, and level of physical activity. $P=0.01$ for the effect of calcium; $P<0.001$ for the effect of lactation; and $P=0.23$ for the interaction between calcium supplementation and lactation. From [45]. Copyright © 1997 Massachusetts Medical Society. Reprinted with permission

concentrations [44]. These results indicate that calcium supplementation in women with habitually low calcium intake does not prevent the bone loss that is observed during lactation and does not influence breast milk calcium concentrations.

11.2.3 Weaning

11.2.3.1 Calcium and Bone Metabolism

During weaning the mother recovers much, if not all, of the bone calcium she lost during lactation by increasing intestinal calcium absorption and decreasing renal calcium excretion (Fig. 11.3). Intestinal calcium absorption is higher in women who are weaning vs. postpartum women who did not breastfeed [23]. An increased fractional calcium absorption was associated with higher serum 1,25-$(OH)_2$D concentrations that also were observed during weaning [23, 50]. Serum PTH concentrations are higher in weaning vs. non-weaning women and are probably the stimulus for the higher serum 1,25-$(OH)_2$D concentrations [50]. Higher PTH concentrations also may be responsible for the decrease in renal calcium excretion [25] that is observed during weaning.

11.2.3.2 Changes in Bone Mineral

The bone loss that occurs early in lactation begins to recover once menses resumes [39–42]. The earlier the resumption of menses, the less bone loss that occurs during lactation and the greater the bone gain during weaning [39]. The length of time to complete recovery is not known, but is probably greater than 6 months postweaning [29, 45]. Factors that may be associated with bone gain during weaning include closely spaced pregnancies, maternal age, and possibly calcium intake.

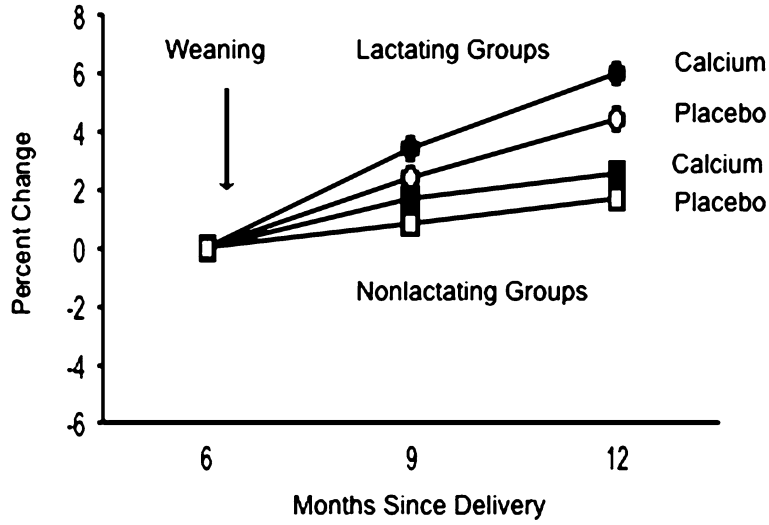

Fig. 11.5 Effects of calcium supplementation and lactation on the mean (+SEM) percent change in the bone mineral density of the lumbar spine during the second 6 months postpartum. Values are adjusted for baseline bone mineral density, height, weight, change in weight, dietary intake of calcium, and level of physical activity. $P<0.001$ for the effect of calcium; $P<0.001$ for the effect of weaning; and $P=0.36$ for the interaction between calcium supplementation and weaning. The lactating women were fully breast-feeding at baseline, and the *arrow* indicates the average time at which breast-feeding was completely ended. From [45]. Copyright © 1997 Massachusetts Medical Society. Reprinted with permission

An early study in the 1970s found that women who had small families (0–2 children) had similar bone density as women who had larger families (seven or more children), supporting the hypothesis that closely spaced pregnancies are not detrimental to bone [51]. Sowers and coworkers found that women who breast-fed for at least 6 months and became pregnant within 18 months of initiating breast-feeding had BMD recovery similar to women who breast-fed for a similar amount of time but did not become pregnant [37]. A more recent study using DXA found that 30 multiparous women (>5 children) who lactated for at least 6 months/child had similar BMD as 6 nulliparous women [52]. Therefore it does not appear that closely spaced pregnancies, which may interfere with bone recovery during weaning, are detrimental to long-term bone health.

Maternal age at the time of lactation may be important in bone recovery following weaning. As described previously, adolescent mothers may adapt to the calcium needs differently than older mothers, especially if their calcium intake is low. Whether there are adaptation differences in adolescent vs. adult mothers during weaning is not known. Hopkinson and coworkers reported that net changes in bone mass following lactation were negatively associated with age: older mothers had less of a recovery during weaning than younger mothers [42]. No other studies have reported differences in bone recovery between younger vs. older adult women.

A randomized study of calcium supplementation among women with habitually low calcium intake (<800 mg/day) studied the effect of calcium supplementation on bone changes during weaning [39]. A group of 95 lactating women and 92 non-lactating women were enrolled at approximately 6 months postpartum and were followed for 6 months. The lactating women were breasting-feeding on average 5.5 times per day when enrolled and weaned their infants during the next 2 months. Within each group half of the women were randomized to placebo and half to calcium supplements (1 g/day). The percent increase in spine BMD in women who were weaning and receiving calcium was of similar magnitude as the percent increase in women who were not weaning but receiving calcium (Fig. 11.5). These results indicate that calcium supplementation in women with habitually low

calcium intakes increases BMD to the same extent in women whether or not they are weaning. Others have recently reported similar findings [43].

Results of a recent randomized trial among Gambian women consuming ≈350 mg calcium/day reported bone changes during lactation based on whether the mother was randomized to calcium supplements (1,500 mg/d) or placebo during the last 20 weeks of pregnancy. The a priori hypothesis was that a greater calcium intake during pregnancy might have beneficial effects on maternal bone outcomes during the first year of lactation. However, the opposite effect was observed: women who received calcium supplements during pregnancy had significantly lower hip BMC throughout the first 12 months postpartum. Additionally, there was evidence of greater BMC loss at the spine and radius in response to lactation (Fig. 11.6) [53]. The authors speculated that the calcium supplement interfered with the adaptive response to a low calcium intake in this population.

11.3 Studies of Parity and Lactation on Osteoporosis Incidence and Fractures

Although there are case reports of pregnancy-induced osteoporosis [54, 55], this is a pathological condition and does not occur during normal pregnancy. The previous sections focused on changes in calcium metabolism and bone mineral status that enable the mother to adapt to the calcium needs of the growing fetus and of milk production for the infant. However, there may be consequences of reproduction on long-term bone health.

Retrospective studies have found lactation history to be associated with both increased BMD [56–60], decreased BMD [61, 62], or no relationship later in life [51, 63, 64]. Numerous methodologies for assessing bone have been used in these studies, ranging from radiographs with aluminum wedges for estimation of bone density to DXA measurements. BMD is used to determine whether someone is "osteoporotic" and is considered a major predictor of future fracture risk [65]. The World Health Organization describes osteoporosis as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture risk," and is defined by a BMD measurement that is 2.5 standard deviations or more below the gender-specific mean value for peak bone mass [66]. As described previously, BMD later in life has not been associated with parity and previous lactation history. Two studies completed in women with large numbers of children (up to 16 births) reported no association between BMD and parity [67, 68], although one study found greater femoral neck bone area and torsional bending strength of the radius with increasing parity [67]. The association between an increase in bone size and bone strength indices following lactation also has been observed up to 16–20 years after parturition [69].

Studies on the relationship between fracture risk and lactation history have found either similar or reduced risk with parity or among women who lactated compared to those who did not lactate [70–73]. One study found no association between fracture risk and parity but did find that fracture cases had breast-fed fewer times and for fewer months than controls, even when age and parity were included as covariates in the statistical analysis [74]. A study of 1,328 incident cases of hip fracture and 3,312 randomly selected controls found that hip fracture risk was reduced by 10 % per child and the odds ratio was statistically significant at parities of 2 or greater (Fig. 11.7) [75]. Hoffman and coworkers found that each birth was associated on average with a 9 % reduction in the odds of hip fracture [72]. In neither study was the fracture risk associated with duration of lactation once parity was taken into account. In summary, an increase in bone size without an increase in BMD should theoretically lead to increased bone strength with higher parity and lactation, and may explain the reduced fracture risk among these women [67].

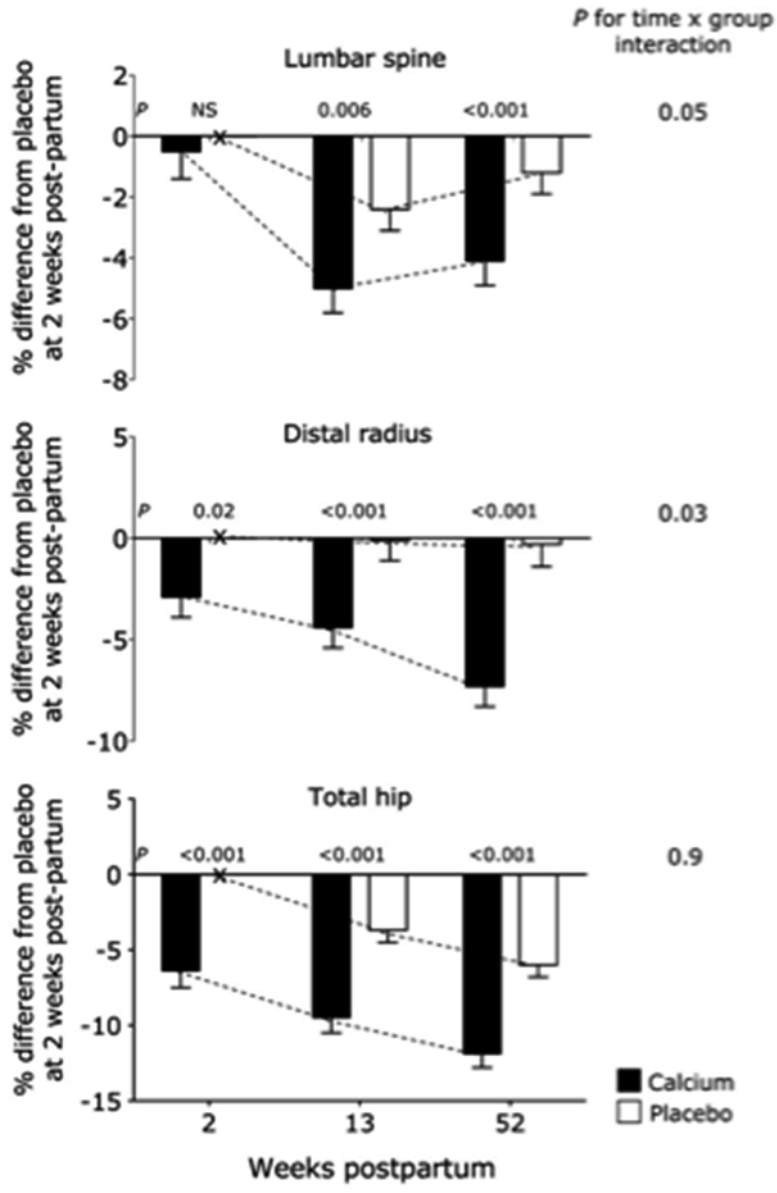

Fig. 11.6 Effect of the calcium supplement in pregnancy on size-adjusted bone mineral content (SA-BMC) of the lumbar spine, distal radius, and total hip at 2, 13, and 52 weeks postpartum. SA-BMC = bone mineral content adjusted for bone area (or bone width), weight, and height. *Bars* and *error bars* represent the mean ± SE percentage differences in SA-BMC relative to the placebo group at 2 weeks postpartum in the calcium group (solid bars) and placebo group (*open bars*). *Dotted lines* represent the apparent time trend within each group. An "*x*" on the *x* axes denotes placebo value at 2 weeks postpartum and is used as the reference and set at zero. Results were obtained from Scheffe post hoc test for time-by-group interaction terms in hierarchical repeated-measures ANOVA models that included subject (nested by group), time, group, and time-by-group interaction. The *P* values depicted are for the comparison of calcium and placebo groups at each time point. Then umbers of subjects at 2, 13, and 52 weeks, respectively, were as follows—for the lumbar spine: 23, 29, and 40 in the calcium group and 27, 29, and 39 in the placebo group; for the distal radius: 53, 53, and 48 in the calcium group and 60, 54, and 45 in the placebo group; and for the total hip: 20, 25, and 39 in the calcium group and 23, 27, and 37 in the placebo group. Figure from [53].

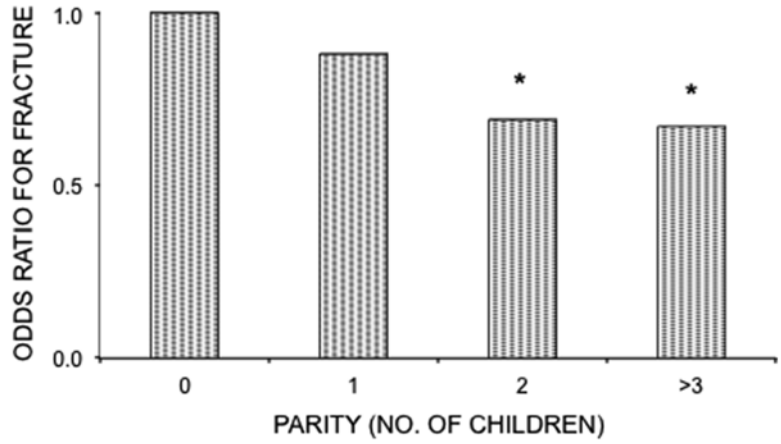

Fig. 11.7 The odds ratio for hip fracture risk was significantly less than 1 at parities of 2 or greater (*asterisk*). Odds ratios were calculated using nulliparous women as the reference. Data from [75]

11.4 Effects of Maternal Diet on the Offspring

Maternal nutrition during pregnancy or lactation may have effects beyond maternal calcium metabolism and bone mineral status. Although dietary calcium intake during lactation does not influence breast milk calcium concentrations, there are some reports that maternal calcium or vitamin D intake during pregnancy may influence bone and growth of the offspring.

11.4.1 Maternal Calcium Intake and Fetal and Neonatal Outcomes

11.4.1.1 Fetal Growth

A recent comprehensive meta-analysis of calcium supplementation for improving pregnancy and infant outcomes found no protective effect of calcium supplementation on low birth weight, birth length, head circumference, or intrauterine growth restriction [76]. However, a significant difference in birthweight was observed with women receiving calcium supplements having heavier infants on average than women receiving placebo. The authors noted that the clinical significance of this difference is uncertain.

With the advent of high-resolution 3-D ultrasound fetal imaging, some studies have begun to investigate how maternal nutrition influences fetal bone growth. A recent longitudinal study among adolescents (aged ≤18 years) found an interaction between maternal calcium intake and vitamin D status with both fetal femur *z* scores and birth length: dietary calcium was associated with fetal femur and birth length only when maternal serum 25(OH)D concentrations were ≤50 nmol/L and maternal serum 25(OH)D concentrations were associated with fetal femur and humerus *z* scores only when maternal calcium intake was <1,050 mg/day [77]. It is possible that adolescents may be more sensitive to low dietary calcium due to the increased requirements resulting from their own continued bone growth. Although these results are of interest, findings from observational studies cannot be used to determine causality. Confounding may be an issue with observational studies since mothers with different calcium and vitamin D intake, or who take vitamin or mineral supplements, are likely to differ in many other factors such as other nutrients, lifestyle, and exercise patterns that may influence the outcome being studied.

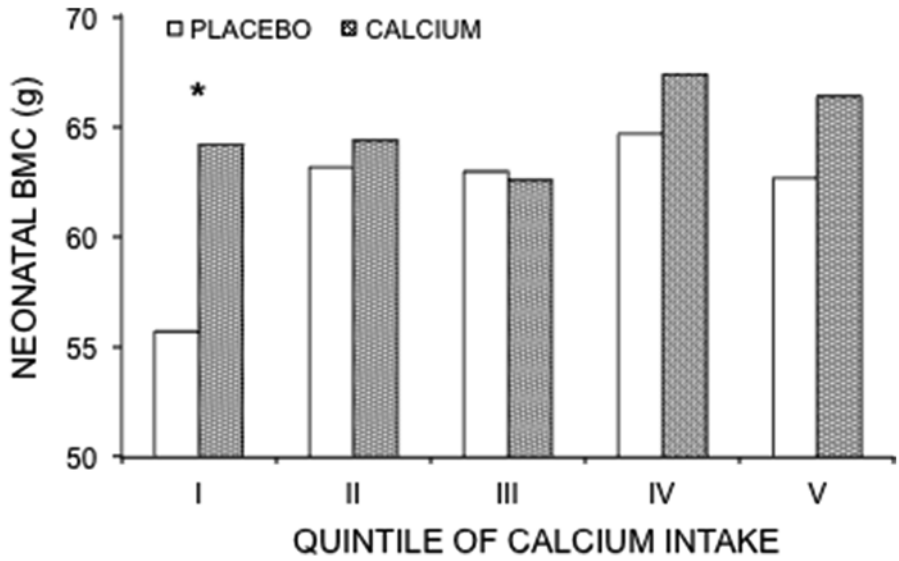

Fig. 11.8 Neonatal bone mineral content (BMC) in infants whose mothers received placebo (*open bars*) and those who received supplemental calcium (2 g/day) (*dark bars*) by quintile of maternal calcium intake. (*asterisk*) Neonates of mothers in the lowest quintile of calcium intake (<600 mg/day) who were randomized to placebo had lower BMC compared to infants in the lowest quintile whose mothers were randomized to calcium supplement. Data from [79]

11.4.1.2 Neonatal Bone

Maternal calcium intake during pregnancy does not influence maternal bone changes, and may only influence neonatal bone density when the mother's calcium intake is very low [78]. Although metacarpal bone density of undernourished pregnant mothers supplemented with 300 or 600 mg calcium/day did not increase compared to unsupplemented mothers, maternal supplementation did increase neonatal bone density [78]. Similar results were recently reported in a large randomized trial of maternal calcium supplementation for the prevention of preeclampsia, but only in mothers with low habitual calcium intake [79]. A total of 256 women were enrolled in a randomized, double-blind, placebo-controlled trial; newborn infants of mothers in the lowest quintile of calcium intake (<600 mg/day) who were randomized to calcium supplement had greater total body BMC compared to newborns in the lowest quintile whose mothers were randomized to placebo (Fig. 11.8). There was no difference in neonatal BMC between placebo and supplemented maternal groups in the other quintiles of calcium intake. A recent meta-analysis on the effect of calcium supplementation for improving pregnancy and infant outcomes found no effect of maternal calcium supplementation on neonatal BMD [76].

11.4.2 Maternal Vitamin D Intake and Offspring Outcomes

Vitamin D is generally considered a prohormone. It may be derived endogenously from the skin or supplied from exogenous sources. Endogenously, provitamin D_3 is converted to vitamin D_3 by ultraviolet radiation and subsequent thermal isomerization. Vitamin D_3 is then transformed in the liver to 25-hydroxyvitamin D (25-OHD) and further hydroxylated in the kidney to 1,25-$(OH)_2$D. A drop in serum concentrations of calcium or phosphorus, or increased serum PTH concentrations, facilitate

this transformation to 1,25-$(OH)_2$D. Serum 25-OHD concentrations serve as the most reliable indicator of vitamin D status in the body, while 1,25-$(OH)_2$D is the hormonally active metabolite, and its concentrations reflect the body's need for calcium and phosphorus.

11.4.2.1 Fetal and Neonatal Outcomes

Maternal vitamin D deficiency during pregnancy may have effects on neonatal calcium metabolism and fetal bone development. Placental transfer of 25-OHD occurs and serum 25-OHD concentrations of newborn infants are approximately 50 % of the mother's concentrations [80, 81]. Maternal vitamin D deficiency is associated with secondary hyperparathyroidism, and vitamin D deficiency and hyperparathyroidism during pregnancy may lead to neonatal hypocalcemia or tetany [82, 83]. In the early 1970s, Purvis and coworkers noted that the occurrence of neonatal tetany among 112 infants was inversely related to the amount of sunlight exposure the mothers had during the last trimester of pregnancy [84]. The authors speculated that the mothers developed hyperparathyroidism secondary to vitamin D deficiency resulting in a transitory hypoparathyroidism in the newborn infants. This neonatal hypoparathyroidism may subsequently lead to hypocalcemia. A recent randomized trial of 400, 2,000, and 4,000 IU/day in pregnant women found cord and maternal serum 25-OHD concentrations were correlated and increased with increasing vitamin D dose [85]. Unfortunately, neonatal PTH concentrations were not reported. Delvin and coworkers conducted a randomized trial of vitamin D supplementation during pregnancy and found that cord serum PTH concentrations were similar in infants of mothers supplemented with 1,000 IU vitamin D/d during the last trimester vs. those mothers not supplemented [86]. However, neonates of mothers supplemented with vitamin D had less of an increase in serum PTH and less of a decrease in serum calcium concentrations between birth (cord blood) and 4 days of age than infants of mothers not receiving vitamin D. These results indicate that adequate maternal vitamin D status during pregnancy ensures appropriate neonatal handling of calcium.

A trial of vitamin D supplementation (1,000 IU/d) among pregnant Asian women at increased risk of vitamin D deficiency reported a higher percent of infants in the placebo group who were small-for-gestational-age compared to infants in the supplemented group (29 vs. 15 %); however, this difference in percent SGA was not statistically significant [87]. Results from observational studies that were not limited to women at high risk of vitamin D deficiency are conflicting with regard to the effect of maternal vitamin D on birth weight and length. A recent longitudinal study of 3,730 multiethnic women in the Netherlands reported a higher SGA risk and lower birth weight in offspring of women with 25-OHD concentrations less than 30 nmol/l early in pregnancy [88], supporting findings from previous studies among women at high risk of vitamin D deficiency. Bodnar and coworkers conducted a nested case-control study of 77 mothers who had serum 25-OHD concentrations determined at 22 weeks gestation and found a significant association between the incidence of SGA in mothers with both low (<37.5 nmol/l) and high (>75 nmol/l) 25-OHD concentrations [89] (Fig. 11.9). This inverse J-shaped relationship remained significant when covariates were included in the analyses. They also found an association between the vitamin D receptor (VDR) genotype and SGA risk, with both maternal vitamin D status and VDR genotype being independent risk factors for SGA.

Morley and coworkers also found an association between VDR FokI genotype and birth size: infants with the FF or Ff polymorphism had lower mean birth weight if their mother was vitamin D deficient compared to infants with the ff polymorphism [90]. The FokI polymorphism did not influence birth size if the mother had adequate vitamin D status.

Two large cohort studies, the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Southampton Women's Study (SWS), found no associations between maternal 25-OHD concentrations and birth weight or length [91–93]. A recent meta-analysis of randomized vitamin D supplementation trials concluded that there was no association between maternal vitamin D supplementation during pregnancy and birth length, birthweight, or head circumference [94].

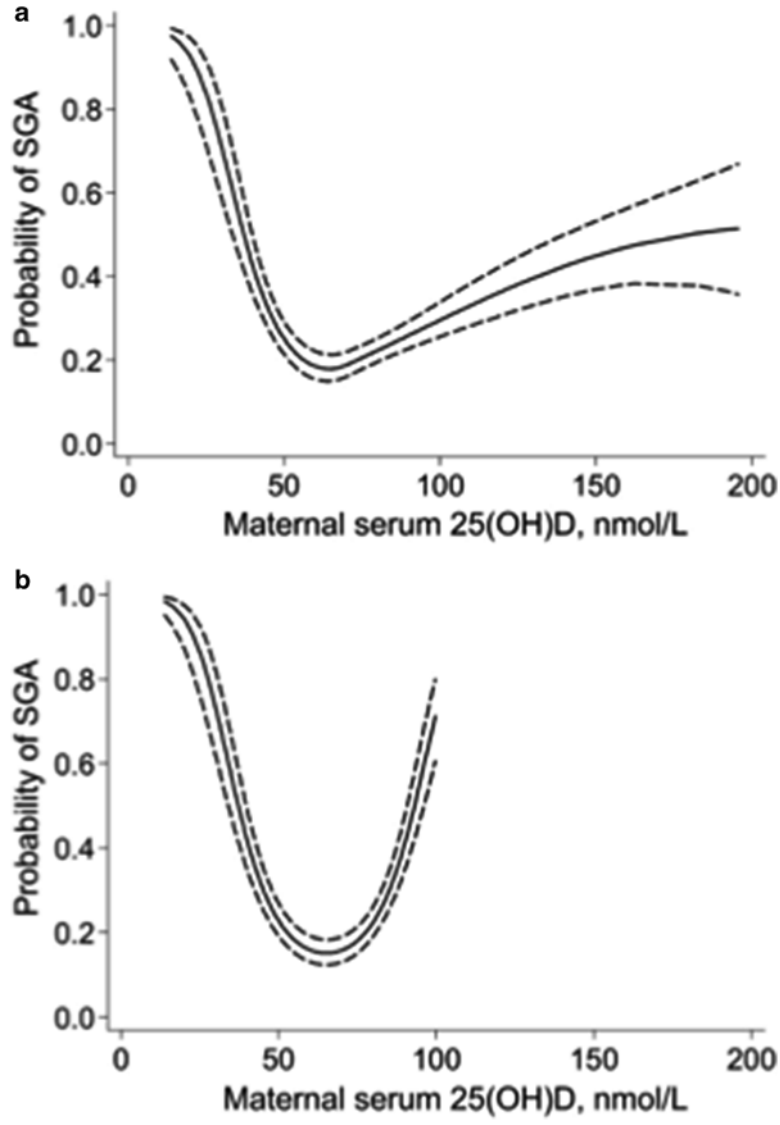

Fig. 11.9 Unadjusted association between the probability of SGA births and serum 25(OH)D concentrations among white women (**a**; $n=273$) and white women with a 25(OH)D ≤ 100 nmol/L (**b**; $n=217$) at <22 weeks. The point estimates were derived from logistic regression models with serum 25(OH)D concentrations specified as a quadratic spline with knot at 70 nmol/L ($P=0.006$; **a**) or quadratic term ($P<0.0001$; **b**). The *solid line* represents the point estimate and the dotted lines represent the 95 % confidence bands. From [89]. Copyright © 2010 American Society for Nutrition. All rights reserved

Fetal or congenital rickets of the newborn are rare, but there are case reports in infants of mothers with severe nutritional osteomalacia associated with vitamin D or calcium deficiency [95–97]. Low cord 25-OHD concentrations have been reported in infants with congenital craniotabes [98], or delayed ossification of the cranial vertex, but these findings have not been replicated in other observational studies or trials [87, 99]. A study conducted in China found that the presence of wrist ossification centers was related to cord serum 25-OHD concentrations [100]. These findings are suggestive of a role of maternal vitamin D on fetal bone development.

Mahon et al. measured femur length and cross-sectional area of the distal femur at 19 and 34 weeks of gestation using 3-D fetal ultrasound in 395 women participating in the SWS [101]. They found that maternal serum 25-OHD concentrations at 34 weeks were inversely correlated to the distal cross-sectional area and a femur splaying index (cross-sectional area/femur length), with no linear relationship to femur length. These results suggest that infants of mothers with low vitamin D status have larger distal femur cross-sectional area than infants of mothers with higher vitamin D concentrations, and the authors speculated based on the increased splaying index that fetal bone changes with low maternal 25-OHD concentrations were similar to what is observed in rickets. An additional report based on the same population ($n = 357$) found maternal 25-OHD concentrations at 34 weeks gestation to be positively associated with proximal metaphyseal diameter at 34 weeks gestation after adjusting for covariates [102]. Maternal serum 25-OHD was not associated with birthweight or neonatal DXA measures of total body BMC and BMD in this study.

There are few studies on the association between maternal vitamin D status and neonatal bone [88, 99, 101, 103, 104]. One of the original studies investigating this relationship found that forearm BMC in 45 Asian and 12 Caucasian newborns measured by single photon absorptiometry did not differ by history of maternal vitamin D supplementation during pregnancy and was not correlated with cord serum 25-OHD concentrations [99]. Weiler and coworkers conducted a study among 50 Canadian infants and found that both birth weight and length were greater in infants with cord 25-OHD concentrations <27.5 nmol/l compared to infants with cord 25-OHD concentrations >27.5 nmol/l [104], findings opposite of those reported by others [87, 88, 91, 92]. Although total body BMC was similar between these two groups of infants, when expressed as BMC per kilogram body weight infants with low cord 25-OHD had lower BMC than infants with higher cord 25-OHD concentrations. Viljakainen and coworkers measured 25-OHD concentrations in 98 Finnish mothers during their first trimester and postpartum period, as well as newborn bone parameters by pQCT [103]. They found that mothers with mean 25-OHD concentrations below 43 nmol/l had newborn infants with lower tibia bone mass and smaller cross-sectional area in the midshaft of the tibia than infants of mothers with mean 25-OHD concentrations greater than 43 nmol/l.

In summary, some studies, but not all, find maternal vitamin D status during pregnancy to be associated with fetal growth and there is some evidence that this effect may be genetically modified. Results from studies suggest a possible relationship between maternal vitamin D status and fetal or neonatal bone.

11.4.2.2 Infant Outcomes

Vitamin D deficiency leads to rickets in infants and toddlers. Infant formula is routinely fortified with vitamin D, but very low vitamin D concentrations have been found in human milk [105, 106] and almost all cases of vitamin D deficiency rickets occur in infants who have been exclusively breast-fed. In addition, the majority of reported cases of rickets have been in black infants, supporting the contention that persons with dark skin have difficulty synthesizing adequate concentrations of vitamin D due to the relative inability of sunlight to penetrate darker pigmented skin. Specker and coworkers found that, based on conservative estimates, exclusively breast-fed infants residing in Cincinnati could maintain serum 25-OHD concentrations above the lower limit of normal (11 ng/ml) with 2 h of sunshine exposure per week if fully clothed except for the face [107]. The cutoff for defining low 25-OHD (11 ng/ml) is based on the concentration that nutritional rickets have been observed, although this may be variable. Other factors such as latitude, season, weather conditions, and use of sunscreens may affect vitamin D status. Large seasonal differences in sunlight exposure and serum 25-OHD concentrations over the first year of life have been observed in infants followed longitudinally [108]. These findings indicate that an infant's sunlight exposure plays a more dominant role in determining their vitamin D status than the mother's vitamin D status or milk vitamin D.

Maternal vitamin D intake is not associated with breast milk calcium concentrations [109] and although vitamin D intake is correlated with breast milk vitamin D concentrations, mothers who consume 600–700 IU vitamin D/day have total vitamin D concentrations ranging from only 5–136 IU/l [106]. Supplementing lactating mothers with 1,000 IU vitamin D/day during winter months did not result in increased serum 25-OHD concentrations among the infants [110]. Infant serum 25-OHD is correlated with maternal vitamin D status early in the neonatal period and is probably a result of placental vitamin D transfer and fetal stores. Beyond the neonatal period, the breast-fed infant's serum 25-OHD concentrations are correlated with neither breast milk vitamin D concentrations nor maternal serum 25-OHD concentrations [107], and the infant is dependent upon endogenous synthesis or other dietary sources for their vitamin D.

Whether maternal vitamin D during pregnancy influences growth and bone accrual later in infancy is not known. Brooke et al. did not find differences in birth weight or length in a vitamin D supplementation trial (1,000 IU/day) among pregnant Asian Indians in Great Britain, but reported significantly greater gains in weight and length during the first year of life among the infants of vitamin D supplemented mothers [111]. Results from the Netherlands ABCD cohort showed different findings in 2,715 infants whose mothers had 25-OHD concentrations determined early in pregnancy: mean infant birth weight was lower in mothers who were vitamin D deficient, but these infants showed accelerated growth in both weight and length during the first year of life [88]. By 12 months of age infants of mothers who had serum 25-OHD concentrations less than 30 nmol/l (standard deviation score [SDS] = +0.12) were longer than infants of mothers who had concentrations greater than 50 nmol/l (SDS = −0.13). Whether this is a result of accelerated growth among the deficient group, or decelerated growth among the high vitamin D group, or a combination of both, was not discussed. These differences in height and weight persisted even after controlling for potential covariates.

11.4.2.3 Child Outcomes

The Southampton Women's Survey (SWS) and the Avon Longitudinal Study of Parents and Children (ALSPAC) have provided information on the role of maternal vitamin D status on growth and bone measures in the offspring through 9 years of age. The SWS enrolled 596 women during pregnancy and of these, 160 children 9 years of age had mothers with serum 25-OHD concentrations measured during the third trimester [92]. No associations between maternal 25-OHD and birth weight, birth length or childhood height and lean mass at 9 years of age were found. However, there was an association between maternal 25-OHD and length at 9 months of age, although a later study by this same group that included 440 mother–child pairs reported no relationship between maternal 25-OHD concentrations and length or weight at 9 months of age [91]. Third trimester 25-OHD concentrations were associated with the child's total body BMC and bone area at 9 years of age and only the relationship with total body BMC remained significant when height was included in the analysis. Potential covariates that could influence bone, such as lean and fat mass [112], were not included in the analysis.

The ALSPAC is a longitudinal study of approximately 14,000 children who are being followed from early pregnancy, and analyses of growth and bone data up to 9 years of age on 6,995 of these children have been reported [93, 113]. A subset of the mothers ($N = 355$) had serum 25-OHD concentrations measured in the third trimester of pregnancy and potential UVB exposure was estimated for all women using local meteorological records [93]. These UVB estimates were found to correlate with serum 25-OHD concentrations in the subset of mothers with available samples [93]. Positive associations were observed between estimated UVB exposure and birth length and weight, as well as height, total body lean mass, BMC, and bone area at 9 years of age; yet no evidence of an association between maternal 25-OHD and any of the offspring's growth or body composition measures was

observed in the 355 mother–child pairs. These investigators previously reported no relationship between maternal vitamin D *intake* during the last trimester of pregnancy and total body BMC at 9 years of age among the offspring, but they did find significant associations between maternal magnesium and potassium intakes and total body BMC [113]. The authors speculated that UVB was a more important determinant of maternal 25-OHD concentrations than maternal vitamin D intake. Magnesium content is high in vegetables, and vegetables may be consumed more often during summer months than winter months. The observed association between BMC at 9 years of age and maternal UVB exposure during pregnancy may be confounded by seasonal differences in magnesium and potassium intakes, or other factors that might vary by season.

In summary, some studies report an association between maternal vitamin D status during pregnancy and growth trajectories during the first year of life, although this is not consistent among studies. Two observational studies reported accelerated growth during the first year of life among infants of vitamin D deficient mothers, while one supplementation trial among women at risk of vitamin D deficiency found decelerated growth among infants of mothers who received placebo. Data on the effect of maternal vitamin D status during pregnancy on offspring growth are inconsistent and future results from recent randomized trials of maternal vitamin D supplementation during pregnancy may answer the question of the role of maternal vitamin D on infant growth.

11.5 Potential Risks of High Maternal Vitamin D

Several recent reports indicate that the relationship between mortality and serum 25-OHD concentrations is not linear, but is elevated at both low and high concentrations which has been described as either a U-shaped curve or an inverse J-shaped curve [114–116]. Similar nonlinear relationships may exist for pregnancy outcomes and maternal 25-OHD concentrations.

Bodnar and coworkers found an inverse J-shaped relationship between SGA probability and maternal serum 25-OHD concentrations, with the probability of SGA increasing at serum 25-OHD concentrations above 70 nmol/l (Fig. 11.9) [89]. Potential adverse effects of high maternal 25-OHD concentrations on growth later in life also has been documented. Leffelaar et al. [88] reported that length at 1 year of age was greater in infants of mothers with low vitamin D during pregnancy (<30 nmol/l; standardized *Z* score of +0.12) compared to infants of mothers with high vitamin D (>50 nmol/l; standardized *Z* score of -0.13). Although the authors interpreted these findings as an accelerated growth trajectory in infants of mothers with low vitamin D, the results also could be interpreted as a decreased growth trajectory among infants of mothers with high serum 25-OHD concentrations during pregnancy.

With regard to bone outcomes, Mahon and coworkers reported no significant linear association between fetal femur length at 34 weeks and maternal 25-OHD concentrations, although the relationship appeared to be nonlinear and more U-shaped, with greater fetal femur length at both low and high maternal serum 25-OHD concentrations [101]. Preliminary results from the SWS presented in abstract form showed a nonlinear relationship between total body BMC in 211 children aged 9 years whose mothers had 25-OHD concentrations determined during pregnancy [117]. This study found lower total body BMC of the child at maternal 25-OHD concentrations <35 nmol/l during the third trimester compared to 25-OHD concentrations >35 nmol/l (~1.06 vs. 1.18 kg respectively), but total BMC appeared to decrease at the higher levels of 25-OHD (~1.10 kg at 25-OHD >77 nmol/l). Since it was analyzed by linear correlation analysis it was not possible to determine whether this was a significant nonlinear relationship. These results suggest that the relationship between maternal vitamin D status during pregnancy and offspring growth and bone accrual may not be a simple linear relationship.

11.6 Conclusion

In summary, adaptive changes occur during pregnancy, lactation, and weaning to insure an adequate calcium supply for fetal growth, milk production, and maternal bone recovery. During pregnancy, serum 1,25-$(OH)_2$D concentrations increase resulting in increased intestinal calcium absorption. Urinary calcium excretion also increases and is probably due to increased glomerular filtration and increased absorptive calcium load. Although bone turnover markers are elevated, it is not apparent whether there are significant changes in bone mass during pregnancy. During lactation, changes in calcium homeostasis are independent of the mother's calcium intake and are more dependent upon ovarian function. As ovarian function returns, serum 1,25-$(OH)_2$D concentrations increase, intestinal calcium absorption increases, a higher renal calcium retention persists, and biochemical markers of bone turnover return to normal concentrations as bone is regained.

Although maternal vitamin D intake during lactation is correlated with breast milk vitamin D concentrations, milk concentrations are too low to provide the infant with sufficient vitamin D. Low maternal calcium intake during pregnancy is associated with low neonatal BMC and maternal vitamin D deficiency affects neonatal calcium homeostasis and fetal bone development. However, these effects are seen at maternal dietary intake levels well below the current recommended amounts and whether supplemental vitamin D above recommended levels affects growth and bone development of the offspring is not known. Due to potential adverse effects of high maternal vitamin D concentrations on the offspring, it is important that all current and future supplementation trials investigate the influence of not just low serum 25-OHD concentrations, but also high concentrations, on these outcomes.

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Chapter 12
Nutritional Requirements for Fetal and Neonatal Bone Health and Development

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Key Points

- Emerging evidence suggests that in pregnancy maternal body composition, diet quality, specific nutrient intake such as vitamin D, calcium and folate, and lifestyle factors such as smoking and exercise may have moderating influences on fetal and child bone development through "developmental programming" mechanisms.
- Specific nutrient needs for fetal skeletal development are currently based on estimates of intrauterine accretion of bone derived from fetal body composition analysis or cross-sectional analysis of preterm infant bone mass at birth but do not account for fetal programming effects.
- Estimates of nutrient needs for skeletal development in premature are higher than for term born infants. For premature infants, delivery of adequate nutrition during early neonatal life to attain intrauterine accretion of bone minerals varies with infant size (appropriate compared to small for gestational age), type of feeding (breast milk compared to formula) and exposure to steroid drugs, but is seldom achieved by term corrected age.
- Future research is required to define maternal nutrition and lifestyle factors in pregnancy and in early infancy in the context of genetic, epigenetic and metabolic factors that will optimize fetal and child skeletal development, achievement of peak bone mass and protect against osteoporosis in later life.

Keywords Developmental origins • Pregnancy nutrition • Fetal bone • Neonatal bone • Infant nutrition • Bone mineral content • Dual energy X-ray absorptiometry

12.1 Introduction

The trajectory for bone mineral deposition through the stages of fetal and infant development is becoming better defined as data emerge on whole-body and regional analysis of bone mineral content (BMC) using dual-energy X-ray absorptiometry (DXA). Previously, knowledge of fetal accretion of nutrients was based on analysis of the chemical composition of fetal and neonatal bodies [1], and more recently for minerals by neutron activation analysis [2]. Accretion of nutrients after birth was derived primarily from metabolic balance studies that yielded retention of mineral. Beginning in the

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early 1980s, BMC of the radius or humerus using single-photon absorptiometry (SPA) was possible to quantitate in small premature infants. Analysis of whole-body BMC by DXA was facilitated by availability of size-specific software beginning in the 1990s, allowing DXA to be validated as a tool [3, 4] for estimating bone, lean, and fat mass in small infants. All three sources of information—fetal tissue analysis, metabolic balance studies, and estimation of BMC by DXA—have contributed to the determination of recommended intakes of minerals and vitamin D to support healthy bone growth. In addition to nutrients, early skeletal development is dependent on genetics, an appropriate endocrine environment including parathyroid hormone (PTH), 1,25-dihydroxycholecalciferol, growth hormone (GH), insulin and insulin-like growth factors (IGFs), as well as physical activity. The integration of these factors is important to skeletal development per se and also the response of such factors to influences in the environment of the growing fetus and child.

12.2 Determinants of Fetal and Infant Bone

12.2.1 Developmental Programming

Observational studies have demonstrated a strong correlation between parental and offspring BMC and bone mineral density (BMD) [5, 6]. Evidence from twin and family studies suggest that the genetic contribution to BMD is between 60 and 90 % [7]. Some skeletal sites are shown to have a higher degree of heritable contribution than others, with the greatest degree of association being the head [7]. However, it is now acknowledged that environmental exposures during fetal and early life may give rise to epigenetic processes that result in developmental "plasticity or the ability of a single genotype to give rise to several phenotypes" [8].

The concept of "developmental programming" embraces the theory that metabolic events during critical time periods of antenatal and postnatal development have moderating effects on later health. Findings from human epidemiological and animal studies support a role for the interaction of gene variants with exposures during pregnancy such as maternal nutrition and other lifestyle variables to influence the programming of fetal, neonatal, and adult bone outcomes [8–11]. Maternal obesity on entering pregnancy has been linked to bone outcomes in the offspring in some but not all studies. In a multivariable regression analysis of 7,121 children at a mean age of 9.9 years, maternal prepregnancy body mass index (BMI) was positively associated with offspring total bone less head (TBLH) BMC and BMD, and spine BMC and BMD [12]. In contrast, maternal weight and gestational weight gain were inversely related to cord blood concentrations of bone formation markers such as osteocalcin and bone specific alkaline phosphatase [13]. Thus, further investigation is needed into the link between maternal weight and offspring bone health, especially in terms of adiposity hormones such as leptin.

In observational studies, a relationship was documented between maternal intake of dairy foods during pregnancy and a positive impact on bone mass in offspring between 6 and 16 years [14–16]. Not only fetal/infant bone mass but bone size (length) is influenced by maternal diet. In adolescent mothers, consumption of less than 2 dairy servings per day during pregnancy was associated with shorter fetal femur length at 20–34 weeks gestation [17]. Fetal femur and humerus length and infant birth length were significantly greater in adolescent mothers whose calcium intake was >1,050 mg/day, especially when vitamin D status was low (<50 nmol/L) [18]. To date, only one RCT in a small sample ($N=36$) investigated the effect of dairy product supplementation on BMD and bone turn-over in pregnant women in China with habitual low calcium intake [19]. A beneficial effect of milk supplementation during pregnancy was demonstrated for bone mass density at the spine and on suppression of bone resorption [19].

Exposure to individual nutrients may also be important to programming of bone in utero. Given the role of folate in methylation processes, a known mechanism of gene silencing, it is not surprising that folate status of women in pregnancy has been associated with indicators of bone programming in the

offspring. In the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study [20], maternal dietary intake of folate was significantly associated with BMD and areal BMC of the spine sub-region at 9 years of age. An association of the maternal MTHFR genotype with the same bone measures was also observed at age 9 years [20].

Maternal vitamin D insufficiency in pregnancy impairs placental calcium transport, thus reducing the trajectory of intrauterine bone accrual [21, 22]. This may occur via vitamin D-induced upregulation of placental mRNA expression of PMCA isoforms 1–4, which predict neonatal bone mineral content independent of other key maternal related variables [23]. In observational studies, vitamin D deficiency in women during pregnancy is a risk factor for vitamin D deficiency in the newborn infant [24], reduced intra-uterine long bone growth [25], lower bone mass at birth [26, 27], lower fetal long-bone growth [28], neonatal rickets [29], and significantly lower whole body bone mineral content (BMC), as well as lower lumbar spine BMC at 9 years of age [30]. Fetuses of vitamin D deficient mothers had a pattern of femoral growth that resembled childhood rickets as measured by 3D ultrasound at 19 weeks of gestation [28]. Programming of fetal bone in relation to maternal vitamin D status in pregnancy may also operate via gene methylation since vitamin D plays a regulatory role in the transcription of DNA methyltransferase enzymes.

Maternal dietary intake of calcium during pregnancy was demonstrated to influence fetal bone mineral accretion. For women with low dietary Ca intakes (<600 mg/day), a supplement of 2 g of calcium from before 22 weeks of gestation resulted in higher BMC of the total body in infants born at term [31]. In a prospective study in humans, higher first trimester maternal intakes of protein, calcium, and phosphorus were associated with higher childhood (6 year) bone mass, while higher carbohydrate intake was associated with lower bone mass [32]. The amount of dietary fat may also be important since an animal study found that after 14 weeks, pups of mothers fed a high fat diet had higher trabecular and cortical bone area [33].

Bone outcomes in the child may also be predicted by fetal growth velocity at specific phases of intrauterine development. Using data from a large ($n = 628$ mother–child pairs) prospective cohort, Harvey et al. [34] demonstrated that early pregnancy abdominal circumference growth defined as 11–19 weeks of gestation, was a stronger determinant of whole body bone area and bone mineral content at 4 years of age while late pregnancy growth defined as 19–34 weeks was more strongly associated with bone mass at birth.

The concept of fetal/neonatal programming of bone status is supported by knowledge of several candidate genes that may explain the genetic basis of adult bone mass such as the vitamin D receptor (VDR) [25], the gene encoding for type 1 collagen and the gene for estrogen receptor [35]. Intrauterine exposure to specific nutritional imbalances may operate via fetal "programming" of candidate endocrine systems that influence skeletal metabolism such as the growth hormone/IGF-1 axis. Cord IGF-1 in term infants was positively associated with whole body bone mineral content of newborn infants but was independent of other proven influences on neonatal bone mass such as maternal body composition and physical activity or birth weight [35].

The genetic variants responsible for BMD variation are beginning to be identified. To date, 66 BMD loci have been identified through candidate gene and genome-wide association studies, confirming the highly polygenic nature of BMD variation [36–38]. These BMD loci have been mostly identified in adult general populations, and their impact on maintenance of BMD post-delivery has yet to be studied.

Other non-dietary factors related to maternal lifestyle such as, smoking, breast feeding and physical activity were identified as determinants of fetal/infant bone status in a population-based cohort study. Infants born at term whose mothers smoked during pregnancy had significantly lower (by 11 %) whole-body BMC than infants of nonsmokers [39]. However, the impact of maternal smoking on bone status of the offspring may not be sustained. In 415 infants in Southern Tasmania who were followed from pregnancy to 16 years of age, maternal smoking was associated with lower bone mass at 8 years but by 16 years there was no effect on bone mass at any site or incidence of fractures [40]. In the latter study, breastfeeding was associated a benefit of 2–3 % higher BMD of the whole body and hip and about a 30 % reduction in fracture risk after adjustment for confounders [40].

Maternal physical exercise is generally promoted during pregnancy and may benefit fetal bone outcomes, although the ideal intensity, duration and type of exercise to achieve a benefit to child bone health are not well prescribed. Maternal thinness as reflected in low triceps skin fold thickness and more frequent and vigorous activity in late pregnancy were also associated with a lower BMC in the infants [39]. Further, analysis by this group reinforced these observations, showing that in a sample of 841 mother–baby pairs lower walking speed in late pregnancy and higher skin fold thickness in mothers during pregnancy were positive predictors of greater neonatal whole body bone and BMC [10]. These maternal predictors of newborn bone mass were independent of placental weight and thus not likely a result of reduced placental delivery of nutrients. However, intrauterine growth restriction leading to small infant size at birth may also be an important marker of ultimate skeletal development, since birth weight is a recognized predictor of bone mass in later life [41, 42].

12.3 Nutrient Needs for Fetal Skeletal Development

Deposition of the key essential minerals for fetal bone development—calcium (Ca), phosphorus (P), and magnesium (Mg)—occurs predominantly in the third trimester of pregnancy, during which approximately 80 % of the mineral of the infant born at term is accrued in the skeleton [43]. A maternal source of vitamin D appears to be important for the transplacental transfer of Ca (and presumably P) to the fetus, and the synthesis of the active metabolite (1,25-dihydroxyvitamin D) that functions in this transport role appears to occur not only in maternal tissues but also in placental tissue and the fetal kidney (reviewed in [21, 44]). After birth, infants are dependent on a normal circulating concentration of 25-hydroxyvitamin D to produce the active metabolite.

12.3.1 Estimates of Intrauterine Accretion of Bone Minerals

Biochemical analysis of body composition of aborted fetuses or infants dying shortly after birth have formed the basis of estimations of accretion of nutrients in the third trimester of pregnancy [1, 43, 45]. From the compositional data, and using weight growth curves of more recent studies, mineral accretion over the period of 24–36 weeks of gestation was estimated to be 90–120 mg/kg fetal body weight/day for Ca, and possibly higher from 36 to 38 weeks of gestation, which is the time of peak fetal accretion of bone mineral [45]. During this intrauterine growth period, P is deposited in amounts of about 60–75 mg/kg/day and Mg is deposited in amounts of about 2.5–3.4 mg/kg/day [45]. The exact amount of maternal vitamin D intake required to optimize fetal bone accretion is unknown. The 25-hydroxyvitamin D accumulates in the fetus and serum concentrations at term birth will reflect maternal vitamin D status, race (African-American being lower than Caucasian), and season of the year [46]. In preterm infants, cord blood concentration of this vitamin D metabolite is variable, with some lower and some higher than the normal reference range (50–215 pmol/L) [47].

12.3.2 Accretion of Bone as a Measure of Calcium Needs During Fetal Life

In vivo intrauterine estimates of fetal bone mineral accretion have not been measured even with DXA technology, due to the small exposure to radiation (of the order of 0.3 mrem per scan). Instead, estimates of BMC at sequential gestational ages have been inferred from cross-sectional measures in preterm infants taken shortly after birth using DXA. For example, in a cross-sectional study of German infants

born at a gestational age of 25–42 weeks, lumbar spine BMC increased 5.5-fold and mid-humerus BMC increased 2.4-fold over the first year of life [48]. In a similar cross-sectional study of US infants [49], accretion of Ca in utero in the third trimester of pregnancy was estimated to be 23.2 ± 35 g (mean ± SD), assuming that bone mineral contains 32.2 % Ca. Reference intrauterine values for BMC in Belgian infants were converted to Ca content of bone and found to compare favorably with the Ca content measured by chemical analysis or with neutron activation analysis [50].

12.3.3 Maternal Nutrition in Pregnancy to Support Nutrient Accretion of Fetal Bone

In pregnancy, maternal vitamin D status is critical to the vitamin D status of the infant at birth and may even program for bone development in childhood. Recommended intakes of vitamin D in pregnancy vary between reports. While the Dietary Reference Intakes from the Institute of Medicine in Washington, DC and Health Canada [51], recommend 600 IU vitamin D day (same amount as for nonpregnant women), the Canadian Pediatric Society recommends 2,000 IU/day [52]. For the prevention of vitamin D deficiency in pregnancy, the American Endocrine Society recommends at least 600 IU/day of vitamin D and states that at least 1,500–2,000 IU/day of vitamin D may be needed to maintain a blood level of 25(OH)D above 30 ng/ml (75 nmol/L) [53]. The latter states that "pregnant women are at high risk for vitamin D deficiency" and that "daily doses of 600 IU vitamin D do not prevent deficiency in pregnant women" [53]. However, claims of "pandemic" or high prevalence of vitamin D deficiency in pregnant and lactating women [52, 53] are not founded on population-based surveys but rather cite literature primarily from Afro-American and Aboriginal groups. The higher recommendations for vitamin D intake in pregnancy are difficult to reconcile based on scientific evidence [54]. In pregnancy, absorption of calcium doubles owing to doubling of the synthesis of the active metabolite 1,25-dihydroxyvitamin D via a non-parathyroid hormone mechanism that upregulates renal 1-alpha-hydroxylase enzyme [21].

To date, evidence to support higher intakes of vitamin D in pregnancy have relied on a single randomized clinical trial in which 494 women were randomized in early pregnancy to vitamin D supplements of 400, 2,000 or 4,000 IU per day and 350 women were followed to term birth [55]. For the groups receiving 2,000–4,000 IU/day, >80 % of subjects achieved a serum 25OHD of >80 nmol/L and both maternal and cord blood 25OHD was significantly higher than for the group randomized to 400 IU/day vitamin D [55]. No adverse effects were reported for hypercalcemia or hypocalcemia, hypercalciuria or parathyroid hormone level [55]. However, the higher vitamin D status was not reported to benefit clinical outcomes of infant growth (including birth weight which was similar across vitamin D supplement groups) or bone size or bone mass; nor was there any association of vitamin D status with outcomes of pregnancy comorbidities [56]. These findings support data from a previous study in 125 Gambian women who all achieved serum vitamin D in pregnancy >50 nmol/L [57]. No differences in birth weight, infant length, whole body, or radius bone measures by DXA at 1 year of age were observed between groups whose mothers had vitamin D status during pregnancy above or below 80 nmol/L [57]. Thus, the need to achieve vitamin D status in pregnancy over 80 nmol/L in order to optimize bone size and mass outcomes in the offspring does not seem warranted based on existing evidence. In addition, there is no evidence that increasing the vitamin D content of breast milk by super dosing the mother produces sufficient transfer of vitamin D to the infant to maintain a normal vitamin D status unless the mother consumes >6,400 IU/day for >6 months [58]. At maternal intakes of 2,000 IU vitamin D/day for 3 months during lactation, milk vitamin D rose only moderately (from 40 to 70 IU/L) and no effect was observed on vitamin D status of the nursing infant [58]. Thus, recommendations for vitamin D intakes beyond the 600 IU/day in pregnant and lactating women do not appear warranted. A prudent approach is to follow current guidelines of directly supplementing the infant with 400 IU/day of vitamin D [51] rather than super dosing the mother.

Table 12.1 Comparison of nutrient recommendations by various groups for mineral and vitamin D intakes of premature infants

	Preterm infants—US [59]	Preterm infants—Europe [60]	Preterm infants—Tsang et al. [61]
Calcium, mg/day	115–220	120–140	160–220
Phosphorus, mg/day	75–140	65–90	78–118
Vitamin D, IU/day	200–400	800–1,000	400

12.4 Nutrient Needs for Skeletal Development in Premature Infants

Recommendations for nutrient intakes for premature infants vary among international sources. A comparison of current recommendations for the bone nutrients—calcium, phosphorus, and vitamin D—for premature infants by the American Academy of Pediatrics in the United States [59], the European Society for Pediatric Gastroenterology, Hepatology and Nutrition [60], and a global consensus [61] is shown in Table 12.1. The recommendations are provided as a range of values reflecting the fact that not all preterm infants are the same and nutrient needs will vary depending of stage of prematurity, size at birth (small versus appropriate for gestational age), and rate of growth.

For vitamin D, the European recommendation is more than twice that of the North American sources. The rationale for this discrepancy is not clear in their report [60]. Premature infants are at risk of poor vitamin D status due to low nutrient stores at birth, low content of vitamin D in human milk and prolonged hospitalization, which prevents endogenous production of vitamin D [47]. For preterm infants or very low birth weight preterm infants, there are three randomized trials supporting the position that 400 IU per day is sufficient supplemental vitamin D for both short term [about 3 months [47]], and long term (9–11 years) normal bone health [62, 63]. After discharge from hospital, premature infants should receive vitamin D supplements as recommended for term infants.

Premature infants fed premature formula or expressed breast milk fortified with human milk fortifiers that have vitamin D added require a vitamin D supplement until they are being fed at least 300–400 ml/day, depending on the product being used. This volume of formula or fortified expressed breast milk is the amount that would supply 400 IU/day of vitamin D. There are few situations where calcium supplementation of infants is indicated. Preterm infants have higher calcium requirements than term infants and preterm formulas and human milk fortifiers are accordingly fortified with added calcium. There is no evidence to indicate, once term corrected age is reached, that higher amounts of calcium need be provided as a supplement.

12.5 Nutrient Needs for Skeletal Development in Term-Born Infants

The most recent nutrient-based recommendations for mineral and vitamin D intakes are those of the Food and Nutrition Board, Institute of Medicine (IOM) [51, 64], which are intended for use by Americans and Canadians (Table 12.2). For infants, the recommended intakes are intended for term-born, healthy, breast-fed infants who are considered the model for normal infant growth.

For vitamin D, setting an AI could not be based on the content of human milk as it contains only marginal amounts of vitamin D. An AI for vitamin D of 400 IU/day was set for infants from 0 to 12 months, based on this intake being associated with maintenance of serum 25-hydroxyvitamin D above 30 nmol/L and likely closer to 50 nmol/L, which represent a vitamin D status that is above that usually associated with clinical rickets in infants. The revised AI for vitamin D of 400 IU/day for infants now set by the Institute of Medicine was previously established by the Canadian Pediatric

Table 12.2 Dietary Reference Intakes for minerals calcium, phosphorus, magnesium, and fluoride for infants [51, 64]

Nutrient	0–6 months	7–12 months
Calcium		
Al (mg/day)	200	260
UL	ND	ND
Phosphorus		
Al (mg/day)	100	275
UL	ND	ND
Magnesium		
Al (mg/day)	30	75
UL	ND	ND
Fluoride		
Al (mg/day)	0.01	0.5
UL (mg/day)	0.7	0.9

AI Adequate Intake, *ND* not determinable due to lack of data of adverse effects in infants, *RDA* Recommended Dietary Allowance, *UL* upper limit

Society [52] and the American Academy of Pediatrics [65]. For breast-fed infants to meet the AI of 400 IU of vitamin D/day they must be provided with a vitamin D supplement. For formula-fed infants, intake of nearly 1,000 ml/day is required to achieve the AI for vitamin D since infant formulas in North America are regulated to contain 400 IU/L of liquid formula.

The DRIs for calcium, phosphorus and magnesium for infants were based on the content of human milk to derive an estimated average intake (AI) for age 0–6 months and with the addition of intake from complementary foods for age 7–12 months. For calcium, the values reflect those provided in the revised DRI report for Calcium and Vitamin D [51]. The AI value of 200 mg/day for breast-fed infants from 0 to 6 months was substantiated by considering that average calcium absorption in infants is around 60 % thus yielding retention of calcium of 120 mg/day, a value about 20 % higher than the estimated accretion of calcium for an infant of about 100 mg/day. For infants 6–12 months of age, recent data on calcium intakes from solid foods for formula fed infants was used to add to the intake from breast milk to yield an AI of 260 mg/day. For fluoride, intake from human milk was the reference for the first 6 months only. After 6 months, the AI for fluoride was set at 0.05 mg/kg/day and adjusted to a reference weight for age, based on the well-documented evidence of the benefit of fluoride intake for the prevention of dental caries (Table 12.2).

12.6 Infants at Risk of Bone Abnormalities due to Nutritional Deficiency/Excess

Sub-optimal bone mineralization still exists in certain infant groups despite widespread recommendations of supplementation with vitamin D. In a survey of rickets by the Canadian Pediatric Society [29], 104 documented of infants with rickets (at a mean age of 1.4 years, range 2 weeks–6.3 years) were identified and associated with vitamin D deficiency. The primary cause was attributed to 94 % of the cases of rickets having been or currently being breast fed, most with no vitamin D supplements. Another causative factor may have exposure to maternal subclinical vitamin D deficiency in utero. As demonstrated in this Canadian surveillance of rickets [29], subclinical maternal vitamin D deficiency was likely since 79 % of mothers did not drink milk during pregnancy and only 12.5 % had taken a supplement with vitamin D. Another key risk factor identified was dark skin colour since 33 % of

infants were Afro-American, 24 % were First Nations or Inuit, 14 % were middle Eastern with only 10 % Caucasian and living in the north (the Territories and Nunavut), Thus, a key issue is not that 400 IU/day vitamin D is not adequate for pregnant women or infants but rather that compliance with taking that recommended amount of vitamin D was almost non-existent [29]. Clearly, the lack of compliance with current recommendations for breast-fed infants to receive 400 IU vitamin D/day from birth is a key issue that needs to be addressed through improved education by health professionals.

In some situations, the recommendation of 400 IU vitamin D per day may not be adequate in the following subgroups of infants: infants born to mothers with sub-clinical or overt vitamin D deficiency due to inadequate placental transfer of vitamin D in utero to build the infant body stores of 25OH-D [29]; infants with liver or renal disease or malabsorption syndromes [66–68]; obese infants due to possible sequestration of vitamin D metabolites in the excess adipose [69]; or those with intermediate or dark skin, who avoid exposure to sun or who cover the majority of their skin outside the home [70]. Such infants may require greater than 400 IU vitamin D per day but intake should not be in excess of the Upper Level (UL) set by the DRI of 1,000 IU/day for infants to 6 months and 1,500 IU/day for infants 6–12 months [51]. For infants living in northern communities (above 40° north), especially those with intermediate or dark skin colour, the Canadian Pediatric Society Nutrition Committee supports the recommendation to supplement term infants with 400 IU/day of vitamin D, but further recommends that infants living in the far north be supplemented with 800 IU/day of vitamin D [52]. However, this recommendation is not based on clinical trial evidence. Rather, risk of vitamin D deficiency has been implied from observational studies in regional communities such as in the Winnipeg area in Aboriginal populations [71].

12.7 Bone Mineral Content in Term and Preterm Infants

Term infants: In term infants, accretion of bone mass in the whole body measured by DXA over the first year of life follows the pattern shown in Fig. 12.1. Longitudinal measures of whole-body bone mineral content (WBBMC) in term-born infants demonstrated that BMC increases by 2.5–3.6-fold over the first year of life [72, 73]. Based on reports of DXA measures of body composition in term infants, Koo [74] estimated that BMC increased by 400 % during infancy while body weight increased by 330 %. However, body weight appears to be the strongest determinant of BMC in growing healthy infants [50, 75]. The independent influence of diet—such as vitamin D or mineral intake or dietary practice of breast feeding compared to formula feeding—on bone mineral accretion in infancy has been addressed in a few studies.

Whole-body BMC in breast-fed term infants has consistently been observed to be lower than in formula-fed infants [73, 75]. In one study [73], a significantly lower BMC was observed at 3 and 6 months of age in breast-fed infants (average milk Ca and P of 300 and 150 mg/L) compared to those fed formula containing moderate amounts of Ca and P (510 and 390 mg/L) but not low Ca and P (430 and 220 mg/L). However, from 6 to 12 months of age, the previously breast-fed infants were fed formula with moderate or high (1,350 mg Ca and 900 mg P/L) mineral content now demonstrated a greater rate of accretion of BMC (81 ± 161 mg/6 months) than the infants fed formula in the first 6 months (73 ± 15 and 71 ± 15 mg/6 months). As a result, by 12 months of age there were no differences in whole-body BMC between feeding groups. If infants were breast-fed beyond 6 months, a lower whole-body BMC than formula-fed infants was maintained to 12 months of age, but such differences were not apparent by 2 years of age [75]. A lower intake of protein and macrominerals from exclusive feeding with breast milk compared to standard infant formulas is the likely explanation for observed variations in growth patterns in early life [75]. Taken together, the available studies indicate that slower accretion of bone mass in early life may represent the biological norm and is not predictive

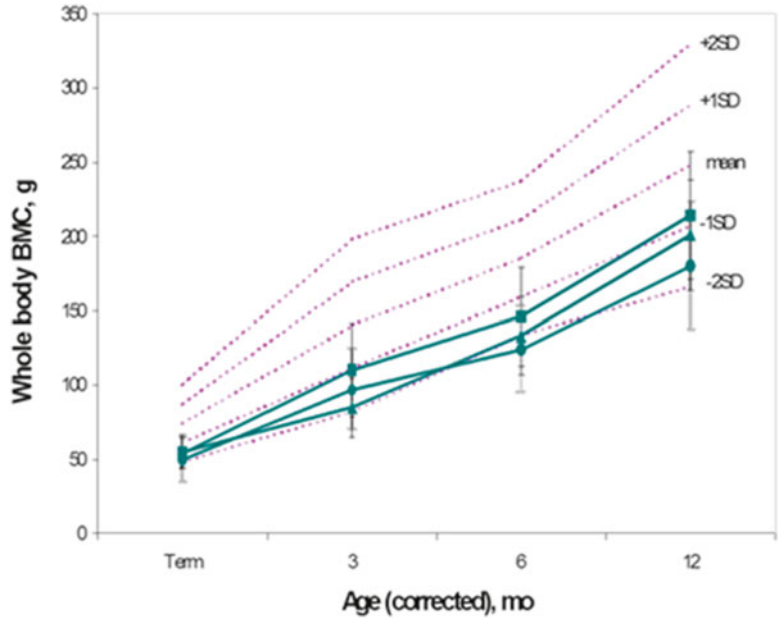

Fig. 12.1 Pattern of whole-body bone mineral content (WBBMC) accretion by DXA in Canadian term born infants fed standard term formula from birth to 1 year is shown in hatched lines (mean ± 2 SD). The *solid green lines* indicate the pattern of change in WBBMC in preterm infants of different birth weight categories and feeding regimens after hospital discharge: *filled circle* Low birth weight (birth weight = 1,154 g, $N = 24$) and breast fed to 6 months and then some term formula to 12 months; *filled square* Low birth weight (birth weight = 1,029 g, $N = 22$) and fed term formula to 12 months; *filled triangle* very low birth weight (birth weight = 866 g, $N = 18$) and fed term formula (Data provided by S Atkinson)

of lower ultimate bone mass in early childhood. It remains to be determined whether differences in patterns of skeletal accretion of bone mineral in fetal and early life influences subsequent metabolic programming of growth and final adult bone mass.

Preterm infants: In utero, the fetus experiences the greatest deposition of calcium and phosphorus into bone during the third trimester of pregnancy, peaking between 32 and 34 weeks of gestation [1, 43]. Thus, preterm infants are born with lower bone mass compared to term born infants. The short-term impact of early nutrition (while in hospital) on whole-body measures of BMC at term-adjusted age have been evaluated in relation to feeding of fortified mother's milk compared to preterm formulas. In some studies in infants less than 1,500 g birth weight and gestational age less than 32 weeks, no differences in whole-body BMC were observed at or near term age between those fed fortified mother's milk or preterm formula in hospital [76, 77]. But the mean BMC of the preterm infants was lower than −1 SD from the mean value for the term infants [76, 77]. In preterm infants of larger birth weight (<1,750 g), feeding of preterm formula compared to fortified human milk for 3 weeks resulted in higher whole-body BMC at 37 weeks gestational age [78]. However, BMC related to bone area or to body weight were similar between the feeding groups and represented about a −2 z-score compared to term-born reference infants [78]. Taken together, the reported studies provide evidence that bone mineral deposition equivalent to that which occurs in utero in the third trimester of pregnancy is not achieved by term age in infants born prematurely, despite the many advances in the delivery of nutrients in early life in this population.

Some of the observed differences in BMC at term age between reported studies may relate to differences in birth size, particularly if the population studied included infants of extremely LBW or small for gestational age [79]. As published previously [80], mean values for whole-body BMC at term-corrected age for preterm infants of varying birth weight and in-hospital feeding regimens fall below

(<−1.5 SD) that of term reference infants (see values at term age in Fig. 12.1). Mean absolute BMC for preterm infants who were appropriate for gestational age (AGA) at term-corrected age was 16–30 % lower, and for those who were small for gestational age (SGA) was 36 % lower than BMC in term-born infants [80]. Since most preterm infants are smaller in both weight and length at term-corrected age than term-born infants, BMC values were expressed as a function of weight or length. Compared to a whole-body BMC for body weight of 20 ± 2 g/kg for term infants, SGA infants (17 ± 3 g/kg) and AGA infants (17 ± 2 g/kg) had lower bone mass at term-corrected age [80]. Expressed as a function of length, BMC g/cm was 1.5 ± 0.2, 1.0 ± 0.3, and 1.1 ± 0.2 for the term, preterm SGA, and preterm AGA infants, respectively [80]. Thus, even with correction for body size, the premature infants did not attain a bone mass comparable to term infants at birth.

The impact of mineral fortification of mother's milk or specialized formulas for preterm infants in early life during hospitalization on accretion of bone mineral measured by bone densitometry has been inconsistent. In a systematic review [81], the effectiveness of multicomponent fortification of human milk on the promotion of growth and bone mineralization in preterm infants was evaluated. Ten trials ($N = 596$ infants) were included in the analysis, which represented random or quasi-random allocation to supplementation of human milk with multiple nutrients or no supplementation within a nursery setting. Unfortunately, only half of the reported trials measured BMC, and there was inconsistency in the use of radial or whole-body measures of bone. The main results of the review showed that BMC was increased by nutrient fortification of formula. However, in four of five studies in which BMC was measured, there were no statistical differences between control and treatment groups. A meta-analysis of BMC measures from the 5 studies (in which one study contributed 59 of 79 infants) showed a positive effect on BMC of fortification of mother's milk with a human milk fortifier (weighted mean difference [WMD] 8.3 mg/cm, 95 % confidence interval (CI) 3.8–12.8 mg/cm) [81]. Plasma alkaline phosphatase, a marker of bone turnover, was not different between treatment groups.

Physical activity, in particular weight-bearing exercise, positively influences bone mass accretion even in preterm infants in early neonatal life as measured using SPA [82] or DXA [83]. Using quantitative ultrasound of the tibia early (about 2 weeks postnatal), intervention with brief daily passive range of motion exercise reduced the usually observed postnatal decline in tibial speed of sound (SOS) measures [84]. The interactive effects of diet and physical activity on accretion of bone mass is just beginning to be addressed in young children [85], but in preterm infants they remain to be elucidated.

The expected time for "catch-up" growth and bone accretion and the nutrients needed to support skeletal development in preterm infants are not well defined. Longitudinal measures of whole-body BMC from term date to 1 year corrected age demonstrated that prematurely born infants [86, 87] experience an increase in BMC of 3.6–4-fold from term corrected age to 12 months, similar to that of term-born infants [80]. However, the velocity of accretion of BMC and capacity for catch-up bone mineral accretion (that which crosses centile or standard deviation lines) in the whole body of preterm infants appears to vary with birth size and nutritional management over the first year of life (Fig. 12.1). By 12 months corrected age, "catch-up" in BMC to above −1 SD was achieved only in infants of mean birth weight over 1,000 g who were AGA (Fig. 12.1). Those infants who were SGA or of ELBW had whole-body BMC at 12 months corrected age between −2 and −1 SD compared to term-born reference infants (Fig. 12.1). Breast-fed premature infants who received more than 60 % of milk intake as breast milk, had lower WBBMC at 3 and 6 months corrected age when compared to formula-fed term infants but by 12 months corrected age their BMC was similar to the formula–fed preterm infants, albeit still below the mean value for term born formula-fed infants (Fig. 12.2).

Catch-up in bone accretion in preterm infants following discharge from hospital was proposed as a benefit of feeding nutrient-fortified formulas. In two randomized trials of a dietary intervention to 3 months corrected age, nutrient-enriched formula that included supplemental dietary protein, Ca, and P after hospital discharge had a positive immediate benefit to BMC when the intervention was

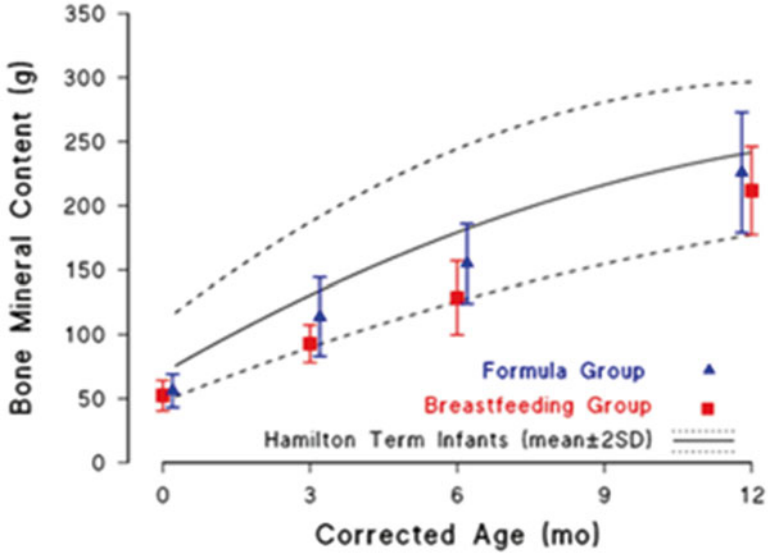

Fig. 12.2 Whole-body bone mineral content by DXA in prematurely born infants fed primarily mother's breast milk or standard term formula. The Breastfeeding Group of preterm infants was fed fortified human milk in hospital and received >60 % of feeding as mother's milk after hospital discharge ($N = 27$, birth weight = 1,187 ± 232 g, gestational age at birth = 29 ± 3 weeks). The Formula Group of premature infants was fed standard term infant formula (FF) from hospital discharge to 1 year corrected age ($N = 26$, birth weight = 1,068 ± 328 g, gestational age at birth = 29 ± 3 weeks). *NOTE*: At 3 and 6 months of age, BMC for the Formula Group was significantly greater than Breastfeeding Group ($p < 0.05$) but not at 12 months corrected age (Data provided by SA Atkinson, McMaster University)

continued to 3 months [88] or 9 months [89] corrected age. However, such a positive effect of early nutrition on BMC is not always sustained [87, 90]. A randomized trial in small-for-gestational age premature infants in response to nutrient-fortified formula feeding to 1 year corrected age, demonstrated that the nutrient enriched formula did not support significantly greater bone mass accretion (Fig. 12.3). Both feeding intervention groups achieved some "catch-up" in BMC as referenced to term infants although neither reached the mean value for term infants by 12 months corrected age (Fig. 12.3). Thus, the whole-body BMC attained in the preterm infants does not catch up to that of term infants at 1 year of age, regardless of receiving protein and mineral intakes that are greater than those fed to term born infants in the first year of life.

Does early nutrition of preterm infants influence bone health in childhood and adolescence? Early "catch-up" of bone mass may not be important for preterm infants if final bone growth and attainment of peak bone mass is not compromised [91]. This question has recently been addressed through observational studies of former preterm infants at various stages of childhood or adolescence. At 8–12 years of age, whole-body BMC in prematurely born infants was significantly lower than for children of similar age born at term [62]. However, the preterm infants were also shorter and lighter, so that their BMC was appropriate for body mass when compared with children born at term. Neither diet in early life (breast milk compared to formula) nor current calcium intake or weight-bearing physical activity was significant determinants of BMC at this peripubertal age [62]. Measures of bone mass of the radius and lumbar spine in 70 preterm infants followed at 9–11 years of age were also lower and height shorter than in term infants; and neonatal diet interventions of Ca, P, or vitamin D were not associated with BMC in childhood [62]. Preliminary evidence suggests that catch-up does not occur during the pubertal growth spurt. Further follow-up of the Fewtrell et al. cohort ($N = 202$) in young adulthood (~20 years), found the former preterm infants to be significantly shorter and with lower

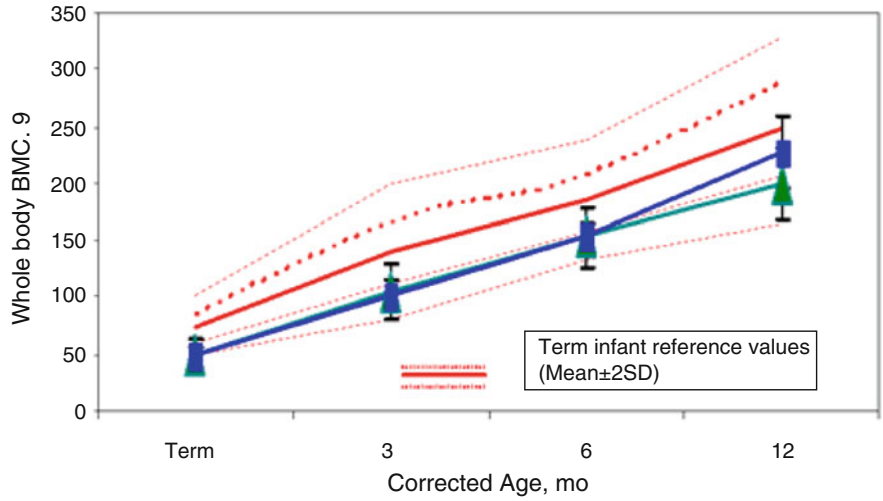

Fig. 12.3 Whole-body bone mineral content (WBBMC) in small-for-gestational age preterm infants randomized from hospital discharge and fed to 1 year corrected age on enriched post-discharge formula (EPDF) ($n=22$, birth weight = 1,410 ± 422 g, gestational age = 33.5 ± 3 weeks) indicated by ■; or standard term formula (SF) ($n=28$, birth weight = 1,411 ± 461 g, gestational age = 33.4 ± 3 weeks) indicated by ▲. Data for SGA preterm infants are plotted against reference data for term infants who were fed standard infant formula. Absolute means and velocity of WBBMC were not different between SF and EPDF groups. (Data provided by SA Atkinson, McMaster University)

lumbar spine BMD compared to reference data [91]. This observation was particularly apparent in those former preterm infants who were born small-for-gestational age [91]. In a study in Canada, former preterm infants ($n=26$) studied at 16–19 years were also found to be shorter and with lower BMC in the whole body, hip, and lumbar spine, although the BMC was appropriate for body size compared to 23 adolescents who were born at term [92]. In the latter study, early life variables such as the amount of human milk fed and duration of time until regain of birth weight was achieved were inversely associated with BMC at adolescence [92].

Birth size and neonatal exposure to steroid therapy may also impact later skeletal development. Extremely low birth weight infants (birth weight = 839 ± 189, $N=47$) who received dexamethasone therapy in early life to low birth weight infants (birth weight = 1,167 ± 215 g, $N=36$) had significantly whole body BMC Z-scores and lower BMD at the lumbar spine at 6–8 years of age compared to term born infants (birth weight 3,470 ± 391 g, $N=36$) [93]. Explanation for the significant delay in bone mass accretion for the extremely low birth weight infants may be multifactorial including lung disease that was treated with steroid therapy in early life, their earlier gestational age, and possibly greater exposure to inhaled steroids in childhood. Delayed early growth may also have contributed to the later delay in bone accretion in childhood [93].

12.8 Conclusion

In summary, evidence is emerging on the significance of exposures during pregnancy including maternal diet and lifestyle factors as well as early nutrition, physical activity and growth patterns in the infant, to achievement of peak bone mass by the end of the second decade and long-term programming of skeletal health. Once such knowledge is confirmed, recommendations for nutrient requirements and lifestyle practices can be better defined in order to optimize skeletal mineralization, achievement of peak bone mass and reduced risk of osteoporosis in later life.

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Chapter 13
Nutrition and Bone Health During Skeletal Modeling and Bone Consolidation of Childhood and Adolescence

Velimir Matkovic and Diane Visy

Key Points

- Bone accretion during childhood is proportional to the rate of growth and height velocity is relatively slow for both boys and girls.
- Retention of calcium in the body of an average child is lower than the calcium retention in an adolescent.
- Bone size, bone mass, and bone mineral density of the regional skeletal sites increase on average by about 4 %/year from childhood to late adolescence and young adulthood.
- Calcium needs are greater during adolescence (pubertal growth spurt) than in either childhood or adulthood.
- According to calcium balance studies the threshold intake for adolescents is about 1,500 mg/day.
- Inadequate calcium intake during growth may increase the risk of childhood fractures and predispose certain individuals to a lower peak bone mass.

Keywords Calcium intake • Growth • Peak bone mass

13.1 Introduction

Adult skeleton evolve from a single cell with a programmed system of constraints on development and mineralization which is under strict genetic control. It has been speculated that genetics contributes about 80 % of the variance in bone mass and the remaining 20 % is affected by one's environment, although the exact contribution of each major determinant of bone mass is unknown. Research data support the hypothesis that peak bone size, bone mass, and to a lesser extent the distribution of bone tissue within the bone as an organ (volumetric bone density) in young individuals are strongly influenced by genetic information by both parents (Fig. 13.1) [1]. This indirectly suggests that bone

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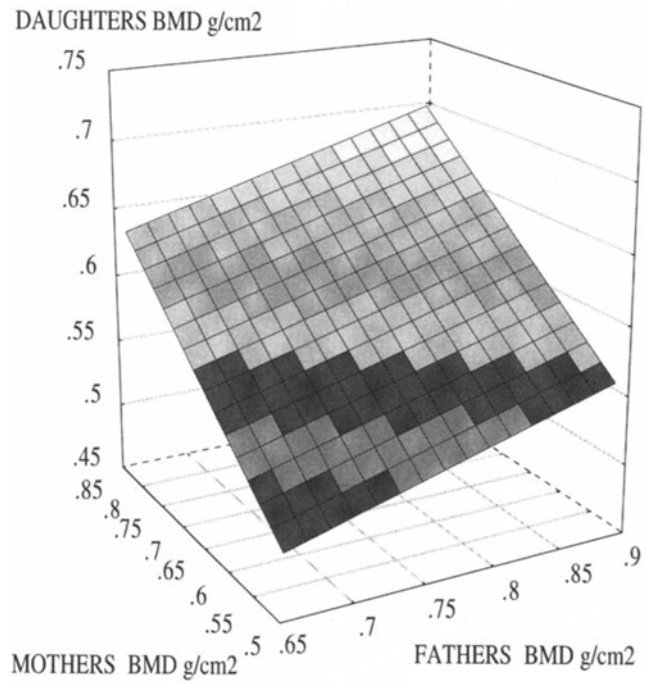

Fig. 13.1 3-D representation (surface plot) of the relation between forearm bone mineral areal density of daughters (z) and their fathers (y) and mothers (x). Mothers and fathers with higher BMD have daughters with higher BMD. Adapted from Matkovic et al. 1990 [1]

candidate genes responsible for bone modeling drifts along longitudinal and periosteal axis in interaction with nutritional factors and physical exercise have an important impact on skeletal development and peak bone mass.

Skeletal tissue not only provides the best example of general growth progress through maturity but also gives us our best approximation of biologic age of humans. The rate of this process and thereby the time required to achieve the mature adult skeleton is variable. Skeletal tissue goes through several developmental stages from fetal life to peak bone mass of young adulthood. Since bone fractures are closely related to the diminished peak bone mass we have to identify all underlying causes responsible for inadequate accumulation of bone tissue during skeletal growth and consolidation [2, 3].

The purpose of this presentation is to define some of the most important nutritional determinants of bone mass during childhood and adolescence (Fig. 13.2), and to discuss the strategy of primary prevention of osteoporosis in the search for better bone health. Over the last few decades, the focus of nutrition research and clinical practices in pediatrics has shifted from the prevention of nutritional deficiencies in young individuals to the establishment of recommended diets to prevent chronic diseases later in life. These priorities may eventually lead to dietary guidelines for the prevention and treatment of osteoporosis by targeting predisposed individuals early in life as emphasized in the Healthy People Act: National Health Promotion and Disease Prevention Agenda [5].

13.2 Skeletal Development and Peak Bone Mass

Peak bone mass is defined as the highest level of bone mass achieved as a result of normal growth [6]. Peak bone mass is important because it determines resistance or susceptibility to fracture [2]. This was first shown in the study of bone mass and hip fracture rates in two farming communities with different dietary habits but the same high level of physical activity over a lifetime. It appeared that both populations were losing bone with age at about the same rate, but those who started with more bone, ended up having higher bone mass and lower incidence of hip fractures. It was concluded that, other

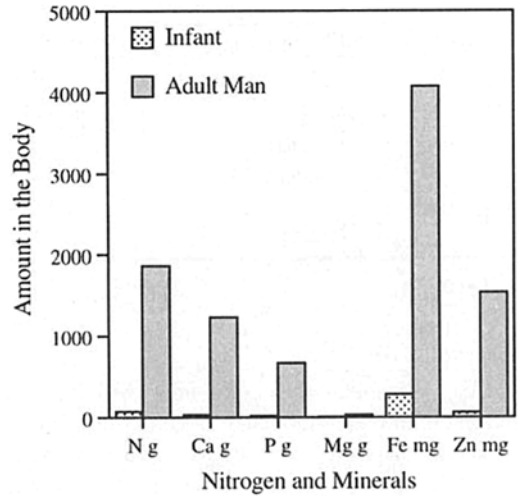

Fig. 13.2 Total body nitrogen and minerals in infants and young adults. The difference between these two phases of life represents the amount of mineral accumulated during childhood and adolescence. Adapted from Widdowson 1985 [4]

things being equal, a high peak bone mass provided a larger reserve later in life. The difference in bone mass and fracture rates were attributed primarily to calcium/protein intake. The differences in bone mass between the communities were established at an early age (30 years), implying that if calcium intake is important, it may be during skeletal growth that it has its greatest impact. This was the first proposal of the hypothesis that increasing peak bone mass by calcium supplementation during skeletal formation may contribute to osteoporosis prevention [2]. Results of a similar ecological study conducted in China on populations accustomed to different calcium intakes over lifetime [7], confirmed the above finding and reiterated the importance of adequate nutrition for skeletal formation and peak bone mass. In a retrospective study, Sandler et al. [8] also showed that postmenopausal women who consumed more milk and dairy products during adolescence had higher bone mineral density at the forearm than those who did not. Overall, it is likely that variations in calcium nutrition early in life may account for as much as a 5–10 % difference in peak adult bone mass. Such a difference, although small, probably contributes to more than 50 % of the difference in the hip-fracture rate later in life [2].

Most of the skeletal mass is accumulated by the average age of 18 years [9–11]. Thereafter, there is a minimal change in bone mass and density with age up to the time of menopause. Some skeletal sites continue to lose bone immediately after the age of 18 (proximal femur, and trabecular bone in the vertebrae) and the other sites show continuous apposition of bone up to the time of menopause (forearm, total spine, head) [11, 12]. The difference in volumetric density (mass/volume ratio in g/cm^3) between childhood and adulthood is minimal, indicating that most of the changes we measure during growth using dual X-ray absorptiometry (DXA, areal density) are predominantly due to the change in bone volume, and to a much lesser extent to increases in true bone mineral density (g/cm^3) [11].

To achieve maximal peak bone mass, dietary calcium and its absorption need to be at/or above the threshold level to satisfy skeletal modeling and consolidation and also obligatory losses in urine, feces, and sweat. Calcium intake thresholds with corresponding threshold balances for growing individuals were reported (Table 13.1) [13, 14]. Growing individuals, contrary to adults, therefore need to be in positive calcium balance to meet the extra needs of skeletal growth and consolidation. The net positive balance of bone tissue contributes to the constant demand for calcium and proteins throughout the developmental process. Except for few isolated reports that calcium deficiency can cause rickets in children, low calcium intake does not have any deleterious effect on bone health of young individuals [15, 16]. It has been suggested, however, that calcium deficiency during growth could contribute to bone fragility fractures during puberty [17, 18]; however, the definitive data to support this hypothesis are still lacking. Calcium deficiency during skeletal formation may negatively impact peak bone mass, therefore reducing the fracture resistance among the elderly [2, 7].

Table 13.1 Calcium intake thresholds and balances for growing individuals

Age group (years)	Threshold intake, mg/day	Threshold balance, mg/day
0–1	+503	1,090
2–8	+246	1,390
9–17	+396	1,480
18–30	+114	957

From Matkovic and Heaney, 1992 [14]

13.3 Childhood

Bone accretion during childhood (2–8 years) is proportional to the rate of growth. During this age interval height velocity is relatively slow and about 5.5 cm/year, for both boys and girls. Bone mineral areal density of the whole body increases at a rate of about 1.0 %/year as compared to adolescence when bone mineral areal density is increasing at about 4 %/year (Fig. 13.3). As a direct consequence of this, retention of calcium in the body of an average child is lower than the calcium retention in an adolescent [13]. Even so, growing children need two to four times the calcium per kilogram of body weight compared with adults. Daily required skeletal calcium retention during this period of life has been estimated at about 100 mg/day. On the average calcium intake of about 1,100 mg/day, children retain about 200 mg/day of calcium, or 18 % of intake. At about 500 mg/day calcium intake children are still in positive calcium balance for about 60 mg/day [13, 14]. In one study from India, children were consuming even less calcium (about 300 mg/day) and were still able to maintain a sufficiently positive calcium balance to assure skeletal development [19]. The results of this study cannot be directly applied to Caucasian children because Indian people are of different ethnic background with lower peak bone mass than their Caucasian counterparts. Calcium intake at about 1,600 mg/day leads to the positive calcium balance of about 300 mg/day. Dietary surveys in this country have shown that calcium intake conforms to the current RDI (800 mg/day) for this age group [20, 21]. With a relatively low urinary calcium excretion, such a calcium intake in this population should provide adequate calcium retention to satisfy the requirements for skeletal growth in children. It therefore appears likely that most children between infancy and puberty are able to meet the daily calcium requirements necessary for adequate skeletal calcium retention. The fact, that children are able to retain more calcium with further increase in calcium intake, is very important one, because it could contribute to higher bone mass and density during this age period. Children who were accustomed to a higher level of dairy product consumption during early growth have higher bone mass at the beginning of puberty [22]. Assuming that those children will maintained their dietary behavior through adolescence they can complete their growth phase with a higher peak bone mass as showed in a 7-year long observational study [23]. A study conducted in New Zealand among 30 girls and 20 boys aged 3–10 years, children who avoided drinking milk had low dietary calcium intakes and poor bone health as represented in the bone mineral areal density of the forearm and total body [24]. There is evidence that calcium supplements (milk) have improved stature in children. In 1927 a series of tests were carried out in Scotland in which about 1,500 children were given additional milk at school for a period of 7 months. Periodic measurements of children showed that the rate of growth in those getting the additional milk was faster than in those not getting the supplement. The increased rate of growth was accompanied by a noticeable improvement in health and vigor [25]. An assessment of bone density was not available at that time. The great increase in the height of young Japanese adults from 1950 to 1970 coincided with a tripling of the national calcium intake from about the lowest in the world to 600 mg/day [26]. In a more recent study a group of British teenage boys who were taking calcium supplementation for 12 months were taller and had a greater bone mass at the end of the intervention than their placebo counterparts [27], however, the same investigators were not able to observe this

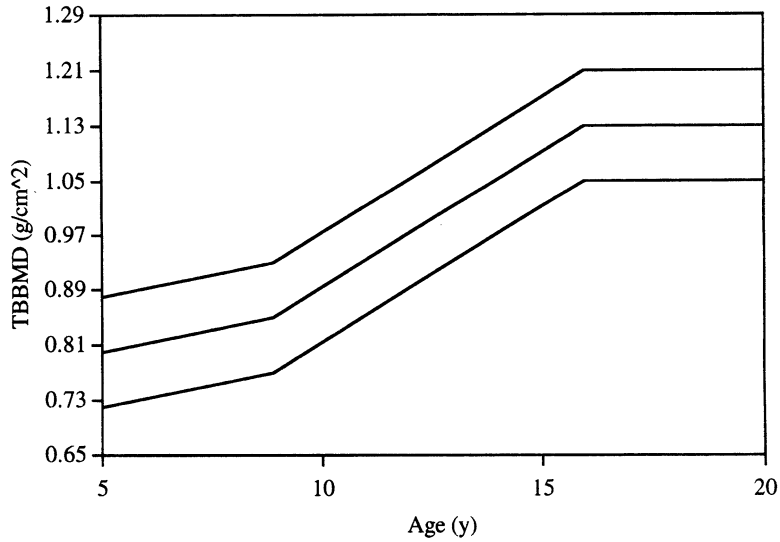

Fig. 13.3 Bone mineral areal density of the total body during growth. Adapted from GE-Lunar standards for boys (GE-Lunar, Madison, WI). These normative data are based on a cross-sectional study and may not accurately represent the change in TBBMD over time, in particular after the inflection point at age 16 years. Total body bone mass should be steadily increasing during late adolescence and throughout young adulthood but at lower rate of change

phenomenon in a cohort of malnourished Gambian children [28] whose bone mineral areal density increased with calcium supplementation but not the stature. It seems that the overall nutritional status is a determinant of the calcium response with regard to longitudinal bone growth.

13.4 Adolescence

Adolescence is characterized by accelerated muscular, skeletal, and sexual development. By the age of 10 the mean height velocity is 6 cm/year in girls and increases to an average peak of 9 cm/year by the age of 12. Peak height velocity for boys starts at 12 years of age (5 cm/year) and reaches a maximum by age of 14 (10 cm/year). Mean height velocity will be close to zero by the age of 15 in girls, and by the age of 17 in boys. The average gain in height in girls between the age of 8 and 17 is about 23 % of the mean adult value. This gain in height is however, dramatically out-paced by the gain in total body calcium; 132 % vs. 19 %, respectively (Fig. 13.4) [11]. Bone size, bone mass, and bone mineral density of the regional skeletal sites increase on average by about 4 %/year from childhood (age 8) to late adolescence and young adulthood when most of the bone mass will be accumulated. This ranged from 1.2 % for the estimate of true density of the body of L_3 vertebra to 6.6 % for the femoral neck [11].

When relating bone mass to pubertal developmental stage, it becomes obvious that most of the bone mass (37 %) is being accumulated between pubertal stage 2 and 4 [11]. This rapid accumulation of bone mass correlates with the rate of growth and probably also requires the concerted action of growth hormone, insulin-like growth factor-I (IGF-I), and sex steroids and its receptors. The increase in circulating IGF-I at early puberty correlates with sexual development and results from the interaction between sex steroids and growth hormone. Specifically, the surge in sex steroids in turn increases the secretion of growth hormone, which stimulates the production of insulin-like growth factor-I [29].

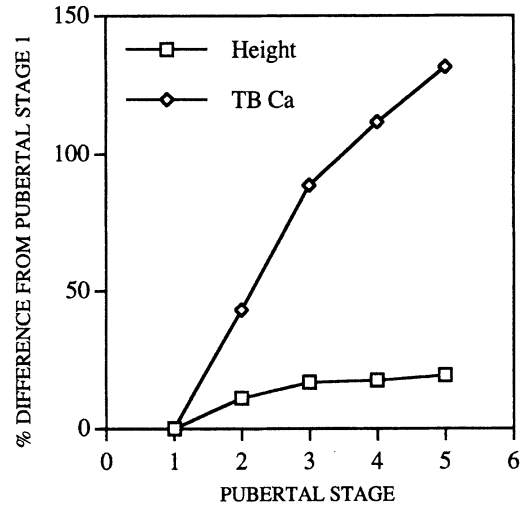

Fig. 13.4 Relative difference in the whole body calcium and stature between pubertal stage 1 and subsequent stages. Based on a cross-sectional study conducted in 80 healthy adolescent Caucasian females, aged 8–17 years. Adapted from Matkovic et al. 1994 [11]

The amount of estrogen required to stimulate longitudinal bone growth is very small. Doses of 100 ng/kg/day produce maximal growth in agonadal individuals. This dosage seems to be insufficient to cause either the development of secondary sexual characteristics or an increase in sex hormone binding globulin. These low dose effects are consistent with the observation that girls attain peak height velocity early in puberty at serum estradiol levels of <30 pg/ml which is one-fifth the mean level found in young adult women [30]. During this phase of rapid skeletal modeling bone mass is not yet consolidated and bone mass per bone volume ratio is relatively low [31]. Due to a high demand for calcium required for skeletal development, pubertal children do have a mild secondary hyperparathyroidism, which is more pronounced among those with lower calcium intakes [32]. Presumably, these events result in the high incidence of the bone fragility fractures (distal forearm) in children reaching the levels observed in women after menopause [33, 34].

Bone consolidation proceeds by the cessation of longitudinal bone growth. This coincides with the increase in estradiol secretion by the beginning of menarche. Time since menarche, therefore, is the best predictor of bone events in young females, and comparable to the time since menopause in older women. Estrogen driven endosteal apposition of bone is responsible for the increase in the relative amount of cortical bone (bone tissue within the bone volume) in premenopausal women as compared to men (higher cortical to total area ratio) [35] (Fig. 13.5). This endosteal apposition of cortical bone starts at menarche and ends at perimenopause. What has been accumulated during the reproductive phase will be lost after menopause, making elderly women more vulnerable to bone fragility fracture in addition to having smaller bones and lower peak bone mass.

Several studies document lower bone mineral density in adult women with a history of late menarche, as indicative of inadequate sex hormone levels during this critical period of skeletal development and/or a short time interval between menarche and menopause. Both young adult women and adolescents with hypothalamic amenorrhea have reduced bone mass at skeletal sites which should not be losing bone [36]. This might lead to the reduction in bone mass at maturity and predispose this population to the increased risk of osteoporosis later in life. The above mentioned menstrual disturbances could be in part due to relative or absolute energy deficiency induced by weight loss or inadequate weight gain as seen in protein–calorie malnutrition, young athletic women, anorexics, and other clinical situations. The onset of menarche is related to serum leptin level and body composition [37].

Calcium needs are greater during adolescence (9–17 years) than in either childhood or adulthood. As a result of the rapid skeletal changes, calcium metabolism in adolescents differs significantly from that in childhood and young adulthood and it has some similarities with calcium metabolism during infancy.

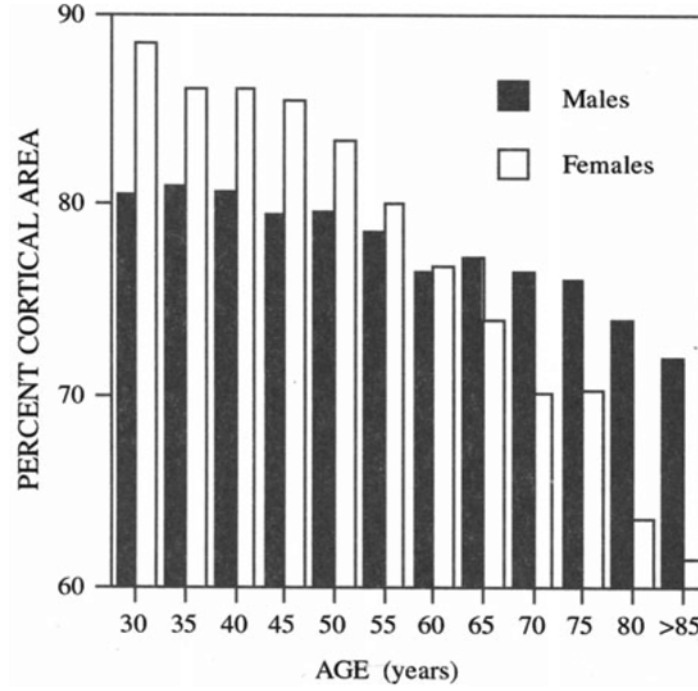

Fig. 13.5 Bar graph of percent cortical area (%CA) of the second metacarpal bone in males and females according to age. Note higher relative cortical bone mass in premenopausal women comparable to men (age < 50 years) Adapted from Matkovic et al. 1980 [35]

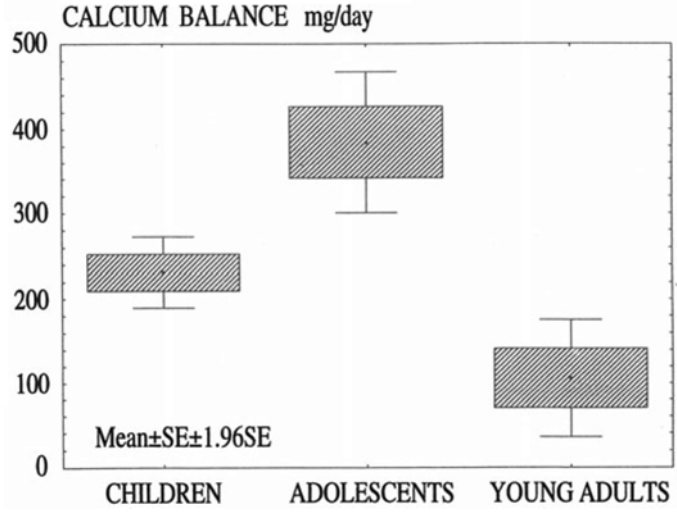

Fig. 13.6 Boxplots of calcium balances in young individuals of different age. Notice high calcium retention during phase of rapid skeletal modeling of adolescence. Calcium balances in all three groups were conducted at intake of 1,186 ± 96 mg/day. Subtracting skin losses of about 60 mg/day from an average calcium balance among adolescents of 400 mg/day leaves skeletal retention of about 340 mg/day. This value corresponds to the average daily skeletal accretion of calcium at the peak pubertal growth (time zero since menarche) as assessed by the whole body bone mineral content measurement by DXA based on a longitudinal study presented in Fig. 13.7. Adapted from Matkovic, 1991 [13]

In general, adolescents retain more calcium than either children or young adults (Figs. 13.6 and 13.7) [13]. According to calcium balance studies the threshold intake for adolescents is about 1,500 mg/day [14]. The corresponding average calcium retention which saturates the skeletons of teenagers is about 400 mg/day. Recommendations for calcium nutrition should take into account that the calcium intake

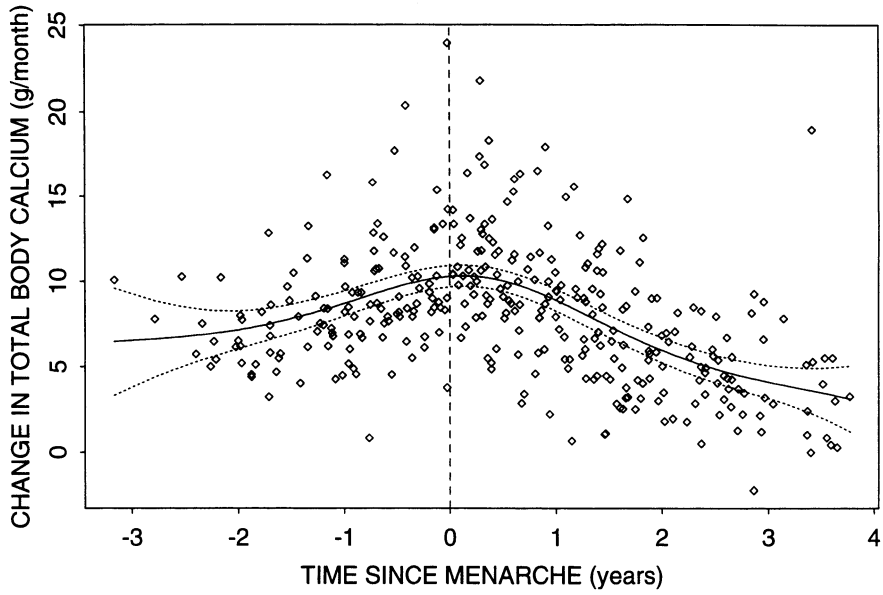

Fig. 13.7 The rate of change in total body calcium (g/month, $n=90$) with time since menarche (years) in a cohort of young females followed annually for 4 years. Total body calcium is considered a fraction (38 %) of total body bone mineral content as measured by dual energy X-Ray absorptiometry (DXA). Scatterplots shown with cubic splines and 95 % confidence intervals. Adapted from Matkovic et al. 2000 [61]

threshold is highly variable and depends on body size as well as stage of human development. When metacarpal cortical bone mass was separately examined in a segment of the population from the high and low calcium districts in Croatia, there was a larger discrepancy in the cortical bone mass per corresponding bone volume for persons of a larger body size than for the smaller individuals (Fig. 13.8) [38]. This indicated that calcium deficiency during growth could disproportionally affect individuals who are genetically predestined to reach a higher level in their peak bone mass, as they require more calcium. Subsequently this has been confirmed in a 7-year long randomized study with calcium supplementation in adolescent females [32]. The study revealed a significant influence of calcium supplementation on bone mineral accretion at the proximal radius only in the tall individuals. A similar observation was observed utilizing metacarpal morphometry measurements over time. In subjects with larger metacarpals, the contrast between the cortical bone mass of the placebo and calcium supplemented individuals was highly significant; however, the effect was not present among subjects with smaller metacarpals [32]. This implies that the habitual dietary calcium intake of ~830 mg/day was adequate for skeletal development of adolescents with smaller metacarpals, but was insufficient for those genetically predetermined to develop larger bones with higher peak bone mass.

The importance of a positive calcium balance during adolescent years is further emphasized by the need to meet not only the rapidly expanding skeletal compartment but also losses of calcium through the skin (not measured in usual balance studies) which may amount to as much as 60 mg/day in adults [39]. Young athletes could lose considerable calcium through sweat; this may be up to 60–80 mg/h of intensive training. Low calcium intake may lead to a negative calcium balance and bone loss, as reported for basketball players [40].

Adolescents, in general, absorb more calcium from their diet than either children or young adults (Fig. 13.9) [13]. The concentration of serum calcitriol is the highest during peak growth; pubertal stages 2–3 (Fig. 13.10) [41]. Urinary calcium increases during the period of adolescence and reaches its maximum by the age of 15–16 years, or by the cessation of puberty. The mean urinary calcium

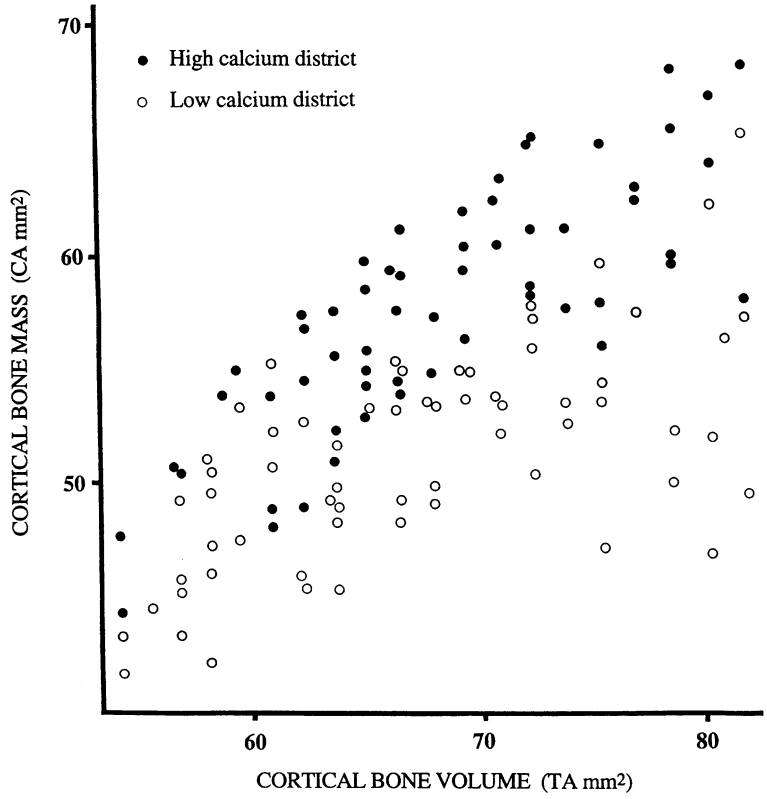

Fig. 13.8 The relationship between metacarpal cortical bone mass (CA) and bone volume (TA) in 40–44 year-old men ($n = 121$) accustomed to different calcium intakes over lifetime. Note a greater disparity between bone mass per bone volume in bigger individuals from high and low calcium regions. Adapted from Matkovic et al. 1979 [2, 38]

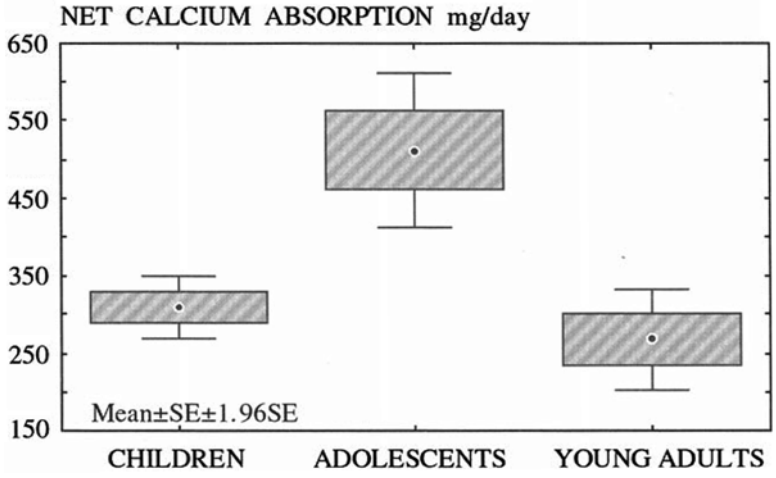

Fig. 13.9 Net calcium absorption in children, adolescents, and young adults at calcium intake of about 1,200 mg/day. Adapted from Matkovic 1991 [13]

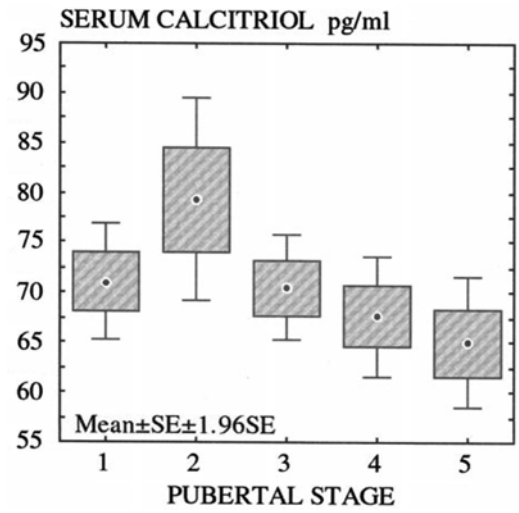

Fig. 13.10 Box plots of serum calcitriol level in young females according to pubertal developmental stage (breast). Highest concentration found during peak pubertal growth in stage 2. Adapted from Ilich et al.1997 [41]

output for young boys and girls aged 9–17 years is about 130 mg/day. Mean urinary calcium excretion at an intake of about 500 mg/day is about 120 mg/day. Further increases in intake up to 1,800 mg/day increase urinary calcium excretion only by 10–20 mg/day. This level of urinary calcium excretion of about 130 mg/day can, therefore, be considered as the mean obligatory urinary calcium loss for the age group 9–17 years [13]. The above indicate that urinary calcium excretion during adolescence is barely related to calcium intake (Fig. 13.11). Body weight and age seem to be the principal determinants of urinary calcium excretion during this phase of life. More powerful relationships between urinary and dietary calcium definitely exist in adults. The explanation for the above is that adolescents retain the absorbed calcium in the skeleton rather than excreting it in the urine [13, 42]. Calcium in the urine is expected to rise, as a result of the increase in the filtered load of calcium, only after the skeletal compartment is being saturated with calcium at intakes above the threshold level. Renal excretion of calcium is believed to be regulated by parathyroid hormone and estrogens and is also influenced by sodium intake. High consumption of salt increases the obligatory calcium loss in the urine which may jeopardize adequate bone accretion during growth (Fig. 13.12) [43].

Nationwide surveys revealed that adolescents, especially females, consume inadequate amounts of calcium [20]. In addition, psychological changes involving the adolescent's search for independence and identity, desire for acceptance by peers, and preoccupation with physical appearance may affect eating habits, food choices, nutrient intake, and ultimately nutritional status. As children reach their teen years they drink less milk and their calcium intake declines far below the current standard (1,300 mg/day) and even more so in comparison to the calcium intake threshold of 1,500 mg/day [14, 21]. Optimizing the calcium intake of young Americans along with increased energy expenditure (physical activity) is, therefore, of critical importance.

The World Health Organization and Food and Agricultural Organization of the United Nations in 2004 increased dietary calcium intake standards for children and adolescents [44] as the previous recommendations from 1962 were very low [45]. It has to be mentioned, however, that the standards for one ethnic group might not satisfy completely the requirements of the other. Each country should develop its own standards specific for the people living in the region. Factors like ethnicity, stature and body frame, dietary habits, determinants of calcium economy in the body (sodium intake, sunlight exposure), and activity level all play a role. Ideally standards should be based on calcium intake thresholds obtained from balance studies and/or whole body bone mass measurements by DXA (38 % of bone mineral content is calcium; worldwide accessible technique) at various calcium intakes. In the absence of metabolic wards or densitometry machines, simple but crude estimates could be based on body weight data.

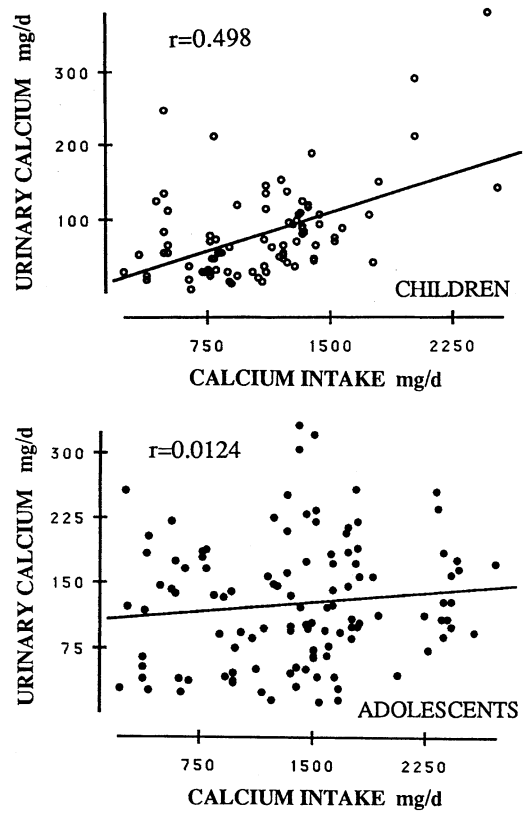

Fig. 13.11 Relationship between urinary calcium and calcium intake in children (*top*) and adolescents (*bottom*). The association is more pronounced during childhood than during adolescence mimicking a "hungry bone syndrome." Adapted from Matkovic 1991 [13]

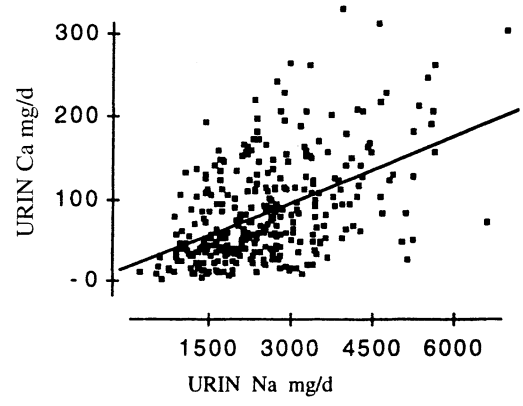

Fig. 13.12 The relationship between urinary calcium and urinary sodium in a cohort of pubertal females ($N=325$, R-squared = 25.0 %, $p<0.0001$). Adapted from Matkovic et al. 1995 [43]

The whole body calcium is about 0.8–1.5 % of body weight irrespective of ethnicity. Minimal variations are related to age (Fig. 13.13). The best example is the comparison of the absolute and relative body calcium (derived from DXA) between two ethnic groups known for the significant differences in body frames: Caucasians vs. Japanese. Japanese have lower amounts of total body calcium but a comparable ratio of calcium to body weight (Fig. 13.14).

Several studies indicated that children and teenagers particularly, may benefit from higher calcium intake with further gain in bone mass. This is important not only with regard to peak bone mass acquisition but also with regard to fracture prevention during growth. The peak incidence of the distal

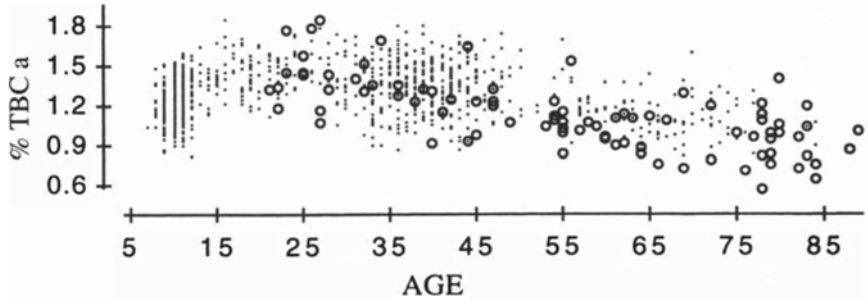

Fig. 13.13 Relative total body calcium content (% of body weight) in 1,218 females (1,128 Caucasian females, dots; 90 Japanese females, *open circles*) aged 7–89 years. The data based on the total body bone mineral content measurements by DXA technology and assuming Ca content is 38 % of the BMC. Relative calcium content increases from about 0.9 % in childhood to about 1.5 % in young adulthood and than declines in elderly women (~1.0 %). The relative calcium content per body weight is the same for Japanese and Caucasian women of different age. The data for Japanese women were kindly supplied by Drs. T. Fujita and A. Tomita

forearm fractures occurs during growth spurt and maximal bone modeling [33]. Fractures of the distal end of the forearm in adolescents may satisfy the criteria of bone fragility fracture comparable to the same in adults. One third of all fractures in children could not be related to a specific activity or environment, but rather to some other contributing factors like nutrition. Chan et al. [17] were first to suggest that low dietary calcium intake might contribute to bone fragility fractures in children. However, as the results of the study were based on a few cases, this fact remains to be confirmed. In a study in Palma de Mallorca, Spain, a significant difference in the fracture rate was found when cities with a high calcium content in their water (282 mg/L) were compared with those with a lower calcium content (86 mg/L) [46].

All of the clinical trials with calcium supplements in children and adolescents completed to date were relatively short in duration (12 months–3 years) and showed a positive effect of calcium on bone mass of young individuals [1, 28, 47–54]. The increase in bone mass observed in those studies could be explained to a large extent by the remodeling transient phenomenon emphasized recently by Heaney [55]. When calcium intervention in some of the studies was discontinued the difference in bone mass between the groups has been diminishing [56, 57], indicating that some of the gain has been lost as the result of the second transient [55]. In some studies the effect of intervention are still maintained 1–3 years upon discontinuation of dairy products, fortified foods, or calcium supplementation. This may be specific to the dairy products [50], the way how calcium supplements were given [52], and/or the level of calcium intake in the habitual diet [28, 58].

It is highly unlikely that calcium deficiency alone as found in the western diets could lead to a reduction in bone size. Calcium deficiency during rapid skeletal growth disturbs the balance in the internal bone remodeling leading to a reduction in bone mass. This defect is transient and can be recovered when bone modeling changes to bone consolidation phase with a rapid decline in calcium requirement (the drop in calcium intake threshold from ~1,500 mg/day to ~900 mg/day). Permanent reduction in bone mass at skeletal maturity (peak bone mass) is expected in young individuals whose internal bone architecture was not developed appropriately or was sacrificed as a result of a relative calcium deficiency to compensate for longitudinal bone growth and periosteal bone expansion (Fig. 13.15) [59]. The results of a long-term intervention study with calcium supplementation extending from childhood to young adulthood confirmed the above statements showing that both models are adequate depending on the relative degree of calcium deficiency in the habitual diet [24, 32]. The number of distal forearm fractures due to moderate trauma in this 7-year long study was higher in the calcium supplemented individuals, although the sample size was not designed to address this outcome variable [60].

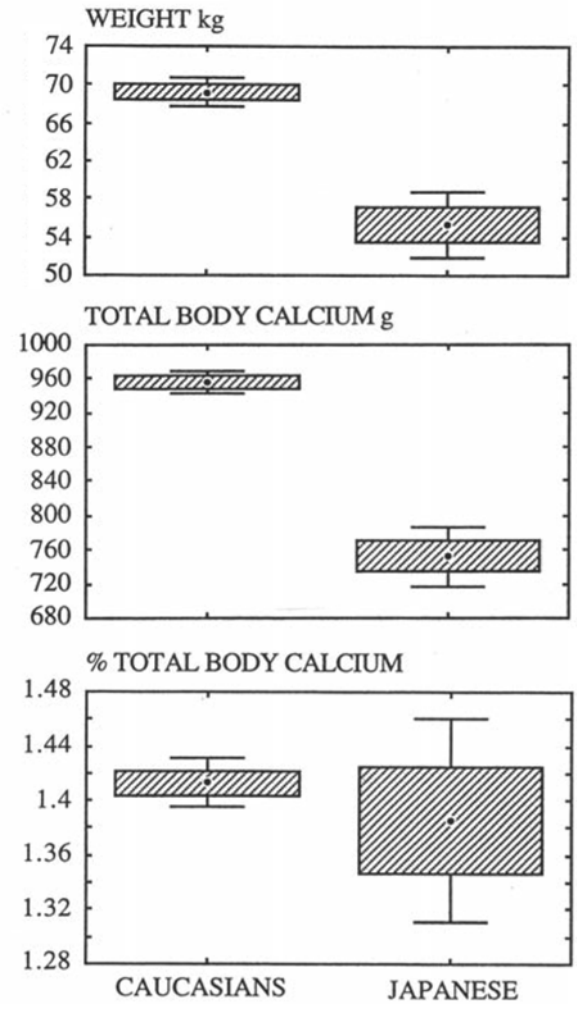

Fig. 13.14 Boxplots of body weight (*top*), total body calcium (*middle*), and percent total body calcium (*bottom*) in the group of young adult (18–50 years) Caucasian ($n=437$) and Japanese ($n=34$) premenopausal women. Results presented as mean ± SE ± 1.96SE. Significant differences are present for body weight and total body calcium, but not for relative body calcium to weight. Data for Japanese women kindly provided by Drs. T. Fujita and A. Tokita. The average total body calcium in the group of Japanese women is 752 ± 107 g while in Caucasians 956 ± 145 g. Subtracting 30 g of skeletal calcium at birth and assuming that most of the bone mass was accumulated by the age 18, the average Japanese and Caucasian women from the group were retaining 109 mg and 141 mg of Ca/day on average, respectively. Adapted from Matkovic et al. 2000 [61]

In addition to calcium, phosphorus is essential for normal bone and tooth formation and, therefore, plays a very important role during skeletal development. Out of about 700 g of phosphorus contained in the human body, approximately 85 % is in the bone while the remaining part is in the soft tissues, where it plays an important role in energy storage and release systems. Phosphorus is a ubiquitous element present in almost all the foods we consume. Most of the consumed phosphorus is excreted in the feces and in the urine. Phosphorus balance studies in adults showed that phosphorus output is equal to input at various intake levels from 700 to 1,800 mg/day indicating excellent adaptation. Due to the lack of balance studies at very low phosphorus intakes, Nordin concludes that it is almost impossible to calculate phosphorus requirements for adults [26]. There is presumably an intake below

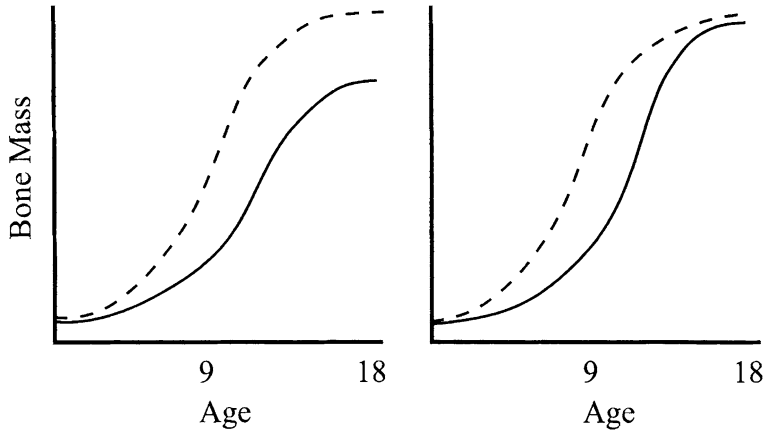

Fig. 13.15 Hypothetical models of bone mass accumulation with calcium supplementation during growth. A very low dietary calcium intake over time results in a permanent deficit in peak bone mass at the time of skeletal maturity (*left*). Recovery of the transient deficit in bone mass during growth by the time of skeletal maturity (*right*). Calcium supplemented individuals will reach peak bone mass earlier than nonsupplemented subjects. The final level of peak bone mass is the same in the two groups. Adapted from Matkovic 1999 [59]

which adult humans go into negative balance; however, this figure is unknown. As phosphorus is an essential component of calcium hydroxyapatite crystal, growing individuals should be in a positive phosphorus balance. As in adults, phosphorus output (urinary and fecal excretion) in adolescents is highly related to phosphorus input (dietary phosphorus). Most of the young females are in positive phosphorus balance of about 97 ± 17 mg/day irrespective of their phosphorus intake [61]. The regression line of phosphorus output on phosphorus intake for the particular intake range (800–2,000 mg/day) has a slope of 0.96 and is almost parallel to the line of equality, with an intercept of −58. The difference between the lines is due to phosphorus retention in the body required primarily for skeletal development. A positive phosphorus balance of about 150–200 mg/day is expected during pubertal growth spurt; age ~12. As sodium contributes to calcium excretion in the urine, it can also influence phosphorus excretion and affect phosphorus balance. As milk and dairy products are the main source of calcium in the diet, they are also a good source of phosphorus with a Ca/P ratio of 1.3. High calcium intake may negatively affect phosphorus absorption as documented for adults [62]. A similar phenomena exist during growth, however, the significance of this for young people has not been established (Fig. 13.16). This may be of importance for adolescents requiring calcium supplements, therefore disturbing Ca–P ratio in the diet with impact on skeletal mineralization due to a relative phosphorus deficiency. The consumption of milk should be encouraged among adolescents because it contains both minerals important for skeletal mineralization in a favorable ratio. The negative Ca–P ratio on the other side has no deleterious effects on bone of young individuals if calcium intakes meet the requirement for growth [61].

Protein–calorie malnutrition during childhood can cause growth retardation and decreased formation of cortical bone, and therefore can interfere with peak bone mass acquisition [63]. This is probably mediated by IGF-I and leptin through its effect on the reproductive function. Serum IGF-I is considered a biochemical marker of nutritional status, primarily protein intake (Fig. 13.17). Excessive protein intake and increase in protein consumption above the recommended allowance level can be associated with hypercalciuria; this, however, has not been confirmed in children [43]. A strong positive nitrogen balance is required for growth and, therefore, the amounts of proteins commonly consumed by young American have no influence on urinary calcium excretion.

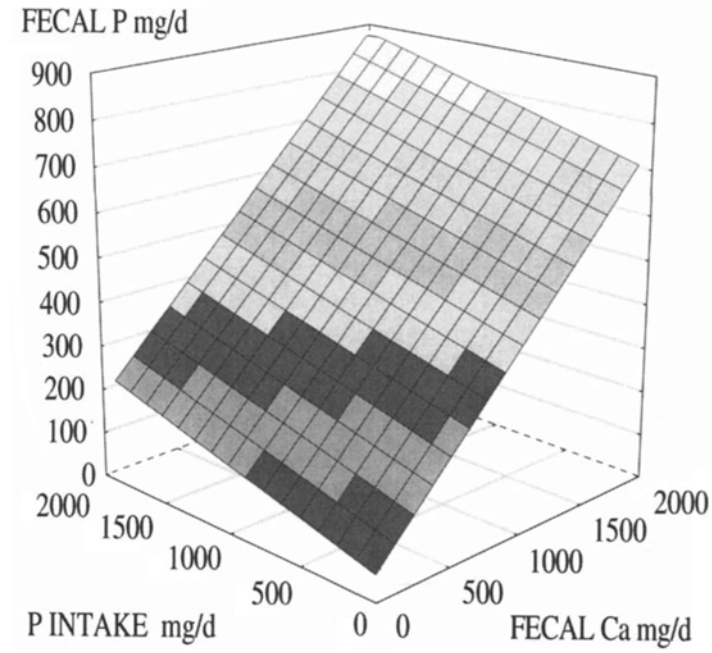

Fig. 13.16 Three-dimensional representation (surface plot) of the relationship between fecal phosphorus (z), fecal calcium (y), and phosphorus intake (x) in a group of adolescent females ($N=42$). Fecal calcium was the main determinant of fecal phosphorus ($p<0.0001$) while phosphorus intake had minimal effect ($p<0.095$). Phosphorus balances were collected from the same sources as calcium balances presented earlier, Matkovic et al.1991 [13]

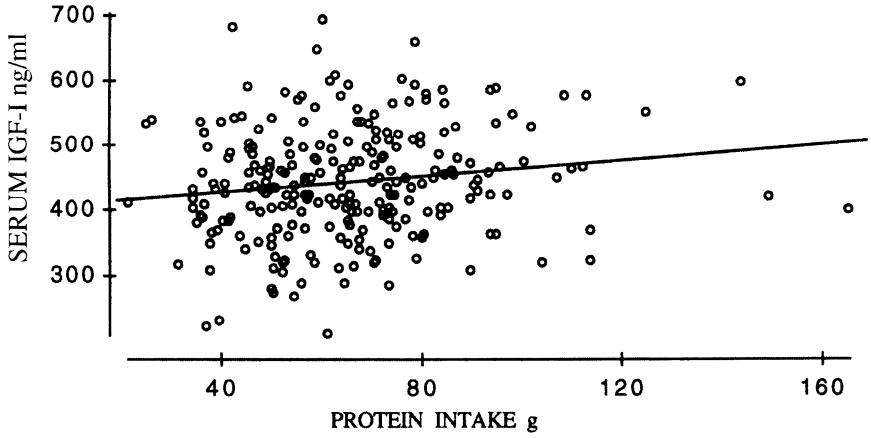

Fig. 13.17 Scatterplot with the regression line of the relationship between serum IGF-I and protein intake in a cohort of healthy adolescent females ($N=229$; In a stepwise regression analysis along with chronological age, skeletal age, and sexual maturity index, the effect of dietary protein on serum IGF-I was found to be significant at $p<0.010$, partial-R = 2.6 %) (IGF-I measured by Nichols-RIA in the laboratory of Dr. C. Rosen, Bangor, Main)

13.5 Nutrient Interactions During Childhood and Adolescence

The National Academy of Sciences, Food and Nutrition Board, as well as the National Institutes of Health Consensus Panel on Optimal Calcium Intake increased calcium intake standards for teenagers to 1,300 mg/day and 1,500 mg/day, respectively [21, 64]. However, there is a concern that high calcium intake may lead to decreased absorption of other important minerals. However, based on the results of several recent studies it can be concluded that high dietary calcium intake does not influence nutritional

status of some of the major minerals and trace elements (Mg, Fe, Zn, Se) in children and adolescents [65–68]. Therefore, public health measures to elevate calcium intakes among young Americans to the new standards (up to 1,500 mg/day) are safe recommendations and should not trigger a concern for the possible induction of iron deficiency anemia, hypomagnesemia, zinc deficiency, and growth retardation, or predispose young people to cardiomyopathy as the result of selenium deficiency.

13.6 Conclusion

Calcium, phosphorus, and proteins are essential for bone growth and skeletal development. Calcium deficiency has been associated with rickets in children, bone fragility fractures during pubertal growth spurt, and with inadequate peak bone mass formation by young adulthood. Mineral and protein requirements are the highest during pubertal growth spurt to allow for longitudinal bone growth and periosteal bone expansion. Calcium is a threshold nutrient, and dietary intake standards have been established for the various phases of growth and development. Public health measure should assure that all children and teenagers can meet those standards to allow for optimal skeletal health early in life.

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Chapter 14
Calcium and Vitamin D for Bone Health in Adults

Bess Dawson-Hughes

Key Points

- The calcium intake requirement is challenging to determine, and the IOM recommendations are based largely on calcium balance studies.
- The IOM recommends a calcium intake of 1,000–1,200 mg per day for older adults to support the preservation of bone mass.
- Food sources of calcium are preferred because higher intakes of calcium in supplement form have been associated with increased risk of kidney stones, and possibly also with increased risk of myocardial infarction and of death from heart disease.
- Vitamin D lowers fracture risk as a result of combined favorable effects on BMD, muscle performance, balance, and risk of falling.
- The magnitude of the risk reduction for falls and fractures with vitamin D supplementation is approximately 20 %.
- Daily, weekly, or monthly dosing of vitamin D_3 is recommended, and high, infrequent dosing should be avoided because it increases risk of falls and fractures.

Keywords Calcium • Vitamin D • Bone density • Falls • Fractures • Nutrition

14.1 Introduction

Osteoporotic fractures are common and devastating occurrences. Many lifestyle risk factors for osteoporosis have been identified and their effects are to a large extent additive. Low calcium and vitamin D intakes are established nutritional risk factors for osteoporosis. This chapter reviews the impact of these nutrients on calcium homeostasis and fracture risk and their role in the prevention and treatment of osteoporosis. It also reviews the impact of vitamin D on risk of falling, which is closely linked to fracture risk. Finally it considers the safety of calcium and vitamin D supplementation.

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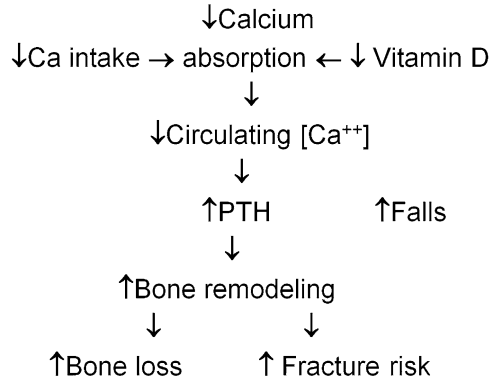

Fig. 14.1 Consequences of calcium and/or vitamin D insufficiency

14.2 Physiology

An inadequate intake of calcium and circulating level of vitamin D, commonly assessed as the serum 25-hydroxyvitamin D [25(OH)D] concentration, alone and in combination, influence calcium-regulating hormone levels. Deficiency of either nutrient results in reduced calcium absorption and a lower circulating ionized calcium concentration. The latter stimulates the secretion of parathyroid hormone (PTH), a potent bone-resorbing agent. Over time, a small increase in the circulating level of PTH leads to measurable and significant bone loss and increased risk of fracture (Fig. 14.1). Vitamin D insufficiency is associated with increased risk of falling, an established risk factor for fracture and other injuries (Fig. 14.1).

14.2.1 Calcium

Intestinal calcium absorption occurs by active transport and passive diffusion. In individuals with low to moderate calcium intakes, absorption occurs largely by active transport, a process mediated by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. As intake increases above approx 500 mg/day, passive diffusion accounts for an increasing proportion of calcium absorbed [1]. Estrogen has a direct effect on intestinal responsiveness to 1,25$(OH)_2$D [2]. There is an age-related decline in calcium absorption efficiency in men and women [3]. This may be related to loss of intestinal vitamin D receptors (VDRs) and/or resistance of gut VDRs to the action of 1,25$(OH)_2$D [4].

Calcium serves as a substrate to support the bone formation phase of bone remodeling in adults. Typically, about 5 mmol (200 mg) of calcium is removed from the adult skeleton and replaced each day. To supply this, one would need to consume about 600 mg of calcium, because calcium is not very efficiently absorbed. This intake estimate is an approximation, since some of the absorbed calcium would be excreted in sweat, urine, and feces and thus not available for deposition in bone and some of the resorbed calcium would be recycled back into bone at new remodeling sites. The amount of calcium needed to replace daily skeletal resorption losses might be thought of as the subsistence requirement. Several studies have demonstrated a significant impact of increasing calcium intake to the subsistence level in subjects with very low usual calcium diets. For example, supplementation with 500 mg/day of calcium had a greater positive effect on change in bone mineral density (BMD) among postmenopausal women with self-selected calcium intakes under 400 mg/day than among those with intakes in the range 400–650 mg/day [5]. Were calcium considered to be solely a substrate

for bone formation, the average calcium requirement might be about 600 mg (and the associated recommended dietary intake therefore about 800 mg/day).

The second mechanism by which calcium affects the skeleton is through its impact on the remodeling rate. A low calcium intake increases the remodeling rate. A high remodeling rate is an independent risk factor for fracture [6], perhaps because it causes more trabecular perforations (or greater architectural deformity). A high remodeling rate may also lead to incomplete mineralization at new remodeling sites. Many FDA-approved treatments for osteoporosis are antiresorptive agents. These treatments lower biochemical markers of bone turnover by 40–50 % and fracture rates by 35–50 %, but increase BMD by only 3–5 %. Dietary calcium at sufficiently high levels, usually 1,000 mg/day or more, lowers the bone remodeling rate by about 10–20 % in older men and women [7–9]. The degree of suppression appears to be dose related, as illustrated by Elders, who treated postmenopausal Dutch women with either 1,000 or 2,000 mg of supplemental calcium [8].

14.2.2 Vitamin D

Vitamin D is acquired from diet and from skin synthesis. Serum 25(OH)D levels decline with aging for several reasons. There is a decline in the amount of 7-dehydrocholesterol, the precursor to vitamin D, in the epidermal layer of skin with aging [10]. In addition to having a decreased capacity to photosynthesize vitamin D, older people often avoid sun exposure or use sun screens that block access of ultraviolet B rays to the skin. There does not appear to be any impairment in the intestinal absorption of vitamin D with aging [11].

Season is a major determinant of 25(OH)D levels in people residing in the temperate zone. At 42° North (the latitude of Boston), skin synthesis of vitamin D does not occur between October and March. In healthy postmenopausal women in Boston, mean 25(OH)D levels ranged from 25.2 ng/ml (63 nmol/L) in March to 38 ng/ml (95 nmol/L) in August [12]. In these women, serum PTH levels varied inversely with the serum 25(OH)D levels [12]. Higher wintertime levels of PTH raise the possibility that bone loss may be increased during the wintertime. In two prospective studies, bone loss from the spine [13] and femoral neck [14] was greater in the 6-month period when PTH levels were highest (winter/spring) than in the 6 months period when PTH levels were lowest (summer/fall). In both of these studies, supplementation with vitamin D preferentially attenuated wintertime bone loss.

14.3 Defining Requirements

14.3.1 Indicators of Calcium Adequacy

14.3.1.1 Intake Associated with Maximal Calcium Retention

Historically calcium balance studies have been performed to identify the intake associated with the zero balance, or the intake at which calcium is neither lost nor gained from the body. Hunt and Johnson compiled balance data from 155 young adults studied on calcium intakes ranging from 415 to 1,740 mg per day [15]. Zero balance was achieved at an intake of 741 mg per day. Balance studies may also be used to identify the intake associated not with zero balance but with the most favorable balance that can be achieved as a result of increasing calcium intake. Increasing intake above the level associated with maximal calcium retention would result in more calcium being absorbed, but that calcium would be excreted rather than retained. Spencer [16] performed balance studies in 181 men,

aged 34–71 years, at six different calcium intake levels ranging from 234 to 2,320 mg per day. Diets were supplemented with either calcium gluconate or with milk for periods averaging 20–38 days. Although the zero balance point in this study occurred at an intake of 800 mg/day, calcium retention increased significantly with increasing intake up to a maximum of about 1,200 mg/day, in both the calcium gluconate and milk groups. If short-term balance studies define longer term patterns, then greater retention would signify greater bone mass.

14.3.1.2 Calcium and Changes in BMD

Many randomized, controlled calcium-intervention trials with change in BMD as the primary end point have been reported. In a meta-analysis of 13 trials, calcium induced significant mean gains (or slowed loss) of 3 % at the spine, 2.6 % at the femoral neck, and 0.6 % at the distal forearm [17]. Mean differences after the first year were also positive, but smaller. The relatively strong initial response to calcium is attributed to closure of remodeling space. A more recent meta-analysis of 15 trials found that calcium caused positive mean percentage changes from baseline of 1.66 % at lumbar spine, 1.64 % at the hip, and 1.91 % at the distal radius [18].

Time since menopause appears to influence the impact of calcium on changes in BMD. In the first 5 years after menopause, rapid bone loss occurs as a result of declining estrogen levels. The increased bone resorption that accompanies estrogen deficiency provides calcium to the blood and other extracellular space. As a result, serum PTH levels decline, $1,25(OH)_2D$ levels decline, and the stimulus to absorb calcium declines. Increasing calcium intake does not entirely reverse this sequence and cannot be relied upon to prevent early menopausal bone loss, although it has in some studies attenuated it [8, 19]. Calcium is generally effective in older postmenopausal women who have completed their skeletal adaptation to the loss of estrogen [5]. In large enough doses, calcium can reverse age-related increases in serum PTH and in bone remodeling [20]. In one trial, the effects of calcium from food (milk powder) and supplement sources on changes in BMD in older postmenopausal women were compared and found to be similar [21].

14.3.2 Indicators of Vitamin D Adequacy

14.3.2.1 Maximal Suppression of PTH and Bone Loss

Declines in serum 25(OH)D trigger compensatory increases in circulating PTH concentrations. Malabanan et al. [22] assessed serum PTH responses to treatment with vitamin D according to the initial 25(OH)D levels of the subjects. In this study, 35 subjects were treated with 50,000 IU/week of vitamin D for 8 weeks. Subjects with 25(OH)D levels below 50 nmol/L (20 ng/mL) had significant declines in serum PTH with treatment, but those with initial 25(OH)D concentrations above this level did not. Vitamin D adequacy in older adults is sometimes defined as the level of 25(OH)D needed to maximally suppress serum PTH levels. Typically, the association is not linear but hyperbolic, as illustrated in Fig. 14.2 [23]. The participants in this study were healthy men and women aged 65 years and older. The data were fitted with several models, the exponential, LOESS, and three-phase models. By the three-phase model, there appeared to be a rapid change in PTH at 25(OH)D levels up to 12 ng/ml (30 nmol/L), slower change in the 25(OH)D range of 12–28 ng/ml, and no change at 25(OH)D levels above 28 ng/ml. In previously reported studies, depending upon the 2-phase model used, two clusters for a single 25(OH)D threshold have been reported, 15–20 ng/ml [24] and 30–32 ng/ml [25]. Inverse associations of serum 25(OH)D with PTH levels have been documented in adolescent girls [26] and in healthy young men and women [27], but inflection points have not been established in these younger populations.

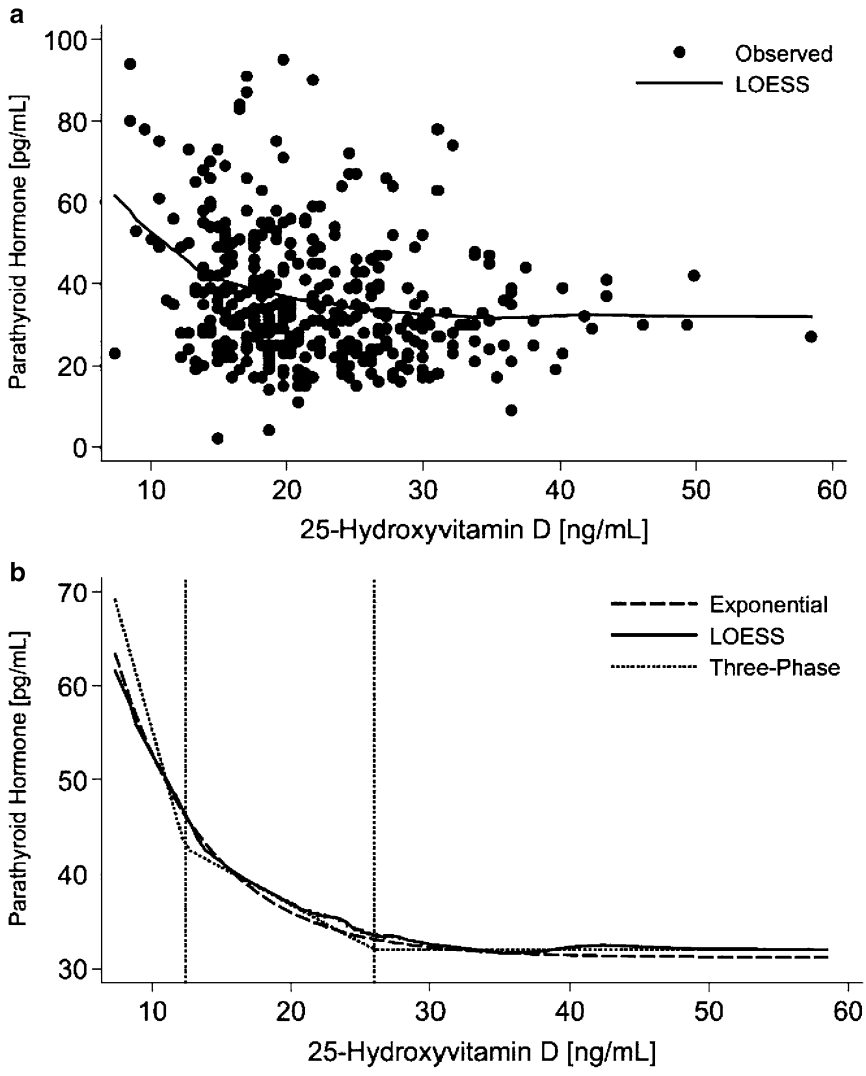

Fig. 14.2 Relation between serum 25(OH)D and parathyroid hormone (PTH). For PTH, 1 pg/mL = 1 ng/L; for 25(OH) D, 1 ng/mL = 2.5 nmol/L (From Durazo-Arvizu et al. [23], with permission.)

Consistent with the reductions in serum PTH, several studies have documented that supplementation with vitamin D lowers rates of bone loss by 1–2 % over 1–2 years in older adults [13, 28], with, as indicated above, most of the reduction occurring during the winter season [13].

14.3.2.2 Vitamin D, Muscle Performance, and Falls

Severe vitamin D deficiency causes proximal muscle weakness and pain [29, 30]. Mild vitamin D insufficiency, although usually asymptomatic, appears to influence muscle performance in the general older population. In 4,100 ambulatory adults aged 60 years and older participating in The Third National Health and Nutrition Examination Survey (NHANES III), lower extremity muscle performance, measured as the 8-ft walk test and the repeated sit-to-stand test, was poorest in subjects with

the lowest 25(OH)D levels and was progressively better at higher 25(OH)D levels throughout and even above the upper end of the reference range [31]. A similar association was observed in a prospective cohort of older Dutch men and women [32]. In the latter study, however, performance reached its maximum at a 25(OH)D level of about 20 ng/ml (50 nmol/L). In a meta-analysis of controlled vitamin D intervention trials with muscle performance end points, Stockton and colleagues found that vitamin D improved lower extremity muscle strength only in individuals with starting serum 25(OH)D levels below 10 ng/ml (25 nmol/L) [33]. The active form of vitamin D, $1,25(OH)_2D$, acts on muscle by binding to classical nuclear VDRs. VDRs have been identified in human muscle and been shown to decline as a function of aging [34]. However, it is not universally agreed that VDRs exist in human muscle [35].

Poor balance is a known risk factor for falling. Balance has been measured in several clinical trials by measuring the degree of sway in the anterior–posterior and medial–lateral directions in subjects standing on a force platform. Two trials compared the effect of 800 IU of vitamin D_3 plus 1,000 mg of calcium per day with 1,000 mg per day of calcium alone on sway in elderly adults. The vitamin D groups had an up to 28 % improvement (reduction) in body sway over periods of 2 and 12 months, when compared with the calcium alone groups [36, 37]. These studies implicate a role for vitamin D supplementation in improving balance in elders, and improved balance may be an important means by which vitamin D reduces risk of falling.

The impact of vitamin D on risk of falling has been examined in several randomized controlled trials and in as many meta-analyses. In one meta-analysis, risk of falling was significantly reduced, by 20 %, in the trials administering 700–1,000 IU per day of vitamin D, whereas doses of 400 IU per day were ineffective [38]. In a different meta-analysis, the risk reduction with vitamin D was 17 % when compared with placebo [39]. Several studies have evaluated the effect of higher doses of vitamin D on risk of falling. In acute hip fracture patients, the proportion falling within the following year did not differ in those randomized to 2,000 IU compared with 800 IU per day of vitamin D_3 [40]. This finding argues against the need for doses higher than 800 IU to lower falls. A trial in 686 women aged 70 years and older reported a null effect of 150,000 IU of vitamin D_3 given orally every 3 months, compared with placebo, on falls and physical function [41]. The effect of this large change on 25(OH)D metabolism is unknown. While further evidence is needed, it appears that vitamin D intake in the range of 700–1,000 IU per day is the amount needed for maximal protection against falling.

14.4 Impact of Calcium and Vitamin D on Fracture Rates

14.4.1 Calcium

Several meta-analyses of randomized, placebo-controlled trials have addressed whether supplemental calcium lowers fracture risk. The Shea meta-analysis [18] of trials in postmenopausal women found that although calcium supplementation increased BMD at every measured site, as indicated earlier, it did not significantly lower risk of vertebral fracture (RR 0.77 [95 % CI 0.54–1.09]) or non-vertebral fractures (RR 0.86 [CI 0.43–1.72]). The 15 trials included in this analysis administered calcium doses of 500–2,000 mg per day over periods of 18 months to 4 years. A subsequent meta-analysis found no significant effect of calcium supplementation on risk of non-vertebral fracture (RR 0.92 (95 % CI: 0.81, 1.05)) and a significant *increase* in risk of hip fracture on calcium (RR 1.64 [95 % CI: 1.02, 2.64]) [42]. A third meta-analysis confirmed the significant *increase* in hip fracture risk (RR 1.50 [95 % CI: 1.06, 2.12]) [43]. Thus the weight of the evidence is that supplemental calcium alone will improve BMD modestly but it has not been demonstrated to be effective in preventing fractures.

Most of the trials have been conducted in Europe and North America where calcium intakes are generally higher than other parts of the world. Consequently, this observation does not necessarily apply to populations with lower usual calcium intakes.

14.4.2 Vitamin D Alone and with Calcium

The effect of vitamin D alone on fracture incidence has been examined in several trials. Heikinheimo et al. [44] reported that an annual intramuscular injection of 150,000–300,000 IU of vitamin D reduced fracture rates in older men and women. Trivedi et al. found that supplementation with 100,000 IU of vitamin D every 4 months (~800 IU per day) significantly lowered risk of non-vertebral fracture risk over 5 years in community-dwelling older men and women [45]. In contrast, a large controlled trial found that supplementation with 400 IU/day of vitamin D did not alter hip fracture rates in elderly Dutch men and *women* [46]. More recently, supplementation with 400 IU/day of vitamin D as cod liver oil was found to have no effect on hip or other fracture rates in very elderly men and women (mean age 85 years) in Norway [47]. Similarly, supplementation of early postmenopausal Finnish women with 300 IU/day of vitamin D had no impact on non-vertebral fracture rates [48]. It appears that a dose of vitamin D higher than 400 IU/day is needed to lower risk of fracture. In the Norwegian and Finnish studies cited above, an inadequate calcium intake, mean ~450 mg, may also have contributed to their lack of skeletal responses to the vitamin D.

A recent meta-analysis pooled individual-subject level data from 11 randomized, double blind, placebo-controlled trials of oral vitamin D given daily, weekly, or monthly in men and women aged 65 years and older [49]. This analysis took into account not only the dose administered but the amount actually taken. Several of the trials including the Women's Health Initiative (WHI) allowed personal supplement use, and many women in that trial took their own vitamin D as well as study pills. The calculated actual dose in this meta-analysis also accounted for the proportion of study pills consumed, according to pill counts. Figure 14.3 displays the relative risk of hip (upper panel) and non-vertebral (lower panel) fracture by quartile of actual intake, compared with control, after adjustment for study, age group, sex, and type of dwelling. Fracture risk reduction was significant only in the highest quartile of vitamin D intake. Individuals in that quartile had a 30 % reduction in risk of hip fracture and a 14 % reduction in risk of non-vertebral fracture when compared with controls ($P<0.0125$ for both).

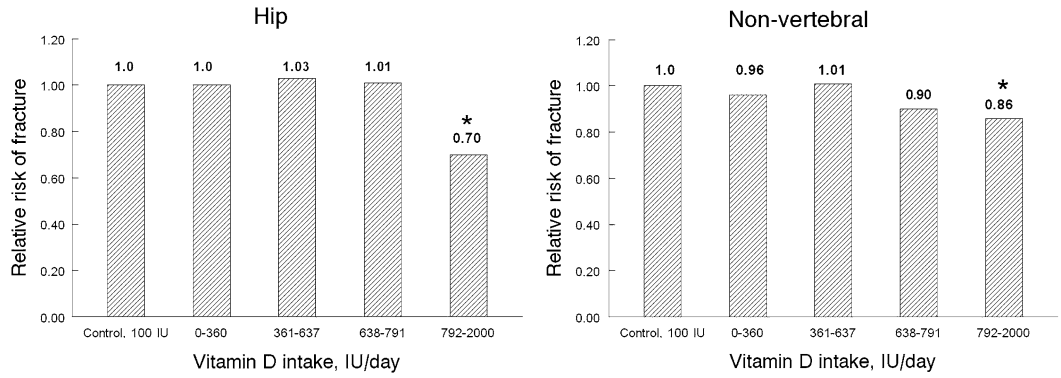

Fig. 14.3 Risk of hip fracture (*left panel*) and non-vertebral fracture (*right panel*) by quartile of actual daily vitamin D intake among 21,241 participants from eight trials that used vitamin D along with any dose of calcium [49]. Analyses were adjusted for study, age group, sex, and type of dwelling. *indicates difference from control, $P<0.0125$

Although the range of intake in that quartile was 792–2,000 IU per day, the median intake was 800 IU per day, indicating that most individuals' intake was in the lower part of that range. Benefits of the highest level of intake were fairly consistent in subgroups defined by age, type of dwelling, baseline 25(OH)D levels, and additional calcium intake. The main conclusion from this study was that an intake of at least 800 IU per day is needed to reduce fracture risk in men and women aged 65 years and older.

14.4.3 Discontinuation of Calcium and Vitamin D

The impact of discontinuing calcium and vitamin D supplements on BMD was illustrated in healthy older men and women treated for 3 years with either 700 IU of vitamin D_3 with 500 mg of calcium or placebo and then follow for 2 years after the study ended [50]. During the 2-year follow-up interval, the subjects were free to take any supplements they chose and were reminded of the current Institute of Medicine (IOM) recommendations for vitamin D and calcium. The subjects returned for BMD measurements at annual intervals. Over the 2-year follow-up period, all gains in BMD at the spine and hip were lost. This occurred despite the fact that half of the subjects reported taking calcium and vitamin D supplements some of the time during the 2-year period. The reversal in BMD was accompanied by a reversal in the suppression of bone turnover markers, and a presumed (but not measured) loss of protection against fractures. In order to sustain the benefits of increased calcium and vitamin D intakes, the higher intakes need to be maintained.

14.5 Calcium and Vitamin D and Pharmacotherapy

In the pivotal trials testing the anti-fracture efficacy of the antiresorptive therapies [51–54] and the anabolic drug, PTH 1-34 [55], calcium and vitamin D supplements were given to both the control and intervention groups. This allows one to define the impact of the drug in calcium and vitamin D-replete patients and to conclude that any efficacy of the drug is beyond that associated with calcium and vitamin D alone. One cannot conclude that these drugs would have the same efficacy in calcium- and vitamin D-deficient patients. Therefore it is important that patients taking pharmacotherapy for osteoporosis have adequate intakes of calcium and vitamin D intakes.

14.6 Intake Recommendations

14.6.1 Calcium

Calcium intake recommendations vary worldwide and the IOM recommendations for the USA are among the highest. The recommended intake of calcium for women aged 51 years and older is 1,200 mg per day [56]. For men, the recommendations are 1,000 mg per day for ages 51–70 years and 1,200 mg per day for age 71 and older [56]. The tolerable upper limit is 2,000 mg per day for men and women [56].

14.6.2 Vitamin D

The IOM vitamin D intake recommendations for men and women are 600 IU per day for ages 51–70 and 800 IU/day for age 71 and older. The safe upper limit is 4,000 IU/day [56].

14.7 Safety

14.7.1 Calcium

Calcium intake affects risk of kidney stones and may also affect risk of coronary artery disease. The association of calcium intake with of kidney stones risk depends upon the source of the calcium. In 1993, Curran and colleagues reported that in older men, higher calcium intake (>1,098 mg compared with <488 mg per day) was associated with a 44 % *lower* risk of kidney stones [57]. They later confirmed the protective effect of calcium from food sources in women in the Nurses' Health Study, but noted that calcium from supplement sources in doses of 500 mg per day and higher was associated with a 21 % *higher* risk of stones [58]. This issue was examined directly in the WHI, a 7-year trial in which women were randomized to daily treatment with 1,000 mg of supplemental calcium plus 400 IU vitamin D or placebo. The women in the supplemented group had a 17 % higher stone risk compared with women taking placebo [59] and that the stone risk was apparent only after 4–5 years of supplementation [60]. This extended time course may explain why stones have not been documented in most trials, which have been of shorter duration.

In 2008, Bolland and colleagues introduced the possibility that use of calcium supplements may increase risk of myocardial infarction (MI) [61]. This resulted from a secondary analysis of their trial in which 1,471 older women with usual mean calcium intake of 850 mg per day had been treated with 1,000 mg of supplemental calcium or placebo, daily for 6 years. This was a surprising observation, in view of earlier observations that calcium supplementation has a favorable effect on two major risk factors for MI, lipid levels [62] and blood pressure [63]. Moreover, in the Framingham cohort, higher calcium intake was not associated with increased coronary artery calcification [64]. Bolland and colleagues then published the results of a meta-analysis of calcium intervention trials in which they concluded that calcium supplements without vitamin D are associated with an increased risk of MI [65]. Like all meta-analyses, this one had its limitations. Among these was the fact that the conclusion relied upon the Grant RECORD Group trial [66] which contributed more than half of the data in the analysis. In this study of men and women aged 70 years and older, MIs were self-reported on a mailed-out questionnaire containing a checklist of possible reasons for hospitalizations; the MIs were not verified. Moreover, the MI data had not previously been reported, and many important details were not described. We do not know the return rate of the questionnaires, but we do know that it was lower in the calcium supplemented group than the group taking no calcium. Also compliance was poor—only half of the participants were taking their study supplements at the midpoint of the 5-year study—and the impact of compliance on the results is unknown. In a similar meta-analysis by Wang that did not include the unpublished data from the RECORD Group trial, calcium had no significant effect on MI risk [67]. In neither of these meta-analyses did the combination of calcium and vitamin D increase risk of MI. Trials identifying no significant effect of calcium supplementation on MI risk include a 5-year trial in 1,460 women treated with 1,200 mg per day or placebo [68] and the large WHI [69]. Bolland subsequently reported that in the subset of women in the WHI who did not take their own personal calcium supplements, study calcium increased their risk of MI [70]. This unplanned subgroup analysis of a secondary analysis is difficult to interpret.

A couple of large observational studies have examined the association of calcium supplement use with cardiovascular risk. In the Heidelberg cohort, Li and colleagues found a positive association between self-reported calcium supplement use and MI [71]. In a large study by Xiao in 388,229 adults age 50–71 years who were followed for 12 years, the men in the highest quintile of calcium supplement use (≥1,000 mg per day) had a 19 % higher risk of death from heart disease than men not taking calcium supplements [72]. In the women, there was no increased mortality with supplement use in any amount. In the men and women, there was no increase in risk of death related to calcium from food sources. In this study, past and current smoking seemed to augment the adverse association of supplemental calcium use with death from heart disease. Collectively the available evidence includes enough of a signal to warrant caution in recommending routine use of high doses of supplemental calcium.

14.7.2 Vitamin D

The IOM has placed the safe upper limit for vitamin D at 4,000 IU per day based on lack of evidence that intake at this level has safety concerns. There is evidence that high infrequent doses of vitamin D (i.e., 500,000 IU orally once per year) is detrimental, in that it increased risk of both falls and fractures in older adults [73]. The reason for this is unknown. It has been observed that a single oral dose of 600,000 IU of vitamin D_3 precipitously raised serum 25(OH)D concentration from 15 to 77 ng/ml (37.5–192.5 nmol/L) 3 days later [74]. Such a shift may have altered the metabolism of the vitamin or precipitated dramatic changes in bone remodeling, but this is speculative.

14.8 Clinical Recommendations

The evidence cited above suggests that to meet the calcium requirement, food sources should be used to the greatest extent possible by adults. For adults in the general population and those on pharmacotherapy for osteoporosis who cannot meet calcium needs with food, supplements may be used to bring the total intake to the recommended level of 1,000–1,200 mg per day. In the unusual event that more than 500 of supplemental calcium is needed, the dose should be split for better absorption [75]. If calcium carbonate is used, it should be taken with a meal for more consistent absorption [76].

For the general adult population, the IOM recommends 600–800 IU per day of vitamin D, based on the effect of vitamin D in lowering fracture risk [56]. Individuals at high risk for low 25(OH)D levels, including obese subjects and those with little effective sun exposure, may need higher intakes to achieve the desired 25(OH)D level. The IOM places that 25(OH)D level at 20 ng/ml (50 nmol/L) [56], whereas clinical guidelines generally recommend a target 25(OH)D level of 30 ng/ml (75 nmol/L) [77, 78]. Vitamin D supplements should be given at daily, weekly, or monthly; high infrequent dosing raises safety concerns [73]. Most supplements are vitamin D_3. This form is preferred over vitamin D_2 because it gives a larger serum 25(OH)D increment [79]. Additionally, with monthly dosing, vitamin D_3 gives a constant increment whereas after vitamin D_2, the serum 25OHD level starts to decline after about 2 weeks.

14.9 Conclusions

Based on evidence from calcium balance studies, the IOM recommends 1,000–1,200 mg per day of calcium in order to support bone mass in men and women aged 50 years and older. Food sources of calcium should be used to the greatest extent possible, with supplements taken only as needed to bring

total calcium intake up to the recommended level. Exceeding the requirement with supplements should be avoided since it offers no benefit and may be associated with increased risk of kidney stones and possibly also heart disease. Vitamin D is needed for bone health and also for muscle performance, balance and for reduced risk of falling. Several controlled trials have documented approximately a 20 % reduction in risk of falling with supplementation. Individuals without regular effective sun exposure will need vitamin D supplements. These supplements should be taken daily, weekly, or monthly; high, infrequent dosing should be avoided because it increases risk of falls and fractures.

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Chapter 15
Nutrition: To Supplement or Not to Supplement the Elderly

Sandra Iuliano

Key Points

- By 2050, people aged over 80 years will number almost 380 million.
- Fracture risk increases with age therefore the elderly will contribute significantly to the fracture burden.
- Dietary modifications, specifically improving protein, calcium, and vitamin D intakes may reduce fracture risk.
- Relationships between protein, calcium, and vitamin D and fracture risk in community-dwelling elderly is inconclusive, although few are truly deficient in these nutrients.
- Nutrient deficiencies are more common in institutionalized elderly in whom fracture risk is high and potential methods of treatment could include supplementation, fortification or dietary changes.
- Combined calcium and vitamin D therapy in institutionalized elderly demonstrated fracture risk reduction of ~30–40 %.
- No studies have specifically investigated the anti-fracture efficacy of oral nutritional supplements in the elderly, but falls reduction has been observed in hospital settings.
- Food fortification with calcium and vitamin D has shown beneficial to bone with reductions in PTH, bone resorptive markers and bone loss reported, but the anti-fracture efficacy has not been investigated.
- Ensuring nutritional adequacy in the elderly may prove vital to curb the fracture burden.

Keywords Bone loss • Calcium • Elderly • Falls • Fractures • Nutrition • Protein • Supplements • Vitamin D

15.1 Introduction

Fracture risk increases with age and is highest in institutionalized elderly, in whom nutrient deficiencies are common, in particular for protein, calcium, and vitamin D. Targeting nutritional interventions at this group is ideal as the high prevalence of nutrient deficiencies, that are amendable to treatment, means fewer will require treatment to prevent fractures.

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This chapter explores the relationships between protein, calcium, and vitamin D, and fracture risk in the elderly. The anti-fracture efficacy of nutritional intervention in the elderly such as supplementation, fortification, and dietary changes is reviewed, with a particular focus on elderly in institutional care. Future research directions are explored in an attempt to offset the growing fracture burden due to the aging of the population.

15.2 Fragility Fractures—A Global Problem

Worldwide, the population is expanding, and life expectancy lengthening. By the year 2050, the number of people aged over 80 years is projected to be almost 380 million, or 4 % of the world's population. One in ten people in developed countries will be 80 years or older [1]. As the population ages, the burden of fractures will also increase. Globally, nearly nine million fragility fractures occur annually [2]. Based on current trends, it is projected that by 2050, the worldwide incidence of hip fractures alone, will increase by ~300 % in males, ~200 % in females, and will total over six million hip fractures yearly [3]. To curb the contribution of the elderly to the total future burden, effective strategies to reduce fracture risk in the elderly are needed.

Aging is characterized by two distinct changes that contribute to fracture risk. Firstly, bone loss accelerates in old age, predominantly in cortical bone, as trabecular bone diminishes. This results in cortical thinning and increased porosity, collectively compromising bone strength and resistance to fracture following minimal trauma [4]. Secondly, muscle mass is lost, increasing the risk for falls. This loss of muscle mass, termed sarcopenia, is associated with loss of strength, functional impairment, disability, and mortality [5]. Loss of muscle mass is more evident in persons over 70 years of age with loss of muscle fibers, particularly atrophy of fast twitch (Type II) fibers, the first to be recruited to avoid a fall [6, 7]. Falls prevention may contribute to fracture risk reduction given that 90 % of hip fractures result from falls [8].

A large portion of the fracture burden comes from the elderly [9, 10]. Drug therapy is not an option, as the cost of treatment would be greater than the disease itself, and side effects are common. Alternative methods of reducing fracture risk that are safe, accessible to all, and cost-effective require investigation. Correcting nutrient deficiencies, especially for protein, calcium, and vitamin D may be one approach. While fracture risk in an individual attributable to deficiencies of these nutrients may be small, the high prevalence of deficiencies in the elderly confers a high attributable risk, so shifting the elderly population to a higher level of intake may have a large net effect [11, 12]. Blood pressure has been lowered by reducing salt intake in the population and is seen as an effective means of reducing the burden of stroke and cardiac events [13, 14]. The principle of using dietary modifications instead of drug therapy to reduce cardiac events can be applied to increasing intakes of protein, calcium, and vitamin D to recommended levels in the elderly to reduce fractures, and may be a realistic option for fracture prevention in the community. This chapter focuses on nutritional methods to reduce fracture risk in the elderly. The specific roles of these nutrients on bone health are covered more extensively in other chapters.

15.3 Nutrition and Fracture Risk in the Elderly

15.3.1 Protein Deficiency and Fracture Risk

No consistent relationship between protein intake and fracture risk has been identified in older adults [15–21]. However, protein deficiency is uncommon in healthy people in community-based studies, with mean protein intakes of ~1 g/kg body weight reported, so the effect of protein deficiency on

fracture risk is difficult to identify. Moreover, it is unclear how accurately single dietary assessments reflect normal long-term dietary protein intake. A further confounder is the source of protein. The acid content of meat-based proteins may exert a detrimental effect on bone, as calcium is released to restore acid-base balance, while vegetable-based proteins are accompanied by basic precursors, so do not generate acidosis and are less detrimental to bone [22].

Protein deficiency is more likely in the elderly, particularly those in institutional care, where rates of malnutrition are high [23–25]. Between 30 and 60 % of elderly in the community are malnourished or at risk of malnutrition, with rates of up to 90 % observed in hospital or rehabilitative settings [24]. About 65 % of elderly in residential care (equivalent to assisted care in the USA) are malnourished [26, 27]. Wikby et al. (2006) observed that between 32 and 38 % of elderly people admitted to residential aged-care were protein–energy malnourished [28]. In a sample of ambulant elderly in residential care, more than 50 % of males and nearly 66 % of female consumed below the recommended intake for protein [27].

A mechanism for an effect of protein on bone is not clearly defined but protein deficiency is associated with accelerated bone resorption, impaired bone formation, and sarcopenia [29, 30]. In the aging animal model, protein deficiency induced in male rats was associated with reduced bone formation, with a less marked change in bone resorption, while in female rats, bone resorption was elevated, with limited formation, resulting in cortical thinning and loss of bone strength [31–33]. Reduced IGF-1 was also observed. Garnero et al. (2000) reported an association between increased fracture risk and low IGF-1 levels, independent of BMD, in healthy postmenopausal women [34, 35].

It has been suggested that an adequate protein intake increases IGF-1, promotes calcium absorption, and stimulate muscle protein synthesis, potentially slowing bone loss and muscle wasting [36]. Current recommended protein intakes in the elderly range from 0.75 to 1.0 g/kg body weight daily however, these recommendations were based on nitrogen balance studies in young adults in whom protein needs may differ to the elderly [36, 37]. Some authors have suggested that moderately higher protein intakes of between 1 and 1.3 g/kg/day are required to maintain nitrogen balance in the elderly, due to observed reductions in protein synthesis efficiency and insulin action in older individuals [38]. The recent recommendations by the PROT-AGE study group suggest for elderly people with acute or chronic diseases protein needs are heightened, and intakes of 1.2–1.5 g/kg/day are well tolerated, expect for those with severe kidney disease or on dialysis [39].

15.3.2 Calcium Deficiency and Fracture Risk

Meta-analyses of calcium studies indicate no clear relationship between total calcium intake (with or without supplementation) and fracture risk [40, 41]. Bischoff-Ferrari et al. (2007) reported no relationship between dietary calcium intake and hip fracture risk in women (RR = 1.01, 95 % CI: 0.97–1.05) or men (RR = 0.92, 95 % CI: 0.82–1.03), non-vertebral fractures and calcium intake (+supplementation) in women (RR = 0.92: 95 % CI: 0.81–1.05), and in fact an increased risk of hip fracture in women with calcium intake (including supplementation) (RR = 1.64, 95 % CI: 1.02–2.64) [40]. Nieves et al. (2008) observed no relationship between calcium intake and osteoporotic fractures over 3 years in 70,000+ postmenopausal women [41]. In contrast, in the Swedish Mammography study involving 60,000+ women followed for over 19 years, an increased risk of first fracture (RR = 1.18, 95 % CI: 1.12–1.25) or hip fracture (RR = 1.29, 95 % CI: 1.17–1.43) was observed for those in the lowest quintile for calcium intake (<751 mg/day) compared to the reference intake (882–996 mg/day), after adjusting for various confounders. Moreover, a 15–17 % increased risk of hip fracture in the highest quintile for calcium (>1,184 mg/day; including supplemental calcium) compared to the reference intake was observed (Fig. 15.1.) [42]. Whether increased fracture risk in high calcium consumers is due to those with a propensity for fractures augmenting their intake or due to suppression of bone turnover delaying fatigue repair of bone is unclear [43].

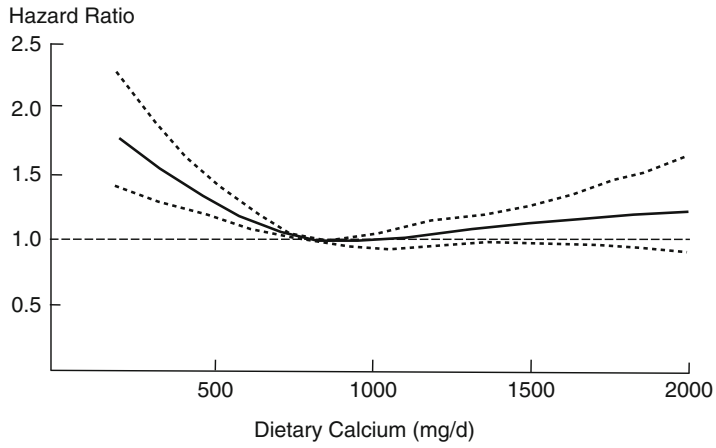

Fig. 15.1 Association between total calcium intake and hip fracture risk in postmenopausal women (adapted from [42])

Calcium intakes below recommended levels are common in the elderly [44, 45]. Median calcium intake for Australian women aged >65 years from the National Nutrition survey was 619 mg/day [12]. Mean calcium intakes of <600 mg/day are reported in institutionalized elderly women [46, 47]. The FAO/WHO report fracture risk is higher in those consuming 400–500 mg/day of calcium [48]. Women with reported lactose intolerance, who avoid consuming milk, have lower calcium intakes than women who drink milk (570 mg/day vs. 850 mg/day) and a 33 % greater risk of fractures [49].

Low calcium intake is reported to increase bone remodelling intensity, and result in an apparent reduction in BMD, and bone loss if BMU balance is negative [50]. Markers of bone turnover are lowered with calcium supplementation, but a reversal of skeletal benefits is observed after calcium (and vitamin D) supplementation is ceased likely resulting from remodelling intensity returning to pre-supplemented levels [51].

Consensus on a recommended calcium intake to reduce fracture risk has not been reached, likely due to lack of agreement between available data, uncertainties regarding calcium balance study methodologies, and insufficient longitudinal data on calcium intake and bone loss [52]. Despite this the FAO/WHO recommended calcium intake for postmenopausal women and men >65 years old is 1,300 mg/day [37]. Evidence for postmenopausal women is based on calcium balance studies, however less data are available for elderly males so their recommendations were increased as a precaution [48].

Recent data from the Women's Health Initiative (WHI) and Swedish Mammography cohort studies suggest the risk of cardiovascular events is increased in those with calcium intakes in excess of recommended levels (with or without supplementation) [53, 54]. While this observation is not definitive, it may need to be considered when promoting calcium intakes in excess of recommended levels as a means of enhancing anti-fracture efficacy.

15.3.3 Vitamin D Deficiency and Fracture Risk

Low serum 25(OH)D levels are associated with increased fracture risk in some but not all studies, and may related to an increased propensity for falls as well as contributing to bone fragility [55–57]. However the serum 25(OH)D level at which fracture risk is heightened is not clearly defined.

In a case-controlled study involving over 20,000 Norwegians aged 65–79 years followed for a mean of 10.7 years, an increased risk of hip fracture was observed in those in the lowest quartile for 25(OH)D (<42.2 nmol/L) compared to the highest quartile (>67.9 nmol/L) (RR = 1.34; 95 % CI: 1.05–1.70) [58]. Comparing 400 female hip fracture cases to controls within the WHI study indicated increased risk of hip fractures with 25(OH)D levels <47.5 nmol/L compared to women with 25(OH)D levels >70.7 nmol/L (RR = 1.71; 95 % CI: 1.05–2.79) [59]. In a cohort of 986 elderly Swedish women followed for 3 years, fracture risk was higher when serum 25(OH)D levels were <64 nmol/L (RR = 2.04; 95 % CI: 1.4–4.04) [60]. De Koning et al. (2013) compared 254 hip fracture patients to 2,402 matched controls, and observed the relationship between serum 25(OH)D levels and hip fracture risk was only evident when 25(OH)D levels were <70 nmol/L [61]. This observation may suggest that fracture risk reduction is no greater when serum 25(OH)D levels are >70 nmol/L, but this requires further investigation.

Most, if not all, non-vertebral fractures follow a fall [62, 63]. The observed relationship between increased falls risk and apparent vitamin D deficiency (e.g., 25(OH)D < 25 nmol/L) in the elderly may relate to muscle function [64]. For example, among 1,200 elderly (>65 years) people in the Longitudinal Aging Study Amsterdam, a higher prevalence of vitamin D levels <25 nmol/L were observed in those who fell ≥2 times during the observation year than those who fell 0–1 times. Those who fell were older and performed less favorably on physical performance tests (walking, chair rises and tandem stands) [65].

The mechanism for an effect of vitamin D deficiency on muscle is not explicit, but vitamin D contributes to cell proliferation and differentiation and so may affect muscle mass and contractile properties, thus compromising muscle function [66]. Vitamin D deficiency is associated with increased body sway, poorer balance, and reduced muscle strength [67–69]. The serum 25(OH)D level at which muscle function is affected is not clearly established. In a community-based cohort of 463 elderly people, quadriceps muscle strength was 20 % lower ($p < 0.001$), gait speed 8 % slower ($p < 0.01$), and diminished balance (14 % more sway) reported in those with serum 25(OH)D <50 nmol/L compared to >50 nmol/L [70]. Amongst other roles, vitamin D is required for active absorption of calcium in the small intestine, the major pathway of absorption when calcium intake is low [71]. If calcium intake is compromised, vitamin D deficiency may result in hyperparathyroidism and accelerated bone loss [72].

Vitamin D deficiency may be compounded in the elderly by reduced mobility, limiting time outdoors, and impaired cutaneous production as UV exposure is the principal source of vitamin D [73]. Institutionalized elderly are at high risk of vitamin D deficiency and in whom fracture risk is high [74]. In a sample of 115 German nursing home residents, 94 % had serum 25(OH)D <50 nmol/L, with similar levels observed in >60 % of elderly female long-term care residents in the USA [75]. Total hours of sunlight exposure was positively correlated with serum 25(OH)D ($r = 0.61$, $p < 0.01$) in 133 Japanese nursing home residents however, mean levels were still low (29.9 ± 13.1 nmol/L) [76]. Even in countries such as Spain, Greece and Australia where cutaneous vitamin D production is not limited by seasons, mean 25(OH)D levels <50 nmol/L are common in institutionalized elderly [74, 77, 78].

Given dietary sources of vitamin D are limited, and vitamin D supplementation is not mandatory, insufficient sunlight exposure contributes to vitamin D deficiency. Increasing sunlight exposure by 15 min/day to augment 25(OH)D levels was effective in hospitalized stroke patients and elderly dementia patients. Mean baseline levels of <25 nmol/L increased to >50 nmol/L, but this strategy required intensive staff support to implement [79, 80]. However, for 600+ ambulatory institutionalized elderly, enhancing sunlight exposure was ineffective in increasing 25(OH)D levels without supervision. Poor adherence (median of 26 %) to the daily sessions resulted in a non-significant change to 25(OH)D levels [81]. Vitamin D supplementation may be required to maintain vitamin D sufficiency in institutionalized elderly [82].

15.4 Supplementation and Bone Health in the Elderly

15.4.1 Oral Nutritional Supplements

Most studies demonstrating health benefits of oral nutritional supplements in the elderly are reported from hospital settings, where minor weight gain, improvements in health outcomes and reduced mortality are observed, but mostly in malnourished patients [29, 83]. A meta-analysis of oral nutritional supplementation in the elderly reported limited benefits to nutritional status in those living in the community, and was not effective as a prophylaxis in well-nourished elderly [84]. Sustainability of oral nutritional supplement use in the elderly is dubious as poor adherence and limited efficacy has been observed in longer-term trials (>6 months), and gastrointestinal disturbances are frequently reported [84].

15.4.2 Oral Nutritional Supplementation in Aged-Care

Insufficient studies in aged-care preclude if providing oral nutritional supplements is effective in reducing fractures, as most studies report changes in mortality, health status, and anthropometry [84]. Gains in weight of ~2.5 % (95 % CI: 1.7–2.7 %), were reported from pooled data in a meta-analysis, but few studies demonstrate favourable changes to lean mass and physical function [84]. Despite improvements in BMI, Bonneyfoy et al. (2003) failed to observed improvements in lean mass assessed from total body water, in retirement home residents (mean age >80 years) over a 9-month period when consuming two oral nutritional supplements daily that provided 400 kcal energy and 30 g protein. Compliance was 54 % [85]. Fiatrarone et al. (1994) observed no change to muscle strength, gait velocity, and stair climb power in nursing home residents (mean age 87 years) when supplemented with a 240 ml oral nutritional supplement daily that provided 360 kcal of energy and 18 g of protein [86]. Gains of ~0.8 kg in fat free mass was observed in elderly Alzheimer disease patients when randomized to receive a choice of oral nutritional supplements (soups, desserts, and drinks; protein content 10–12 g) over a 3-month period [87]. Supplement administration and monitoring was performed by caregivers.

Compliance with, and acceptance of oral nutritional supplements by elderly people is often poor, and so likely influences efficacy [88]. Furthermore in aged-care it has been observed that oral nutritional supplements are often not delivered according to treatment plans and waste is considerable [89, 90]. Kayser-Jones et al. (1998) reported that <1/3 of nursing home residents were provided with the correct type and dosage of oral nutritional supplements and mean intake was ~55 % [91]. Moreover elderly with limited appetites may have difficulties consuming supplements in addition to regular meals so normal intake may be compromised. Fiatarone et al. (1994) observed during a 10-week exercise and nutrition supplementation trial in frail nursing home residents, that habitual dietary intake decreased by ~250 kcal/day in the supplement arm, so no change to total energy intake was observed. Manders et al. (2006) observed during a 24-week randomized double-blind placebo-controlled study of oral nutritional supplements in 176 nursing home residents that mean food intake in the supplemented group declined by ~0.5 MJ/day resulting in no difference in change to energy intake between intervention and controls [92].

15.4.3 Oral Nutrition Supplements and Falls and Fractures in the Elderly

To date the anti-fracture efficacy of oral nutritional supplements has not been reported. However, the anti-falls benefits of oral nutritional supplements have been investigated in hospital patients. A randomized intervention of 210 elderly malnourished patients (mean age 74 years) reported a significant

reduction in patients who fell during the 3 months following discharge [93]. Patients were provided a protein–energy enriched diet during admission, oral nutritional supplements plus vitamin D and calcium supplementation post discharge, and support by telephone counselling by dieticians. Protein intake was 11 g/day ($p<0.05$) greater and serum 25(OH)D 11 nmol/L higher ($p<0.01$) with intervention compared to controls. Falls risk in patients prior to intervention was not determined. The basis for the observed benefits is unclear as no improvements in fat free mass, hand-grip strength or physical performance were observed. In another 16-week randomized trial involving 253 undernourished elderly patients (mean age 82 years) no effect of oral nutritional supplements was observed on falls or time to first fall despite improved hand-grip strength in those supplemented. Adherence to supplementation was <40 %. No post-discharge counselling or support was provided to encourage compliance [88].

15.4.4 Vitamin D Supplementation and Fracture Risk Reduction in the Elderly

Vitamin D deficiency has been implicated in falls and fractures however, benefits of vitamin D therapy alone on risk reduction is inconclusive [94, 95]. Supplementing over 3,700 elderly aged-care residents with 2.5 mg ergocalciferol (or placebo) quarterly (~1,100 IU/day) for ~10 months, did not reduce falls or fractures [96]. Baseline 25(OH)D levels ($n=18$) were 59 nmol/L. Meyer et al. (2002) supplemented over 1,000 nursing home residents with cod liver oil daily; with (~400 IU) or without vitamin D, for 2 years, and observed no reduction in fractures with supplementation [97]. Baseline 25(OH)D levels were ~50 nmol/L. Of 540 aged-care residents (mean age 83.4 years) who completed a 2-year vitamin D intervention (~1,000 IU/day) or received a placebo, the incidence of falls with intervention was reduced (0.73 %; CI:0.57–0.95), but not the number of fallers (RR = 0.82; 95 % CI; 0.59–1.12) or fractures (RR = 0.69; 95 % CI: 0.40–1.18) [98]. Bischoff et al. (2003) supplemented elderly women in long-care stay for 5 months with 800 IU Vit D/1,200 mg calcium daily or calcium alone and observed a 49 % reduction in the number of falls, but not number of fallers (RR = 0.7; 95 % CI: 0.3–1.5) in the vitamin D supplemented arm [99].

Annual high-dose vitamin D as an intramuscular injection (300,00 IU) or orally (500,000 IU) was ineffective in reducing falls or fracture risk in older people, however increased risk was observed [100, 101]. Smith et al. (2007) randomized over 9,000 people (>75 years old) to an annual 300,000 IU vitamin D injection or placebo for 3 years and observed an increased risk of hip fracture (HR = 1.49; 95 % CI: 1.02–2.18, $p=0.04$), in particular for women (HR = 1.80; 95 % CI: 1.12–2.9) with supplementation [100]. In a randomized 3–5 year intervention using an annual oral dose of 500,000 IU vitamin D, or placebo in over 2,000 healthy women (mean age 76 years), 15 % more falls and 26 % more fractures were reported with supplementation. The authors speculated that the single high-dose regime, more than the total dose received (~1,300 IU/day) may have contributed to increased falls and fracture risk, as falls and fractures peaked during the first 3 months after supplements were administered [101].

The observed efficacy (or lack there of) of vitamin D may be influenced by study durations (insufficient time to observe adequate events), treatment strategies (daily, monthly, or single bolus), vitamin D type (D2 vs D3), baseline 25(OH)D (many of whom are not deficient), and the level of 25(OH)D achieved with supplementation [102]. Some trials report outcomes of combined calcium and vitamin D therapy, so isolating the effect of vitamin D is challenging. Meta-analyses often yield positive benefits to falls or fracture risk despite few individual studies achieving statistical significance [103]. For example the pooled relative risk reduction of high dose vitamin D (792–2,000 IU/day) studies was 0.72; 95 % CI:0.59–0.89, but only one of the studies reported a significant fracture risk reduction with vitamin D (+calcium) therapy [62, 104].

The optimal 25(OH)D level for falls and fracture risk reduction is not clearly established, as too the optimal supplement dose [105]. Doses <800 IU/day vitamin D were deemed ineffective in reducing falls and fractures risk however, these lower dose studies were conducted in nursing home residents

in whom mobility may be limited, compared to ambulatory elderly in residential care in whom falls and fractures rates are high [102]. However, based on current evidence 800 IU+/day vitamin D is suggested for institutionalized elderly with limited sunlight exposure, to benefit falls and fracture reduction [104].

15.4.5 Calcium Supplementation and Fracture Risk Reduction in the Elderly

Calcium supplementation trials in older adults provide limited evidence of fracture risk reduction with calcium therapy alone [40]. In a 4-year trial, no fewer fractures were observed in healthy postmenopausal women (mean age 66 years) when supplemented with 1,600 mg calcium/day compared to placebo. Mean dietary intake was ~700 mg/day [106]. Prince et al. (2006) observed no fracture risk reduction over 5 years in 1,460 postmenopausal women (mean age 75 years) when supplemented with 1200 mg/day calcium compared to placebo (RR = 0.87; 95 % CI: 0.67–1.12), but fewer fractures were observed in compliers ($n = 830$, RR = 0.66; 95 % CI: 0.45–0.97). Non-compliers were older and had poorer physical function [107]. Results from the RECORD trial involving over 5,000 elderly fracture patients indicated no fracture risk reduction over 24–62 months when supplemented with 1,000 mg/day calcium (and/or vitamin D) [108]. During a 5-year intervention involving over 1,400 women (mean age 74 years) supplemented with 1,000 mg/day calcium, or a placebo, no fracture risk reduction was observed with treatment. Compliance ranged from 55 to 58 % [109]. Dawson-Hughes et al. (1997) randomized 389 community dwelling older men and women for 3 years to 500 mg calcium (+700 IU vitamin D) daily or a placebo and observed a non-significant reduction in first osteoporotic fractures with supplementation (OR 0.4 95 % CI: 0.2–1.1) [110].

The lack of anti-fracture efficacy may be influenced by a number of factors. Few participants are calcium deficient, with dietary intakes >700–800 mg/day often reported, and methods of dietary assessment may not reflect true long-term calcium intake. Compliance with calcium supplements was often poor, frequently due to gastrointestinal disturbances, and tends to worsen over time [107, 108]. Poor compliance even with osteoporosis medications limits anti-fracture efficacy [110]. High dropout rates may also hinder inferences made [50]. For example, only ~1 % (n = 53) of the initial RECORD cohort of >5,200 were available at 60+ months [50, 108]. High attrition would erode the power to detect a treatment effect.

15.4.6 Food Fortification and Fracture Risk Reduction in the Elderly

Food fortification may enhance nutritional intake in the elderly by improving nutrient density (e.g., calcium), or by acting as a vehicle to provide nutrients (e.g., vitamin D) that have limited dietary sources, but are of importance to bone. Enhanced skeletal benefits are generally observed in those with low habitual calcium intakes and serum 25(OH)D levels (e.g., <50 nmol/L). Short-term interventions (<16 weeks) in postmenopausal Asian women (mean calcium intake 260–480 mg/day) randomized to receive calcium or calcium/vitamin D fortified milk or serve as controls demonstrated reductions in PTH and bone turnover, of ≥10 % and ≥20 % respectively with intervention [112, 113]. In an 18-month randomized intervention in postmenopausal Chinese women receiving calcium-fortified milk, providing 900 mg calcium and 256 IU vitamin D daily, or placebo, BMD at the hip was improved by ~2 % with treatment, but no change was observed at the lumbar spine [114].

Total body BMD was maintained in 50 postmenopausal women over 30 months when provided calcium/vitamin D fortified dairy foods (~1,200 mg calcium and 300 IU vitamin D) daily, relative to

controls (0.3 % vs. −1.8 %, $p<0.001$) [115]. The basis of these observations is unknown given that no treatment effect was observed for IGF-1, 25(OH)D, PTH or bone turnover markers at month 12 [116]. Moreover, the intervention involved nutrition counselling and education, so adherence without additional support in unknown.

Malnutrition is common in institutionalized elderly. Given their limited access to foods, or in some cases, ability to consume sufficient quantities of food, fortification would improve nutrient intake and could potentially reduce fracture risk. Indicators of bone metabolism have improved using food fortification. Bonjour et al. (2009) observed a 12.3 % decrease in PTH and a 16.9 % increase in IGF-1 in elderly institutionalized women (mean age 85 years) after 1-month of supplementation using calcium (151 g/100 g) and vitamin D (50 IU/100 g) fortified soft cheese [117]. Bonjour et al. (2013) subsequently compared the efficacy of vitamin D- (400 IU) and calcium-fortified (800 mg) yoghurt to regular yoghurt (calcium content 280 mg) over 56 days in 60 vitamin D deficient elderly institutionalized women, and observed 25(OH)D was ~20 nmol/L higher ($p<0.0001$) and PTH and CTX, 20 % ($p<0.001$) and 8 % ($p<0.05$) lower with fortification [118]. Residents consumed two 125 g serves daily, with two flavors provided. Products were rated as satisfactory, so long term adherence uncertain.

A 1-year intervention using high dose vitamin D (5,000 IU/day) and calcium (320 mg/day) fortified bread in institutionalized elderly improved 25(OH)D levels from <30 nmol/L to >120 nmol/L; PTH over halved, rates of bone turnover were lowered by ~27 % and increased BMD at the lumbar spine and total hip observed [119]. Greiger et al. (2009) provided calcium and vitamin D fortified milk to elderly people in residential care and observed that without guidance for food service staff to modify the menu to increase milk intake, no changes were observed for PTH, bone turnover markers, physical function or bone ultrasound measures and mean serum 25(OH)D levels remained below 50 nmol/L [120].

Iuliano-Burns et al. (2012) conducted a 2-year randomized cluster intervention using a dairy-based protein, calcium, and vitamin D supplement in elderly people in residential care. Staff were trained to incorporate the supplement into foods, or pre-fortified foods were provided. Among the 813 residents (mean age 85.5 years), supplementation reduced the number of residents who fell by 42 % (OR = 0.58, 95 % CI: 0.44–0.78, $p<0.001$). Supplementation slowed bone loss at the proximal femur, which declined in the control group by 2.5 % ($p<0.05$), maintained serum 25(OH)D, which declined in the control group by 22 % ($p<0.001$) and reduced PTH by 16 % ($p<0.05$) [121]. Despite the observed reduction in falls, a limitation of the study was high staff turnover, and staff having difficulties preparing the fortified foods. This highlights the difficulties within residential aged-care when implementing interventions that require additional effort by staff.

Staff play an important role in ensuring nutritional adequacy of age-care residents. Simmons et al. (2006) observed that even with feeding assistance, nursing home residents did not achieve desired levels for energy intake. Staff assisted residents during meals for ~6 min each, which is less than the 30+ min estimated to ensure an adequate intake [122]. In a subsequent 24-week cross over intervention, nursing home residents at risk of malnutrition were assisted at meals and snacks. Energy intake increased by ~300 kcal/day, and weight was maintained or increased during intervention, but lost during the control period. Staff assisted each resident for 42 min during meals and 13 min during snacks compared to usual care of 5 min and less than 1 min per residents for meals and snacks respectively [123].

Of the methods explored to enhance nutritional intake in institutionalized elderly such as enhancing food flavors, provision of snacks, staff education, and altering dining environment, the greatest improvements are when residents are assisted with eating [124]. As dementia is a risk factor for falls, and those with dementia often require feeding assistance, ensuring an adequate nutrient intake has even greater importance [125]. Whether these changes to food intake translate to falls and fracture risk reduction require further investigation.

15.5 Dietary Approach to Improving Bone Health in the Elderly

Many interventions to increase energy intake in aged-care residents, give insufficient regard to the nutritional quality of the foods being served. Often the actual foods served to residents do not meeting recommended levels for key bone-related nutrients, and the required food groups to delivery these nutrients not provided in sufficient quantities [27]. Energy dense, nutrient poor 'extra' foods provided to institutionalized elderly contribute to energy intake but do not contribute substantially to the nutritional quality of the foods served, especially for nutrients such as calcium, protein and vitamin D that are important for bone [46].

A 12-week cluster-randomized dietary-based intervention targeting 41 aged-care residents with BMI < 18.5 was unsuccessful in improving total energy or protein intakes but fat intake was augmented. Energy intake was enhanced with butter and cream, with the only change to diet quality being substitution of water-based drinks with milk-based beverages. Intake of most micronutrients, including calcium and vitamin D, failed to increase with intervention. Residents consuming the energy enriched foods ($n = 16$) gained on average 1.3 kg body weight, but as protein intake was not increased it is unclear if this constituted gains in lean mass [126].

Focusing on food quality (in addition to quantity) may be effective in reducing malnutrition risk, and have potential anti-fracture efficacy. For example dairy foods are a good source of protein and calcium, and a common vehicle for vitamin D through fortification. To test a food-based approach Iuliano et al. (2013) undertook a feasibility study to determine if improving dairy intake from the 2 serves/day that is currently consumed, to the recommended 4 serves/day could be a potential anti-fracture strategy in institutionalized elderly [127]. One hundred and thirty residents (mean age 86.5 years) in four aged-care facilities participated in the 4-week trial. Two facilities underwent intervention while two served as controls so consumed from their normal menu. Recipes were modified, low calcium beverages substituted for milk-based drinks and dairy foods added to meals (Table 15.1).

Following intervention, daily increases in mean energy intake (900 kJ, $p < 0.001$), protein intake (+25 g, $p < 0.0001$), proportion of energy from protein (+4 %, $p < 0.0001$), and proportion of estimated energy requirements (EER) (+18 %, $p < 0.0001$) were observed, while proportion of energy from fat decreased (−3 %, $p < 0.0001$). In controls mean energy intake remained below the EER, and protein

Table 15.1 Calcium content from example menus before (regular) and after (high dairy) modifications, that increased dairy food consumption from two to four serves per day in low-level aged care residents

Food item	Regular menu (mg Ca/serve)	Food item	Modified menu (mg Ca/serve)
Beverages			
White coffee	24	Milk coffee	171
Cordial	2	Cold milk	212
Soups			
Tomato (water-based)	15	Tomato (milk-based)	122
Mushroom	25	Mushroom (milk-based)	173
Meal items			
Broccoli	11	Broccoli baked	122
Mashed potato	11	Scalloped potatoes	108
Desserts			
Jelly and cream	14	Rice pudding	279
Added cream	10	Added custard	283
Snacks			
Savoury biscuit	10	Cheese and Biscuit	146
Sweet biscuit	18	Yoghurt	296

intake remained unchanged. Increases in mean daily intakes of calcium (+679 mg, $p<0.0001$), vitamin D (+1.4 μg, $p<0.0001$), phosphorus (+550 mg, $p<0.0001$), and zinc (+2.8 mg, $p<0.0001$) were observed, which remained unchanged in controls. Calcium and zinc intakes achieved recommended levels with intervention, but remained below recommended levels in controls. Mean sodium intakes remained unchanged.

The mean increase in protein intake of 25 g/day was greater than that reported using supplements (6 g/day) or food fortification/snacks (12 g/day) [92, 128, 129]. This level of protein supplementation is similar to amounts suggested to maximize muscle protein synthesis and prevent sarcopenia [130]. Moreover intake of leucine in dairy foods has been shown to increase IGF-1 expression, lean muscle mass and muscle protein synthesis in older women [131, 132].

Given that dairy foods are readily available and require minimal staff time to prepare, improving dairy food intake to recommended levels may be effective in preventing malnutrition, and potentially reducing fracture risk in institutionalized elderly. Larger and more extensive trials in aged-care residents are required to determine the long-term skeletal and anti-fracture benefits of food-based interventions.

15.6 Why Target Elderly People in Aged Care

Targeting elderly residents in aged-care is likely cost effective given that falls and fracture risk is highest in this group, so fewer require treatment to prevent a fracture [9, 62, 133, 134]. Mandatory reporting of falls and fractures ensures accurate monitoring of treatment efficacy, and compliance is more likely as staff administer medications [105]. Risk factors such as medical conditions and medication use are closely monitored, and environmental risks for falls and fracture remain relatively constant. Most notably rates of nutritional deficiency are high, so residents are more likely to benefit from treatment. These factors may have contributed to the success of the landmark calcium and vitamin D intervention reported by Chapuy et al. (1992), in which over 3,000 elderly institutionalized women were randomized to received 1,200 mg calcium and 800 IU vitamin D daily, or a placebo. Total non-vertebral and hip fractures were 32 and 43 % lower in the supplemented group compared to controls [62]. Serum 25(OH)D levels increased from a mean of 40 to 100+ nmol/L with treatment, but remained below 33 nmol/L in controls. In a subset of 56 residents, a 2.7 % increase in BMD at the total proximal femur in the treated group was observed, with a 4.6 % loss reported in controls ($p<0.001$). The mechanism responsible for the reduction in all non-vertebral fractures was not examined, but the early observed benefits were more likely indicative of a muscular effect and so a reduction in falls, but falls were not measured and muscle function not tested.

The contribution of elderly residing in aged-care to the fracture burden is substantial with 22 % of all low trauma fractures and 37 % of hip fractures admitted to hospital arising from this high risk group [135]. As the population ages, more elderly people will rely on institutionalized care. The need for effective fracture prevention strategies in this high-risk segment of the population is growing in importance.

15.7 Conclusion

As the population ages, the number of elderly people and those relying on institutionalized care will rise, as too will the number of fractures. The sustainability of this aging population will be dependant on the elderly maintaining suitable health to minimize fracture risk. Nutritional adequacy helps maintain health, yet still rates of malnutrition are high and intake of key bone-related nutrients suboptimal

in the elderly, in particular those in institutional care. Targeting fracture risk reduction in institutionalized elderly is likely cost-effective given the burden of fractures arising from this group. Ways of optimizing nutrition to reduce fracture risk requires attention.

Further research is needed to elucidate the potential benefit of protein intake on the aging process and fracture risk reduction. The delivery of protein via oral nutritional supplements appears most effective in improving health outcomes in the elderly in short-term clinical settings such as during hospital admission and post-recovery, but evidence is limited that long-term use benefits health, likely due to diminishing compliance over time. Individually calcium and vitamin D supplementation is less effective at reducing fracture risk in the elderly, but collectively anti-fracture efficacy has been observed in institutionalized elderly perhaps because vitamin D deficiency is widespread and calcium intakes commonly below recommended. Controlled sunlight exposure was ineffective in improving vitamin D status in institutionalized elderly so supplementation may be necessary. However, the optimal dose is not clear.

Alternative methods of enhancing nutrition in the elderly require further exploration. Fortification with calcium and vitamin D was effective in improving measures of bone metabolism in institutionalized elderly, but appears to require the use of pre-fortified foods to ensure the correct delivery of food by staff. Results from research investigating the benefits of improving the nutritional quality of foods offered to residents in aged-care is promising but large-scale trials are needed to determine if providing more nutritious food choices is an effective anti-fracture strategy. Improving the nutritional status of the elderly, to potentially reduce fracture risk may prove paramount, to offset the growing fracture burden due to the aging of the population.

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Chapter 16
Nutrition and Skeletal Health in Blacks

Susan S. Harris

Key Points

- Vitamin D deficiency is much more common among African Americans than other American groups.
- There is little evidence that improving vitamin D status in this group will have an important benefit on skeletal health.
- It is possible that some African Americans have adaptive physiologic responses to low vitamin D and calcium intakes that result in better bone acquisition and maintenance.
- It would be premature to suggest that vitamin D or calcium requirements are different in this group from others or to suggest that vitamin D deficiency among them carries no increased health risks.
- African Americans should aim for the same dietary intakes of calcium and vitamin D and the same blood targets for 25(OH)D as other groups until both the skeletal and nonskeletal benefits to them of correcting vitamin D deficiency have been further explored.

Keywords African Americans • Skeletal health • Osteoporosis • Nutrition • Vitamin D • Calcium • Nutritional supplements • Parathyroid hormone • 25-Hydroxyvitamin D • 1,25-Dihydroxyvitamin D

16.1 Introduction

Cultural and socioeconomic differences across race and ethnic groups in the US affect dietary intakes of key nutrients and other behaviors that affect skeletal health. There may also be genetically determined differences in the physiological response to varying nutritional states. Population groups in Africa are extremely diverse genetically. The focus of this chapter is on African Americans, a group whose African ancestors came predominantly from West Africa and that also reflects a variable admixture of European and other genetic influences [1]. The bone density values and fracture rates of at least some African populations differ from those of US blacks [2], and the nutritional and other environmental influences in the USA are quite different from those in many African countries.

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16.2 Bone Mineral Density and Fracture Risk

It is well established that US blacks have a reduced risk for osteoporosis compared with US whites and others [3, 4]. Medicare data suggest that about 5 % of black women and 3 % of black men will have a hip fracture by age 90, compared with 16 % of white women and 6 % of white men [5]. In addition, the prevalences of osteoporotic fractures [6] *and* of all nonvertebral fractures [7] among blacks is about half that of whites. Although other factors, including bone geometry [8, 9], may also play a role, the lower fracture rates of blacks probably result primarily from their higher bone mineral density (BMD) [4, 6, 10–15], particularly from achievement of a higher peak density by young adulthood [13, 16]. Racial comparisons of BMD or size-adjusted bone mineral content generally show higher values in prepubertal black compared with white children [17–21]. In addition, black children appear to have greater increases in BMD than white children during puberty [22–25].

BMD of young black adults is reported to be from 4 to 13 % higher than that of young white adults at various skeletal sites [10, 26–30]. Early postmenopausal bone loss may be modestly slower in black women than in white women [14], but bone loss in later years appears to be about the same for blacks and whites [14, 31, 32].

16.3 Vitamin D Status and Calcium Intake

This skeletal advantage exists despite nutritional factors that would appear to put blacks at an *increased* risk compared with other groups. The most notable of these differences is a far higher prevalence of vitamin D insufficiency [11, 12, 33–40]. For example, data from NHANES-III show that, among residents of the Southern states who were measured in winter, more than 53 % of non-Hispanic blacks had 25-hydroxyvitamin D [25(OH)D] levels indicative of vitamin D insufficiency compared with only 8 % of non-Hispanic whites [39]. This difference may result in part from lower vitamin D intake owing particularly to less use of supplements [41] including multivitamins [42] and other vitamin D supplements [42], but the principal reason is that skin pigmentation in blacks sharply reduces the amount of vitamin D that is produced during sunlight exposure. Among healthy, free-living young women living in New England, 25(OH)D concentrations in blacks were only half as high as those in whites, and the increases from winter to summer were smaller [35]. This difference probably does not result from any absolute limit in the production of vitamin D or 25(OH)D, because, when given adequate exposure to ultraviolet light, black adults can reach mean concentrations of both parent vitamin D [43] and 25(OH)D [44] that are similar to those of whites. The 25(OH)D response to increased dietary vitamin D is similar in blacks and whites [45]. Reduced vitamin D acquisition, from either skin production or diet, is partially compensated for by an increased rate of 25(OH)D production [37], and the extent of this adaptive mechanism appears to be similar in blacks and whites when both have low initial 25(OH)D concentrations [37]. Some of the black/white difference in 25(OH)D remains unexplained by differences in diet, sun exposure, body size and other behavioral factors [46] suggesting potential genetic influences.

Total calcium intake, although below recommended levels in most adult Americans, is even lower among blacks because of lower dietary calcium intakes (compared with whites and Hispanics) [47, 48] and also because of less calcium supplement use [42, 47]. Despite a higher prevalence of lactose intolerance among blacks [49], dairy consumption appears to be only modestly lower in blacks than whites [42, 50]. Milk is the most important single source of dietary calcium among black adolescents and adults [51], and lactose digestion can apparently be improved with prolonged consumption of dairy products [52].

16.4 Body Size and Composition

In the USA, obesity is more prevalent among black women than white women [53], and their reduction in osteoporosis risk despite relatively poor calcium and vitamin D nutrition can be explained in part by differences in body size and body composition. The contributions of these factors to BMD differences is difficult to assess because of the fact that the densitometric methods used to measure bone density are themselves affected by body size variables, including bone size [54] and the thickness and composition of soft tissue [55]. Nevertheless, it is a fairly consistent finding that *adjusting* for body weight, height, or related measures reduces but does not eliminate the black–white difference in BMD [13, 30].

16.5 Calcium Regulating Hormones

16.5.1 1,25-Dihydroxyvitamin D [1,25(OH)$_2$D] and Parathyroid Hormone (PTH) Concentrations

Concentrations of the calcium regulating hormones 1,25(OH)$_2$D and PTH tend to differ by race. Specifically, both young [34, 35, 58] and old [12, 36, 38] blacks have increased PTH concentrations compared with whites. Blacks also tend to have higher concentrations of 1,25(OH)$_2$D, the active form of vitamin D, despite substantially lower concentrations of its precursor, 25(OH)D [12, 56]. This inverse association is observed in other populations as well [57], and likely results from an increased rate of kidney synthesis of 1,25(OH)$_2$D stimulated by elevated PTH. The higher PTH and higher 1,25(OH)$_2$D of blacks are both consistent with their lower calcium intake and poorer vitamin D status. When young black and white women who had fairly similar calcium intake and 25(OH)D concentrations were observed, no differences in 1,25(OH)$_2$D or PTH were observed [33]. However, among older black and white adults with similar calcium intakes, PTH was higher in blacks than whites for a given 25(OH)D concentration [36].

16.5.2 Association of 25(OH)D with PTH

There is extensive evidence in young [34, 35, 58] and old [36, 40, 45, 58, 59] blacks that, as in whites, reduced 25(OH)D is associated with increased PTH. Two large observational studies have compared the curvilinear associations of 25(OH)D with PTH in blacks and whites. One of these studies included 359 older blacks and 2,565 older whites seeking advice on osteoporosis [40] and the other included 1,579 black and 1,203 young and old white adults in NHANES [58]. In both of these studies, PTH in blacks decreased with increasing 25(OH)D only up to 25(OH)D concentrations of about 20–25 ng/ml but, in whites, continued to decline, though at a slower rate, with higher 25(OH)D concentrations.

16.5.3 Skeletal Resistance to PTH

It has been suggested that the higher BMD of blacks despite a higher prevalence of secondary hyperparathyroidism may result from a relative skeletal resistance to the effect of PTH [60]. This would theoretically allow them to benefit from the kidney effects of high PTH [increased synthesis of

$1,25(OH)_2D$ and increased reabsorption of calcium] without suffering from the increased skeletal calcium release that is also associated with elevated PTH. Adult black women have generally been reported to have reduced rates of bone turnover compared with whites [12, 61, 62]. Consistent with a skeletal resistance to PTH, reduced bone turnover in blacks has been observed despite higher baseline PTH, higher stimulated PTH levels, or similar high PTH concentrations during PTH infusion [60, 63]. Furthermore, blacks have lower calcium excretion than whites even after multiple factors including calcium intake and PTH are controlled for [33].

16.5.4 Intestinal Resistance to $1,25(OH)_2D$

Another potential explanation for the higher BMD of blacks is that they may have an intestinal resistance to the actions of $1,25(OH)_2D$. This is supported by a study of calcium retention in black and white women given high- and low-calcium diets [64]. The black women had lower 25(OH)D and higher $1,25(OH)_2D$ than the white women throughout the study, and had greater increases in $1,25(OH)_2D$ when calcium intake was decreased. Despite the greater $1,25(OH)_2D$ response of the black women to the diet change, the calcium retention fraction did not differ by race, consistent with an intestinal resistance to the actions of $1,25(OH)_2D$. This hypothesis was further supported by an experiment in which the effect of administered calcitriol on calcium absorption was observed in black and white women. The groups had similar increases in $1,25(OH)_2D$, but the resulting increase in calcium absorption was smaller among the black women [65]. Such an adaptation could benefit the skeleton because $1,25(OH)_2D$ may have a positive effect on bone that is independent of its effect on calcium absorption [66], perhaps through stimulation of osteoblast activity [67]. It may be that such a direct effect requires a relatively high $1,25(OH)_2D$ concentration, as may be more readily achieved if intestinal resistance to the hormone protects against hypercalcemia [68].

16.6 Vitamin D, Calcium, and Skeletal Health

16.6.1 Observational Studies

Two cross-sectional studies in older African-American men reported positive associations of 25(OH)D with tibial BMC [69] and hip and spine BMD [70] but only below threshold 25(OH)D concentrations of 15–19 ng/ml. Another showed no association of 25(OH)D with BMD of the hip, spine or radius in black men with a mean age of 48 years and a mean 25(OH)D of 25 ng/ml [71]. In contrast to these, NHANES III showed a significant positive association with total hip BMD in older blacks and this association appeared to persist through levels as high as 40 ng/ml [72]. With respect to fracture, a nested-case control study conducted within the Women's Health Initiative (WHI) Observational Study reported an *increased* risk of hip fracture among black women with 25(OH)D levels over 20 ng/ml compared with lower levels [73]. As the authors point out, this inverse association may reflect confounding due to a greater degree of European admixture among subjects with higher 25(OH)D. European admixture may have been a confounder in the other observational studies as well. Since people of European descent tend to have both lower BMD and higher 25(OH)D than those of African descent, a positive association would tend to be found between 25(OH)D and BMD in African-American groups with varying degrees of European admixture.

16.6.2 Intervention Studies

Even large well-designed observational studies of vitamin D and skeletal health may not be able to fully account for confounding by genetic and lifestyle factors that are associated with both 25(OH)D status and skeletal health. For this reason, randomized, controlled intervention studies are needed to determine the potential benefits of improved vitamin D and calcium nutrition in blacks. Several such trials provide some insight regarding potential skeletal benefits of vitamin D and calcium supplementation in blacks, but important questions remain.

16.6.2.1 Two Trials in Postmenopausal Black Women

Two randomized, controlled trials, both conducted in New York State, examined the effect of vitamin D supplementation on changes in BMD in postmenopausal women [74, 75]. In the first, Aloia and colleagues studied 208 healthy postmenopausal black women with a mean age of 60 years [74]. Subjects were randomly assigned to placebo or 800 IU/d of vitamin D_3 and all subjects received calcium supplements as needed to bring calcium intake to 1,200–1,500 mg/d. After 2 years of follow-up, the vitamin D dose was increased to 2,000 IU/d and the subjects were followed for an additional year. Mean 25(OH)D in the vitamin D group was 19 ng/ml at baseline, increased to 28 ng/ml after supplementation with 800 IU/d, and increased to 35 ng/ml after supplementation with 2,000 IU/d. At the four skeletal sites measured (spine, mid-radius, total hip and total body) the percentage changes in BMD over 3 years in the two groups were within 0.05 percentage units of each other and not statistically significant. Thus, this study provides no evidence of skeletal benefit from providing supplemental vitamin D to calcium-replete black women with starting 25(OH)D levels of 19 ng/ml at baseline.

In the second trial, Nieves and colleagues studied 103 healthy postmenopausal black women with a mean age of 62 years [75]. Subjects were randomized to placebo or 1,000 IU/d of vitamin D_3 and all subjects received calcium supplements as needed to bring calcium intake to 1,000 mg/d. Mean 25(OH)D in the vitamin D group was 12 ng/ml at baseline and was 22 ng/ml at the study end 2 years later. The investigators observed slightly lower rates of bone loss over 2 years in the vitamin D supplemented group compared with placebo but none of these differences achieved statistical significance. The placebo group lost 0.11–0.19 percentage units more BMD from the spine, total hip and trochanter, and 0.61 more from the femoral neck than the vitamin D supplemented group. The femoral neck difference is within the range of clinical benefit, and, had the sample size been larger, may have been statistically significant.

16.6.2.2 The Women's Health Initiative

In a study of women enrolled in WHI clinical trials [76], investigators examined the effect of supplementation with vitamin D plus calcium compared with placebo on fracture incidence in 36,282 postmenopausal women aged 50–79 of whom 3,317 were black. Subjects were randomly assigned to receive 400 IU/d vitamin D_3 plus 1,000 mg/d calcium or placebo and followed an average of 7 years for the occurrence of fractures. The study was not powered to detect effects of supplementation within the subset of blacks, and the number of fractures among them was small. The hazard ratio for fracture was 0.73 in the supplement relative to place groups but the confidence interval around the estimate was wide (0.16–3.32). Thus, while the findings from this subset analysis are consistent with a 27 % reduction in hip fracture risk among blacks given 1,000 mg/d calcium and 400 IU/d vitamin D, the uncertainty around this estimate is very high and the result may be due to chance.

Furthermore, the low vitamin D dose given would suggest that, if any benefit were present, it likely came from the substantial increase in calcium that was provided rather than from the vitamin D component of supplementation.

16.7 Conclusion

In summary, vitamin D deficiency is much more common among African Americans than other American groups, but there is as yet little compelling evidence that improving vitamin D status in this group will have an important benefit on skeletal health. It is possible that some African Americans have adaptive physiologic responses to low vitamin D and calcium intakes that result in better bone acquisition and maintenance. However, for several reasons it would be premature to suggest that vitamin D or calcium requirements are different in this group from others or to suggest that vitamin D deficiency among them carries no increased health risks. First, any genetically conferred protection among African Americans is likely to vary across subsets of differing African ancestral lineage and differing European admixture. There is currently no way to identify which individuals may suffer more than others from the consequences of vitamin D or calcium deficiencies. Second, clinical trials, though they avoid the potential confounding of observational studies, are limited in their ability to study multiple vitamin D doses and to study adequate numbers of subjects who are vitamin D deficient at baseline. Finally, it now appears that vitamin D deficiency may affect cardiovascular disease and type 2 diabetes as well as other important health conditions that are highly prevalent among African Americans. Thus, African Americans should aim for the same dietary intakes of calcium and vitamin D and the same blood targets for 25(OH)D as other groups until both the skeletal and nonskeletal benefits to them of correcting vitamin D deficiency have been further explored.

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Chapter 17
Nutrition and Skeletal Health in Other Racial/Ethnic Groups

Elaine Cong and Marcella Donovan Walker

Key Points

- Osteoporotic fractures are a major public health problem and skeletal health varies widely across ethnic, racial, and geographic groups.
- Understanding factors contributing to skeletal health in non-white racial/ethnic populations is imperative.
- The highest aBMD is found in African Americans followed by Hispanics, whites, and Native Americans, with the lowest aBMD in Asian Americans.
- Racial differences in fracture risk do not necessarily parallel racial differences in aBMD, with the lowest fracture risk among African Americans, followed by Asian Americans and Hispanics, with the highest fracture risk in non-Hispanic whites and Native Americans.
- The paradoxical low fracture risk in Asian Americans despite low aBMD may be due to differences in bone microarchitecture, bone geometry, or non-skeletal factors.
- Racial differences in skeletal health are influenced by weight, bone size, and lifestyle factors such as physical activity and nutrient intake.
- There are racial differences in calcium absorption and retention. Limited data suggests that the relationship between 25(OH) D, parathyroid hormone and BMD may vary by race.
- Cross-sectional studies in Asians and Asian Americans have indicated an association of higher soy intake with higher aBMD and reduced fracture risk, but soy isoflavone intervention studies have shown conflicting results.

Keywords Race • Ethnicity • Asian American • Hispanic • White • Nutrition • Diet • Skeletal health • Bone microarchitecture • Bone density • Fracture

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17.1 Introduction

Osteoporotic fractures represent a major public health problem with enormous societal and economic burdens [1–3]. One in two women over age 50 will experience an osteoporotic fracture [4]. Race and ethnicity affect the incidence, risk factors, diagnosis and treatment of osteoporosis and fragility fractures [5]. Non-white populations represent a rapidly growing group at risk for osteoporotic fracture [6]. Given the vast diversity of racial and ethnic groups as well as the variation in nutrition across the globe, this chapter primarily focuses on nutrition and skeletal health in non-white populations in the USA, other than African-Americans (see Chap. 19). The term race refers to the concept of dividing individuals into groups based on physical or biologic characteristics with a presumed genetic basis. Ethnicity is often used, not only to suggest a similar genealogy, but common cultural and religious behaviors and experiences, as well as shared language and geography. The National Institutes of Health and US Census bureau categorize non-white populations into four racial groups (African Americans, Asian Americans, American Indian/Alaska Natives, and Native Hawaiian/Other Pacific Islanders) and two ethnic groups as either Hispanic or non-Hispanic. While some have suggested that such classifications are not useful given limited genetic variation across the human species, such categories are supported by genetic marker cluster analyses. For example, in the US Family Blood Pressure Program study, genetic cluster analysis demonstrated four distinct, non-overlapping genetic clusters (white, African American, East Asian, and Hispanics) which corresponded almost perfectly to self-identified race/ethnicity [7].

Minority groups in the USA represent a growing population at risk for fragility fracture. According to the 2010 US Census, the greatest growth in the total US population over the last decade resulted from increases in those who reported their race as non-white and those who characterized their ethnicity as Hispanic [8, 9]. As a result of changing demographics, the distribution of fractures in the USA is also expected to shift. While 12 % of fractures in the USA occurred among non-white minorities in 2005, this percentage is predicted to exceed 20 % by 2025 [8]. Adding to this burden, mortality and disability rates after fracture tend to be higher among minorities than whites [5, 9]. Therefore, improving our understanding of skeletal health and modifiable risk factors for osteoporosis and fragility fracture including nutrition among minorities is an important public health initiative for the twenty-first century.

17.2 Racial Differences in Areal Bone Mineral Density

Most data pertaining to racial differences in bone mineral density (BMD) have been attained with dual-energy x-ray absorptiometry (DXA). Because DXA provides a two dimensional BMD measurement (i.e., areal BMD in g/cm^2), it is affected by bone size and/or weight. DXA tends to underestimate areal BMD in those with small body/bone size while overestimating it in those with large body/bone size [10–13]. Racial differences in areal BMD, therefore, must be evaluated with respect to racial differences in skeletal size and weight.

Studies in adult Asians living in Asia or the USA have typically demonstrated lower areal BMD (aBMD) in comparison to whites and other racial/ethnic groups [10, 14–17]. Weight or bone size explains some of these differences [10, 18]. For example, data from the Study of Women's Health Across the Nation (SWAN) suggest that when adjustments are made for weight and other covariates [10, 19], aBMD is actually greater in Chinese and Japanese Americans versus whites. In contrast, compared to African-Americans, lower aBMD persisted in Chinese and Japanese Americans even after adjusting for weight [10].

Most studies indicate that Hispanic Americans have similar [20–22] or slightly higher [23] aBMD as compared to non-Hispanic whites. In contrast, some recent studies noted lower aBMD in Hispanic Americans compared to non-Hispanic whites [17, 24]. The majority of investigations in the Hispanic

community have focused on Mexican Americans, with much less data available in other Hispanic groups. However, in the National Osteoporosis Risk Assessment (NORA) study, a subgroup analysis of Hispanics by heritage (including Mexican, Puerto Rican, and Cuban Hispanics) did not reveal any significant differences in aBMD [17].

Areal BMD data in American Indians and Native Alaskans is far from complete but limited information indicates similar aBMD compared to white women [17, 25]. There are almost no published data in Native Hawaiians/Pacific Islanders in the USA. In a study of women aged 25–34 years living in Hawaii, Hawaiian women tended to have the highest aBMD by single-energy X-ray absorptiometry at the radius and calcaneous and DXA of lumbar spine compared to white, Japanese, and Filipino women [26]. Most differences were attenuated or eliminated when adjusted for height and weight, though differences between Hawaiian and Filipino women persisted at the spine. Likewise, differences between Hawaiians and whites persisted at the radius after adjustment for height and weight and were due to greater bone mineral content rather than differences in bone size [26].

The available information on unadjusted aBMD in adults can be roughly recapitulated by a hierarchy in the following manner, with higher aBMD in men versus women in all groups: African Americans > Hawaiians/Pacific Islanders > non-Hispanic whites, Hispanics and Native Americans > Asian Americans. Although there are fewer data available, these racial patterns in aBMD are also apparent in children, adolescents, and young adults [27–29].

17.3 Racial Differences in Fracture Risk

Racial differences in fracture rates do not necessarily parallel racial differences in aBMD. Population studies in the USA demonstrate that Asian and African Americans share a similarly low risk of fractures although these two groups represent opposite ends of the spectrum with respect to aBMD. Despite low aBMD, Asians and Asian-Americans have relatively low rates of hip fractures compared to white and Hispanic individuals [17, 30–32]. Likewise, wrist/forearm osteoporotic fractures were lower in Asian versus white, Hispanic, and Native American postmenopausal women in one study [17]. In contrast, vertebral fracture rates are similar or higher in Asian versus white women [5, 33]. Few studies have examined differences in aBMD or fracture risk among Asian American subgroups. However, one such study of Medicare enrollees indicated that hip fracture rates tended to be the lowest in Chinese Americans compared to Japanese or Korean Americans [31]. In a retrospective study in Northern California comparing South Asian, Chinese American, and white postmenopausal women, femoral neck osteoporosis prevelence was highest among Chinese Americans and lowest in whites. In this study, despite low aBMD, Chinese Americans had the lowest fracture rates as compared to South Asian and whites who had similar fracture rates at the wrist and other non-vertebral sites [34].

In Hispanics, some studies suggest that hip and spine fracture rates are decreased by about 25–35 % in both men and women versus non-Hispanic whites, but are similar at the distal radius and tibia/fibula [32, 35, 36]. However, studies in US Hispanics are not all directly comparable due to differences in Hispanic subgroups enrolled and uncertainty whether fracture risk is equivalent between different Hispanic heritages. For example, Mexican Americans predominate in California and Texas, whereas elsewhere in the USA, Puerto Rican and Cuban Americans are also well represented. In addition, Hispanics may identify as either white or African American race. In the NORA study, which included Mexican, Cuban, and Puerto Rican postmenopausal women, Hispanics demonstrated a similar risk of fracture compared to whites at all skeletal sites [17]. By contrast, another study, which utilized Medicare claims records, found that age-adjusted hip fracture rates differed based on heritage in both genders. Rates were lowest and similar to African Americans in African American Hispanics from Puerto Rico and the Caribbean, and highest and similar to whites in Mexican Americans. Cuban Americans had intermediate rates of hip fracture [31].

Fracture risk data in Native Americans is sparse and limited to fracture rates derived from the Women's Health Initiative (WHI) and NORA studies of postmenopausal women. Both these studies suggest that non-vertebral fracture rates are similar between Native Americans and non-Hispanic whites [37, 38]. These results differ from that in First Nation Canadians, which demonstrate significantly higher rates of hip and other fractures versus non-First Nation Canadians [39]. Very limited fracture risk data in children exist. One racially diverse cohort of healthy children in the USA indicated that upper and lower extremity fracture rates were higher in white children compared with children of all other races/ethnicities including black, Hispanic, Asian, and "other" race/ethnicities [40].

In summary, the available data on fracture rates across adult racial and ethnic groups in the USA suggests the following hierarchy, with some variability between skeletal sites [6, 41, 42]:

- *Distal radius, tibia, and fibula*: non-Hispanic whites, Hispanics, Native Americans > African Americans > Asian Americans.
- *Hip*: non-Hispanic whites, Native Americans > Hispanics > Asian Americans > African Americans.
- *Vertebral Spine*: non-Hispanic whites, Native Americans, Asian Americans > Hispanics > African Americans.
- *Overall*: non-Hispanic whites, Native Americans > Hispanics > Asian Americans > African Americans.

Notably, in whites, African Americans, Hispanics, and Native Americans, variation in aBMD tends to correspond to and account for at least some of the ethnic/racial differences in observed fracture rates. By contrast, the low aBMD in Asian Americans does not effectively explain the reduction in non-vertebral fracture rates seen compared to whites. The incongruent relationship between aBMD and fracture rates in Asians had remained puzzling but has been further elucidated with recent advances in imaging technology. Prior to the advent of high resoluton imaging, non-skeletal factors such as shorter height [43], lower incidence or severity of falls [44], shorter hip axis length [45, 46] and differences in soft tissue thickness [47, 48] were hypothesized to account for the low fracture rates seen in non-white groups such as Asians. While these factors may contribute to racial variation in fracture rates, differences in bone microstructure and macrostructure and mechanical competence are now clearly also recognized as important.

17.4 Racial Differences in Bone Size, Microarchitecture, and Other Aspects of Bone Quality

Other bone characteristics, incompletely captured by standard DXA, also contribute to fracture risk. These include bone size and geometry [18, 45 49–53], microarchitecture [54–61], and bone turnover [10, 60] among others. While DXA is the gold standard for assessing BMD and fracture risk, it has several limitations. DXA provides a two-dimensional depiction of BMD rather than a true volumetric density. As noted above, this is particularly problematic when studying racial differences in skeletal health given racial variations in bone size, which affects the measurement of aBMD. Further, DXA cannot distinguish the cortical and trabecular bone compartments, nor can it assess bone microarchitecture which is an independent determinant of bone strength [58–61]. Some of these elements of bone quality can now be measured noninvasively via high resolution peripheral quantitative computer tomography (HRpQCT) of the forearm and distal tibia sites, central quantitative computed tomography (cQCT) of the spine and hip or micro magnetic resonance imaging (μMRI). To date, only the QCT methodologies have been used to assess racial differences in bone quality. While both cQCT and HRpQCT have the ability to assess bone size and volumetric density (which is not influenced by bone size) with separate measures for the trabecular and cortical compartments, only HRpQCT has high enough resolution to assess trabecular microarchitecture.

In the MrOs study, which included older white, African-American, Hispanic, and Asian men, cQCT analyses indicated that Asian and black men had greater integral and trabecular volumetric BMD (vBMD) as well as greater cortical thickness at the hip site compared with white men while Hispanic men did not differ from whites. The authors suggested that these differences might account for the lower rates of hip fracture in Asian and black men compared to whites [63]. Utilizing HRpQCT, Walker et al. demonstrated that premenopausal Chinese American women have smaller bone size, but thicker and denser cortical bone, greater trabecular density and thickness, greater trabecular connectivity, and a higher trabecular plate-to-rod ratio versus white women. Similar findings were observed in a study of white and Chinese women in Australia [64]. These characteristics conferred greater finite element analysis-estimated bone stiffness or strength as compared to whites [64–68]. Likewise, postmenopausal Chinese Americans had thicker and denser cortical bone as well as a higher trabecular plate-to-rod ratio which translated into greater whole bone stiffness, despite similar trabecular density as compared to white women [65]. Similar microstructural advantages have also been observed in Asian adolescents and young adults [69]. After adjusting for covariates, Canadian adolescent and young adult South and Southeast Asian males had greater cortical bone density and thickness and lower cortical porosity compared with white males [69]. Asian females also had greater cortical vBMD and thickness and lower trabecular spacing than whites after adjusting for covariates. Thus, microstructural advantages in Asians may compensate for smaller bone size and are consistent with the lower rates of non-vertebral fractures versus whites. To our knowledge, there are no published reports comparing bone quality using HRpQCT in Native Americans, Hispanics, or Hawaiians/Other Pacific Islanders to date.

Studies utilizing DXA-based methods for assessing hip geometry and strength indicate racial differences in these parameters that are generally consistent with racial differences in hip fracture rates [70–72]. For example, in the SWAN study, composite hip strength indices were higher (better) in Chinese, Japanese, and African American women compared with whites [71]. In a WHI sub-study, buckling ratios were similar at the femoral neck in white, Mexican-Americans, and Native Americans but lower (better) in African-American women [70]. In sum, new technologies are providing insight into racial differences in bone geometry, microstructure, and strength which tend, in contrast to DXA, to be consistent with observed racial differences in fracture risk.

17.5 Racial Differences in Bone Acquisition and Loss

Racial differences in bone mineral density are apparent in children and adolescents. While few longitudinal studies have been performed, some work has suggested differences in the tempo of bone mass accrual, with black and Asian females and Asian males tending to reach a plateau in aBMD earlier than other groups [29]. Data from cross-sectional studies that attempt to characterize bone accrual may be subject to cohort effects. However, one cross-sectional study demonstrated Alaskan Native and white children and young adolescents shared a similar bone mineral content (BMC) and ratio of BMC to bone width (BMC/W; an adjustment for bone/body size) at the humerus, distal radius, and ulnar shafts, suggesting similar rates of bone acquisition [73].

Racial differences in rates of age-related and postmenopausal bone loss have also been reported. In the longitudinal SWAN study, Asian Americans had the most rapid vertebral spine BMD loss over the perimenopausal period, as compared to non-Hispanic whites and African Americans [74]. However, the statistically significant absolute difference in bone loss by race or ethnicity was small (1–2 %) and was accounted for by the lower weight among Asian Americans [74]. In a cross-sectional study in Australia, Asian women had greater vBMD "loss" at the spine versus white women while rates were similar in men [19]. Another cross-sectional study in Alaskan Natives found accelerated rates of bone "loss" most notably at the mid-shaft radius versus whites with 10–15 % lower BMC and ratio of BMC to width at age 40, and 15–30 % lower at age 70 [73].

17.6 Racial Differences in Nutrition and Skeletal Health

Although aBMD and fracture risk differ by race/ethnicity, both can vary markedly across geographic location even within the same ethnic or racial group. The extent to which racial differences in bone density and fracture risk are related to genetic or environmental factors is unclear. A number of studies, however, do indicate the importance of environmental factors. For example, despite the fact that >90 % of the population of Beijing, Hong Kong, and Taiwan are Han Chinese, fracture rates are much higher in Hong Kong and Taiwan than in mainland China [42]. Other studies indicate that immigration from Asia to the USA and associated changes in diet, weight, exercise, menarche age, estrogen exposure, body fat, and smoking affect aBMD. For example, against a common genetic background, Japanese immigrants to the USA have higher aBMD than their native Japanese counterparts but lower aBMD than American-born Japanese [75, 76]. Similarly, a cross-sectional study in Chinese immigrants to the USA demonstrated that older age of immigration to the USA was a risk factor for lower aBMD [77]. These findings imply that the environmental milieu can modify aBMD and fracture risk among a genetically similar population.

Among the various environmental factors affecting bone mass, nutritional intake is known to influence both peak bone mass and subsequent post menopausal bone loss [78]. Nutrients act both directly and indirectly on bone metabolism and structure. For example, high protein intake directly results in high amino acid levels which increase bone resorption and urinary calcium excretion acutely, but indirectly stimulates insulin-like growth factor I production which increases osteoblast proliferation and activity over the long term, resulting in net positive bone balance [79–81]. Other indirect nutritional influences include low calcium intake and vitamin D intake, which stimulates parathyroid hormone secretion and increased bone turnover [82].

However, few studies have assessed the contribution of nutrition to ethnic and racial differences in bone mineral density and fracture risk. Further, almost no data exist regarding whether there is variability in the response to nutrient intake on skeletal health across ethnic and racial groups. In the SWAN study [83], racial and ethnic groups had statistically but not necessarily clinically significant differences in nutrient intake. For example, this study reported higher energy-adjusted protein intake and lower energy-adjusted fat intake in Chinese Americans versus other races/ethnicities (protein: 76.3 ± 1.4 g per day versus 65.0 ± 0.6 g per day in African Americans to 73.1 ± 1.3 g per day in Japanese Americans; fat: 59.5 ± 1.4 g per day versus 63.3 ± 1.4 g per day in Japanese Americans to 69.5 ± 0.6 g per day in African Americans). In addition, energy-adjusted calcium intake was higher in whites and Hispanics versus Japanese, Chinese and African American women (835 ± 9 mg per day in whites, 813 ± 33 mg per day in Hispanics, 684 ± 31 mg per day in Chinese Americans, 652 ± 30 mg per day in Japanese Americans, 635 ± 14 per day in African Americans). However, the SWAN study did not report any associations between racial differences in nutrient intake and aBMD, with the exception of soy isoflavone consumption (discussed below) in Asian American subgroups [84].

Calcium. Data from other studies, in addition to SWAN, support the concept that non-white populations, particularly Asian Americans, have lower dietary calcium intake versus whites [67, 85–87]. The extent to which these differences might contribute to either racial variation in peak bone mass attainment or postmenopausal/age-related bone loss is unclear. Studies in Asian and Hispanic children and young adolescents do demonstrate calcium intake to be a small but significant predictor of total body bone mass and bone mineral content [88, 89]. A meta-analysis of 19 intervention studies involving 2,859 white, Asian, Gambian, and mixed race/ethnicity children with doses of daily calcium supplementation varying between 300 and 1,200 mg found only a small effect on total body BMC and upper limb aBMD but not other skeletal sites [90]. Further, ethnicity did not modify the effect of calcium supplementation. However, few studies included in the aforementioned meta-analysis were performed in children with low baseline calcium levels. A subsequent randomized, placebo-controlled study in 96 white adolescents in the UK suggested that calcium supplementation resulting in mean

daily calcium intake >1,000 mg versus <1,000 mg is associated with higher BMC and aBMD at all skeletal sites, as well as decreased bone resorption markers and parathyroid hormone level [91]. This finding persisted after adjusting for height and weight.

Racial differences in calcium retention may also be important. Similar to results from studies in African Americans, data suggest that maximal calcium retention occurs at a lower daily calcium intake in Chinese American girls and boys versus whites. No further increase in calcium retention was observed with calcium intakes exceeding 1,100 mg per day in boys and 970 mg per day in girls, suggesting a lower daily calcium requirement in Asian Americans [92]. By comparison, in a study done in white children and adolescent females aged 9–18, maximal calcium retention was not observed until a calcium intake level of 1,300 mg per day [93].

In the postmenopausal period, calcium may attenuate bone loss indirectly via inhibition of parathyroid hormone [82]. Indeed, a meta-analysis of 32 controlled trials of calcium supplementation indicated that calcium supplementation of about 1,000 mg per day prevented postmenopausal bone loss [94]. Further, a meta-analysis of nine randomized controlled clinical trials found that combined supplementation with vitamin D and calcium reduced the risk of hip and non-vertebral fracture by about 20 % versus supplementation with either alone [95]. However, analysis of the National Health and Nutrition Examination Survey (NHANES III) data in white, African American, and Mexican American men and women aged 50–79 years did not indicate an association between a low daily calcium intake (<300 mg) and low aBMD in any racial/ethnic group [94]. Whether racial differences in calcium intake account for racial variation in rates of bone loss or fracture risk is unclear. Given that fracture risk is low among Asian and African Americans despite low calcium intake, other factors may predominate.

25-Hydroxyvitamin D (25(OH)D). Vitamin D plays a central role in calcium homeostasis and bone health. Multiple studies have demonstrated that non-white minorities have lower 25(OH)D levels compared to whites [97–101]. These differences may be due to reduced vitamin D intake [102–104] and/or variation in skin pigmentation [104–106]. In two New Zealand studies comparing Asian, European, Pacific, and indigenous Maori, 25(OH)D levels varied significantly between ethnic groups in both children and adults, with Asians having the lowest levels and Europeans the highest levels [105, 106]. A significant inverse correlation between 25(OH)D and skin pigmentation was also noted, with Europeans having the lowest skin pigmentation and Asians having the highest skin pigmentation. As in whites, reduced sun exposure and obesity also contribute to low 25(OH)D levels among non-whites [107, 108]. However, in one study, variation in self-reported sun exposure did not reflect 25(OH)D levels within Hispanic subgroups [109]. For example, despite similar levels of self-reported sun exposure in Puerto Rican and South American men, Puerto Rican men had a much higher rate of vitamin D deficiency. The study, however, did not report or control for differences in dietary vitamin D intake.

Although the optimal vitamin D level for bone health is controversial, many define the threshold for vitamin D sufficiency as the lowest serum concentration of 25(OH)D that maximally suppresses parathyroid hormone (PTH) levels. This threshold was derived from studies predominantly consisting of non-Hispanic whites, and therefore it is unclear whether the current recommendations for 25(OH)D levels pertain to other ethnic and racial groups. In a cross-sectional analysis of 2003–2004 and 2005–2006 NHANES data, Mexican Americans had significantly lower 25(OH)D and higher PTH levels versus whites [110]. In both Mexican-Americans and whites, serum 25(OH)D and calcium intake was significantly correlated with aBMD after adjusting for age, gender, BMI and renal function, and an inverse relationship between 25(OH)D and PTH was seen at a 25(OH)D level below 20 ng/ml. These results suggest that the goal 25(OH)D level in Mexican Americans is similar to that in whites. In the Boston Area Community Health Bone study (BACH/Bone), which recruited white, black, and a heterogeneous group of Hispanics including Puerto Ricans, Dominicans, Central Americans, and South Americans, vitamin D levels were lower in Black and Hispanic men versus white men. No correlation between 25(OH)D and aBMD was observed among Hispanics or Blacks.

This contrasted with results in white men, in whom there was a positive, albeit weak, correlation between 25(OH)D and aBMD [109]. The differences between these two studies may be due to differences between the Hispanic subgroups or range of vitamin D levels.

Data in Asian populations are limited. One study of Chinese men in Hong Kong indicated a positive association between 25(OH)D and aBMD at all sites [111]. Another study in Japanese American men and women living in Hawaii found that dietary intakes of milk, calcium, and vitamin D were significantly and positively associated with BMC after adjusting for age, weight, height, exercise, history of fracture, thiazide use, and estrogen use [103]. Data regarding the relationship between vitamin D and PTH levels in Asians are lacking. In aggregate the results suggest that the relationship between 25(OH)D, PTH, and BMD may differ by race. We speculate that this may be secondary to racial differences in calcium retention or differences in the vitamin D receptor.

Differences in vitamin D receptor alleles may contribute to the lower risk of osteoporotic fractures despite higher prevalence of vitamin D deficiency in Africans and Asians versus whites, with Gambians and Chinese having higher prevalence of an allele associated with increased aBMD [112]. However, this finding was not reproduced in a study of African Americans versus whites [113] and no studies have analyzed vitamin D allele frequency in other US non-white minorities.

PTH. PTH is critical for calcium and phosphorous homeostasis and its regulation is important in bone health. In the only US study examining PTH and dietary factors in an ethnically and racially diverse group, results showed that PTH levels in Mexican Americans, African Americans, and whites were positively associated with age, female gender, current smoking, serum uric acid, and BMI, and negatively associated with dietary calcium, serum calcium, and serum 25(OH)D. In whites only, serum retinol was inversely correlated with PTH level. No association was found between PTH and phosphorous, protein, magnesium, alcohol and caffeine intake, physical activity, season of blood draw, menopausal status, or use of hormone replacement, bisphosphonates, or birth control [114]. In cross-sectional studies of elderly Japanese women, there was no correlation between PTH, 25(OH)D, or calcium intake with forearm BMD [115, 116]. By contrast, a cross-sectional study done in young Japanese college students found that mild secondary hyperparathyroidism, lower weight and lower physical activity were associated with low aBMD at the lumbar spine and femoral neck, with low calcium intake also associated with low aBMD at the femoral neck [117]. In this cohort, 32 % of women had vitamin D deficiency, 15.7 % had mild secondary hyperparathyroidism, and calcium intake was lower than average. While data are very limited, these discordant results could suggest that in this native Japanese population, PTH, 25(OH)D and calcium play a larger role in peak bone mass attainment than in postmenopausal bone loss.

Protein. Data suggest that protein is likely to contribute to the attainment of peak bone mass. A prospective observational study in German (presumably white) male and female children and adolescents aged 9–19 years demonstrated a positive correlation between protein intake and periosteal circumference, cortical area, BMC, and calculated strength strain index by pQCT [118]. The only randomized controlled studies evaluating protein and bone health in white children and adolescents have used milk as the intervention rather than protein itself. The results demonstrate higher whole body BMD, bone mineral content and IGF-1 in the milk-supplemented groups [119]. There are no data regarding whether differences in protein intake in children contribute to racial differences in skeletal health.

In adults, controversy exists over whether protein intake has a positive, negative or neutral impact on bone [120–124]. While amino acids derived from proteins stimulate anabolic growth factors, theoretically amino acids can also lead to acid production and increased calcium excretion [81]. A review of 34 cross-sectional studies from 16 countries demonstrated that countries with higher animal protein consumption had higher fracture rates [125]. However, data are confounded by the fact that these countries also had longer life expectancy. In contrast, cohort studies comparing high meat and low meat diets did not show any significant difference in serum or urinary calcium balance [126]. Furthermore, a meta-analysis of 18 cross-sectional and 6 randomized placebo controlled studies

found a small but significant increase in lumbar spine aBMD but no effect on hip fractures or aBMD at other skeletal sites [127]. In another meta-analysis of 46 studies assessing dairy food intake and bone health, only one had significant ethnic and racial diversity but it did not show any contribution of dairy intake on the decreased fracture risk observed in African Americans and Hispanics versus whites [128, 129]. Overall, the impact of protein intake on bone appears to be modest and there are very limited data regarding racial differences in protein intake and its effect upon skeletal health.

Soy. Soy isoflavones, a subclass of plant-derived chemicals with a molecular structure similar to that of estrogen, weakly bind both the α and β estrogen receptor [130]. A number of cross-sectional population studies in Asia have suggested an association between higher soy isoflavone intake (average 25–50 mg per day) and higher aBMD as well as a reduced risk (up to 60 %) of fracture [131–134]. In the SWAN study, only the Chinese and Japanese American subgroups had clinically significant median intakes of soy isoflavones. When these subgroups were analyzed, there was no association between genistein (a soy isoflavone) and aBMD in the Chinese group with a mean intake of 5.8 mg per day. In contrast, in premenopausal Japanese women who had a higher mean genistein intake, genistein intake was positively associated with lumbar spine and femoral neck aBMD, after adjustment for age, menopausal status, dietary calcium, protein and alcohol intake, BMI, smoking, physical activity, duration of residence in the USA, and hyperthyroidism [84]. However, randomized controlled trials of soy isoflavone components versus placebo in US white, European Caucasian, and Asian populations have demonstrated safety but not necessarily efficacy upon aBMD [135–140]. Two studies by the same group in Italy suggest that supplementation with 54 mg of genistein daily versus placebo leads to improved lumbar spine and femoral neck aBMD, reduced bone turnover markers, and increased bone formation markers in white postmenopausal osteopenic women [141, 142]. By contrast, studies in white postmenopausal women without osteopenia or osteoporosis have not uniformly shown a beneficial effect of soy isoflavones versus placebo upon aBMD or bone turnover [143–145]. Currently, it is unclear whether the beneficial effect of soy isoflavones indicated by observational data is due to racial characteristics specific to Asians, behavioral characteristics (i.e., habitual whole soy consumption), or other unmeasured confounding factors that may be present in Asians who consume whole soy. Further, no studies have compared the effect of soy isoflavones upon skeletal health in whites versus Asians in the same geographic location.

Body Mass Index (*BMI*). Body weight is a major determinant of BMC and aBMD. A meta-analysis of 12 prospective population based cohorts demonstrated that low body weight or low BMI is associated with increased risk of fracture at any skeletal site in both men and women globally [146]. Conversely, the MrOS study found that high BMI was also associated with increased risk of nonvertebral fractures. However, differences did not persist after adjusting for variation in mobility, suggesting that worse physical function in obese men may explain the results [147]. Analysis of the NHANES data showed that low BMI was the strongest risk factor for low aBMD in all racial/ethnic groups studied [96]. In the BACH/Bone cross-sectional study, controlling for six categories of covariates was sufficient to account for the majority of racial and ethnic variation in age- and height-adjusted bone mineral content, with lean to fat mass ratio having the largest influence [148]. The other covariates were: serum 25(OH)D levels, osteocalcin, estradiol and socioeconomic factors (born in the USA and income). While differences in body weight may explain some racial differences in BMD, low body/weight appears to be a risk factor for low BMD within all race/ethnic groups.

17.7 Conclusion

There is racial and ethnic variability in aBMD. Studies of aBMD generally indicate the greatest aBMD in African Americans followed by whites, Hispanics, and Native Americans, with the lowest aBMD in Asian Americans. However, differences in fracture risk do not exactly parallel differences

in aBMD. In general, the lowest fracture risk is observed in African Americans, followed by Asian Americans, Hispanics, with the highest fracture risk in non-Hispanic whites and Native Americans. The paradoxical decrease in fractures despite low aBMD in Asian Americans may be due to differences in bone microarchitecture, bone geometry, bone size, or non-skeletal factors.

Racial differences in skeletal health are influenced by weight, bone size, and lifestyle factors such as physical activity and nutrient intake. However, more research is needed to assess for racial differences in nutrition and its effects on skeletal health across minority populations. There are racial differences in calcium intake and retention, which may affect skeletal health. In Mexican Americans as in Caucasians, 25(OH)D level <20 ng/ml is inversely correlated with PTH and directly correlated with aBMD. In contrast, this may not be the case in blacks and other Hispanic subgroups while no data exist in Asians. These preliminary results suggest the possible need for different vitamin D normal ranges for different race/ethnicities. The effect of racial differences in protein intake upon bone mass has not been sufficiently studied. Soy isoflavone intervention studies have demonstrated conflicting results despite cross-sectional studies in Asians that indicate an association of higher soy intake with greater aBMD and reduced fracture risk. More data regarding the effect of racial differences in nutrition upon skeletal health are needed. In particular, intervention trials assessing the effect of various nutrients upon aBMD and/or fracture risk in large, multiethnic populations are needed.

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Part III
Effects of Dietary Macronutrients

Chapter 18
Food Groups and Bone Health

Andrea L. Darling and Susan A. Lanham-New

Key Points

It is important to study the effects of food groups and whole foods on bone health. This is because nutrients may interact in a synergistic manner to influence bone health and osteoporosis risk. This whole diet and food based approach has yielded many insights into the relationship between nutrition and bone health, including the following:

- Cohort and cross-sectional data suggest that diets that are higher in fruit, vegetables, milk and cereal are associated with increased bone mass as compared with diets high in processed and snack foods.
- Consumption of milk and other dairy products appears to have beneficial effects on building bone mass in childhood and adolescence, and may also help offset bone loss after the menopause. However, more research is required to assess whether milk and dairy product consumption can prevent fractures in later life.
- The effects of veganism and vegetarianism on bone health, as compared with omnivorous diets, are not yet clear, with conflicting results being found from different research studies.
- Some research suggests that diets rich in fruit and vegetables may benefit bone health via increased physiological alkalinity. However, conflicting results have been found from recent intervention trials that have attempted to assess the effect of fruit and vegetable supplementation on bone.
- Alcohol, caffeine and soda intakes have the potential to influence bone health. Currently there is evidence that alcohol may be beneficial to bone in moderation, but toxic to bone at higher doses. There is also concern about the potential negative effects of soda on bone health. However, data are difficult to interpret due to the strong interactions between soda intake and lifestyle factors that are detrimental to bone health.

Keywords Diet • Bone • Protein • Calcium • Dairy products • Vegetarianism • Dietary acidity • Food groups • Fruit • Vegetable • Milk • Cereal • Dairy • Alkaline • Alcohol • Caffeine • Soda

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18.1 Introduction

Studies aimed at determining the relationship between key nutrients (especially calcium) and bone health have examined directly the effect of a specific nutrient (or in some cases a variety of nutrients) commonly consumed in the human diet. Consideration of the "foods" we actually consume rather than the nutrients contained within them is an alternative strategy, which has been adopted in other disciplines for examining the relationship between diet and disease. Because site sensory characteristics and the direct hedonic pleasure derived from food are two of the key determinants of food choice, such a "food-orientated" approach is logical way forward [1]. Interestingly, such a procedure has been used to examine associations between dietary factors and a variety of diseases. Examples include breast cancer [2], stomach cancer [3], ischemic heart disease [4], and chronic disease [5].

18.1.1 The Balance of Good Health Plate/Pyramid

The commonality of "nutrients" that exist within food groups is the critical factor to be considered in determining which "food groups" may be important to the etiology of bone health within populations and groups of individuals. Incredibly, there is a considerable level of agreement within and between countries concerning the proportions with which we should be eating food/food groups. A number of countries use different formats, but essentially the focus of the message is predominantly the same. For example, in the UK a plate model is used (see Fig. 18.1), and in the USA, a pyramid model is used. The agreed guidelines are shown in Table 18.1.

Fig. 18.1 UK plate model for food group consumption

Table 18.1 Specified guidelines for a healthy diet

Eat a variety of different foods.
Eat plenty of foods rich in starch and fiber.
Eat plenty of fruit and vegetables.
Do not eat too many foods that contain a lot of fat.
Do not have sugary foods and drinks too often.

18.1.2 Specified Food Groupings and Bone Health

Five food groups exist, with recommendations to consume in the following proportions in the diet: (1) bread, other cereals, and potatoes (35 %, 6–11 servings per day); (2) fruit and vegetables (30 %, at least 5 servings per day); (3) meat, fish, and alternatives (15 %, 2–3 servings per day); (4) milk and dairy foods (15 %, 2–3 servings per day); (5) fatty and sugary foods (5 %, used sparingly). In reviewing the current evidence for an effect of such food groupings on bone health, emphasis will be given to the following areas: the impact of dietary "quality" in general on bone; the effect of milk and dairy products on the skeleton; the influence of meat/animal products on bone integrity; a fruit and vegetable link to skeletal health; and the impact of other food types on bone. Areas will be reviewed in the context of the available research information, and areas for future research will be discussed.

18.2 Impact of Dietary "Quality" on Bone

Two large population-based studies have examined the specific impact of dietary "quality"/food groups directly on indices of bone health, namely, the Aberdeen Prospective Osteoporosis Screening Study (APOSS) [6, 7] and the Framingham Osteoporosis Study [8]. Cluster analysis on 904 women (mean age 54), premenopausal, perimenopausal, and postmenopausal, showed that a number of foods groups, including fried foods, cakes, processed meats, and puddings, were associated with worsening hip bone loss (Spearman rank correlation coefficients ranged from −0.07 to −0.08, respectively, $p<0.05$) [6]. In the Framingham study, analyses of food groups were divided into the following categories: (1) fruit, vegetables, milk, and cereal (termed the "healthy group"); (2) soda, pizza, and salty snacks; (3) cheese and other dairy; (4) meat, bread, and potatoes; (5) baked goods and sweets; and (6) alcohol. The hip bone mass in both males ($n=601$) and females ($n=905$) was found to be significantly higher in the "healthy group." For a total of three of the four hip sites measured, male subjects in the candy group were found to have significantly lower bone mineral density (BMD) than those in the fruit, vegetable, and cereal group, and women in the candy group were also found to have significantly lower BMD than all but one other group at the radius [8]. Similar findings were also found in the new APOSS analysis [7] and that of an Australian study [9] with healthy diets associated with higher bone mass than diets containing high proportions of processed or snack foods [7, 9].

These more recent data support the findings of both the original APOSS baseline study [10, 11] and the older Framingham cohort [12] and indicate that a high fruit and vegetable intake is protective to the skeleton, whereas high candy consumption is associated with lower bone mass, regardless of gender. However, a recent 8-year intervention study using the Women's Health Initiative Cohort showed no association between a healthy diet compared with normal diet in the risk of hip fracture [13]. More long term intervention trials are required to assess the significance of this finding.

18.3 Milk and Milk Products and the Skeleton

18.3.1 Milk Consumption: General Comments

Because milk and milk products provide more than 50 % of the total calcium in the Western diet as well as a number of other key nutrients including phosphorus, magnesium, and zinc, they have a fundamental role in bone health determination. Milk may affect bone health in a different way to that of calcium alone [14]. As noted by Heaney (2000), dairy products are complex, containing many essential nutrients, and thus their effects on bone health are likely "more than can be accounted for by any single constituent and the totality of their effects may be more than the sum of parts" [15].

18.3.2 Milk Studies and Peak Bone Mass Development

Only a few studies have used food sources of calcium (particularly dairy products; either fortified or non-fortified) as the supplementary vehicle to investigate the relationship between calcium and bone mass attainment. Of those studies that have been published, the results demonstrate a clear positive effect on BMD [16–21]. In the prospective, randomized, placebo-controlled, double-blind study published by Bonjour et al. [18], the effects of calcium-fortified foods on bone mass at different skeletal sites were investigated in a cohort of prepubertal girls aged 7–9 years. The foods were enriched with calcium salts from milk extract (in the form of phosphate salts). Benefits were seen in both bone mass and in height between the supplemented and unsupplemented groups, and the effects were greatest in girls with a spontaneous intake below the median. One year after discontinuation of the intervention, the differences in the gain in BMD and in the size of some bones were still detectable, and after 3.5 years, this difference was also still there [20]. In the milk supplementation trial by Cadogan et al. [17], teenage girls consuming a 300-mL milk supplement every day for 18 months had significant increases in total body BMD (9.6 % vs. 8.5 %) and total body bone mineral content (BMC) (27 % vs. 24 %) compared with the control group. Raised insulin-like growth factor (IGF)-1 levels were also reported in the children supplemented with milk, which may point to a stimulation of periosteal bone apposition with the resultant effect of a larger skeletal envelope. Follow-up of these subjects 1 year after discontinuation of the supplement showed a sustained benefit of the milk supplement on bone mass. Finally, a recent meta-analysis suggests that baseline calcium intake may be important, with the largest gain from dietary calcium and dairy product supplementation seen in children with the lowest intakes of calcium at baseline [22]. There is also evidence in the literature that milk basic protein (MBP) directly suppresses osteoclastic-mediated bone resorption, resulting in the prevention of bone loss in the animal model [23].

Finally, a number of retrospective studies have been reported that show a link between low milk consumption during the childhood and adolescence and lower BMD in a number of age groups. Results are consistent for young women [24], premenopausal and postmenopausal women [25] and older postmenopausal women [26, 27]. In the study of Chinese adolescent girls aged 12–14 years ($n = 649$), milk intake was found to be positively associated with distal radius and ulna bone mass ($p < 0.05$) [28]. Milk intake was found to account for 3.2 % of the variation in BMD.

18.3.3 Milk Studies: Effects on Postmenopausal Bone Loss

Of the milk supplementation studies published, there is evidence of a positive relationship with bone health in premenopausal and postmenopausal women. In a 24-month investigation by Prince et al. [29], a daily supplement of fortified skimmed milk powder was found to prevent bone loss in postmenopausal women

and was comparable with a calcium supplement. Favorable effects on markers of bone metabolism have been demonstrated in postmenopausal women following supplementation with dairy products [29, 30].

In the supplementation study by Lau et al. [31], the effect of milk supplementation on postmenopausal bone loss in Chinese women accustomed to a low-calcium diet was investigated. A total of 200 Chinese women aged 55–59 years were randomly assigned to receive 50 g of milk powder per day that contained 800 mg of calcium, or were assigned to a control group. The milk-supplementation group was found to have significantly reduced height loss as well as BMD loss at the three sites measured. Serum parathyroid hormone (PTH) was also found to be lower and serum 25-hydroxyvitamin D [25(OH)D] significantly higher in the milk group compared with the control group at 12 months.

The mechanisms for reduced bone loss in the postmenopausal stage remain to be fully quantified. The study by Lau and colleagues [31] suggests a reduction in PTH levels, which were found to be significantly lower in the milk-supplemented group, is likely to have resulted in a reduction in bone turnover, although no specific measurements were taken. The findings of this study are particularly relevant given the low-calcium characteristic of the diet consumed by the Asian population and the rising public health problem of osteoporosis in this region.

Other recent cross-sectional studies also suggest an impact of milk intake on bone health in this age group. Lifetime milk consumption was found to be positively associated with bone mass at the lumbar spine, hip, and mid-radius in 581 postmenopausal women [32], and similar positive results for milk and bone mass have been found in 965 Japanese men [33].

18.3.4 Milk Intake and Risk of Fracture

Findings of studies concerning milk intake and fracture risk are conflicting: Cumming and Klineberg [34] found that a higher level of consumption of dairy products at 20 years of age was associated with an increased risk of hip fracture in both elderly men and women (aged 65 years and over) [34], and similar results have been found in the USA Nurse's Health Study. In contrast, Johnell et al. [35] found that low milk intake was a significant risk factor for fracture in a large study of European women (mean age 78.1) [31], and a low intake of milk and cheese was found to be associated with an increased risk of fracture in elderly men [36]. More recently, a case control study in Taiwain found milk intake to be predictive of fracture risk in women, but not men [37]. In contrast, a follow-up of postmenopausal women in Nurse's Health Study showed that there was no relationship between hip fracture risk in later life and amount of milk consumed in teenage years (aged 13–18 years) [38]. The Framingham Offspring study [39] found that intake of dairy products was associated with spine and hip BMD, although the association varied by dairy product type. However, there was no association between diary product use and risk of fractures, perhaps because the number of fractures was small ($n=43$). In confirmation of these study findings, a recent meta-analysis of six cohort studies found no association between fracture risk and milk intake [40]. Therefore there is still ongoing debate as to the effect of dairy products on fracture risk in populations, although more recent data suggests that there is no association.

18.4 Impact of Meat/Animal Protein on the Skeleton

Few data are available on populations consuming a diet highly dependent on animal foods, particularly meat. Mazess and Mather [41] examined the BMC of forearm bones in a sample of children, adults, and elderly Eskimo natives of the north coast of Alaska. After the age of 40 years, the Eskimos of both sexes were found to have a deficit of bone mineral of an order of magnitude between 10 and 15 % relative to white standards. In a further study on Canadian Eskimos, ageing bone loss was found to occur at an even greater rate [42].

The hypothesis that a high dietary ratio of animal to vegetable protein increases bone loss and risk of fracture has been studied in a prospective cohort of 1,035 women who participated in the Study of Osteoporotic Fractures (SOF) [43]. Community-dwelling white women aged over 65 years were recruited into the study. Recent dietary history (over the preceding 12 months) was assessed using a "validated" food-frequency questionnaire. BMD was measured using dual-energy X-ray absorptiometry (DXA) at the total hip and sub regions. Two BMD measurements were taken, with an average of 3.6 years (SD 0.4 year) between each assessment, and the rate of bone loss was calculated as the percent difference between two BMD measurements in a subset of the participants ($n = 742$). Hip fractures were assessed prospectively for 7 years (SD 1.5 years), and fracture data were available for all 1,035 women for whom dietary data was collected. Fractures were confirmed with radiographs and a review of radiologists' reports [43].

Women with a higher ratio of animal to vegetable protein intake had a higher rate of bone loss at the femoral neck than did those with a low ratio, as well as a greater risk of hip fracture (relative risk = 3.7). These findings remained significant after adjustment for important confounding factors including age, weight, estrogen use, tobacco use, physical activity, and total calcium and protein intake. These findings suggest that a reduction in animal protein and an increase in vegetable protein may decrease bone loss and risk of hip fracture [43].

However, a recent systematic review and meta-analysis showed that increased total protein intake had a small benefit for bone mass [44], although there was no association between any type of protein intake (animal, vegetable) with risk of hip fracture. The results for bone mass may be explained by the beneficial effects of protein intake on IGF-1 production and calcium absorption to offset any detriment of increased physiological acid load. Moreover, protein may be beneficial for bone health when adequate fruit and vegetables as well as calcium are consumed [45]. Therefore, it is difficult to predict the effect of animal/meat protein on bone health without consideration of the context of the whole diet.

18.5 Fruit and Vegetables and Bone Health

18.5.1 Early Work Linking Acid–Base Imbalance and the Skeleton

As early as the 1880s, the skeleton was considered a potential a source of buffer, contributing to both the preservation of the body's pH and defense of the system against acid–base disorders [46]. Studies more than three decades ago showed the detrimental effects of "acid" from the diet on bone mineral in humans and animals. There is evidence that in natural (e.g., starvation), pathological (e.g., diabetic acidosis), and experimental (e.g., ammonium chloride ingestion) states of acid loading and acidosis, an association exists with both hypercalciuria and negative calcium balance [47]. Because the majority of calcium is contained in bone, it is likely that increased urinary calcium excretion is from an osseous source, and it has been demonstrated that acidosis decreases renal calcium reabsorption [48].

The role of bone in acid–base homeostasis is complex. The skeleton has been referred as being "a giant ion-exchange column loaded with an alkali buffer" because 80 % of body carbonate, 80 % of body citrate, and 35 % of body sodium are contained in solution within the hydration shell of bone and released in response to metabolic acid [49, 50].

18.5.1.1 Dietary Link to Osteoporosis: A Hypothesis

In 1968, Wachman and Bernstein put forward a hypothesis linking the daily diet to the development of osteoporosis: "the increased incidence of osteoporosis with age may represent, in part, the results of a life-long utilisation of the buffering capacity of the basic salts of bone for the constant assault

Table 18.2 Vegetarianism and bone health: summary of studies

Author	Year (reference)	Source	Findings	Summary
Ellis et al.	1972 [54]	Am J Clin Nutr 25:555–558	BMD ↑ in vegetarian group	✓
Ellis et al.	1974 [55]	Am J Clin Nutr 27:769–770	BMD ↓ in vegetarian group	X
Marsh et al.	1980 [56]	JAMA 76:148–151	Bone loss ↑ in omnivores	✓
Marsh et al.	1983 [57]	Am J Clin Nutr 37:453–456	BMD ↑ in vegetarians	✓
Marsh et al.	1988 [58]	Am J Clin Nutr 48:837–841	BMD ↑ in elderly vegetarians	✓
Tylavsky et al.	1988 [59]	Am J Clin Nutr 48:842–849	No difference in BMD between groups	–
Hunt et al.	1989 [60]	Am J Clin Nutr 50:517–523	No difference in BMD between groups	–
Lloyd et al.	1991 [61]	Am J Clin Nutr 54:1005–1010	No difference in BMD between groups	–
Tesar et al.	1992 [62]	Am J Clin Nutr 56:699–704	No difference in BMD between groups	–
Reed et al.	1994 [63]	Am J Clin Nutr 59:1997–1202	Bone loss rates similar	–
Chui et al.	1997 [64]	Calcif Tissue Int 60:245–249	BMD ↓ in vegan group	X
Lau et al.	1998 [65]	Eur J Clin Nutr 52:60–64	Hip BMD lower in vegetarian group	X

Source: Adapted from [56] with permission
BMD bone mineral density

against pH homeostasis" [51]. On a Western diet, adult humans produce roughly 1 meq of acid per day. The more acid precursors a diet includes, the greater is the degree of systemic acidity. There is also good evidence to show that with increasing age, overall renal function declines and acidity increases and thus humans become (albeit slightly) more acidic [52].

18.5.2 Effect of Vegetarianism on Bone Health

Studies of populations following a lacto-ovo-vegetarian diet and their effects on bone mass published prior to 1990 found bone mass higher in the vegetarian group compared with omnivores [53] (see Table 18.2; [54–65]). Results of several of these studies are likely to have been subject to bias because the data were based on Seventh Day Adventists (SDAs), who had a significantly different lifestyle from that of the omnivorous group (e.g., higher physical activity levels and abstaining from smoking, caffeine, and alcohol). Recently published studies suggest no differences in BMD between vegetarians (or vegans) and omnivores [66–68] with one exception [69]. In one longitudinal study, no differences were seen in bone loss rates between the lacto-ovo vegetarians and the omnivorous group [63] as part of a 5-year prospective study of changes in radial bone density of elderly white US women (mean age 81 years) living in residential communities. Also, no difference in bone loss was seen between vegans and omnivores in another longitudinal study [67]. In contrast, a meta-analysis suggests that vegetarian diets are associated with reduced bone mass, with the strongest association seen for vegan diets [70]. However, the strength of the association was not clinically significant, and the studies included in this meta-analysis were heterogeneous, representing the varying nature of studies in this field [70].

18.5.2.1 Acidity in Foods: Importance of PRAL (Potential Renal Acid Load)

Vegetable-based proteins generate a considerable amount of acid in the urine [71]. The potential renal acid load (PRAL) is a useful marker characterizing the acidity of foods, and it has been shown that many grain products and some cheeses have a high PRAL level. The PRAL concept may provide an explanation for the lack of a positive effect on bone health indices in studies comparing vegetarians and omnivores because these foods are likely to be consumed in large quantities by lacto-ovo vegetarians (Table 18.3).

Table 18.3 Potential renal acid load (PRAL) values of a variety of foods and food groups

Food/food group	PRAL (mEq/100 g edible portion)	Food/food group	PRAL (mEq/100 g edible portion)
Fruit and fruit juices		Milk, dairy products and eggs	
Apples	−2.2	Milk (whole, pasteurized)	0.7
Bananas	−5.5	Yoghurt (whole milk, plain)	1.5
Raisins	−21.0	Cheddar cheese (reduced fat)	26.4
Grape juice	−1.0	Cottage cheese	8.7
Lemon juice	−2.5	Eggs (yolk)	23.4
Vegetables		Meat, meat products, and fish	
Spinach	−14.0	Beef (lean only)	7.8
Broccoli	−1.2	Chicken (meat only)	8.7
Carrots	−4.9	Pork (lean only)	7.9
Potatoes	−4.0	Liver sausage	10.6
Grain products		Beverages	
Bread (white wheat)	3.7	Coca Cola	0.4
Oat flakes	10.7	Coffee (infusion)	−1.4
Rice (brown)	12.5	Tea (Indian infusion)	−0.3
Spaghetti (white)	6.5	White wine	−1.2
Cornflakes	6.0	Red wine	−2.4

Source: From [55]

18.5.3 Fruit and Vegetable Intake and Bone: A Review of Population-Based and Intervention Studies

A variety of population-based studies have recently been published, with remarkable similarities between two of the largest nutrition and bone health surveys [72]. A beneficial effect of fruit and vegetable/potassium intake on bone mass has been shown in children; premenopausal, perimenopausal, postmenopausal, and elderly women; on bone loss in men; and on markers of bone metabolism and peripheral skeletal sites in women (Table 18.4) [73–79]. Since the publication of these studies, others have also lent support to a beneficial association between fruit and vegetable intake and bone mass [80]. Results of the Dietary Approaches to Stopping Hypertension (DASH) intervention trial lend further support to the view that diets high in fruit and vegetables may be important to bone health. Diets rich in fruit and vegetables were associated with a significant fall in blood pressure compared with baseline measurements [81]. However, of particular interest to the bone field was the finding that increasing fruit and vegetable intake from 3.6 to 9.5 daily servings decreased urinary calcium excretion from 157 to 110 mg/d [81]. More recently, it has been reported that the DASH diet (which emphasizes low-fat dairy products, fruit and vegetables, and a reduced amount of red meat) was found to significantly reduce markers of bone metabolism [82]. However, a recent meta-analysis of eight observational and intervention studies in women over 45 years has shown no association between fruit and vegetable intake and bone health [83]. Many of the studies had significant risk of bias and there was significant between study heterogeneity, with more good quality trials needed in this area [83].

18.6 Other Food Groups: Hot and Cold Beverages

Other foods remaining on the balance of the good health plate/pyramid have received relatively scant attention in the literature with respect to a direct effect on bone health. Data for alcohol consumption are intriguing: excessive alcohol intake is associated with osteoporosis and osteoporotic fractures [84], and it is well known that alcohol is directly toxic to bone-forming cells [85] and may disrupt

Table 18.4 Impact of fruit and vegetables on bone: a review of population-based studies showing a positive link

Author	Year (reference)	Source	Details	Findings
Eaton-Evans et al.	1993 [73]	Proc Nutr Soc 52:44A	77 Females, 46–56 years	✓ Vegetables
Michaelsson et al.	1995 [74]	Calcif Tissue Int 57:86–93	175 Females, 28–74 years	✓ K Intake
New et al.	1997 [10]	Am J Clin Nutr 65:1831–1839	994 Females, 45–49 years	✓ K, Mg, fiber, vitamin C ✓ Past intake: fruit and vegetables
Tucker et al.	1999 [8]	Am J Clin Nutr 69:727–736	229 Males, 349 females, 75 years	✓ K, Mg, fruit and vegetables
New et al.	2000 [11]	Am J Clin Nutr 72:142–151	62 Females, 45–54 years	✓ K, Mg, fiber, vitamin C ✓ Past intake: fruit and vegetables
Jones et al.	2001 [75]	Am J Clin Nutr 73:839–844	215 Boys, 115 girls, 8–14 years	✓ K, urinary K
Chen et al.	2001 [76]	J Bone Miner Res 16:S386	668 Females, >48–62 years	✓ Fruit
Miller et al.	2001 [77]	J Bone Miner Res 16:S395	300 Males, 50–91 years	✓ K, Mg
Stone et al.	2001 [78]	J Bone Miner Res 16:S388	1,075 Men, 65 years and over	✓ K, lutein
New et al.	2002 [79]	Osteopor Int 13:S77	164 Females, 55–87 years	✓ K, fruit and vegetables

Source: Adapted from [56], reproduced with permission

bone metabolism in humans [86]. However, moderate alcohol consumption may not be detrimental to bone health. A positive association between moderate alcohol intake and BMD has been reported in premenopausal women [10], postmenopausal women [87], and elderly women [88, 89], as well as men [87]. Moreover, a recent study in Korean women suggests that moderate, (but not high or low) consumption of alcohol is associated with reduced bone strength [90]. The mechanisms for the positive relationship between alcohol and bone at moderate intakes require further clarification but point to (1) alcohol affects endogenous hormone levels and induces adrenal production of androstenedione and its adrenal conversion to estrone; and (2) alcohol stimulates the secretion of calcitonin, which is likely to favor an increase in bone mass [86].

Varied results have been obtained in studies examining coffee consumption and bone density, but data appear to suggest a negative association only when high caffeine intake is accompanied by very low calcium intake [91]. A recent study suggest that high coffee consumption may be associated with slightly reduced bone mass, but showed no association with bone fracture [92]. Recently, a positive association has been noted between tea drinking and bone mass in postmenopausal women and may point to the influence of flavonoids contained in tea on bone health, but this is an area requiring further attention [93]. Finally, there has been some concern regarding the rising consumption of soda drinks in Western societies, and the impact this may have on bone health. Recent studies have shown that soda consumption is negatively associated with bone mass [94, 95] but not associated with osteoporosis risk or fracture risk [96, 97]. This is a complex issue to interpret due to differences between soda types and the interaction between soda consumption with other lifestyle factors which are detrimental to bone health [94–97]. More research is needed in this area.

18.7 Conclusion

The approach of using food groups to examine the relationship between diet and disease is an appropriate and logical approach to examining the relationship between diet and osteoporosis. [98]. There is somewhat remarkable agreement among countries as to the proportions with which we should be

eating food groups. The data suggest that milk and milk products (as providers of more than 50 % of total dietary calcium) and fruit and vegetables are beneficial to bone health across the age ranges, although clearly more work on fracture reduction is required. Experimental work is required to determine the exact mechanisms of action of these two specific food groups on the skeleton. Further research is required concerning the effect of other food groups on both indices of bone health and in fracture risk reduction [99].

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Chapter 19
Vegetarianism and Bone Health in Women

Susan I. Barr

Key Points

- Vegetarian diets typically exclude meat, fish, and poultry (lacto-ovo vegetarian) and may also exclude dairy products and eggs (vegan).
- Bone mineral density (BMD) and/or fracture risk may be affected by nutrients present in lower amounts in vegetarian versus omnivorous diets and/or that vary across the range of vegetarian diets (e.g., calcium, vitamin D, vitamin B_{12}, protein), as well as substances that may be present in higher amounts in vegetarian diets (e.g., oxalates, phytates, flavonoids).
- Physical activity and weight status may also vary between vegetarians and omnivores, and need to be considered when comparing these two groups.
- A meta-analysis of studies assessing BMD at the spine and/or hip reported slightly lower BMD in vegetarians than omnivores, but concluded that the difference was not clinically relevant. However, many of these studies included Asian vegans, most of whom had calcium intakes and similar to omnivorous controls.
- Two studies have prospectively examined fracture risk between vegetarians and omnivores. The EPIC-Oxford study followed almost 35,000 subjects (77 % female) for 5 years, and reported that vegans (but not lacto-ovo vegetarians or semi-vegetarians who ate fish) had ~30 % higher fracture risk than omnivores.
- Long-term adherence to vegan diets appears to be associated with increased fracture risk in Western settings, where calcium intakes of vegans are much lower than those of omnivores.

Keywords Vegetarian diets • Bone mineral density • Fracture • Calcium • Vitamin D • Vitamin B_{12} • Protein • Oxalates • Phytates • Flavonoids • Physical activity • Weight status • Omnivores • Vegan

19.1 Introduction

In recent years there has been an increased interest in possible health impacts of plant-based diets, including whether or not they affect bone health (i.e., bone mineral density (BMD) and fracture risk). Accordingly, the purpose of this chapter is to review possible mechanisms whereby dietary and

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Table 19.1 Definitions of Vegetarian Diets

Type of diet	Characteristics
Semi-vegetarian	No use or infrequent use of red meat Some individuals may include chicken (pollo-vegetarians) and/or fish (pesco-vegetarians) Dairy products and eggs are included
Lacto-ovo vegetarian	Do not consume meat, fish, or poultry Include dairy products and eggs Some individuals may exclude eggs (lacto-vegetarian) or dairy products (ovo-vegetarian)
Vegan	Do not consume meat, fish, or poultry Do not consume dairy products or eggs Some vegans will consume dairy products and/or eggs as ingredients in other foods (e.g., baked goods), while others also exclude foods containing these ingredients Some vegans also exclude foods or ingredients that are derived from animals (e.g., honey, foods containing gelatin)
Macrobiotic	Diet is based on cereals, pulses, and vegetables Small amounts of seaweeds, fermented foods, nuts, seeds, and seasonal fruit are consumed Fish may be consumed occasionally, but meat and dairy products are usually avoided

lifestyle factors associated with vegetarianism could influence bone health, and to review the available literature comparing bone health between vegetarians and omnivores. Because relatively few studies have been conducted with men, this review is confined primarily to women, unless noted otherwise.

19.2 Vegetarian Diets

Vegetarian diets are often described as excluding tissue protein sources (meat, fish, and poultry) [1]. According to this definition, a relatively small percentage of the population in Western countries (e.g., ~4 % in the USA) would be classified as vegetarian [2]. However, there is considerable variability among the diets followed by vegetarians, as shown in Table 19.1. Accordingly, the nutrient composition of the diet would vary depending on the type of diet followed. For example, if use of dairy products was increased to replace tissue protein sources, a lacto-ovo-vegetarian diet could contain more calcium than a traditional omnivorous diet, whereas a vegan diet would likely contain less calcium. Other key nutrients that vary among vegetarian diets include protein, vitamin D, and vitamin B_{12} [1]. These differences in nutrient composition suggest that associations with bone health could be heterogeneous.

19.3 Possible Mechanisms by Which Vegetarianism Could Influence Bone Mineral Density

There are a number of mechanisms whereby components of a vegetarian diet or aspects of a vegetarian lifestyle could influence BMD, and thus fracture risk, in either a positive or negative manner. Most of these possible mechanisms are discussed in depth elsewhere in this volume (e.g., Chaps. 14, 20, 22, 31, 32, 33, and 34); accordingly, only brief overviews are presented here.

19.3.1 Dietary Factors

19.3.1.1 Calcium

Calcium is a major component of bone mineral, and must be provided in the diet for bone mineralization to occur. It has been suggested that calcium is a threshold nutrient: higher intakes of calcium are related

to increased mineralization during growth (or decreased loss during aging) up to the putative threshold, above which higher intakes have no additional effect [3]. Thus, diets that differ in calcium content at levels below the threshold could be hypothesized to lead to differences in bone mineralization.

Vegetarian diets have the potential to contain either more, similar amounts, or less calcium than omnivorous diets. Several studies suggest intakes are similar [1, 4–6], although lacto-ovo vegetarians who increase their use of dairy products to replace meat, fish, and poultry may have higher intakes [7, 8]. Conversely, vegans typically have calcium intakes that are lower than those of omnivores [9–12].

19.3.1.2 Vitamin D

Although the data are not always consistent [13], vitamin D is well-recognized as playing a role in bone health [14]. Naturally occurring dietary sources of vitamin D are limited to animal foods. The richest sources are fish liver oils; other important sources are fatty fish such as herring and salmon, and small amounts are found in chicken and beef liver, shrimp, egg yolk, and butter. In some countries (e.g., the USA and Canada) fluid milk and some other foods are fortified with the vitamin. Recently, it has been recognized that small amounts of 25-hydroxyvitamin D present in meat, fish, poultry and eggs also make meaningful contributions to vitamin D status [15]. Globally, most people rely on endogenous synthesis to provide the majority of their needs. However, sunlight exposure may not be adequate as distance from the equator increases, or if sunscreen or protective clothing blocks endogenous synthesis.

Dietary vitamin D intakes are likely to be low in North American vegetarians who exclude fluid milk and do not use vitamin D supplements. In countries that do not fortify milk or other foods, vegetarians may also have lower intakes than omnivores because of the exclusion of meat, fish, and poultry. Compared to omnivores, lower circulating vitamin D levels have been reported in premenopausal vegan women living in Finland [16] and in postmenopausal vegan Buddhist nuns [11], but did not differ between vegetarians (predominantly lacto-ovo) and meat-eaters among Seventh-day Adventists living in the USA [17].

19.3.1.3 Protein And Acid/Alkali Balance

The role of protein in the maintenance of bone has engendered considerable debate, and evidence exists to suggest adverse effects of both inadequate and excessive protein intakes [18]. On the one hand, bone acts as a metabolic buffer, and it is known that metabolic acidosis leads to loss of bone mineral [19]. Thus, diets that result in a net acid load have the potential to lead to bone mineral loss, whereas diets with a low acid load could be less likely to do so. Most high-protein foods lead to acid end products, whereas most fruits and vegetables are high in organic anions and yield net alkali. Accordingly, the "acid-ash" hypothesis suggests that high protein diets would have adverse effects on bone through increased urinary calcium loss, while diets rich in fruits and vegetables (sources of alkali) would enhance calcium balance. However, recent reviews and meta-analyses indicate that a causal association between dietary acid load and osteoporosis is not supported by the evidence [20, 21]. Conversely, protein is required for synthesis of bone matrix proteins, is needed to support an anabolic environment, and also acts to enhance calcium absorption [18]. A recent meta-analysis reported a small positive impact of protein supplementation on spinal BMD in randomized trials, but did not observe an impact of protein intake on hip fracture risk in cohort studies [22]. Although it appears likely that higher protein intakes within the normal range do not adversely affect bone—and may be beneficial—additional research is needed to clearly delineate the "optimal range" and how it is influenced by calcium and perhaps other dietary variables [23].

Compared to omnivorous diets, most vegetarian diets (and in particular, vegan diets) contain lower amounts of protein [4–6, 9, 11, 24, 25].

19.3.1.4 Vitamin B_{12}

Over the past decade, a growing body of research has provided suggestive evidence of an association between vitamin B_{12} status and bone health, although the mechanism has not been clarified [26, 27]. Specifically, increased serum homocysteine levels (which increase when folate and B_{12} status are poor) have been associated with fracture risk, and there appears to be a weak inverse association between serum B_{12} and fracture risk. Overall, high homocysteine and/or low B_{12} status appear to be associated with increased bone resorption and decreased bone formation. Few intervention studies have been conducted: In elderly Japanese stroke patients with high homocysteine levels, supplementation with folate and vitamin B_{12} versus placebo reduced the relative risk of hip fracture [28]. Conversely, in another study of community-dwelling individuals (in a country with mandatory folate fortification and thus lower homocysteine levels), there was no effect on fracture risk of supplementation with folic acid, vitamin B_6, and vitamin B_{12} versus placebo [29].

Vitamin B_{12} intakes and status are known to be lower in vegetarians than omnivores, and vegans tend to have the lowest levels [1]. In contrast, folate intakes are generally similar, or may be slightly higher in vegetarians.

19.3.1.5 Phytates/Oxalates

Calcium may be poorly absorbed from foods rich in oxalic acid [30] or phytic acid [31], both of which are found in plant foods (oxalates in spinach, sweet potatoes, rhubarb, and beans; phytates in unleavened bread, raw beans, seeds, nuts, and grains, and soy isolates). Although specific intake data could not be located, it is likely that vegetarian diets contain higher amounts of phytates and oxalates than omnivorous diets.

19.3.1.6 Flavonoids

Soy products, used by many vegetarians, are rich in the isoflavones genistein and daidzein, which act as naturally occurring selective estrogen receptor modulators and thereby have the potential to have effects on bone [32]. Lower rates of hip fractures in Asian women have been associated with soy consumption, and some (but not all) intervention trials with isolated isoflavones have had positive findings. It is possible that outcomes may vary depending on age or life stage. Another consideration is that soy product intake and vegetarianism are not necessarily synonymous. Although average intakes are likely higher in vegetarians, some omnivorous women may use large amounts of soy products, and not all vegetarians incorporate them in their diets.

19.3.2 Lifestyle Factors

A vegetarian lifestyle may be associated with other health-promoting behaviors, such as not smoking and being physically active, that also have the potential to influence bone and could therefore confound comparisons of bone density based on diet. This possibility can be illustrated by comparisons of overall mortality rates between vegetarians and omnivores [33]. When comparisons were made to population data, standardized mortality ratios for all causes of death for vegetarians were significantly below the reference value of 100. However, when comparisons were made to controls with similar socioeconomic status and health behaviors, no differences were seen [33]. This emphasizes the need to choose appropriate omnivorous controls when assessing the possible impact of a vegetarian diet on bone.

In contrast to the positive lifestyle behaviors referred to above, some weight-conscious young women may adopt a vegetarian diet because of concerns about body weight, in essence using vegetarianism as a socially acceptable way of limiting food intake [34–36]. To the extent that dieting or weight concerns can compromise nutrient intake, increase cortisol levels, and/or interfere with menstrual function, the potential for adverse effects on bone exists [37, 38]. It should be emphasized that these effects are not specific to vegetarians, and that a vegetarian diet per se does not appear to interfere with normal menstrual function in otherwise healthy women [39]. Although the association between weight-related concerns and vegetarianism is unlikely to be apparent among those who follow vegetarian dietary patterns for religious reasons (e.g., Seventh-Day Adventists, Buddhists), it may be a factor among some who adopt vegetarian diets for other reasons. Again, attempting to control for this would be important when assessing the effects of vegetarianism on bone.

19.4 Studies Assessing BMD and Fracture Risk in Vegetarians and Omnivores

The previous section described some of a number of mechanisms whereby vegetarianism could either have a protective or an adverse effect on BMD. In this section the results of available studies comparing bone density and fracture risk between vegetarians and omnivores are presented. Interpretation of these studies is not straightforward: First, many of the vegetarians were members of religious groups that advocate a vegetarian diet. They may differ in several respects, other than diet, from those who adopt vegetarian diets because of concerns about animal rights, for example. Second, particularly for vegetarians whose vegetarianism is not based on membership in a religious group, the characteristics of the vegetarian diet—and even whether a vegetarian diet is followed—may change over time [40]. And finally, as indicated earlier, there is great heterogeneity among vegetarian diets, and in particular, the extent to which intakes of nutrients such as calcium is impacted by the diet.

19.4.1 Early Studies of Bone Density or Bone Mineral Content

A number of early studies comparing bone density of vegetarians and omnivores were conducted with single-photon or single-energy X-ray absorptiometry [4–8, 41, 42]. This technique was used to measure BMD of peripheral bones, but could not assess clinically relevant central sites such as the lumbar spine and hip [43]. Most studies examined Caucasian postmenopausal women who had followed lacto-ovo-vegetarian diets for a lengthy period of time; the number of vegans was not large enough to provide meaningful data.

Two studies reported higher bone density in postmenopausal lacto-ovo vegetarians than in omnivorous controls: Ellis et al. [7] observed higher bone density at the center of the third metacarpal medulla and the proximal phalanx in 25 long-term British lacto-ovo vegetarians aged 53–79 years, and Marsh et al. [41] found that mean BMD of the radius was higher in long-term lacto-ovo-vegetarian women aged 50–87 years. Unfortunately dietary intakes were not assessed in these studies. However, Marsh et al. subsequently reported on 7-day weighed diet records kept by ten lacto-ovo vegetarians and ten omnivores aged 52–87 years from the same geographic area as the initial study, to assess whether dietary factors contributed to the observed difference in bone density [8]. Mean calcium intakes (lacto-ovo vegetarian = 898 mg/day and omnivorous = 712 mg/day) did not differ significantly, and mean phosphorus intakes were almost identical (lacto-ovo vegetarian = 1094 mg/day and OMNI = 1103 mg/day). However, the Ca:P ratio was significantly higher in lacto-ovo vegetarians ($p < 0.001$), suggesting that the difference in calcium intakes likely approached significance.

Three other groups did not detect differences in BMD between postmenopausal lacto-ovo vegetarians and omnivores [4–6, 42]. Tylavsky et al. compared bone density at the mid and distal radial sites between 88 lacto-ovo-vegetarian and 287 omnivorous postmenopausal women aged 60–98 years [4]. Vegetarians, who were members of the Seventh-Day Adventist religious group, had adhered to their diets for at least 16 year. No difference in age-adjusted bone density or bone mineral content (BMC) was observed between groups at either bone site, nor did calcium intakes differ. Five years after the initial bone measurements, follow-up measurements were made on 189 members of this group (49 lacto-ovo vegetarians and 140 omnivores) [42]. Although all women lost bone, BMD loss rates did not differ between lacto-ovo vegetarians and omnivores. Similarly, Hunt and colleagues reported that BMC and bone width (BW) of the nondominant radius were comparable between groups of 146 Methodist omnivores and 144 Seventh-day Adventist lacto-ovo-vegetarian women [5]. The two groups were similar in age, height, weight and calcium intakes, although protein intakes were higher in omnivores. Finally, both axial and peripheral bone density were compared between 28 matched pairs of postmenopausal lacto-ovo vegetarians and omnivores studied by Tesar and colleagues [6]. All women were members of religious groups, and vegetarians had followed a vegetarian diet for at least 10 years (mean = 35 years). Single-photon absorptiometry was used to measure BMC and BMD at the midshaft and distal radius, and dual-photon absorptiometry was used to measure whole-body, regional, and lumbar spine BMC and BMD. Diet was assessed using a 24-h recall and a 6-day record, and supplement use was recorded. Dietary and supplemental intakes of calcium, phosphorus, and the Ca:P ratio were very similar between groups, although protein intake was higher among omnivores. Twenty comparisons of BMC and BMD were made between groups, and only head BMD differed, being higher among vegetarians ($p < 0.05$). Since p values were not adjusted for multiple comparisons, this difference would be expected based on chance alone.

Only very limited data on vegan women was available in these early studies. Eleven vegan women were included among approximately 300 vegetarians aged 52–90 studied by Marsh et al. [8]. Calcium intake was not assessed, but milk intake averaged 450 mL/day for lifetime lacto-ovo vegetarians and 0 mL/day for vegans. Although statistical analysis of the results was not reported, the radial BMD of the vegan women appeared to be lower than that of lifetime lacto-ovo vegetarians (0.465 vs 0.571 g/cm^2).

Taken together, these early studies suggested that, compared to omnivores, BMD was either not different or slightly greater among postmenopausal women who had followed lacto-ovo-vegetarian diets for many years. The studies reporting similar BMD values also reported similar calcium intakes, whereas those reporting higher BMD values did not assess calcium intake, although the authors suggest it may have been higher in lacto-ovo-vegetarian women.

19.4.2 More Recent Studies of Bone Mineral Density

More recently, studies have assessed BMD at clinically relevant sites such as the lumbar spine and femoral neck [11, 16, 24, 25, 44–49]. A meta-analysis by Ho-Pham et al. [50] reported findings from nine of these studies, published between 1991 and 2009. The studies included were observational comparisons of vegetarians and omnivores aged ≥18 years of age, assessed BMD, were written in English and published in peer-reviewed journals. Only two studies included men, and five were conducted in Asian populations. Random-effects meta-analysis indicated that mean BMD at both the femoral neck and the lumbar spine averaged 4 % lower in vegetarians: in both cases the ratio of the means (RoM) in vegetarians compared to omnivores was 0.96, with a 95 % CI of 0.93–0.98 ($p < 0.001$). In subanalyses, the RoM was lower among vegans than lacto-ovo vegetarians (RoMs of 0.94 and 0.98, respectively, both compared to a value of 1.0 for omnivores), and in Caucasian than Asian vegetarians (0.90 and 0.97, respectively, again compared to omnivores). The authors also used a Bayesian

analysis, and determined that the probability of BMD ≥ 5 % lower in vegetarians than omnivores was 42 % for the femoral neck and 32 % for the lumbar spine. They interpreted this as being clinically insignificant, and unlikely to result in a clinically important difference in fracture risk [50].

While this meta-analysis was carefully conducted, its findings need to be interpreted within the context of the participant characteristics and diets. Specifically, among 1,286 vegetarians in the nine studies, 1,206 were Asian (from China, Taiwan, Korea, or Vietnam) and only 80 were Caucasian (from Europe or North America). Among the Caucasians, only 30 were vegan. It seems probable that the composition of Asian and Caucasian vegetarian diets differed. For example, among the five studies of Asians, four provided data on calcium intakes [24, 45, 47, 49]. In the study of Chiu et al. [45], calcium intakes did not differ between subjects defined as "long-term vegan vegetarians" ($n = 71$, who had followed a strict vegan diet for ≥15 years) and "others" ($n = 187$, who were lacto-ovo vegetarians, alternated between vegan and omnivorous diets, or who had been vegan for <15 years). Kim et al. [47] reported that calcium and intakes were similar between vegetarians and omnivores, while Lau et al. [24] reported higher calcium intake in vegans than omnivores. Only one study [49] reported higher calcium intakes in omnivores than vegans. Conversely, in North America and Europe, vegan diets are considerably lower in calcium than diets of lacto-ovo vegetarians and omnivores [1, 9, 12]. Along the same lines, the majority of studies of Asians reported similar BMIs between vegetarians/vegans and omnivores, whereas in Western countries, lower weights are frequently reported for vegetarians [1, 12]. Thus, while it may be appropriate to conclude that BMD differences between Asian vegetarians (vegans and lacto-ovo vegetarians) and omnivores are not clinically meaningful, this conclusion may not be warranted in other contexts.

19.4.3 Fracture Risk

To date, only two published studies could be located in which fracture risk was directly compared between vegetarians and omnivores [11, 12]. The first, and most comprehensive, was a prospective study conducted among the Oxford cohort of the European Prospective Investigation into Cancer and Nutrition [12]. The sample, which was 77 % female, included 19,249 meat eaters, 4,901 fish eaters (who excluded meat), 9,420 vegetarians (who consumed dairy products and/or eggs) and 1,126 vegans. Approximately 5 years after recruitment, data on incident fractures of bones other than the digits or ribs were obtained by questionnaire. During the follow-up period, 343 men and 1,555 women reported one or more fractures. Fracture incidence was examined by diet group among women, men, and both sexes combined, and three sets of analyses were conducted. The first adjusted only for age, the second adjusted for age and non-dietary factors (smoking, alcohol, body mass index, recreational and occupational physical activity, marital status, and for women, number of children and use of hormone replacement therapy), and the third adjusted for age, non-dietary factors, and energy and calcium intakes. Sex was also adjusted for when both sexes were combined.

Compared to meat eaters, fracture incidence rate ratios (IRR) adjusted for sex, age, and non-dietary factors were similar for fish eaters (IRR = 1.01; 95 % CI 0.88–1.17) and vegetarians (IRR = 1.00; 95 % CI 0.89–1.13), but were 30 % higher for vegans (IRR = 1.30; 95 % CI 1.02–1.66). Further adjustment for energy and calcium intakes had no effect on IRRs for fish eaters and vegetarians, but attenuated the risk for vegans (IRR = 1.15; 95 % CI 0.89–1.49). The authors also conducted an analysis restricted to those with reported calcium intakes above 525 mg/day, which included 94–95 % of meat eaters, fish eaters and vegetarians, but only 42 % of vegans. In this analysis, the IRR for vegans was 1.00 (95 % CI 0.69–1.44). Overall, the authors concluded that fracture risk was ~30 % higher in vegans, and that this appeared to be a result of their lower mean calcium intakes as risk was not increased among vegans with higher calcium intakes [12].

The other study reporting fracture risk data was a 2-year follow-up of 88 vegan and 93 omnivorous Buddhist nuns in Vietnam [11]. At follow-up, incident vertebral fractures had occurred in five vegan

and five omnivorous women, as assessed from spinal X-rays. There was clearly no difference in fracture risk based on diet group, nor were differences observed in BMD change or markers of bone turnover [11]. This study, however, was limited by the small number of subjects, the short duration of follow-up, and the very low number of fractures.

19.5 Conclusion

The potential exists for characteristics of vegetarian diets to have beneficial or adverse effects on bone density. Following a lacto-ovo-vegetarian diet with adequate calcium, protein, and vitamin D may have potential to favorably affect BMD, although it seems likely that an omnivorous diet with similar characteristics would be equally favorable. Long-term adherence to a vegan diet, however, appears to be associated with lower bone density and increased fracture risk, particularly in Western settings. The mechanism(s) underlying these observations is not yet known with certainty, but it is reasonable to suggest that suboptimal intakes of nutrients such as calcium, protein, and vitamin D may contribute. Accordingly, it would be prudent for vegans to choose fortified foods and/or supplements to ensure they meet current recommendations for these nutrients.

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Chapter 20
Protein Intake and Bone Health

Jean-Philippe Bonjour, Thierry Chevalley, Patrick Amman, and René Rizzoli

Key Points

- Dietary protein represents a key nutrient for bone health and thereby for the prevention of osteoporosis.
- During growth, protein under nutrition from infancy to childhood and adolescence results in reduced bone mass and strength.
- High protein intake, particularly when associated with physical activity, favors healthy development and peak bone mass acquisition enabling individuals to reach their genetic potential.
- There is a positive interaction between dietary protein, calcium–phosphate economy, and bone metabolism mediated by the anabolic bone trophic factor IGF-I.
- Amino acids such as arginine can exert a direct positive effect on the IGF-I production by bone forming cells.
- In young adulthood energy deficit, as observed in anorexia nervosa, can be associated with insufficient protein supply, low circulating IGF-I and bone loss.
- With aging, the reduction in the protein intake is associated in both genders with a decrease in the serum level of IGF-I, lower femoral neck aBMD, and poor physical performance.
- Protein under nutrition is often present in patients experiencing hip fracture and outcome after hip fracture can be significantly improved by normalizing protein intake.
- An adequate intake of proteins should be recommended in the prevention and treatment of postmenopausal and age-dependent osteoporosis.

Keywords Dietary protein • Bone acquisition • Physical activity • Mineral metabolism interaction • Protein malnutrition • Anorexia Nervosa • Elderly • Hip fracture patients • Protein repletion

20.1 Introduction: Proteins as Bone Matrix Constituent

Bone is a composite tissue, made up of mineral, organic matrix, water, and cells. The major constituent of bone mineral is an impure form of hydroxyapatite located within and between collagen fibrils. Collagen Type I represents about 98 % of total bone proteins. The main non-collagenous proteins are osteocalcin, osteopontin, sialoprotein, and osteonectin [1].

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In the process of bone modelling, mainly during growth, and remodelling during adulthood, the organic matrix is formed and resorbed. Molecular products of these two processes, particularly from Type I collagen, are released into the systemic extracellular compartment. They can be chemically analyzed and used as markers of bone formation and resorption [2–4]. Other non-collagenic bone proteins such as tartrate-resistant acid phosphatase-5b, specific bone alkaline phosphatase or osteocalcin are also released during the process of bone remodelling. They are detectable within the systemic extracellular compartment, and are also used to estimate the rate of bone remodelling, as well as its changes in response to physical, pharmaceutical, or nutritional interventions [2–4].

20.2 Effect of Protein Intake on Calcium–Phosphate Economy and Bone Metabolism

Protein from food is required to promote bone formation. As for any other organs of the body, the synthesis of intracellular and extracellular bone proteins and other nitrogen-containing compounds is dependent upon the supply of amino acids. Besides this role as "brick supplier," proteins, through their amino acid content, can influence bone mineral economy and metabolism. Thus, nutrition [5], and particularly dietary protein, increases the circulating level of insulin-like growth factor-I (IGF-I) [6–8]. IGF-I is expressed in most tissues of the body. Nevertheless, liver-produced IGF-I is the main source of circulating IGF-I [9] (Fig. 20.1). In response to variations in protein intake, the changes in circulating IGF-I can be observed in absence of any difference in dietary energy supply as observed in both animal experiments [10] and in human subjects [6].

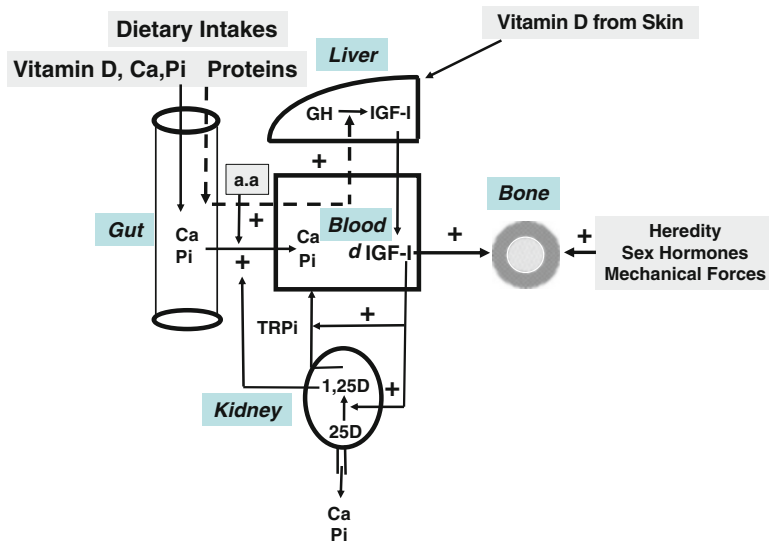

Fig. 20.1 Role of dietary protein on calcium and inorganic phosphate (Pi) economy, and bone health. The hepatic production of insulin-like growth factor-I (IGF-I), which is under the positive influence of the growth hormone (GH), is also stimulated by amino acids (a.a.). IGF-I exerts a direct action on bone anabolism. In addition, at the kidney level, IGF-I increases both 1,25,dihydroxyvitamin D (1,25D) formation from 25-hydroxyvitamin D (25D) and the tubular reabsorption (TR) of Pi. By this dual renal action, IGF-I favors a positive balance of calcium and Pi. Moreover, amino acids can directly stimulate the intestinal absorption of calcium that can account for the increased urinary calcium excretion observed with high protein diet. 25D is formed in the liver from vitamin D, which is supplied from both dietary and cutaneous sources

Enhanced IGF-I production linked to food protein can also exert a favorable impact on bone mineral economy by a dual renal action. IGF-I stimulates the kidney production and increases the circulating level of 1,25 dihydroxyvitamin D (1,25D) [11–14], the active form of vitamin D (Fig. 20.1). This vitamin D metabolite in turn boosts the intestinal absorption of both calcium and inorganic phosphate (Pi). The second action of IGF-I at the kidney level is to increase the tubular reabsorption of Pi. Through this dual activity of IGF-I, the concentration of calcium and Pi in the systemic extracellular compartment rises and thereby positively influences the process of bone mineralization [15]. The indirect positive effect of protein intake on intestinal calcium absorption, via the IGF-I—1,25D link, is combined with a direct stimulatory effect of amino acids such as arginine and lysine on calcium translocation from the luminal to the contra-luminal side of the intestinal mucosa [16, 17] (Fig. 20.1). Amino acids could activate the calcium-sensing receptors (CaRs) [18] and thereby increase the translocation of calcium through channels (TRP)V5 and-or TRPV6 localized in the intestinal epithelium [19, 20] (see also for review [21–23]). The enhanced intestinal calcium absorption quantitatively to a large extent explains the associated calciuria, making very unlikely that any other source than gut would contribute to the dietary protein-induced increase in urinary calcium [24, 25]. This notion is corroborated by the fact that relatively high protein intake is not associated with a negative calcium balance [24, 25]. Furthermore, reduced intestinal calcium absorption and increased serum level of both PTH and 1,25D is observed when healthy women are exposed to a relatively low (0.7 g/kg/day) as compared to medium (1.0 g/kg day^{-1}) or high (2.1 g/kg day^{-1}) protein diet [26, 27]. By using calcium kinetics in human there was no increase in bone resorption in response to a high protein diet inducing a marked elevation in both intestinal absorption and urinary excretion of calcium, [17]. The net bone balance corresponding to the difference between bone resorption (Vo^-) and bone formation (Vo^+) was not reduced by the high protein diet [17]. Thus, a series of animal experiments and human clinical trials underscore the positive effect of increased dietary intake of protein on calcium–Pi economy and bone balance. In sharp contrast, it has been alleged that dietary proteins, particularly those from animal sources, might be deleterious to bone health by inducing chronic metabolic acidosis leading eventually to osteoporosis. Over the last decades, this apparently attractive hypothesis has prompted several investigators to explore in epidemiologic studies whether consumption of high animal protein intake would be associated with either decreased areal (a) bone mineral density (BMD) or content (BMC), or increased incidence of fragility fractures, particularly those occurring at the level of the proximal femur (see below). Nevertheless, several arguments have been raised against the dietary protein-induced acidosis hypothesis of osteoporosis [19, 25, 28, 29], a theory that disregards the essential homeostatic role of the kidney in the regulation of the acid–base balance [30]. Furthermore, there is no consistent evidence for superiority of vegetal over animal protein on calcium metabolism and bone health. In fact, animal protein could be more efficacious than soy or vegetable protein for promoting bone growth in mice [31] or for preventing hip fracture in postmenopausal women [32].

At the bone level, there is also direct evidence that amino acids such as arginine can stimulate the local production of IGF-I by osteoblastic cells [33]. This effect is associated with increased osteoblastic cell proliferation and collagen synthesis [33]. IGF-I is probably the main mediator of the anabolic effect of parathyroid hormone (PTH) [34]. This PTH-IGF-I link explains, at least in part, the marked positive effect of intermittent PTH therapy on bone formation and bone mass as well as on fragility fracture reduction, observed in a randomized controlled trial (RCTs) carried out in osteoporotic women [35].

20.3 Protein Intake and Bone Acquisition

Bone mass and strength achieved by the end of the growth period, simply designated as “peak bone mass (PBM),” plays an essential part in the risk of osteoporotic fractures occurring in adulthood. It is considered that an increase in PBM by 1.0 standard deviation would reduce by 50 % the fragility

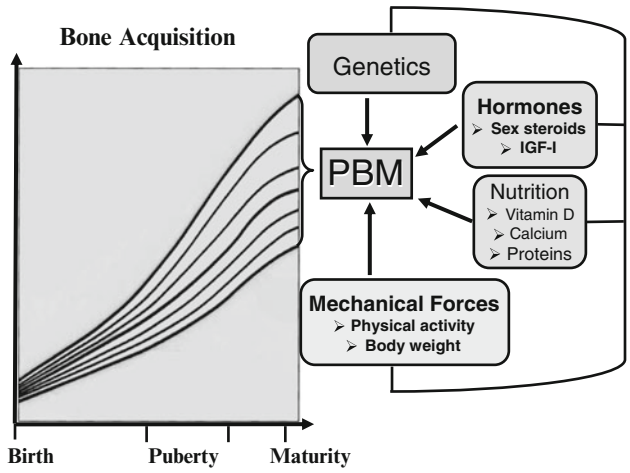

Fig. 20.2 Determinants of bone mass and strength development from birth to maturity. In healthy human subjects four main determinants -genetics, hormones, nutrition and mechanical forces- influence bone mass and strength from birth to the end of the second decade. At this time maximal value, the so-called peak bone mass (PBM), is virtually attained. As depicted on the *right*, these four factors are interconnected, as for instance an increased protein intake enhances the positive impact of physical activity on bone acquisition during growth. The *curves* of the diagram on the *left* illustrate the wide range of individual PBM values that can be assessed at maturity among young healthy subjects of both genders. The genetically predetermined trajectory can be modified by environmental factors particularly nutrition and physical activity

fracture risk (see for review [36]). The genetically determined trajectory of bone mass development can be, to a certain extent, modulated by modifiable environmental factors (Fig. 20.2). Among these factors physical activity and nutrition are key determinants in the acquisition of bone mass during growth. Growing bones are usually more responsive to mechanical loading [37] and bone trophic nutrients [36] than adult bones. Furthermore, the impact seems to be stronger before than during or after the period of pubertal maturation. Among nutrients that can specifically interact with bone metabolism, calcium supplementation has been extensively studied from infancy to the end of pubertal maturation. Much less consideration has been given to protein intake, although this macronutrient is essential for adequate accumulation of bone tissue during growth, as well as maintenance of the skeletal structural integrity throughout life.

Both animal and human studies indicate that low protein intake per se could be particularly detrimental to bone acquisition. Under nutrition, including inadequate supplies of energy and protein during growth, can severely impair bone development [38]. An inadequate protein supply appears to play a central role in the pathogenesis of the delayed skeletal growth and reduced bone mass that is observed in undernourished children [38]. Low protein intake could be detrimental to skeletal integrity by lowering the production of IGF-I [39]. Variations in the production of IGF-I could explain some of the changes in bone and calcium–Pi metabolism that have been observed in relation to dietary protein intake. Indeed, the plasma level of IGF-I is closely related to the growth rate of the body. In humans, circulating IGF-I progressively rises from 1 year of age to the onset of pubertal maturation. Then, serum IGF-I markedly increases to reach maximal values before declining, this in relatively close correspondence to pubertal maturation, peak height velocity and acceleration in bone mass accumulation [40–42]. As mentioned above, IGF-I appears to play a key role in calcium–Pi metabolism during growth by stimulating at the kidney level both the tubular Pi reabsorption and production of 1,25D [15]. Furthermore, IGF-I is considered as an essential factor for bone longitudinal growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate [43–45]. It also has a role on trabecular and cortical bone formation. IGF-I also affects bone mass positively,

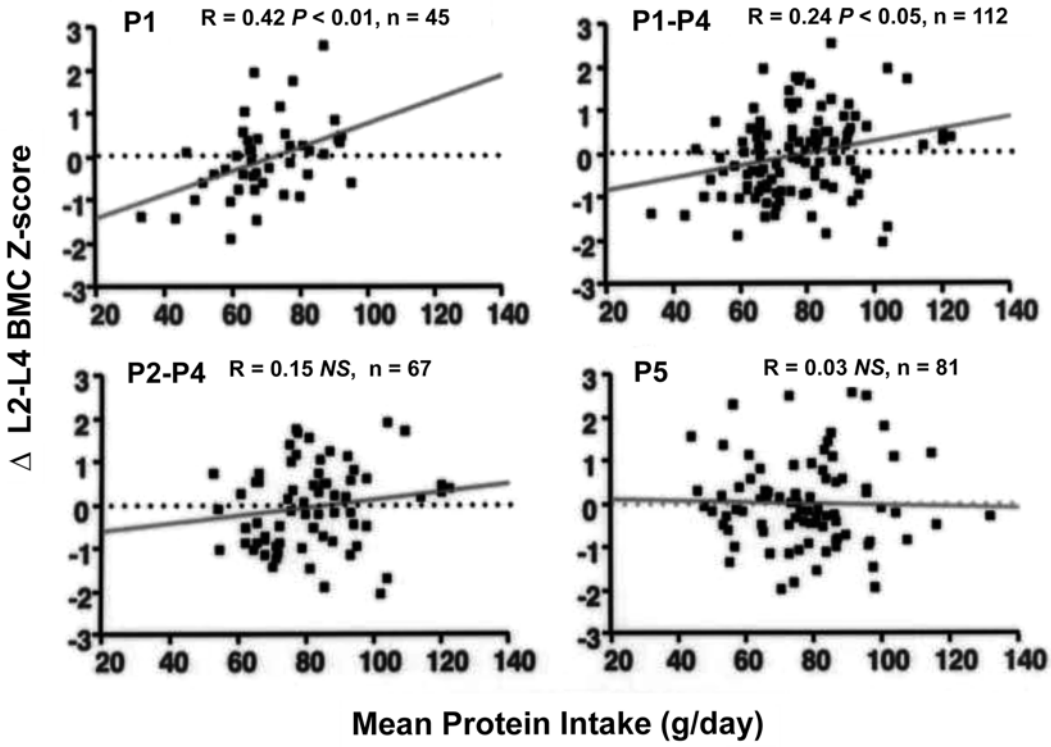

Fig. 20.3 Relation between protein intake and bone acquisition during pubertal maturation. The mean protein intake from dairy and vegetable sources was recorded in two 5-day diet diaries at 1-year intervals. A positive correlation was found in prepubertal (P1), but not in post pubertal (P5) subjects. Each dot corresponds to the change (*triangle*) in lumbar spine (L2–L4) bone mineral contend BMC adjusted for age and gender (*Z*-score) in 193 subjects aged from 9 to 19 years. Adapted from Theintz et al. *J Clin Endocrinol Metab* 1992;75:1060–65 and Clavien et al. *J Adolesc Health* 1996;19:68–75

increasing the external diameter of long bones, probably by enhancing the process of periosteal apposition [43–45]. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action may result in a reduction in both longitudinal and cross-sectional bone development [43–45].

In "well" nourished children and adolescents, the question arises whether or not variations in the protein intake within the "normal" range can influence skeletal growth and thereby modulate the influence of genetic determinants on peak bone mass attainment [46]. Considering the relationship between protein intake and bone mass gain, it is not surprising to find a positive correlation between these two variables [46]. The association appears to be particularly significant in prepubertal children (Fig. 20.3) [42, 47].

20.4 Interaction of Protein Intake and Physical Activity

Growing bones are usually more responsive to mechanical loading than adult bones. Increased physical activity was shown to stimulate mineral mass accumulation in children and adolescents [48]. Adequate nutritional supply can be expected to sustain the anabolic effect of mechanical loading on bone tissue, as it does on skeletal muscle development. Among nutrients, high calcium intake was shown to enhance the response to physical activity in healthy children aged 3–5 years [49]. Long-term

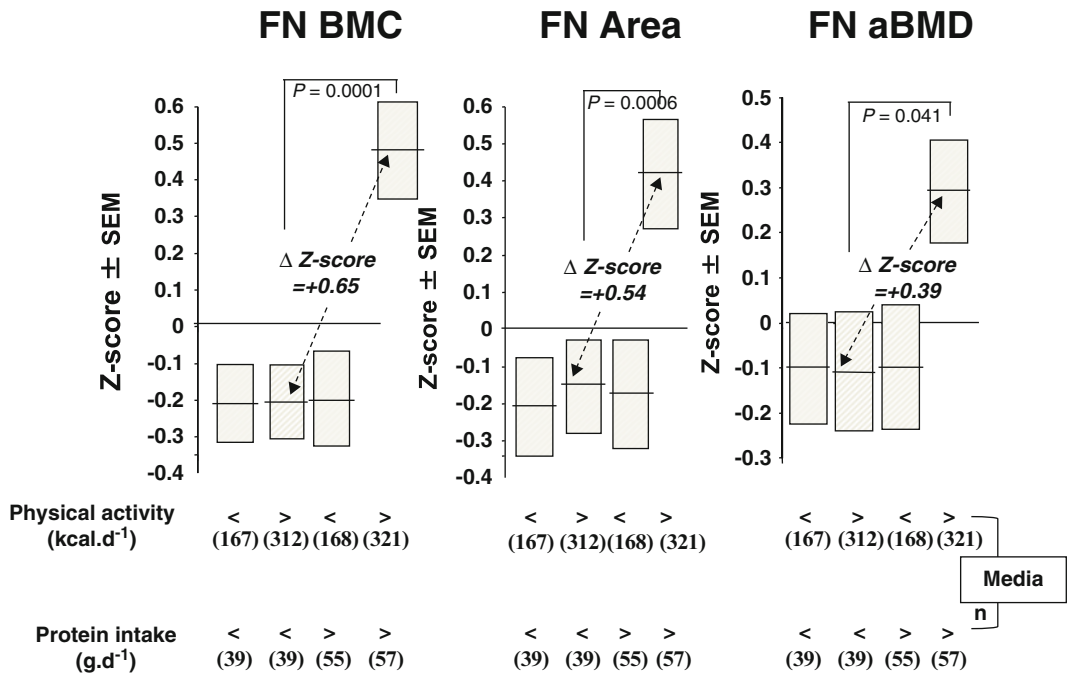

Fig. 20.4 Influence of protein intake on increased physical activity on femoral neck (FN) BMC, Area and aBMD in healthy prepubertal boys. Relatively high physical activity (above the median of energy expenditure, kcal day^{-1}) is associated with significant increase in FN BMC, Area and aBMD in subjects having protein intake above but not below the median. The statistical difference in *Δ Z*-score between these two groups was 0.66, 0.54 and 0.40 in FN BMC, Area and aBMD, respectively. Both mean energy expenditure and protein intake for each subgroup are indicated below the respective bars. Adapted from Chevalley et al. *J Bone Miner Res* 2008;23:131–42

protein consumption exerts a stronger impact than calcium intake on bone mass and strength acquisition in healthy children and adolescents aged 6–18 years [50]. In 8-year-old prepubertal boys, high protein intake was shown to enhance the bone response to increased physical activity [51]. At the femoral neck level, the increased aBMD, BMC, and AREA (Fig. 20.4) were associated with a wider external perimeter [51], a macro-architecture feature that should confer greater resistance to mechanical load [52].

20.5 Protein Malnutrition and Bone in Relation with Intensive Exercise or Anorexia Nervosa

A positive correlation between protein intake and bone mass has been found in premenopausal women [53]. In women on a low-calorie diet, insufficient protein intake could be particularly deleterious for bone mass integrity. In athletes or ballet dancers, intensive exercise can lead to hypothalamic dysfunction with delayed menarche and disruption of menstrual cyclicity and bone loss [54, 55]. The combination of an eating disorder, menstrual dysfunction, and osteopenia has been called “female athlete triad” [56]. Nutritional restriction likely plays an important role in the disturbance of the female reproductive system resulting from intense physical activity. The propensity to nutritional restriction is more common when leanness confers an advantage for athletic performance. Insufficient energy intake with respect to energy expenditure is supposed to impair the secretion of GnRH and thereby

	Anorexia Nervosa n=34	Controls n=33
Age (yr)	15.9	15.0
BMI (kg/m^2)	16.6*	22.3
IGF-I (ng/ml)	294*	556
LS aBMD (mg/cm^2)	893*	971
Z-score	–0.74*	+0.13

* $P < 0.001$ vs. Controls

Fig. 20.5 IGF-I and lumbar spine (LS) aBMD in anorexia nervosa. The marked reduction in BMI and in serum IGF-I reflect the deficient nutritional status documented in a group of anorexic adolescent girls as compared to age-matched controls. The bone consequence of anorexia nervosa is expressed by a severe deficit in lumbar spine (LS) aBMD, of –0.87 *Z*-score. Data from Misra et al. *J Clin Endocrinol Metab* 2008; 93:1231–37 and 1292–97

leads to a state of hypoestrogenism. However, the relative contribution of insufficient protein intake with low IGF-I remains to be assessed, since it is frequently associated with reduced energy intake.

Anorexia nervosa is a frequent condition in young women [57–59]. Reduced aBMD can be measured at several skeletal sites in most women with anorexia nervosa [60]. It is not surprising that young women with anorexia nervosa are at increased risk of fracture later in life. Body weight, but not estrogen use, is a significant predictor of aBMD in women with anorexia nervosa [61]. With estrogen and calcium deficiency, low protein intake very likely contributes to the bone deficit observed in anorexia nervosa. Circulating IGF-I, a marker of protein nutrition [5, 62], is low in anorexia nervosa (Fig. 20.5) [63]. In this situation, serum osteocalcin and bone specific alkaline phosphatase, two biochemical markers of bone formation, are significantly reduced [64]. In mature adolescents with anorexia nervosa, circulating IGF-I was linked to variations in the nutritional state and was the major correlate of bone formation markers [64].

20.6 Effects of High Protein Diet on Calcium and Bone Metabolism During Energy Deficit

Energy deficit (ED), from either reduced dietary intake or increased expenditure is used to induce weight loss in overweight or obese subjects [65]. Periods of ED due to intense physical activity can also be experienced by healthy, normal-weight individuals such as athletes or army trainees [66]. Fat mass loss by ED in overweight and-or obese individuals, can also be detrimental to skeletal muscle mass and strength as well as to bone integrity. During ED the adverse effect on skeletal muscle can be attenuated by high protein diet. There has been some concern whether high protein diet may aggravate ED-induced bone loss [67, 68]. This issue has recently been examined in a short time study in young healthy adults [65]. Increasing the protein consumption from 0.8/kg bw day^{-1} (Recommended Dietary Allowance) to 2.4 g/kg bw day^{-1} did not negatively affect calcium homeostasis and bone turnover [65]. This observation is in keeping with a long term study in postmenopausal women with elevated BMI showing that a higher protein diet during weight reduction increases circulating IGF-I and attenuates total and trabecular bone loss at several skeletal sites including ultra distal radius, lumbar spine

and total hip [69]. Results from other trials indicate that high protein diet induces neither a negative calcium balance nor an acceleration in bone resorption rate [17, 70, 71]. The hypothesis that dietary protein, at an intake level leading to increased urinary titrable acid and decreased pH, would cause systemic acidosis and induce deleterious consequences on calcium economy and bone integrity has been refuted in several recent reviews and meta-analysis [19, 25, 28, 30, 72–74]. Furthermore, two recent original reports did not sustain the hypothesis that high dietary acid load might be detrimental to bone by accelerating the age-related decline in aBMD and increase the incidence of fragility fracture [75, 76].

20.7 Epidemiological Studies on Protein Intake in Adult Women and in the Elderly

An early small but often quoted cross-sectional study suggested that high protein diet might be detrimental to forearm area bone mineral density (aBMD) in limited number of healthy young women [77]. However, in several later reports this negative association between protein intake and aBMD or content (BMC) was not confirmed in both premenopausal and postmenopausal women. Furthermore, in a large number of studies, a positive relationship between protein intake and aBMD or BMC has been found (see for review [19, 28]). In the Framingham Osteoporosis Study, increased protein intake was protective against spinal and femoral bone loss in a large cohort of elderly women and men prospectively followed over a period of 4 years (Fig. 20.6a, b) [78]. As in hospitalized elderly patients, those with a higher protein intake had a greater aBMD, particularly at the femoral neck level [79]. Whereas a gradual decline in caloric intake with age can be considered as an adequate adjustment to the usual progressive reduction in energy expenditure, the parallel reduction in protein intake is certainly detrimental to both structure and function of several organs or systems including skeletal muscle and bone. With aging there is a decline in both the intake of protein (Fig. 20.7a) and the circulating level of IGF-I (Fig. 20.7b). As mentioned above, dietary protein is crucial for bone and muscle development. Recent evidence suggests a significant underestimation or protein requirements in adult human, particularly in elderly [19, 80–82]. Thus, increasing protein above the Recommended Dietary Allowance (RDA) may help prevent the loss of bone and muscle mass in elderly [19, 80–82].

There is evidence that the favorable effect of increasing protein on aBMD or BMC is better sustained when the supply of both calcium and vitamin D are adequate [83–85]. Reciprocally, in postmenopausal women with low calcium intake (600 vs. 1,500 mg/day), a relatively high protein consumption (20 vs. 10 % of energy intake) enhanced calcium retention. Likewise, in healthy older women and men, protein supplements increasing the daily intake from 0.78 to 1.55 g/kg day^{-1}, when isocalorically substituted to carbohydrates, were associated with higher circulating levels of IGF-I and lowered levels of urinary N-telopeptide, a marker of bone resorption [86]. These results are compatible with a preventive effect of relatively high protein intake on bone loss in elderly.

20.8 Cross-Cultural Comparison of Hip Fracture Incidence

Some cross-cultural studies comparing protein intake and hip fracture incidence in women living in various countries have been interpreted as suggesting that high protein intakes from animal source exert deleterious effects on bone health [87, 88]. However, the way both terms of this putative relationship between protein intake and hip fracture incidence were deduced is highly questionable. First, the use of per capita food supplies provided by the FAO of the United Nations is not a reliable estimate

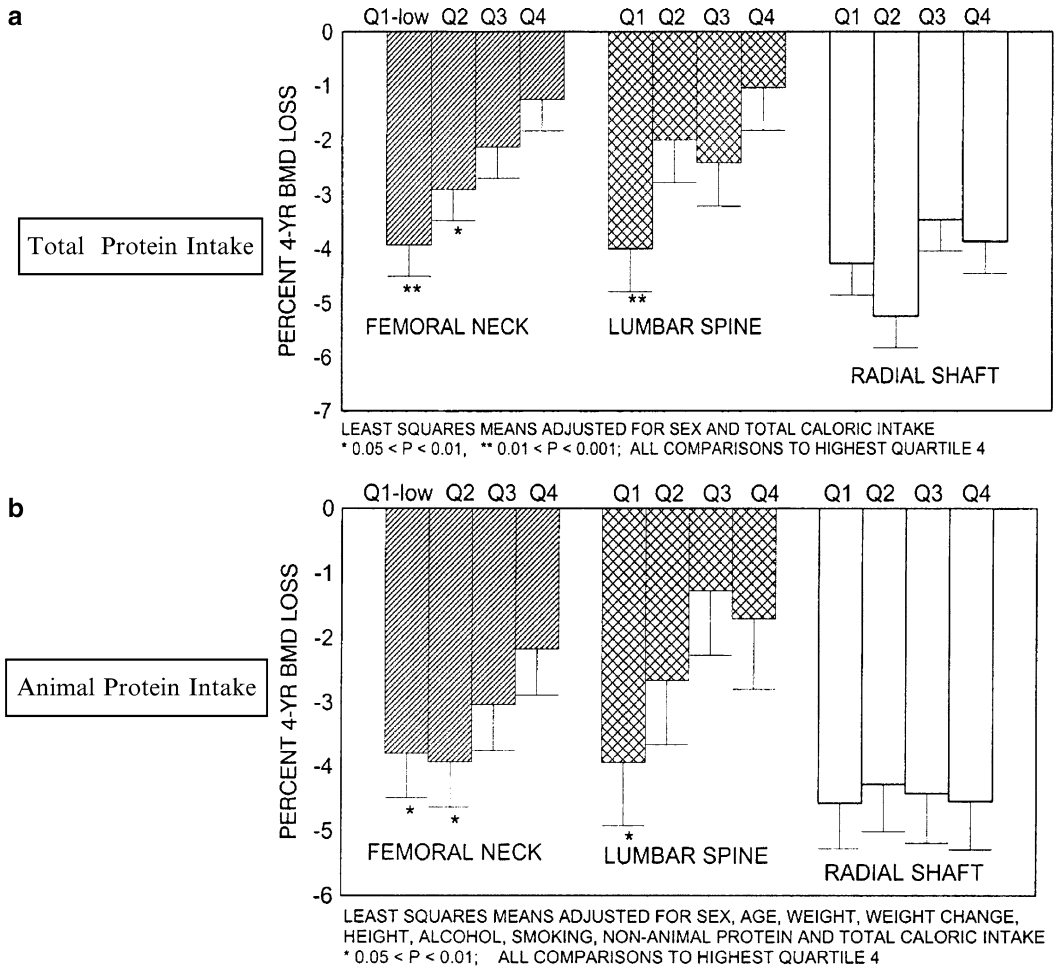

Fig. 20.6 Effect of low protein intake on bone loss in elderly women and men. The relation between baseline protein intake and subsequent 4-year change in aBMD was assessed in 615 women and men from the Framingham Osteoporosis Study, aged 75 years (range 68–91 years) at baseline. Lower protein intake (both total and from animal sources) was significantly related to bone loss at femoral (**a**) and spine sites (**b**), but not in the radial shaft (not shown). These results were obtained after controlling for several potential confounders including age, weight, height, weight loss, total energy intake, current estrogen use, smoking, alcohol intake, caffeine and physical activity. An important strength of this study was that a wide range of dietary protein was consumed. Thus, the lowest (Q1) and highest (Q4) quartile of total protein intake were 17–51 and 84–152 g day^{-1}; the lowest (Q1) and highest (Q4) quartile of animal protein intake were 4–32 and 58–132 day^{-1}. Adapted from Hannan et al. *J Bone Miner Res* 2000;15:2504–12

of the protein intake of the population at risk of hip fracture. It is derived from the total amount of animal protein available for the whole population, i.e., the amount produced plus the amount imported minus the amount exported by a given country, divided by the number of inhabitants. In this rough average estimate of the whole population intake, any selective decline in protein consumption with aging is not taken into account, as reported in several reviews [19, 84, 89, 90]. Secondly, as expected, countries with the highest incidence of hip fracture are those with the longest life expectancy. Age adjustment to the 1977 or 1987 distribution of the US women population [87, 88] does not correct the marked difference in life expectancy between populations of various socioeconomic conditions.

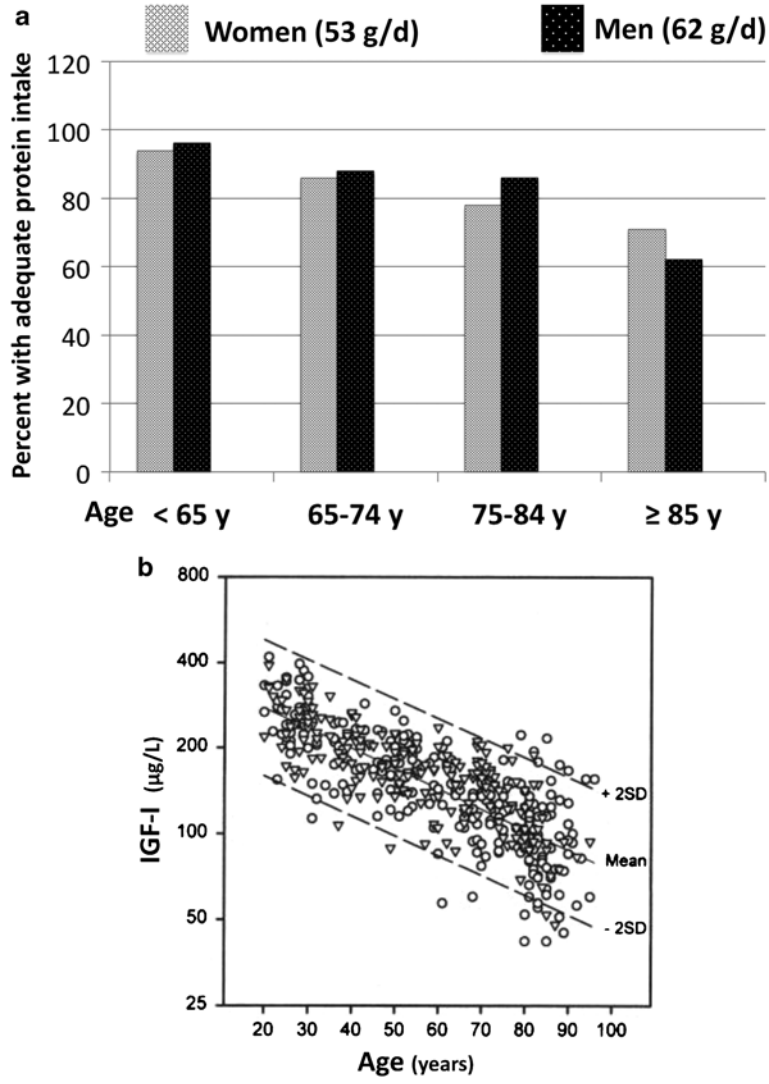

Fig. 20.7 Protein intake, serum IGF-I in relation with aging. The decline with aging in the protein intake and in the serum level of IGF-I is depicted in (**a**) and (**b**), respectively. (**a**) The protein consumption was recorded from a representative sample ($n = 1{,}453$) of persons living in two Italian cities. The protein intake considered as adequate is indicated above the diagram for both women and men. Data adapted from Bartali et al. *J Nutr* 2003;133:2868–73. (**b**) The progressive decline in serum IGF-I was recorded in 448 healthy subjects from young adulthood to old age. The correlation coefficient R between IGF-I and age was −0.74 ($P < 0.01$). The regression lines from the mean and 2 SD are shown. Adapted from *Lewitt MS, Hall K 2005 The Insulin Growth Factor System and Nutrition in Adulthood and Aging. In: Houston MS, Holly JMP, Feldman EL (eds.) IGF and Nutrition in Health and Disease. Humana Press, Totowa, New Jersey, pp 157–74*

20.9 Prospective Observational Studies on Protein Intake and Hip Fracture

In contrast to this "negative" aspect of protein intake hypothesized from cross-cultural analysis, several prospective observational studies have rather shown either a protective effect of relatively high protein consumption or, at least, no detrimental effect on hip fracture incidence. Low protein intake has been

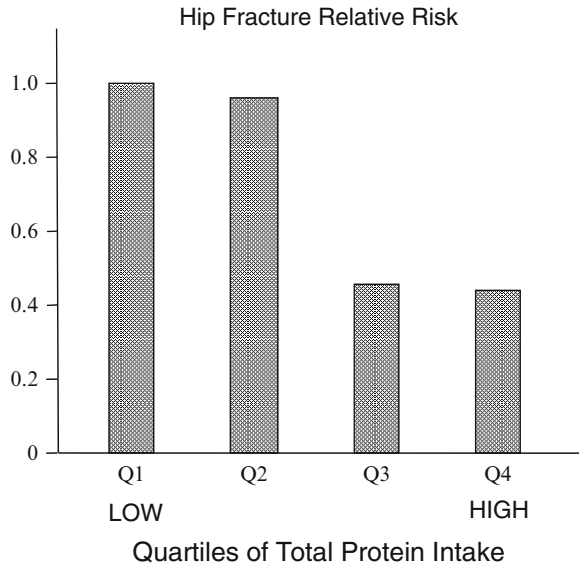

Fig. 20.8 Reduced incidence of hip fracture with relatively high protein intake. The diagram represents the relative risk of hip fracture according to quartiles of total protein intake. The nutrient intake was assessed in a cohort of Iowa women aged 55–65 at baseline. The incident hip fractures were ascertained in the follow-up analysis of 104,338 person-year. The risk of hip fracture was negatively associated with total protein intake (Q4 > 12.05 g/MJ vs. Q1 < 9.56 g/MJ, age-adjusted relative risk: 0.31), especially from animal sources (Q4 > 9.26 g/MJ vs. Q1 < 6.48 g/MJ, age-adjusted relative risk: 0.21) after adjustment for age, body size, parity, smoking, alcohol intake, estrogen use and physical activity (*P* trend = 0.037). Adapted from Munger et al. Am J Clin Nutr. 1999;69:147–52

documented in elderly subjects at risk of fragility fractures, and more so in those experiencing hip fracture (see for review [89]). It is associated with low body mass index (BMI) as clearly documented in a meta-analysis gathering 12 prospective worldwide multicenter studies including 60,000 men and women with a total follow-up of 250,000 person-years [91]. In elderly, low BMI is correlated with protein under nutrition, that in turn is associated with low bone and skeletal muscle mass [19, 90].

In a large prospective study (Iowa Women's Health Study) including about 32,000 women aged 55–69, total protein intake was inversely associated with the risk of hip fracture (Fig. 20.8) [32]. Thus, the risk reduction in hip fracture incidence was 67 and 79 % for the highest vs. the lowest quartile in total and animal protein intake, respectively [32]. In a smaller case-control study including both women and men residing in Utah, higher total protein intake was associated with a significant reduced risk of hip fracture in 50–69-year-old subjects [92]. In older, 70–89-year-old residents of this county, however, protein intake was not significantly associated with a decreased or an increased risk of hip fracture [92]. As discussed by the authors, it is unclear whether the lack of protective effect in the 70–89-year group would reflect a functional difference in nutritional protein metabolism or merely an artifact due to methodological limitations of the case–control study design in the oldest subjects [92]. In both Iowa and Utah studies, calcium intake did not modify the risk evaluation of hip fracture in relation with protein intake [32, 92]. These observations somehow contrast with an analysis [93] of results obtained in a large French postmenopausal women cohort study initiated in 1990 to identify most frequent cancer-associated risk factors [94]. Overall, no association was found between fracture risk and either total protein (from animal or vegetable sources) or calcium intake [93]. However, further cross-tabulation analysis that subdivided the population in four subgroups revealed a slightly but significant increased risk when the highest quartile of protein intake was combined with the lowest quartile of calcium intake [93]. Of note, in this population of relatively young postmenopausal women, the daily protein intake was normal to high (mean about 1.45 g/kg day^{-1}) and the calcium intake fairly

high (mean about 1,045 mg/day) [93]. Therefore, this epidemiological study does not concern elderly women at risk of under nutrition as observed in hip fracture patients [95]. In another relatively young cohort aged from 35 to 59, the "Nurses' Health Study," a trend for hip fracture incidence inversely related to protein intake has been reported [96]. In the same prospective epidemiological study, however, forearm fracture incidence was slightly increased (RR = 1.18, 95 % CI 1.01–1.38) in the highest (>95 g day^{-1}) as compared to the lowest (<68 g day^{-1}) quintile of age-adjusted total protein intake [96]. The reason for this skeletal site difference in the recorded association might be related to physical activity and mode of falling that differs for hip vs. forearm fracture [52]. In contrast to the French study discussed above [93], as well as to a retrospective Norwegian survey [97], no significant relation with the calcium–protein ratio was found with either hip or forearm fracture incidence in the "Nurses' Health Study" [96].

20.10 Meta-analysis of Protein Intake, aBMD or BMC and Hip Fracture

Studies reported from 1966 to 2008 on the relation between protein and bone integrity in healthy human adults were systematically reviewed and meta-analyzed [98]. From the 18 studies that could be quantitatively analyzed a significant positive pooled correlation was computed between protein intake and aBMD or BMC measured at the main clinically relevant skeletal sites [98]. Four suitable hip fracture studies [32, 96, 97, 99] were also meta-analyzed [98]. In contrast to cross-cultural ecologic studies mentioned above [87, 88], no negative association was found between the relative risk of hip fracture and the protein intake [98]. In relation with protein under nutrition and fragility fractures, the risk of spinal and hip fractures was associated with low circulating levels of IGF-I [100, 101]. Furthermore, in the elderly at risk of osteoporotic fractures, marginal dietary protein intake results in loss of muscle mass, which is associated with reduced IGF-I plasma, level [102]. Muscle mass and strength are important determinants of the risk and consequence of falling in elderly [89]. There is evidence that the anabolic response of muscle to dietary protein is attenuated in elderly and, consequently, the amount of protein required to enhance muscle mass is greater [19]. Several epidemiological and clinical studies point to a beneficial effect of increasing the protein intake in elderly above the current RDA of 0.8 g to approx. 1.2 g/kg day^{-1}; short-term studies indicated beneficial effects of protein intake up to 1.6–1.8 g/kg day^{-1} [19].

20.11 Intervention Study on the Impact of Protein Repletion After Hip Fracture

In a randomized, double-blind, placebo-controlled trial, oral protein supplement providing 20 g of casein/day during 6 months, as compared to an isocaloric supplement was given to patients with a recent hip fracture [6]. Both protein supplemented and placebo-controlled groups were vitamin D replete, and received daily 500 mg of elemental calcium. The protein supplemented group displayed a significantly greater increase in plasma IGF-I level (Fig. 20.9a) and lessened loss of bone mineral mass at the contra lateral proximal femur (Fig. 20.9b), with a trend for less vertebral fracture [6]. Muscle strength improved in the protein supplemented group as compared to the isocaloric placebo-controlled group [6]. Furthermore, in the protein supplemented patients there was also an improvement in clinical outcomes that were associated with a reduced length of stay in rehabilitation hospital [6].

Thus, dietary protein, by impacting on both bone and skeletal muscle anabolism have a key role in the prevention of bone loss and sarcopenia, thus reducing the propensity to fall and the risk of fragility fractures (Fig. 20.10). The positive action of dietary proteins requires adequate supply of both vitamin D and calcium.

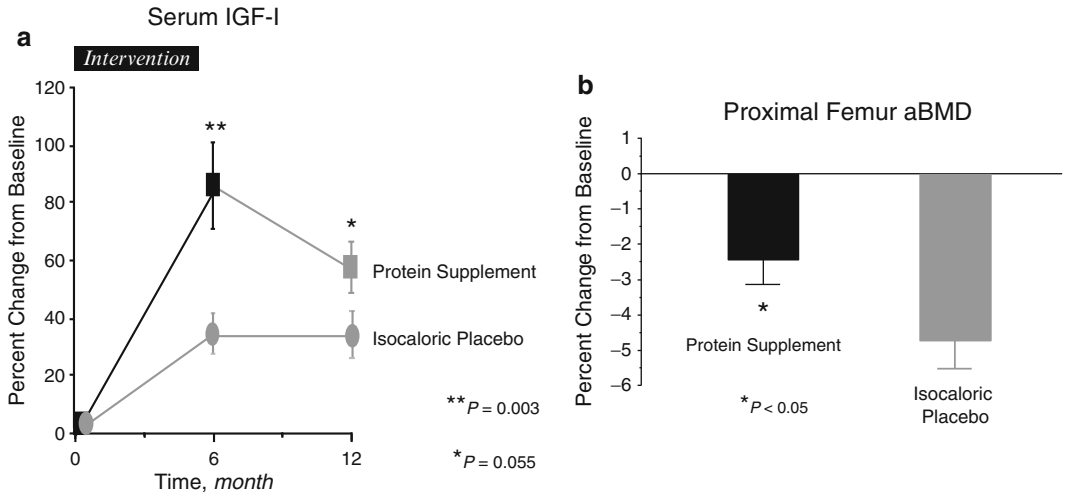

Fig. 20.9 Effects of protein supplements on serum IGF-I and proximal femur in patients with a recent hip fracture. The design was a 6-month, randomized, double-blind, placebo-controlled trial with 6-month post-treatment follow-up. All patients ($n = 82$, mean age 80.7) received calcium supplementation, 550 mg/day and one dose of vitamin D, 200,000 at baseline. They were randomized to consume either a protein (casein) supplement, 20 g/day or an isocaloric supplement (placebo-controls). (**a**) Change in serum IGF-I from baseline to the end of intervention at month 6 and follow-up at month 12. (**b**) Change in femoral neck aBMD from baseline to month 12, showing the significant attenuation of bone loss in the protein supplemented group as compared to the iso-caloric placebo group

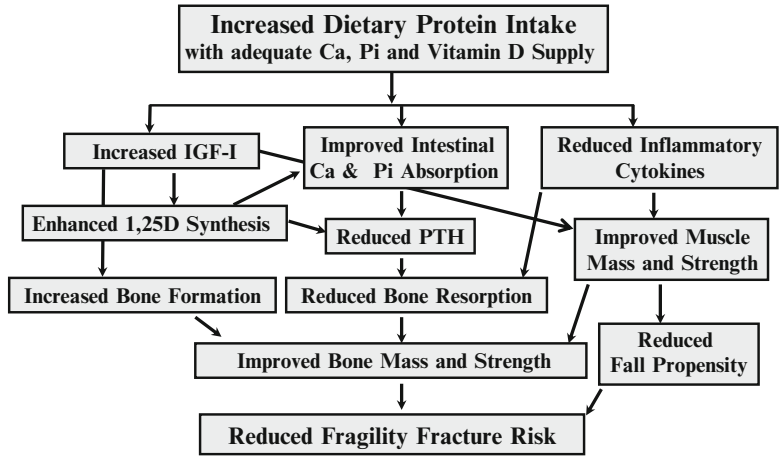

Fig. 20.10 Positive influence of protein intake on bone and skeletal muscle health in elderly. With aging, the impact of dietary protein on both bone and skeletal muscle anabolism plays a important role in the prevention of osteoporosis and sarcopenia. Dietary protein with calcium and vitamin D contribute to attenuate both age-dependent bone loss and the propensity to fall, thereby reducing the risk of fragility fracture

20.12 Conclusion

In the development and maintenance of bone structures resistant to usual mechanical stresses, adequate nutrition plays an important part. In addition to calcium associated with an adequate supply of vitamin D, dietary protein represents a key nutrient for bone health and thereby for the prevention of osteoporosis. During growth, protein under nutrition from infancy to childhood and adolescence

results in reduced bone mass and strength, thereby increasing the risk of fragility fracture in later life. On the contrary, high protein intake, particularly when associated with physical activity, favors healthy development and peak bone mass acquisition, thereby enabling individuals to reach their genetic potential. There is a positive interaction between dietary protein, calcium–phosphate economy, and bone metabolism. This interaction appears to be mediated by the anabolic bone trophic factor IGF-I, the hepatic production of which is stimulated by amino acids supplied by dietary proteins. Amino acids such as arginine can exert a direct positive effect on the IGF-I production by bone forming cells. In young adulthood energy deficit, as observed in anorexia nervosa, can be associated with insufficient protein supply, low circulating IGF-I, bone loss, and increased risk of fragility fracture. With aging, the reduction in the protein intake is associated in both genders with a decrease in the serum level of IGF-I, lower femoral neck aBMD, and poor physical performance. Protein under nutrition is often present in patients experiencing hip fracture. Furthermore, clinical outcome after hip fracture can be significantly improved by normalizing protein intake, which is associated with a rise in the serum IGF-I level. Thus, dietary protein contributes to bone health from early childhood to old age. An adequate intake of proteins should be recommended in the prevention and treatment of postmenopausal and age-dependent osteoporosis.

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Chapter 21
Fat and Bone

Francisco J.A. de Paula and Clifford J. Rosen

Key Points

- Nutrients are in the cornerstone position for the prevention of metabolic diseases such as diabetes mellitus and are gaining increasing relevance on the approach of cardiovascular disturbances.
- Conversely, only recently obesity started to be considered a threat for bone.
- Fat has direct and indirect influence on bone mass development and maintenance.
- The mechanisms of fat action on bone are discussed.
- Excess bone loss occurs in individuals on fast body weight loss after bariatric surgery.
- Preliminary results regarding the effect of PUFAs on bone are insufficient.

Keywords Bone • Fat • Osteoporosis • Polyunsaturated fatty acids • PPAR

21.1 Introduction

Nutrients and hormones, each having their proper role, are essential elements for life. Nutrients are the basis of energy supply for all physiological activities, including body growth, cell proliferation and environmental adaptations. Hormones share with the neural system the coordination of vital circuits as well as all physiological functions to maintain quality of life. Consequently, energy metabolism is affected directly or indirectly by all classes of hormones (protein or steroid, central or peripheral-originated, membrane or nuclear receptor signaling hormones). The interplay between hormones and nutrients, are interconnected to insure either health maintenance or disease expression. Bone, a very sophisticated mesenchymal tissue, structured by an amalgamation of hydroxyapatite and proteins, can have intense cell activity during growth (bone modeling) and for renewal (bone remodeling). New functions have been recently recognized in bone cells. Osteocalcin a protein produced by osteoblasts, after decarboxylation by osteoclast activity, becomes a molecule able to modulate insulin

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and adiponectin secretions by β-cell and adipocyte, respectively. Thus, hard tissue not only depends on an appropriate energy supply to maintain its properties, but is an active player in the modulation of energy metabolism [1–3]. Expectedly, nutrients and their indirect by-product, body weight, deeply affect bone strength; however, the exact connection still challenges physicians and researchers to uncover their optimal combination for peak bone mass acquisition and maintenance.

In recent decades, body composition emerged as a new determinant of endocrine function within the organism. The plasticity of the adipose tissue is not only linked to its capacity to accumulate fat during periods of nutrient surplus [3–6]. As adipocytes fill with fat droplets, the endocrine/paracrine/autocrine profile of adipose tissue transmutes and favors a constellation of metabolic and cardiovascular diseases as well as cancer [7, 8]. Conversely, bone mass usually is preserved or increased in obese individuals, simulating an insulation of bone [9]. However, recent evidence indicates that obese individuals are not protected from fracture [10, 11]. The role of nutrient components on cardiovascular disease has been thoroughly scrutinized since the Framingham Study disclosed that dietary profile is an independent risk factor for cardiovascular disease and mortality [12]. The investigation of the impact of nutrients on bone strength has historically been directed to minerals, vitamin D, and protein, while the other components received less attention.

Here, we intend to give to the reader an overview of the principal aspects involving the influence of fat on bone, including an overview of the systemic and bone effects of dietary fat and mechanisms of fat influence on bone mass.

21.2 Dietary Fat: Systemic and Bone Effects

21.2.1 Systemic

The bulk of diet is constituted of the so-called macronutrients, carbohydrate, protein and fat. Medical Societies recommend that individuals consume carbohydrates at a rate of 50–60 % of total calorie intake with proteins and lipids completing the requirements with 10–15 and 30 %, respectively [13, 14]. Due to three characteristics of mammals (their intermittent eating pattern, their limited capacity to store carbohydrates and the continuous use of glucose by the central nervous system) a complex arrangement, involving insulin-dependent tissues and endocrine adaptation, is necessary to allow the organism to keep glucose support during fasting and exercise. In spite of this, fat is the prime substrate for energy storage [15]. The advantage of being able to store energy as fat is primarily due to the efficiency of its energy production during oxidation and the fact that fat does not need water to be stored. In mammals, fat can be endogenously synthesized from glucose, lipids and amino acids while the main supply of fat is exogenous.

In previous times, when food was not readily available, thrifty genes were a welcomed advantage, allowing more efficient storage of fat [16, 17]. Improvements in socioeconomic conditions along with increasing availability of high-energy industrialized foods in combination with a more sedentary lifestyle have led to the emergence of the obesity epidemic. The positive imbalance of energy and consequent hypertrophy and hyperplasia of the adipose tissue concomitantly attracts macrophages that ultimately create a chronic inflammatory state [18, 19]. While monocytes and macrophages are the main source of MCP-1 (i.e., inflammatory chemokine), adipocytes might also produces MCP-1 [20]. Obese individuals have higher expression of MCP-1 within adipose tissue than controls and visceral adipose tissue has greater production of MCP-1 than the other fat pads. In addition, most likely there is a functional loop involving FFA from adipocytes and macrophage-derived TNF-α to sustain an active inflammatory state in adipose tissue from obese. Clinical and experimental investigation shows that once fat is accumulated within the body, it is easier to maintain the stock of energy than to

dissipate it [21]. A vicious cycle to preserve body weight creates the conditions to feed-forward the emergence of comorbidities associated with obesity, including insulin resistance, inflammation and pro-thrombotic states. Obesity appears to have both detrimental and positive effects on the skeleton, this complex relationship is currently undergoing thorough investigation [22, 23].

Life insurance companies have documented that mortality rates show a U shaped correlation with body weight [24, 25]. While death in thin individuals is related to infectious diseases, for the obese this outcome results from diabetes mellitus, arterial hypertension, thromboembolic phenomena, cardiac ischemia and cancer. Fortunately, famine has become outmoded in western countries, but the current challenge is to avoid unnecessary energy intake. Two fronts have been exhaustively investigated in the approach to obesity: reduction in total calorie consumption as well as the substitution of specific dietary components that facilitate the maintenance of obesity leading to comorbidities.

At the beginning of the last half of the twentieth century, major focus was posed on fat profiles based on data showing that fat consumption was a risk factor for cardiovascular disease. These studies indicated the adverse effects of saturated fat on total and LDL-cholesterol. At that time, replacement of saturated with polyunsaturated fat was considered a more appropriate approach [26]. A different position was assumed during the 1980s, when major importance was projected to shift from fat to carbohydrate consumption. The impact of this approach was questioned in face of the emergence of two alterations in the lipids profile: the stimulus in the synthesis of triglycerides and the decrease in the serum levels of HDL-cholesterol. Consequently, after replacement of fat with carbohydrates, the circulatory levels of lipids might not improve the development of cardiovascular disease. On the other hand, a better ratio of HDL-cholesterol/LDL-cholesterol results when saturated fat is substituted for either polyunsaturated or monounsaturated fat. Other ingredient is the effect of *trans*-unsaturated fatty acids (derived from the hydrogenation of vegetable oils), which elevate the circulatory levels of LDL and triglycerides and decrease HDL-cholesterol. In the Nurse's Health Study diet was evaluated during a period of 14 years with a catalogue of 61 items semiquantitative food frequency questionnaire [27]. The cohort evaluated 80,082 women without CVD, detecting 939 occurrences of acute myocardium infarct or CVD death. The relative content of fat energy components was assessed in comparison to the correspondent intake of energy from carbohydrate. While it was observed a strong correlation between *trans* fatty acids in diet with CVD, this association was weak and non-significant regarding total fat [27].

Several studies indicate that before the ingress of nutrients into the circulation, their absorption is not only dependent on digestion by stomach acid, bile and pancreatic enzymes. Intestine microbiota appears to be far more than a passive commensal inside the lumen of gastrointestinal tract, it take part on the efficiency of absorption. The gut houses a large and diverse community of microorganisms, which correspond to the main and earliest source of microbial exposure in human. Recently, experimental and clinical investigation indicated an association between gut microbiota and obesity [28–30]. Furthermore, in a dual pathway the quality of diet participates on the determination of the profile of intestine microbiota. Wit and colleagues investigated in C57Bl6 mice the effect of dietary fat, differentiated by the rate of polyunsaturated-to-saturated fatty acids [31]. The high saturated fat diet reduced microbial diversity in intestine and increased the ratio of Firmicutes-to-Bacteroidetes. They observed that saturated fat stimulates more weight gain and hepatic steatosis than unsaturated fat. The overflow of fat to the distal intestine on the saturated fat diet induced changes in gut microbiota composition and the expression of genes related to lipid metabolism in the distal small intestine (Bcmo1 and Scd 1) [31].

The impact of dietary fat on the incidence of cancer has been also evaluated. Several studies compared the correlation of individual consumption of fat in different countries with the occurrence of different neoplasias (e.g., prostrate, breast, and colon) found positive association. On the other hand, more well designed studies in which the variables received more convenient treatment, it was observed less consistent association between fat intake (total or fractions) and cancer of colon and breast. For example in the Women's Health Initiative it was not observed that decreased total fat intake impacts

Table 21.1 Metabolites of n-3 and n-6 polyunsaturated fatty acids (PUFAs) with anti-inflammatory properties

n-6 PUFA	n-3 PUFA	
Anti-inflammatory		
AA	EPA	DHA
Lipoxin B4	Leukotriene B5	Maresins
Lipoxin A4	Prostaglandin E3	Protectin D1
	Resolvin E1	Resolvin D
	Resolvin E2	Resolvin D1
		Resolvin D3
		Resolvin D4

on breast and colon cancer [32, 33]. Although limited, there are studies showing positive association between ingestion of animal fat and prostate cancer [34]. The benefits of vegetable oil for prostate cancer have also been suggested for in some studies [35]. Currently, it is more appropriate to conclude about the effect of fat diet based on data of cardiovascular disorder than with tumor emergence.

The unfavorable results obtained with the high-carbohydrate diet on CVD reinforced the interest on the study of the impact of fat quality on degenerative disorders, namely, monounsaturated and polyunsaturated fat. For review see the following references [26, 36, 37]. Long-chain polyunsaturated fatty acids (LCPUFAs) have at least 18 carbons and 2 double bonds and are subdivided into two categories depending on the position of the first unsaturated carbon (i.e., n-3 and n-6). α-Linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18-2n-6) are two essential fatty acids (EFA), due to the incapacity of humans to synthesize a fatty acid with a double bond among the first nine carbons. Oily Fish is the major source of n-3 LCPUFAs, whereas n-6 LCPUFAS are found in vegetable oils, including soybean and corn. LCPUFAs are substrates for an array of metabolites, in a process of non-enzymatic oxidation as well as enzymatic oxidation by lipooxygenases (LOX), cyclooxygenases (COX), and cytochrome P450-like epoxygenases. ALA and LA compete for the same enzymes in the process of elongation, desaturation and for COX. ALA is substrate for the synthesis of various long-chain n-3 fatty acids, including docosahexaenoic acid (DHA; 22:6n - 3) and eicosapentaenoic acid (EPA, 20:5n-3), which reportedly have anti-inflammatory. LA is processed in arachidonic acid (ARA; 20:4n-6). Table 21.1 shows the metabolites derived from n-3 and n-6 PUFAS with anti-inflammatory action. The relative amounts of these fatty acids also define the availability of substrates for synthesis of eicosanoids and autacoids (e.g., resolvins). In addition to the anti-inflammatory actions these fatty acids work as effectors on nuclear receptor, namely peroxisome proliferator-activated receptor (PPAR) and retinoid X acids. Therefore, PUFAs potentially affect different systems and organs driving cellular responses to physiological and environmental stimuli. Previous studies indicated that DHA and EPA might influence the susceptibility of cardiovascular disease and other degenerative disorders such as Alzheimer [38]. Here our major interest is the discussion of the evidence of the role of PUFAs on the skeleton (see below).

21.2.2 Lipids and Bone

21.2.2.1 Adipose Tissue and Bone

Body weight is a major determinant of BMD, and thinness is an independent risk factor for low BMD and fracture. Moreover, in young women, exercise does not compensate the combination of scarce adipose tissue associated to amenorrhea. The so-called Female Athlete Triad, coincidently FAT, comprises exercise, low energy availability, amenorrhea and as complication, low bone mineral density. Therefore, physical activity does not overwhelm the detrimental effects of insufficient energy

availability and low body weight [39]. Especially the practice of High-impact activity increases BMD in women. In prepubertal children, exercise might cause a 4–5 % gain in bone mass gain. On the other hand, BMD in amenorrheic athletes is lower than their eumenorrheic counterparts despite doing similar weigh-bearing exercise. Bone mineral density was evaluated in 93 female adolescent runners. Multiple regression analysis indicated that in addition to menstrual irregularity, BMI, and lean tissue mass were independent predictors of low BMD [40]. Low body weight may also contribute to bone loss in several diseases. For instance, diverse studies verified that HIV infection is associated with low BMD. In a review of data published from 1966 to March 2007, Boland and colleagues analyzed data of nine studies among 40 identified studies and one of 68 identified abstracts reporting BMD and weight or body mass index in adult patients with HIV and a healthy age- and sex-comparable control group [41]. The authors conclude that HIV-infected patients are lighter than controls and low body weight may largely account for the high prevalence of low BMD reported in HIV-infected patients [41]. The relevance of body weight for bone mass development and maintenance is further underpinned in studies exhibiting accentuated bone loss in individuals on fast body weight loss after bariatric surgery [42].

On the other hand, population studies show a positive influence between body fat and bone density, creating an expectative of positive association between adiposity and bone strength. However, remains controversy as to whether fat has a positive or detrimental effect on bone in both the pediatric and adult populations. Especially in children and adolescents there are several cross-sectional studies suggesting that fat mass may have negative effect on bone [43, 44]. Furthermore, during the first two decades of life, this detrimental effect is reinforced by studies showing increased fracture occurrence in obese children [45–47]. Remarkable results were obtained in the Avon Longitudinal Study of Parents and Children (ALSPAC) developed in Bristol (UK) as a long-term health research project that enrolled more than 14,000 mothers during pregnancy in 1991 and 1992, and subsequently studied the health and development of their children [48]. A cross-sectional analysis of the relationship between fat mass and bone in this cohort of children at 9.9 years demonstrated a strong positive relationship between total body fat mass and total body (minus the skull) bone mass and area. Additionally, it was observed that as girls advanced through puberty, the positive relationship between fat and bone mass was attenuated and thereafter overturned; the same pattern was not observed in boys, but they were in small number [48].

Another aspect to be taken into account in the evaluation of fat's influence on bone strength is adiposity distribution. Three decades ago the role of different fat pads on the emergence of metabolic disorders was highlighted. At that time simple measurements of biochemical parameters and the ratio of waist to hip circumferences demonstrated an association of abdominal fat and insulin resistance. In a recent study magnetic resonance image was used to quantify visceral adipose tissue, and bone mineral density to measure bone mass, with determinations of serum levels of adiponectin, leptin, and inflammatory markers (E-selectin, soluble intercellular adhesion molecule, interleukin-6) [49]. The main objective was to evaluate the relationship of regional fat mass and adipokines with BMD. The results showed that VAT is an independent inverse determinant of bone density in obesity and suggested that this association may be mediated by adipokines and a chronic inflammatory state [49]. Surplus of energy leads to fat accumulation not only on subcutaneous and visceral pads, ectopic deposition of fat in muscle and liver are relevant ingredients in the degenerative disorders associated to obesity [50] (Fig. 21.1).

Bone marrow is another niche for fat accumulation and common conditions associated with osteoporosis, i.e., aging, menopause, and glucocorticoid therapy, show increased marrow adiposity [51, 52]. However, the nutritional relationship between bone mass, bone marrow fat, and white adipose tissue is much more complex and incompletely understood. For instance, anorexia nervosa a complex disorder, involving hormonal and psychiatric disorders triggering self-imposed diet avoidance is clinically marked by emaciation and scarce global white adipose tissue. Unexpectedly, fat accumulation in bone marrow and decreased bone mass are the hallmark of bone disorders in anorexia nervosa [53].

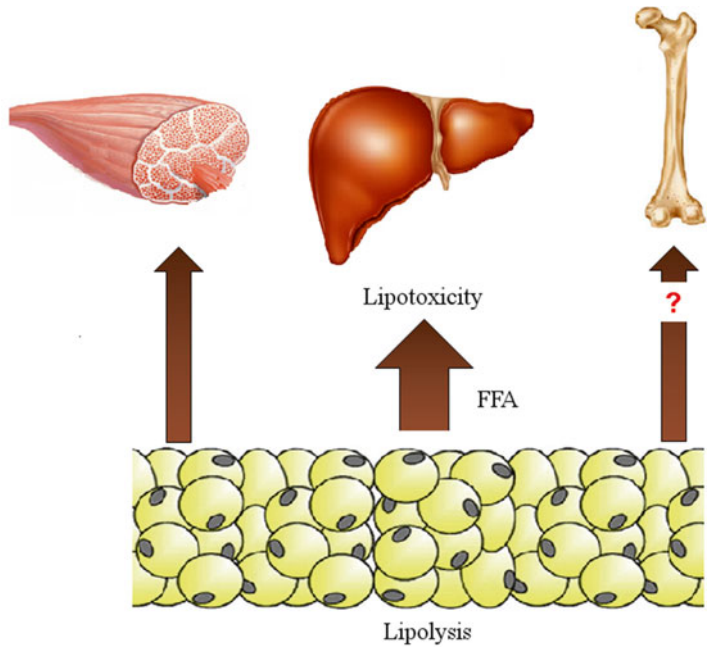

Fig. 21.1 When adipocytes are overwhelmed with energy surplus that surpasses their storage capacity there is an overflow of lipids to other tissues. Visceral adipocytes have greater β-adrenergic lipolytic sensitivity than subcutaneous adipocytes. They undergo lipolysis more easily, releasing greater amounts of free fatty acids (FFAs) into the portal circulation. Lipotoxicity in consequence of fat accumulation in liver and in the intracellular and extracellular compartments of muscle is an important ingredient within the complex metabolic disturbance of insulin resistance. Although there are results showing negative association between central obesity and bone mass, the impact of lipids overflow on bone cells is still to be elucidated

Moreover, equivalent results were acquired in mice submitted to diet restriction, which shows that diet has a unique relationship with bone marrow fat [54].

21.2.2.2 Circulatory Lipids and Bone

As mentioned above, circulatory lipids have commonly been used as a surrogate parameter for the investigation of the impact of fat on diverse disorders. Previous population-based studies showed that LDL-Col has a negative association with BMD in men, premenopausal and postmenopausal women. Hsu and colleagues evaluated 7,137 men, 4,585 premenopausal women, and 2,248 postmenopausal women aged 25–64 years in a community-based, cross-sectional study. They found significant negative relations between whole-body BMC and cholesterol, triacylglycerol, LDL, and the ratio of HDL to LDL in all groups [55]. Additionally the authors also observed a negative relationship between fat mass and bone mineral content in the whole body and total hip. In another study, a small group of postmenopausal women was evaluated regarding the role of atherogenic lipid profile on lumbar and femoral bone mineral density (BMD). Atherogenic lipid profile or hyperlipidemia was defined as hypercholesterolemia (> or = 240 mg/dl) or high low-density lipoprotein cholesterol (high-LDLc > or =160 mg/dl) or high lipoprotein (a) [high-Lp (a) > or =25 mg/dl]. The results showed that women with hyperlipidemia had lower mean-adjusted BMD (mean ± SEM) at lumbar spine (0.865 ± 0.020 vs. 0.958 ± 0.028 g/cm^2, $p = 0.007$) and femoral neck (0.712 ± 0.015 vs. 0.796 ± 0.021, $p = 0.004$ g/cm^2) than those with normal lipid levels [56]. Similar results were obtained in a South Korean population-based sample of 375 premenopausal and 355 postmenopausal rural women aged 19–80 years [57].

Recently, the link between marrow adiposity with serum lipids levels was investigated in 16 patients (eight females and eight males) with type 1 diabetes mellitus and 12 controls (seven females and five males) [58]. In this study, quantification of marrow adiposity was performed by an alternative method of magnetic resonance that allows for multislice imaging, including iterative decomposition of water and with echo asymmetry and least-squares estimation (IDEAL) instead of the most common used technique of spectroscopy. Measurements of marrow fat were performed in lumbar spine (L4), distal femur and proximal tibiae. Diabetic patients had $HbA1_c$ significantly higher than controls (7.7 ± 0.4 vs 5.5 ± 0.4 %). In diabetic patients HDL serum levels were negatively associated with body weight and body mass index (BMI). In general marrow fat was lower in L4 (54 %) than in long bone (85 %). The authors observed a positive correlation between marrow adiposity and serum levels of cholesterol, LDL, cholesterol/HDL ratio and triglycerides. Serum HDL levels, alone, did not correlate with any marrow adiposity measures. Marrow adiposity was positively correlated with age, but there were no correlation between marrow adiposity with weight and diabetes disease or severity parameters. There was no correlation between marrow adiposity and $HbA1_c$. Serum levels of osteocalcin was significantly lower in diabetic patients, as observed in other studies [2, 59], but there were no correlation between osteocalcin with serum lipids, BMD and marrow adiposity. These intricate results challenge more investigations to study the role of serum levels of lipids on the determination of BMD and marrow adiposity.

21.2.2.3 FAT and PPAR-γ2

Peroxisome proliferator-activated receptor-gamma (PPARs) are members of a especial group of molecules, the ligand-activated nuclear hormone receptor transcription factor superfamily, which also includes the receptor of vitamin D, retinoic acid, thyroid, and steroid hormones. The endocrine molecules able to signaling through this machinery are pleiotropic hormones acting in multiple systems and affect the majority of cells. Other coincidences between these hormones are their significant effects on bone and energy metabolism. For instance, while thyroid hormone deficiency and glucocorticoid excess are secondary cause of obesity, both hypercortisolism and thyrotoxicosis induce bone loss [60–63]. Obesity has an intricate relationship with vitamin D; considered a state of vitamin D deficiency due to sequestration of cholecalciferol and ergocalciferol in fat tissue, in spite of this is associated with increased bone mass [64]. Additionally, animal models harboring severe deficiency in vitamin D action (VDR knockout) and synthesis (Cyp27B1 knockout) have an unexpected thin phenotype and increased insulin sensitivity [65, 66]. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a member of the PPAR family of transcriptional factors, which has multiple roles not only in cell fate determination, but lipid biosynthesis, mitochondrial biogenesis, inflammation, neoplastic growth and insulin sensitivity [67–69]. PPAR-γ 1 and 2 are the two major forms of PPAR-γ protein, which are produced by differential usage of promoters and alternative splicing [70]. PPAR-γ1 is widely expressed in several tissues including the liver, skeletal muscle, adipose tissue and bone, while expression of PPAR-γ2 is almost restricted to adipocytes [71]. The expression of PPAR-γ2 seems to have a crucial role in adipogenesis, as stem cells with deletion of PPAR-γ2 gene in are unable differentiate in adipocyte, whereas overexpression of PPAR-γ2 in fibroblast leads to adipogenesis. During the initial steps of adipogenesis, the expression of CCAT/enhancer binding protein (C/EBP) transcription factors C/EBPβ and -δ is enhanced by adipogenic hormones. In parallel, these factors stimulate PPAR-γ2 expression, which in turn promotes the expression of C/EBP-α, generating a self-perpetuating loop for adipogenesis [72].

The organism can produce an array of endogenous ligand, including polyunsaturated fatty acids and modified fatty acids, such as prostaglandins [73], denoting that ligand availability does not modulate PPAR-γ2 activity. It has been hypothesized that the control of PPAR-γ2 expression is the pivotal mechanism for the control of PPAR-γ2 activity [74]. However, PPARγ responds poorly to native fatty acids compared to PPARα and PPARδ, driving speculation that modified fatty acids may be the

biological ligands. Certain prostanoids, including 15-deoxy-Δ12,14 prostaglandin J2 (15-dPGJ2), are excellent activators of PPARγ [75]. However, it is unlikely that 15-dPGJ2 is present at sufficient levels in vivo to be a biologically significant ligand. Other options like oxidized fatty acids, (e.g., 9-HODE and 13-HODE) found in oxidized low-density lipoprotein (LDL), activate PPARγ with increased potency and efficacy relative to native fatty acids and are present at significant concentrations in atherosclerotic lesions (39). Whether oxidized fatty acids serve as activators in other tissues, however, is not clear. It is possible that different ligands for PPARγ may be of primary importance in other contexts. For example, the ligand responsible for PPARγ activation in adipogenesis may be distinct from those that activate PPARγ in macrophages in the artery wall.

The exogenous ligands thiazolidinediones (TZDs) shed light on PPARγ due to their antidiabetic properties [76, 77]. TZDs affect the transcriptional activity of PPARγ, influencing the expression of target genes, e.g., LPL, FAT/CD36, GLUT4, acyl-CoA synthase [78–80]. In addition to improved insulin sensitivity, TZDs promote fat storage in white adipose tissue and at the same time reduce the overflow of FFA to muscle and the expression of adipokines and inflammatory cytokines. It is likely that the transcriptional network of marrow adipogenesis is governed by the same mechanisms used to regulate white adipocyte differentiation, and PPARγ is certainly critical in this process. Marrow adiposity is induced by treatment with PPARg agonists (TZDs), although this effect is quite variable and dependent on the type of TZD [81, 82].

Bone marrow fat can be affected positively or negatively by physiological and pathological stimuli, varying from mechanical loading, menopause, aging, diet restriction, hypercortisolism, hormone replacement therapy, and drugs [52, 54, 83–85]. Although the major causes of osteoporosis; i.e., aging, menopause and glucocorticoid-induced osteoporosis are associate with increased bone marrow fat [51]; bone mass accrual take places concomitantly with expanding bone marrow fat [3, 86]. The mechanistic interaction between bone cells and adipocytes, which define bone mass gain or loss, still remains enigmatic.

Previous studies showed that bone marrow stromal cells, collected from old mice (i.e., >24 months), exhibit increased expression of PPAR-γ2 and decreased expression of gene-related indicators of osteoblastogenesis, respectively [87, 88]. This scenario reflects an ideal environment for adipogenesis, which is consequent to oxidative stress. During aging, increased production of oxidized lipids is a well-known cause of oxidative stress [89]. Experiments in vitro demonstrate that oxidized lipids are able to both, hamper osteogenesis and stimulate adipocyte differentiation. In accordance, oxidized metabolites of LDL are ligands for PPAR-γ2 and during the aging process the production of these molecules is augmented [90, 91].

To further highlight the relevance of oxidative stress for the increment of bone marrow adipogenesis, several studies investigate the role of antioxidants on adipocyte differentiation. For instance, oxysterols including 20(S)-hydroxycholesterol (20S) induce the osteogenic differentiation of pluripotent mesenchymal cells, while inhibiting their adipogenic differentiation [92]. Two structural analogues of 20S, Oxy34 and Oxy49 are also able to induce the osteogenic and inhibit the adipogenic differentiation of bone marrow stromal cells through activation of Hedgehog (Hh) signaling [93]. In the same vein, there are data showing the effects of olive oil, accepted to have antioxidant properties, on the promotion of osteogenesis and inhibition of adipogenesis in the bone marrow by downregulating PPAR-γ2 [94, 95]. Thus, dietary lipids can take part in the determination of bone mass development and maintenance; the next section is specifically dedicated to that point.

21.2.2.4 Dietary Fat and Bone

Although the influence of dietary fat on bone has been rarely studied, there is some evidence that it impacts hard tissue. Experimental investigation supports the above cited clinical study in type 1 diabetes mellitus, when the effect of dietary fat on bone was directly investigated in rats. Bielohubi et al.,

[96] submitted rats to three different kinds of diet composition: normal chow (CH, 9 % fat, 33 % protein, and 58 % carbohydrates), low-carbohydrate–high-fat diet (LC-HF)-1 (66 % fat, 33 % protein, and 1 % carbohydrates), or LC-HF-2 (94.5 % fat, 4.2 % protein, and 1.3 % carbohydrates). Rats fed LC-HF diets accumulated significantly more visceral and bone marrow fat and showed increased leptin but decreased insulin-like growth-factor 1 (IGF-1). Both, peripheral quantitative computed tomography (pQCT) and micro-CT (microCT) independently revealed significant reductions in BMD of tibiae in the two groups submitted to LC-HF diet. Moreover, the force necessary to fracture bone in biomechanical testing was decreased in specimen from animals fed with a high-fat diet. The determination of the bone remodeling marker N-terminal propeptide of type I procollagen suggested reduced osteoblastic activity with high fat intake, whereas the bone resorption marker CrossLaps remained unchanged. Real-time PCR analysis revealed significant reductions by 70–80 % of transcription factors influencing osteoblastogenesis (Runx2, osterix, and C/EBPb) in bone marrow of rats fed LC-HF diets.

In a study of dietary induced changes in bone mass, investigators asked whether bone development in foals is affected by seasonal changes in pasture and dietary supplementation with concentrates rich in sugar and starch or in fat [97]. Forty foals were examined during two years, 20 each year. In each year, ten mares and their foals were fed a corn and molasses supplement (SS) and ten others were fed a corn oil and fiber supplement (FF). The animals were evaluated by monthly dorsopalmar radiographs for the assessment of the left metacarpus from birth to weaning and then every other month until 1 year of age. Bone density was estimated using imaging software and an aluminum stepwedge. The results showed that bone mineral content was lower in weanlings and yearlings fed the FF supplement than in those fed SS. Regression analysis indicated positive relationships between bone mineral content and body weight, age. The authors hypothesized that fat and fiber, may alter the availability of elements necessary for bone development [97].

In 2006, the relation of dietary fat to hip bone mineral density (BMD) was assessed in men and women using NHANES III data ($n = 14{,}850$) [98]. Models were adjusted for age, sex, weight, height, race, total energy and calcium intakes, smoking, and weight-bearing exercise. Data from women were further adjusted for use of hormone replacement therapy. Analysis of covariance was used to generate mean BMD by quintile of total and saturated fat intake for four sex/age groups. Saturated fat intake was negatively associated with BMD at several hip sites. The greatest effects were seen among men <50 year old (linear trend $p = 0.004$ for the femoral neck). For the femoral neck, adjusted mean BMD was 4.3 % less among men with the highest compared with the lowest quintile of saturated fat intake (BMD, 95 % CI: highest quintile: 0.922 g/cm^2, 0.909–0.935; lowest quintile: 0.963 g/cm^2, 95 % CI: 0.950–0.976). The authors concluded that there is a negative association between saturated fat intakes with bone mineral density and that men are particularly susceptible to manifest this undesirable effect [98].

The role of diet PUFAs on bone turnover is controversial. Currently, there is no consensus about the effects of PUFAs on bone strength; most data are based on cross-sectional studies, using bone mineral density as a surrogate end point. Rousseau and colleagues assessed the relationship between self-reported omega-3 fatty acid (O3FA) intake, bone mineral density (BMD) and lower extremity function in older adults [99]. They evaluated 247 individuals over aged 60 years (male = 118 and female = 129) residing in the community or an assisted living facility. They found a mean reported intake of O3FA equals to 1.27 g/day. Subjects with lower recorded O3FA intake (less than 1.27 g/day) had lower BMD than those with higher reported O3FA intake. In addition, the authors assessed the results through a multiple regression analysis with femoral neck BMD as the dependent variable and reported intake of O3FA, O6FA, protein, and vitamin D as independent variables. The analysis showed that reported O3FA intake was the only significant variable, accounting for 6 % of the variance in BMD. The authors also assessed the lower extremity functionality by the tests of chair rise time, walking speed, Timed Up and Go, and frailty. There was no independent association between reported O3FA intake and lower extremity function. While the above mentioned study did not take into account

several important points, including confounding parameters such as age, sex and the type of different fatty acids, another investigation addressed these aspects [100].

The Kuopio OSTPRE Fracture Prevention Study developed in Finland was designed to investigate the relationship between dietary PUFAs [total, n-3 (EPA and DHA) and n-6 (AA and LA)] and bone mineral density (BMD) among elderly women. The study comprised 554 postmenopausal women subdivided regarding hormone replacement therapy. At baseline they filled a 3-day food record and a questionnaire on lifestyle factors, diseases and medications. The authors verified a positive association between the dietary PUFAs (total, total n-3 and total n-6) and BMD at lumbar spine and in total body but not at femoral neck in women without HRT. Specific analysis of the effects of individualized PUFAs reveals in those women without HRT a positive association between LA (n-6) e ALA (n-3) with total body and lumbar spine BMD. In the subgroup with HRT was not observed association between diet components and BMD.

The impact of PUFAs and fish (tuna, dark fish, white fish, shellfish and total fish) intake on bone was evaluated in the Framingham Osteoporosis Study, which included individuals of both gender at baseline (1988–1989; $n=854$) and changes 4 years later in adults ($n=623$) with a mean age of 75 years [101]. No significant cross-sectional associations were observed for intakes of the individual essential fatty acid intakes in women or men. However, a significant interaction was observed between AA intake and EPA + DHA intake (below or at or above the median) at the FN in women, but not in men. Moreover, in women with intakes of EPA + DHA at or above the median, those women with the highest intakes of AA had a higher mean baseline FN-BMD than did those with the lowest intakes. Different results were obtained in male subgroup; in men with the lowest EPA + DHA intakes (quartile 1), those with the highest intakes of AA (quartile 4) lost more FN-BMD than did men with the lowest intakes of AA. The authors concluded that fish consumption may protect against bone loss and that the protective effects of a high AA intake may be dependent on the amount of EPA + DHA intake.

The beneficial effect of PUFAs and fish in diet was not confirmed in other studies. For example, the association of Fish and EPA + DHA consumption with bone mineral density (BMD) and hip fracture risk was evaluated in Cardiovascular Health Study [102]. The authors were also interested to investigate whether high linoleic acid (LA) intake, the major dietary n-6 PUFA, modifies the associations. The study included 5,045 participants aged 65 years and older and BMD were available for 1,305 participants. After multivariable adjustment, femoral neck BMD was 0.01 g/cm^2 lower in the highest versus lowest tuna/other-fish intake category ($p=0.05$, for trend). EPA + DHA intake (higher vs. lower median of 0.32 g/day) was associated with lower femoral neck BMD (0.66 vs. 0.71 g/cm^2, $p<0.001$) among those with LA intake greater than the median 12.1 g/day ($p=0.03$ for interaction). No significant associations were found with total-hip BMD. In addition, during the following up period of 11.1 years 505 hip fractures occurred. Fish or EPA + DHA consumption was not significantly associated with fracture incidence [hazard ratio (HR) for extreme categories: HR = 1.23, 95 % confidence interval (CI) 0.83–1.84 for tuna/other fish; HR = 1.16, 95 % CI 0.91–1.49 for fried fish; and HR = 0.98, 95 % CI 0.71–1.36 for EPA + DHA].

Few studies were performed having a randomized controlled configuration and none assessed a large number of individuals. A small number of recently postmenopausal women were investigated in relation to the effect of synthetic genistein in combination with other potential bone-protective dietary molecules on bone mineral density (BMD) [103]. During six months, 58 women were randomized; one received daily calcium only ($n=28$) and the other ($n=30$) was treated with genistein (30 mg/days), vitamin D3 (800 IU/days), vitamin K1 (150 µg/days) and polyunsaturated fatty acids (1 g polyunsaturated fatty acids as ethyl ester: eicosapentaenoic acid/docosahexaenoic acid ratio = ~2/1). The authors verified that while the calcium-treated group exhibited bone loss the combination of genistein induced a significant bone gain. Also, it was observed an elevation in the serum levels of bone specific alkaline phosphatase and N-telopeptide of type 1 collagen. It is necessary to consider in this study that

the gain in bone mass occurred in the site of ward's triangle and that there was no significant change in femoral BMD. The other relevant aspect is that the group on PUFA treatment actually received a mixture containing bone active components, including vitamin D and K [104–107]. Thus, it is not possible to attribute the bone effects to PUFAs in that context.

The literature reveals conflicting results in relation to the effects of PUFAs on biochemical bone markers. A clinical protocol was developed to evaluate changes in bone biomarkers in hyperlipidemic subjects submitted to replacement of regular milk with fortified milk during 1 year. The fortified milk was enriched with eicosapentaenoic acid and docosahexaenoic acid from fish oils, oleic acid, vitamins A, B(6), and E, as well as folic acid. The study comprised 72 patients (age range: 35–65 years) and they were randomly divided into two groups. The supplement group ($n = 39$) consumed 0.5 L/day of fortified milk, whereas the control group ($n = 33$) consumed 0.5 L/day of semiskimmed milk containing the same amount of total fat. At the end of the study the subgroup with enriched milk showed a significant increase in plasma eicosapentaenoic acid (42 %), docosahexaenoic acid (60 %), vitamin B6 (38 %), OPG (18 %), RANKL (7 %), OPG/RANKL (10 %), red blood cell folate (21 %), serum folate (53 %), calcium (4 %), vitamin D (11 %), and osteocalcin (22 %). Different from the results obtained in the previous study [103], significant differences were not observed in the serum levels of C-telopeptide of type 1 collagen a biochemical marker of bone resorption. The authors concluded that dietary supplementation with the fortified milk drink improved nutritional status and bone formation markers in adult hyperlipidemic patients. Unfortunately, Martin-Bautista and colleagues did not measure bone mass in their study. Although suggestive it is not possible to conclude that their volunteers have gained bone mass based only on biochemical bone markers, especially in a small group of individuals. Disorders associated with osteoporosis such as primary hyperparathyroidism as well as drug-treated osteoporosis with teriparatide or bisphosphonate exemplified how difficult is the interpretation of circulatory levels of bone markers. In other words, increased or decreased marker of bone formation does not mean respectively net bone gain or loss (e.g., primary hyperparathyroidism and bisphosphonate treatment). Also, there are several studies showing that circulatory concentration of osteoprotegerina and RANKL does not reflect the correspondent production in bone microenvironment [108]. It was detected increased serum OPG levels in postmenopausal women with osteoporosis [109]. In addition, there are data showing a negative correlation between serum OPG levels and forearm BMD [110]. Regarding the measurement of soluble RANKL, there are published data showing that low serum RANKL levels are predictors of the risk of fracture [111]. Additionally, a point that cannot be neglected in the last study is the multiple factorial interventions. In parallel with increased PUFAs intake patients were submitted to elevated consumption of linoleic acid (monounsaturated fat) and decreased of saturated fat.

21.3 Conclusion

The last decade repositioned the relationship between bone and other physiological systems. Instead of being a passive element on the complex network that assures metabolic homeostasis in different conditions, actually the skeleton is relevant for the modulation of energy disposal. Not unexpectedly bone tissue also is influenced by several endogenous and exogenous factors, including ample neuronal and endocrine modulation as well as impact of diet, climate and lifestyle. Fat, the diet component with highest caloric density not necessarily is a villain for obesity development and emergence of disorder. Regarding cardiovascular disorder, mounting evidence indicate that unsaturated has beneficial effects, protecting arterial wall from the process of atherosclerosis. Preliminary results regarding the effect of PUFAs on bone are insufficient, but satisfactory enough to challenge the development of large randomized clinical trial.

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Chapter 22
Acid–Base Balance and Bone Health

David A. Bushinsky and Nancy S. Krieger

Key Points

- On a typical Western diet, humans generate metabolic acids which must be excreted, through renal mechanisms, to maintain a stable physiologic systemic pH.
- Any impairment of kidney function will lead to a fall in systemic pH which is termed metabolic acidosis. During metabolic acidosis, the bone buffers acid (protons) and also releases calcium, a response that has been observed both in vivo and in vitro.
- In metabolic acidosis there is direct proton-mediated physicochemical release of calcium from bone.
- Sodium and potassium are also released from the mineral surface in exchange for the hydrogen ions.
- With metabolic acidosis of longer duration (>24 h), release of bone calcium occurs by a cell-mediated stimulation of bone resorption and inhibition of bone formation.
- The cell-mediated response to metabolic acidosis involves changes in specific gene expression and is primarily due to a stimulation of endogenous osteoblastic prostaglandin E_2 production, leading to production of RANKL and subsequent activation of osteoclastic bone resorption.
- The initial signaling event in the osteoblast appears to be activation of a specific proton receptor, OGR1.
- In addition to a net increase in bone resorption, metabolic acidosis has also recently been shown to stimulate production of osteoblastic FGF23.
- Respiratory acidosis, due to an increase in the partial pressure of CO_2, does not alter proton or calcium flux in bone.
- As renal function decreases with age, kidneys cannot excrete the daily acid load and this mild metabolic acidosis can lead to a significant decrease in bone mineralization potentially contributing to osteoporosis and fracture.

Keywords Metabolic acidosis • Bone resorption • Calcium • Osteoblasts • Bone formation • Prostaglandins • FGF23

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22.1 Introduction

On a daily basis humans eat substances that during metabolism generate or consume protons [1–4]. Acid may be generated by the following reactions:

$$\text{methionine or cystine} \rightarrow \text{glucose} + \text{urea} + SO_4^{2} + 2H^+$$

$$\text{arginine}^+ \rightarrow \text{glucose} + \text{urea} + H^+$$

$$R^- H_2PO_4 + H_2O \rightarrow ROH + 0.8HPO_4^{2-} / 0.2H_2PO^{4-} + 1.8H^+$$

Acid may be consumed, which is equivalent to base being generated, by the following reactions:

$$\text{glutamate}^- + H^+ \rightarrow \text{glucose} + \text{urea}$$

$$\text{lactate}^- + H^+ \rightarrow \text{glucose} + CO_2$$

$$\text{citrate}^- + 4.5O_2 \rightarrow 5CO_2 + 3H_2O + HCO_3^-$$

The net result is that when consuming a typical Western diet adult humans generate approximately 1 meq of acid per kg per day [5, 6]. The more acid precursors contained in the diet, the greater degree of systemic acidity [5]. This acid must be completely excreted by the kidney. Any impairment in kidney function will lead to an increase in systemic acidity which is termed metabolic acidosis. The maintenance of a stable physiologic systemic pH is of critical importance to the survival of mammals [6–8]. While only net loss of hydrogen ions can ultimately correct acidosis [6], bone appears to be instrumental in the maintenance of a stable physiologic systemic pH during metabolic acidosis; however, this homeostatic function is often at the expense its mineral content [2, 9–13].

22.2 In Vivo Effects of Metabolic Acidosis on Bone

During in vivo acute metabolic acidosis (a primary decrease in bicarbonate concentration ($[HCO_3^-]$)), ~60 % of the administered protons (hydrogen ions) are buffered outside of the extracellular fluid [14] by soft tissues [15–17] and by bone [7, 8, 18–24]. The in vivo evidence that bone acutely buffers protons, and in the process releases calcium, derives principally from the loss of bone sodium and/or potassium [25–31], carbonate [23, 29, 32, 33] and the increase in serum calcium [34] observed during acidosis. Bone sodium (or potassium) loss implies proton for sodium (or potassium) exchange and carbonate loss suggests consumption of this buffer by the administered protons. As at least 98 % of body calcium is contained within bone [35, 36], the increase in serum calcium likely derives from mineral stores.

Chronic metabolic acidosis increases urinary calcium excretion [37–39] without an increase in intestinal calcium absorption [40, 41], resulting in negative calcium balance [12, 42], which appears to reflect proton-mediated dissolution of bone mineral [6, 8, 10, 18, 37, 43]. Indeed, chronic metabolic acidosis appears to decrease mineral content in most in vivo studies [10, 12, 13, 42].

The independent effect of acidosis to suppress bone formation was elegantly demonstrated in children with renal tubular acidosis [44–46]. Children with renal tubular acidosis have stunted linear growth. The provision of base to these children has been demonstrated to dramatically increase their growth. There is ample clinical evidence that acidosis adversely affects bone during renal failure [47–50] which may be corrected by bicarbonate administration [51–53]. Bone carbonate is decreased in acidic, uremic patients [54–56]. This decrease may represent dissolution of bone

carbonate stores or replacement by phosphate resulting in the incorporation of H^+ into the mineral [23, 32, 33]. In a radiographic study the majority of patients with proximal renal tubular acidosis had rickets or osteopenia [57].

Adults with distal renal tubular acidosis and normal renal function were recently shown to have a lower bone mineral density than normal controls [58]. Bone histomorphometry demonstrated that these patients with renal tubular acidosis had a significantly decreased bone formation rate and an increased osteoid surface and osteoid volume when compared to normal controls. These patients were then treated with potassium citrate, which is metabolized to bicarbonate, to correct the acidosis [59]. The treatment resulted in a significant increase in bone mineral density at the trochanter of the femur and in the total femur. There was an increase in bone formation rate. Interestingly the level of parathyroid hormone rose significantly in the treated patients.

Chronic acid ingestion, in the form of the common North American high-protein diet, coupled with the known effects of acid on bone, have led to the suggestion that this acid production may play a role in the etiology of osteoporosis [10, 60, 61]. As we age there is a decrease in overall renal function, including the ability to excrete acid [62]. With increasing age, humans become slightly, but significantly, more acidic [62, 63]. Supporting this hypothesis is the observation that administration of base appears to decrease the negative calcium balance induced by high-protein diet [64–66].

22.3 In Vitro Observations

Although in vivo evidence strongly suggests that bone is involved in the systemic response to acid–base disorders, until fairly recently there was little direct in vitro confirmation [7]. Neuman et al. found that a reduction of medium pH produced a marked increase in hydroxyapatite solubility [67]. Dominguez and Raisz [68] determined that an acid medium induced movement of prelabeled calcium from bone.

We undertook a series of studies to test the hypothesis that cultured bone exposed to a physiologically acidic medium would release calcium into the medium and buffer the increased medium hydrogen ion concentration [2, 4, 6, 8, 9, 21–24, 30, 32, 69–77]. We utilized the model of cultured neonatal mouse calvariae as the calvariae (frontal and parietal bones of the skull) have functioning osteoclasts and osteoblasts [78, 79], respond to hormones and synthesize DNA and proteins as does bone in vivo [80]. Calvariae can be cultured in the physiologic carbon dioxide-bicarbonate buffer system [24] where medium pH can be regulated precisely by independently altering the partial pressure of carbon dioxide or bicarbonate concentration, simulating either "respiratory" or "metabolic" (respectively) acid–base disorders [1, 2, 6, 9, 24].

22.3.1 Acute Acidosis

22.3.1.1 Calcium Release

Calvariae cultured in acidic medium exhibit proton-dependent net calcium efflux during both acute (3 h) and more chronic (>24–99 h) incubations [2, 3, 9, 23, 24, 26, 30, 32, 69–71]. During acute incubations there was net calcium efflux from the calvariae when medium pH was decreased to less than the physiologic normal of 7.40 by decreasing the $[HCO_3^-]$, no net flux at a neutral physiologic pH and an influx of calcium into bone when pH was greater than 7.40 [24] (Fig. 22.1).

The hypothesis that the mechanism of proton-mediated net calcium efflux from bone during these acute incubations was direct physicochemical (non-cell-mediated) calcium release was next tested.

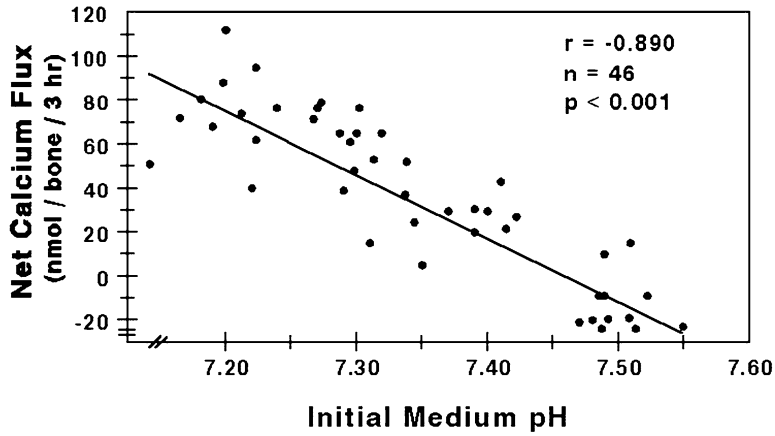

Fig. 22.1 Effect of initial medium pH on net calcium flux in neonatal mouse calvariae cultured for 3 h. A positive flux indicates net calcium movement from the bone into the medium. Medium pH was adjusted with concentrated HCl or NaOH at a partial pressure of carbon dioxide of 40 mmHg. Calvariae were preincubated in neutral pH medium for 24 h prior to this 3-h incubation. ($r = -0.890$, $n = 46$, $p < 0.001$) (data from [69])

Calvariae were cultured calvariae with agents that would stimulate or suppress bone cell activity but not affect the mineral directly [69]. It was found that bone cells contributed a constant, pH-independent net calcium flux from the mineral during these acute (3 h) experiments, thus acute proton-mediated calcium release was due to physicochemical and not cell-mediated mechanisms [69] (Fig. 22.2). To confirm that acidic medium could alter physicochemical forces and promote dissolution of the bone mineral, synthetic carbonated apatite disks were cultured in physiologically acid medium [81]. The carbonated apatite disks are an accurate, cell-free, model of bone mineral [82–87]. Net calcium flux from cultured carbonated apatite disks was stimulated in response to a physiologic acidosis, similar to that of cultured calvariae, supporting the hypothesis that acidic medium can induce physicochemical calcium release from bone [81].

The type of bone mineral in equilibrium with the medium, and thus altered by the physicochemical forces, might be carbonate or phosphate in association with calcium. To determine which, calvariae were cultured in medium in which the driving forces for crystallization with respect to the solid phase of the bone mineral were altered by changing medium pH [23]. With respect to calcium and carbonate, but not calcium and phosphate, there was bone formation in a supersaturated medium, no change in the bone mineral when cultured in a saturated medium and bone dissolution into an undersaturated medium. Thus bone carbonate appears to be solubilized during an acute reduction in medium pH leading to a release of calcium. When calvariae are cultured in acidic medium there is a progressive loss of total bone carbonate during a model of metabolic acidosis [32]. Further support for the role of carbonate in acid-mediated bone mineral dissolution comes from studies in which it was demonstrated that at a constant pH, whether physiologically neutral or acid, bone net calcium flux is dependent on the medium bicarbonate; the lower the bicarbonate, the greater the calcium efflux from bone [73]. Bone carbonate appears to be in the form of carbonated apatite [85, 87, 88].

22.3.1.2 Hydrogen Ion Buffering

The in vitro evidence for proton buffering by bone is derived from studies of acidosis-induced proton influx into bone [22–24] and microprobe evidence for a depletion of bone sodium and potassium during acidosis [25, 26, 30, 31, 89]. When calvariae are cultured in medium acidified by a decrease in

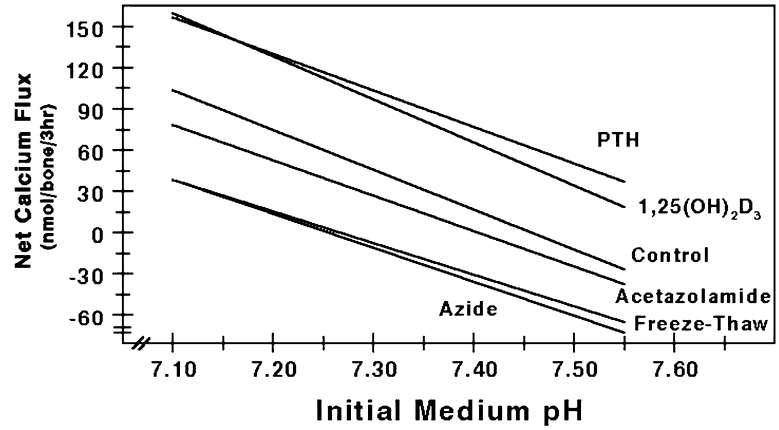

Fig. 22.2 Comparison of regressions of initial medium pH on net calcium flux for six separate groups of calvariae. Calvariae were incubated for 24 h in similar medium prior to this 3-h incubation. Abbreviations: Control, calvariae incubated in control medium (pH ~ 7.40); PTH, calvariae incubated in control medium with parathyroid hormone 1×10^{-8} M; $1,25(OH)_2D_3$, calvariae incubated in control medium with 1,25 dihydroxyvitamin D_3 1×10^{-8} M; Acetazolamide, calvariae incubated in control medium with acetazolamide 4×10^{-4} M; Freeze–Thaw, calvariae incubated in control medium after 3 successive freeze–thaw cycles. Regressions are different due to a difference in intercepts of all groups except PTH and $1,25(OH)_2D_3$, which are similar and azide and freeze–thaw, which are similar. Slopes are similar in all six groups (data from [69])

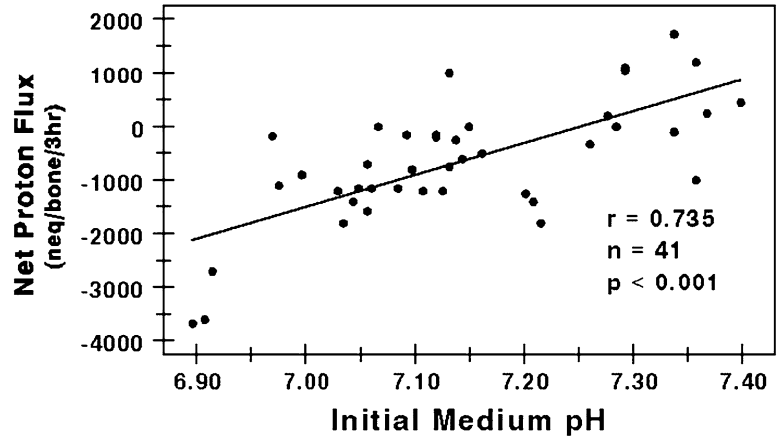

Fig. 22.3 Effect of initial medium pH on net proton flux in calvariae cultured for 3 h. A positive flux indicates net proton movement from the calvariae into the medium, a negative flux the opposite. pH was adjusted for the 3-h incubations with concentrated HCl or NaOH at a partial pressure of carbon dioxide of 40 mmHg (data from [24])

the concentration of bicarbonate (metabolic acidosis), there is a net influx of protons into the bone, decreasing the medium proton concentration and indicating that the additional hydrogen ions are being buffered by bone [22–24] (Fig. 22.3). This leads to an increase in the pH of the culture medium.

22.3.1.3 Proton for Sodium and/or Potassium Exchange

Bone is a reservoir for sodium and potassium and its surface has fixed negative sites that normally complex with sodium, potassium, and hydrogen ions; the sodium and potassium appear to exchange

freely with the surrounding fluid [35, 67] Using a high-resolution scanning ion microprobe with secondary ion mass spectroscopy it was found that the surface of the bone is rich in sodium and potassium relative to calcium [25, 26, 89–92]. After incubation in acidic medium there is loss of surface sodium and potassium relative to calcium [25, 26, 30, 31, 89] in conjunction with buffering of the additional protons, suggesting sodium and potassium exchange for hydrogen ions on the bone surface resulting in a decrease in medium acidity [25, 27, 28]. When osteoclastic function is inhibited with calcitonin, microprobe analysis indicates that physicochemical proton buffering by bone causes relatively equal calcium and sodium loss [26]. In acidic medium, osteoclastic function is necessary to support the enriched levels of bone potassium [31].

22.3.1.4 Fall in bone carbonate

Bone contains ≈ 80 % of the total carbon dioxide (including CO_3^{2-}, HCO_3^-, and CO_2) in the body [93]. Approximately 2/3 of this is in the form of carbonate (CO_3^{2-}) complexed with H^+ (as HCO_3^-), calcium, potassium and sodium, and other cations, and is located in the lattice of the bone crystals where it is relatively inaccessible to the systemic circulation. The other third is located in the hydration shell of hydroxyapatite where it is readily available to the systemic circulation. Acute metabolic acidosis decreases bone total carbon dioxide [33]. Acidosis induces the release of calcium and carbonate from bone [23] leading to a progressive loss of bone carbonate during metabolic acidosis [32].

When both the in vitro and in vivo studies are considered together there is strong evidence that bone is a H^+ buffer capable of maintaining the extracellular fluid pH near the physiologic normal. The loss of both bone sodium and carbonate suggests that in addition to sodium for H^+ exchange there is a progressive loss of carbonate in response to acidosis.

22.3.2 Chronic Acidosis

22.3.2.1 Calcium Release

Chronic metabolic acidosis induces the release of bone calcium, predominantly by enhanced cell-mediated bone resorption and decreased bone formation [70, 72, 74, 94–96]; however, there is a component of direct physicochemical acid-induced dissolution, as in acute metabolic acidosis [23, 24, 26, 69]. In vivo rat studies have shown stimulation of cell-mediated bone Ca resorption during prolonged acidosis [34, 97].

There is cell-mediated resorption of bone calcium after 99 h of culture in acidic medium produced by a decrease in medium bicarbonate [70] (Fig. 22.4). Acidosis has also been shown to increase osteoclastic and inhibit osteoblastic activity [72]; release of the osteoclastic enzyme β-glucuronidase was stimulated (Fig. 22.5) while osteoblastic collagen synthesis (Fig. 22.6) and alkaline phosphatase were inhibited. Conversely an increase in [HCO_3^-], metabolic alkalosis, decreases net calcium efflux from bone through an increase in osteoblastic bone formation and a decrease in osteoclastic bone resorption [95]. Further evidence that metabolic acidosis inhibits osteoblastic function was obtained utilizing primary osteoblasts in culture. Isolated osteoblasts cultured for 3 weeks synthesize collagen and form nodules of apatitic bone [98–101]. Metabolic acidosis leads not only to fewer nodules, but decreased calcium influx into the nodules [94] (Fig. 22.7). Thus, it appears that both augmentation of osteoclastic bone resorption and inhibition of osteoblastic bone formation have a prominent role in the hypercalciuria of chronic metabolic acidosis [72, 94].

During renal failure there is often increased parathyroid hormone (PTH) in addition to acidosis [102, 103]. To determine if acidosis and PTH have additive effects on net calcium efflux, mouse calvariae were cultured in acidic medium containing PTH [104]. Acidosis and PTH were found to

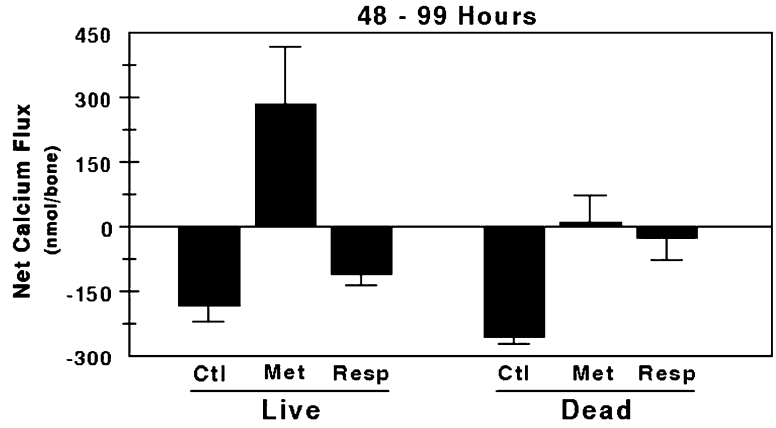

Fig. 22.4 Net calcium flux during the final 51 h of a 99-h incubation for the six groups of calvariae indicated. *Live* calvariae cultured in living state, *dead* calvariae subjected to three free-thaw cycles before culture, *Ctl* calvariae culture in unaltered medium, *Met* medium acidified by lowering the bicarbonate concentration, *Resp* medium acidified by increasing the partial pressure of carbon dioxide. Values are mean ± SEM (data from [70])

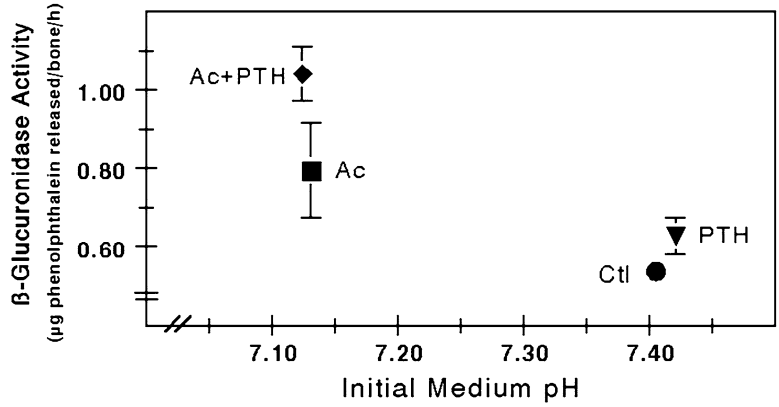

Fig. 22.5 Effect of acidosis and PTH, alone and in combination, on osteoclastic β-glucuronidase activity. Calvariae were incubated in control medium (Ctl), medium acidified to pH ~ 7.10 (Met); medium with parathyroid hormone 10^{-10} M final concentration (PTH), or with PTH added to acidic medium (Met + PTH). Calvariae were incubated for 24 h and then transferred to similar fresh medium for an additional 24 h. At the end of the second 24-h incubation, aliquots of medium were removed for assay of β-glucuronidase activity. Values are mean ± SEM. $^{*}p < 0.05$ vs. Ctl; $^{+}p < 0.05$ vs. Met; $^{o}p < 0.05$ vs. PTH (data from [104])

independently stimulate net calcium efflux from bone (Fig. 22.8), inhibit osteoblastic collagen synthesis (Fig. 22.6) and stimulate osteoclastic β-glucuronidase secretion (Fig. 22.5) while the combination had a greater effect on each of these parameters than either alone.

22.3.3 Acidosis-Induced Alterations in Gene Activity

Based on the H^+-induced increase in osteoclastic bone resorption and decrease in osteoblastic bone formation [72, 74], it was hypothesized that acidosis affects the pattern of gene expression in osteoblasts. As a model system primary neonatal mouse calvarial cells were used, which are principally

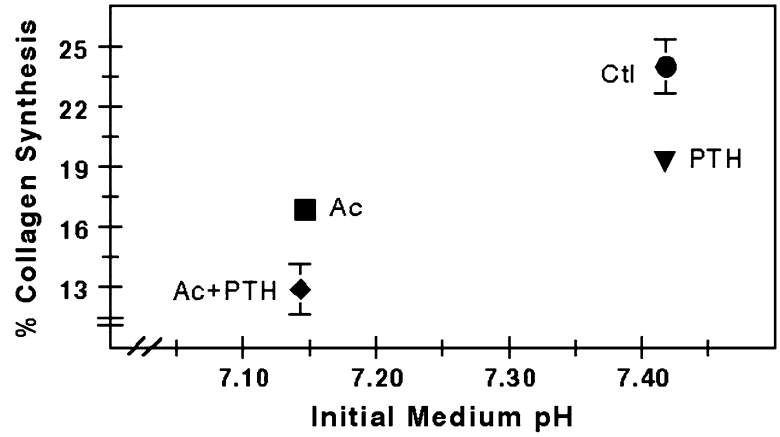

Fig. 22.6 Effect of acidosis and PTH, alone and in combination, on osteoblastic collagen synthesis. Calvariae were incubated in control medium (Ctl), medium acidified to pH ~ 7.10 (Met); medium with parathyroid hormone 10^{-10} M final concentration (PTH), or with PTH added to acidic medium (Met + PTH). Calvariae were incubated for 24 h and then transferred to similar fresh medium for an additional 24 h. Incorporation of [^{3}H]proline into collagenase-digestible protein in calvariae was measured during the final 3 h of the second 24-h incubation. Values are mean ± SEM. *$p < 0.05$ vs. Ctl; $^+p < 0.05$ vs. Met; $^\circ p < 0.05$ vs. PTH (data from [104])

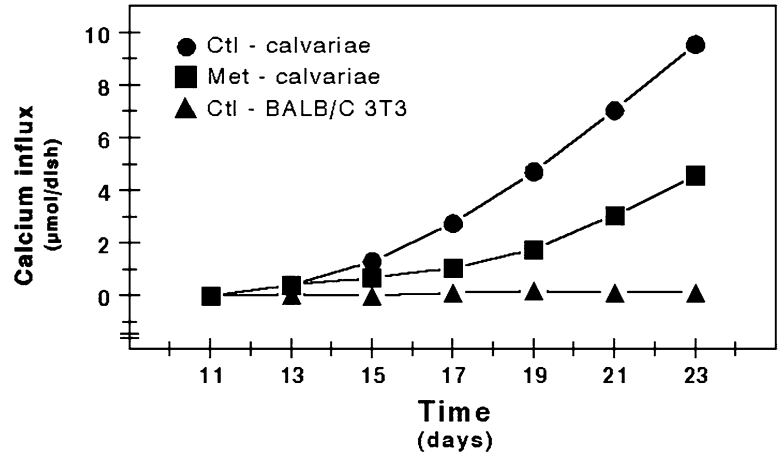

Fig. 22.7 Cumulative calcium influx as a function of incubation time for cultured neonatal mouse calvarial cells. Cells, which are predominantly osteoblasts, were incubated in control medium until confluent (day 9) and then cultured for an additional 14 days in control medium (Ctl—calvariae), medium acidified by decreasing the medium bicarbonate concentration (Met—calvariae). Balb/C 3T3 mouse fibroblasts were also incubated in control medium (Ctl—BALB/C 3T3). Values are mean ± SE. Changes in medium calcium concentration were calculated by subtracting the final from the initial calcium concentration and correcting for volume. Results are summed over the 14-day incubation period and represent calcium influx into the cultured cells (data from [94])

osteoblasts [105]. To assay acute effects of acidosis on gene expression cells were cultured in physiologically neutral pH medium until confluent and then stimulated with fresh medium at either neutral or acidic pH. RNA was harvested at various times after stimulation. Among a group of immediate early response genes, including *Egr-1*, *junB*, *c-jun*, *junD*, and *c-fos*, only the magnitude of *Egr-1* stimulation was altered by medium pH. A progressive decrease in pH to 6.8 led to a parallel decrease in *Egr-1* stimulation and an increase in pH to 7.6 led to an increase in *Egr-1* stimulation [96].

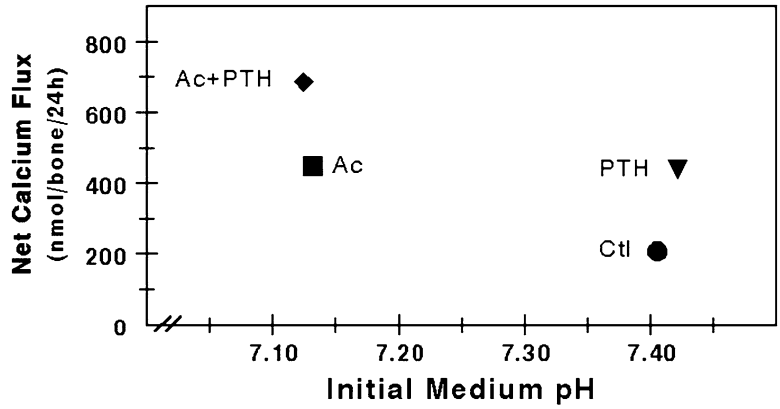

Fig. 22.8 Effect of acidosis and PTH, alone and in combination, on net calcium efflux from cultured neonatal mouse calvariae. Calvariae were incubated in control medium (Ctl), medium acidified to pH ~ 7.10 (Met); medium with parathyroid hormone 10^{-10} M final concentration (PTH), or with PTH added to acidic medium (Met + PTH). Calvariae were incubated for 24 h and then transferred to similar fresh medium for an additional 24 h. At the end of the second 24-h incubation, aliquots of medium were removed for assay of net calcium flux. Values are mean ± SEM. $^{*}p < 0.05$ vs. Ctl; $^{+}p < 0.05$ vs. Met; $^{o}p < 0.05$ vs. PTH (data from [104])

Osteoblasts express type 1 collagen as the major component of the bone extracellular matrix which subsequently becomes mineralized. Type I collagen RNA was stimulated approximately three- to fivefold, 40 min after medium change; the stimulation was again decreased by acidosis and increased by alkalosis [96].

Cultured primary mouse calvarial cells differentiate and form sites of mineralization, known as bone nodules [94, 98–101]. During this process, osteoblasts express a number of matrix proteins distinct to bone, including bone sialoprotein, osteocalcin, osteonectin, osteopontin, and matrix gla protein [106]. Metabolic acidosis is known to decrease bone nodule formation and subsequent mineralization [94]. It was hypothesized that acidosis would alter the pattern of matrix gene expression in chronic cultures of bone cells resulting in a matrix that mineralizes less extensively than that from cultures incubated at neutral pH. After 3–4 weeks in neutral pH medium there was a dramatic increase in osteopontin RNA (Fig. 22.9)[107]. In contrast there was no increase in osteopontin RNA in acidic cultures. Osteopontin contains RGD domains and serves as an anchoring protein for macrophages and osteoclasts; osteopontin may also be a chemoattractant for these cell types [108, 109]. Downregulation of osteopontin expression may serve to limit recruitment of bone-resorbing cells during acidosis, perhaps a cause of low turnover renal osteodystrophy [8]. RNA for matrix Gla protein is also induced by neutral differentiation medium, reaching levels 20- to 30-fold greater than those before differentiation. Again, acidosis almost totally prevents the increase in matrix Gla protein RNA levels (Fig. 22.10). While matrix Gla protein expression is not limited to bone, matrix Gla protein comprises about 10 % of the carboxyglutamic acid found in bone [110]; the Gla residue coordinates with calcium and may serve to direct calcification [110]. The levels of RNA for the housekeeping gene GAPDH did not vary with pH, nor did the level of a second bone-associated RNA species, osteonectin or TGFβ1 indicating that there is not overall cellular toxicity. To determine if acidosis reversibly impairs cellular production of osteopontin and matrix Gla protein, cultures of primary calvarial bone cells were put in acidic differentiation medium at day 8, then switched to neutral medium at either days 15, 22, or 29 [107]. One week of exposure to acidic medium had no lasting effect on osteopontin and matrix Gla protein expression, while a 2 week exposure had a small inhibitory effect. There was partial recovery of RNA for osteopontin and matrix Gla protein after 3 weeks of acidosis. In the same samples, osteonectin and GAPDH RNA expression were not affected.

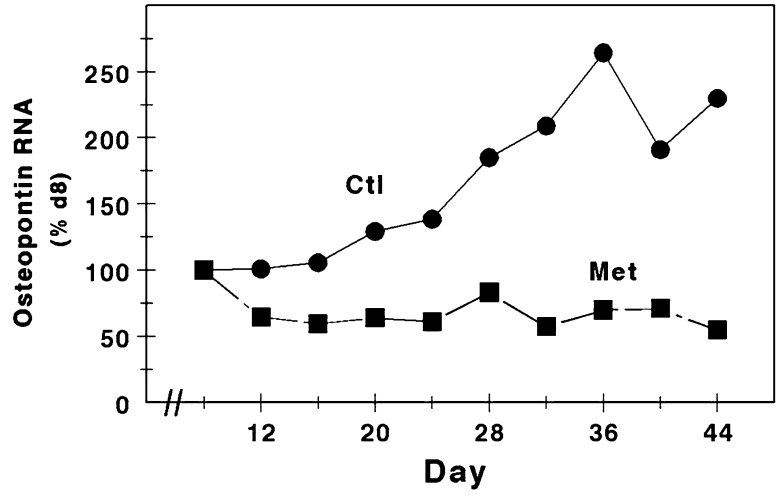

Fig. 22.9 Time course of osteopontin gene expression. Cells were grown for 8 days in control medium (pH = 7.5), prior to incubation in neutral (pH = 7.5, Ctl) or acidic (pH = 7.1, Met) differentiation medium, and were harvested for RNA at indicated times. After Northern blotting and hybridization with an osteopontin probe, the filter was quantitated using a Molecular Dynamics PhosphorImager. To correct for variations in loading, the filter was stripped and rehybridized with GAPDH. Values expressed on *day 8 (d8)* are the ratio of osteopontin RNA to GAPDH RNA. All values on subsequent days are expressed as the ratio of osteopontin RNA to GAPDH RNA on that day divided by the ratio on *day 8* (predifferentiation). Plot is of a single, representative experiment (data from [107])

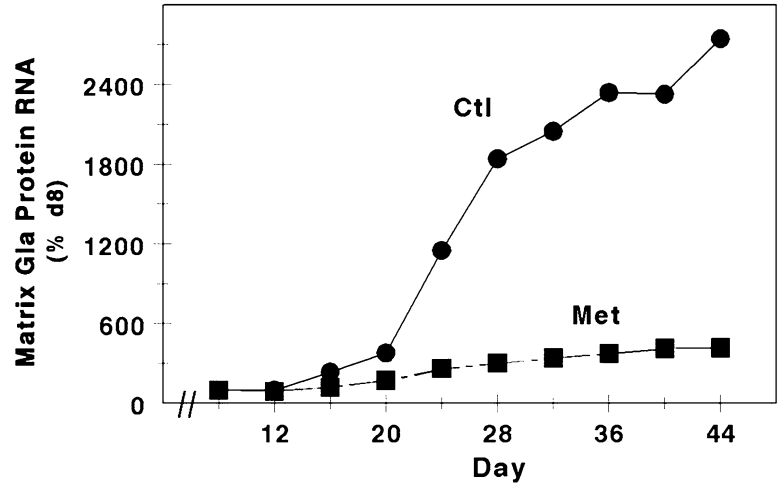

Fig. 22.10 Time course of Matrix Gla Protein RNA expression. Cells were grown for 8 days in control medium (pH = 7.5), prior to incubation in neutral (pH = 7.5, Ctl) or acidic (pH = 7.1, Met) differentiation medium, and were harvested for RNA at indicated times. After Northern blotting and hybridization with a Matrix Gla Protein probe, the filter was quantitated. To correct for variations in loading, the filter was stripped and rehybridized with GAPDH. Values expressed on *day 8 (d8)* are the ratio of Matrix Gla Protein RNA to GAPDH RNA. All values on subsequent days are expressed as the ratio of Matrix Gla Protein RNA to GAPDH RNA on that day divided by the ratio on *day 8* (predifferentiation). Plot is of a single, representative experiment (data from [107])

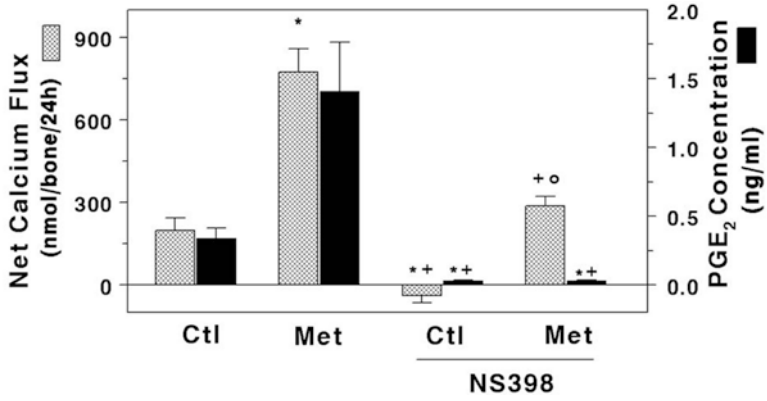

Fig. 22.11 Pharmacologic inhibition of prostaglandin E_2 production decreases acid-induced calcium efflux. Neonatal mouse calvariae were incubated in neutral (NTL) medium (pH = 7.4) or acidic (MET) medium (pH = 7.1) for 48 h in the absence or presence of 1 μM NS398, a specific inhibitor of COX2, with a medium change at 24 h. Medium PGE_2 concentration is presented on the right ordinate (*solid bars*) and net calcium flux is on the left ordinate (*hatched bars*) for the 24–48 h time period. Data are the mean ± SE for 11 pairs of calvariae in each group from. $^*p < 0.001$ vs. NTL; $^+p < 0.001$ vs MET; $^\circ p < 0.001$ vs NTL + NS398 (data from [113])

22.3.4 Intracellular Signaling in Response to Acidosis

Prostaglandins, especially prostaglandin E_2 (PGE_2), mediate bone resorption induced by a variety of hormones. To test the hypothesis that acid-induced bone resorption is mediated by prostaglandins, neonatal mouse calvariae were cultured in neutral or physiologically acidic medium with, or without, NS398, a specific inhibitor of cyclooxygenase 2 (COX-2) [111] (refs.), the rate-limiting inducible enzyme in prostaglandin production [112].

Net calcium efflux and medium PGE_2 levels were determined. Compared to neutral pH medium, acid medium led to an increase in net calcium flux and PGE_2 levels after 48 h, a time at which acid-induced net calcium flux is predominantly cell-mediated and NS398 inhibited the acid-induced increase in both net calcium flux and PGE_2 (Fig. 22.11) [113]. Net calcium flux was correlated directly with medium PGE_2. Exogenous PGE_2, at a level similar to that found after acid incubation, induced net calcium flux in bones cultured in neutral medium. Acid medium also stimulated an increase in COX-2 RNA and PGE_2 levels in isolated bone cells (principally osteoblasts), which was again inhibited by NS398. In addition, calvariae from mice with a genetic deficiency in COX-2 had significantly less acid-induced calcium flux. Thus acid-induced stimulation of cell-mediated bone resorption appears to be mediated by endogenous osteoblastic PGE_2 synthesis.

Growth and maturation of osteoclasts are dependent on the interaction of the osteoclastic cell-surface receptor RANK with a ligand expressed on the surface of osteoblasts, RANKL [114]. The RANK/RANKL interaction initiates a differentiation cascade which leads to mature, bone-resorbing osteoclasts and increases their resorptive capacity and survival. To test the hypothesis that metabolic acidosis increases expression of RANKL, neonatal mouse calvariae were cultured in acidic or neutral medium, and the relative expression of RANKL RNA was determined by RT-PCR and quantitated by Northern analysis. Metabolic acidosis significantly increased the expression of RANKL RNA at 24-h and at 48-h compared to respective controls (Fig. 22.12). Bone calcium efflux was increased in acidic, compared to control, medium. At 48-h calcium efflux was correlated directly with RANKL expression. Indomethacin, an inhibitor of prostaglandin synthesis prevented the acid-induced increase in RANKL RNA indicating a pivotal role for prostaglandins in the induction of RANKL by metabolic acidosis. The acidosis-induced increase in osteoblastic RANKL would augment osteoclastic bone

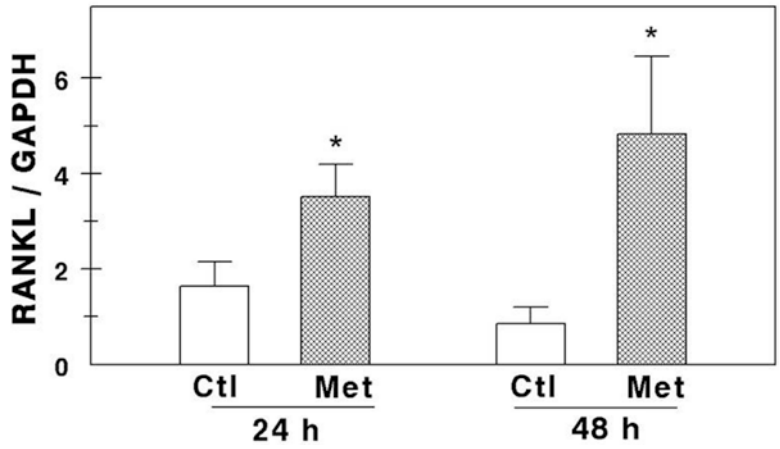

Fig. 22.12 Effect of metabolic acidosis on RANKL expression. Neonatal mouse calvariae were incubated in neutral medium (Ctl) or in medium acidified by a primary reduction of the bicarbonate concentration, to model metabolic acidosis (Met), either for 24-h or for 48-h. In the 48-h experiments, after the initial 24-h, calvariae were moved to similar fresh preincubated medium for the final 24-h. Calvariae from each culture dish were pooled for isolation of total RNA using a Qiagen RNeasy kit. First strand cDNA was synthesized from total RNA. Aliquots were amplified using gene specific primers and then were electrophoresed on agarose. Filters were autoradiographed and quantitated using a densitometer and then were stripped and reprobed for GAPDH. To control for differences in RNA loading each signal for RANKL was normalized to its respective GAPDH RNA. Data are mean ± SE, $n = 7–11$ pairs of calvariae in each group. Compared to CTL, with MET there was a significant increase in the ratio of RANKL to GAPDH at 24-h and at 48-h. Data are mean ± SE. *RANKL* receptor activator of NFκB ligand; *different from CTL, $p < 0.05$. (data from [114])

resorption and help explain the acidosis-induced coupling of osteoblastic and osteoclastic activity (Fig. 22.13) and the increase in bone calcium efflux.

Most recently we have found that metabolic acidosis regulation of osteoblastic activity, ultimately leading to osteoclastic bone resorption is initiated by activation of a proton (H^+) receptor, OGR1 [115, 116], leading to Ca_i signaling [117] upstream of COX2 stimulation [113]. OGR1 is a member of a small family of G-protein coupled proton-sensing receptors [118] and is expressed in osteoblasts [119] and osteoclasts [120, 121]. Ludwig et al. demonstrated that decreasing pH in osteoblasts containing OGR1 led to accumulation of phosphoinositide metabolites [119] and we found that in response to metabolic acidosis, OGR1 mediates an increase in intracellular Ca in mouse osteoblasts [115]. Further support for this mechanism comes from the observation that pharmacologic inhibition of inositol phosphate-mediated calcium signaling blocks acid-induced bone resorption in the neonatal mouse calvariae as well as acid stimulation of COX2 and RANKL in isolated osteoblasts [117].

22.3.5 Acidosis Regulation of Fibroblast Growth Factor 23

In patients with chronic kidney disease (CKD), acid production continues while acid excretion diminishes, leading to metabolic acidosis [11]. As described above, during metabolic acidosis bone buffers the increase in hydrogen ions through direct physicochemical dissolution as well as by inhibiting osteoblastic bone formation and stimulating osteoclastic bone resorption, leading to calcium and phosphate release into the systemic circulation. As renal function declines there also is an incremental increase in the phosphaturic hormone, fibroblast growth factor 23 (FGF23) [122, 123]. The increase in FGF23 results in decreased renal tubule phosphate reabsorption [123, 124] and reduced 1,25-dihydroxyvitamin D_3 [122, 124] with subsequent reduced intestinal phosphate absorption [122].

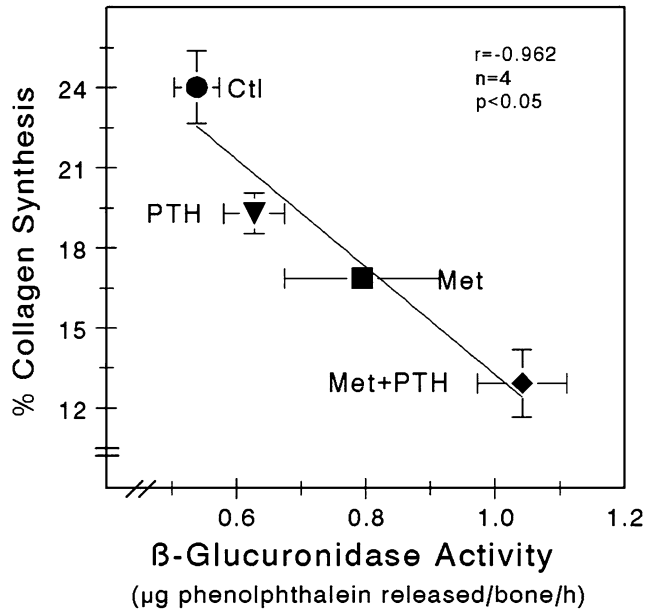

Fig. 22.13 Correlation between percent osteoblastic collagen synthesis and osteoclastic β-glucuronidase activity. Calvariae were incubated in Ctl, Met, PTH, 10^{-10} M final concentration, or with Met + PTH. Calvariae were incubated for 24 h and then transferred to similar fresh medium for an additional 24-h incubation. Incorporation of [^{3}H] proline into collagenase-digestible protein in calvariae was measured during final 3 h of second 24-h incubation. At end of second 24-h incubation, aliquots of medium were removed for assay of β-glucuronidase activity (data from [104])

Elevated levels of FGF23 induce left ventricular hypertrophy [125] and are associated with a significant increase in mortality in patients with CKD [126] and those on dialysis [127]. FGF23 is produced in osteocytes and osteoblasts [124] and phosphate, 1,25-dihydroxyvitamin D_3 and PTH have all been shown to regulate FGF23 [128–130], though it is not certain what is the primary regulator.

As acidosis is often associated with CKD and leads to bone buffering by regulating osteoblast activity, we tested the hypothesis that acidosis directly regulates FGF23 production. We have demonstrated in mouse calvarial cultures as well as cultured mouse osteoblasts, metabolic acidosis directly increases FGF23 RNA and protein levels [131]. As this stimulation was observed in isolated cells (Fig. 22.14) it is not secondary to a release of mineral phosphate from the bone. This suggests that the elevated levels of FGF23 observed in CKD may be due, at least in part, to metabolic acidosis directly stimulating osteoblast production of FGF23.

22.3.6 Acidosis-Induced Changes in Bone Ion Composition

A high-resolution scanning ion microprobe with secondary ion mass spectroscopy was utilized to determine how [H^+] alters the ion composition of the bone mineral [25, 26, 30, 31, 89–92, 132, 133]. Studies to date have shown that the calvarial surface is rich in sodium and potassium relative to calcium [25, 26, 30, 31, 89–92, 133]. The excess bone potassium is maintained through cell-mediated processes [91]. Loss of bone cell function produces an influx of calcium and marked release of bone potassium; there is a fall in the ratio of potassium/calcium, and to a lesser extent sodium/calcium, at the superficial surface of the mineral [91]. Metabolic acidosis causes release of mineral calcium and leads to a reduction in the surface ratio of sodium/calcium and potassium/calcium, indicating a greater

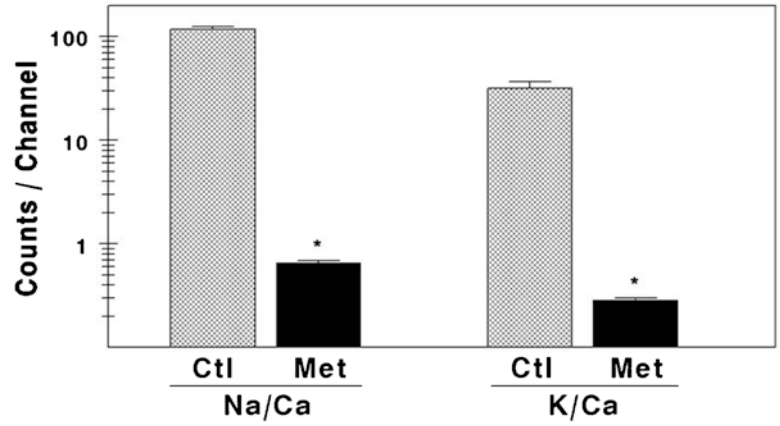

Fig. 22.14 Ratio of sodium to calcium (Na^+/Ca^{2+}) and potassium to calcium (K^+/Ca^{2+}) in the mid-cortex of neonatal mouse femurs after drinking only distilled water (Ctl) or water with 1.5 % NH_4Cl (Met) for 7 days. Values are expressed as mean plus the upper 95 % confidence limit. Compared to Ctl, there was a significant fall in the ratios of Na/Ca and K/Ca after acid treatment. $*p < 0.05$ vs. Ctl (data from [134])

relative release of mineral sodium and potassium than calcium [25]. However, the mineral and medium are in equilibrium [23] and there is movement of ions between the two [67] making it difficult to interpret the apparent ion fluxes, especially with respect to potassium and sodium. To help better understand the effects of acidosis on potassium relative to calcium, bone mineral was labeled in vivo with the stable isotope ^{41}K to determine the response of the bone mineral to acidosis. The mineral was found to be rich in potassium relative to calcium; acidosis caused a fall in the ratio of ^{41}K to calcium indicating loss of this stable isotope from the bone mineral [31].

Since mineral in live bone is rich in potassium relative to calcium it was unclear if the osteoclasts selectively removed potassium or if they nonselectively removed the surface of the bone mineral. Neonatal mouse bone cells were isolated and cultured on bovine cortical bone slices in the presence of parathyroid hormone [30]. The ion microprobe was then utilized to compare the unresorbed bone to that at the base of the osteoclastic resorption pits. In the presence of parathyroid hormone the osteoclasts nonselectively removed the potassium rich surface of the bone mineral [30].

The microprobe was also used to study acute physiochemical bone mineral dissolution caused by acidosis [26]. When calvariae were cultured with the osteoclastic inhibitor calcitonin there was a fall in the ratio of sodium/calcium coupled to an influx of calcium into bone, indicating little change in bone sodium. When calvariae were cultured in acidic medium with calcitonin there was calcium release with no change in sodium/calcium, indicating that physicochemical bone mineral dissolution causes relatively equal calcium and sodium release [26].

All of the previous work using the ion microprobe to study the effects of acidosis on bone used bone cultured in vitro [134]. To better understand the effects of acid on bone, an in vivo model was established. The microprobe was used to determine the mass spectra of important ion groups from femurs of mice acidified with oral ammonium chloride compared to mice drinking only distilled water. An area in the midcortex (midway between the marrow space and the superficial cortex of the longitudinally split femur) midway down the bone shaft was studied. Compared to mice given only oral distilled water the addition of NH_4Cl to the drinking water led to a marked change in the positive ion spectrum. In control mouse femurs the peak for potassium and sodium is far higher than that for calcium indicating that there is more potassium and sodium then calcium in the midcortex of the bone (Fig. 22.14). However after oral ammonium chloride, there is a fall in the ratios of potassium and sodium relative to calcium. With respect to the negative ions in the midcortex of the control femurs there was almost as much phosphate as carbon and as much phosphate as the carbon nitrogen bond.

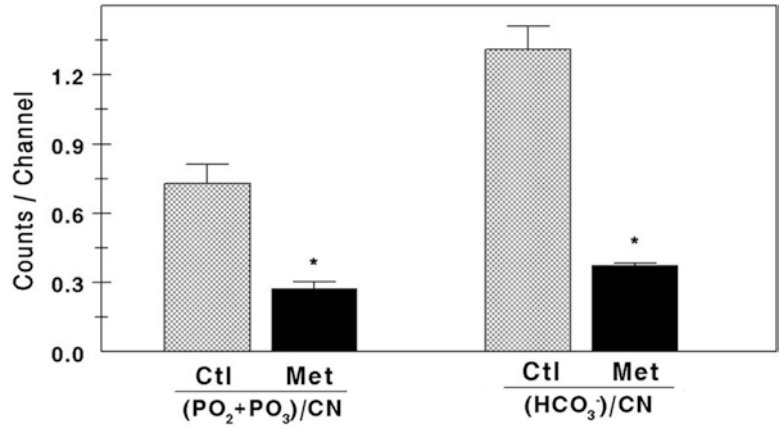

Fig. 22.15 Ratio of total phosphates (PO_2+PO_3) to the carbon nitrogen bond (CN) and total phosphates to carbon (C_2) in the mid-cortex of neonatal mouse femurs after drinking only distilled water (Ctl) or water with 1.5 % NH_4Cl (Met) for 7 days. Values are expressed as mean plus the upper 95 % confidence limit. Compared to Ctl, there was a significant fall in the ratios of (PO_2+PO_3)/CN and (PO_2+PO_3)/C_2 after acid treatment. *$p<0.05$ vs. Ctl (data from [134])

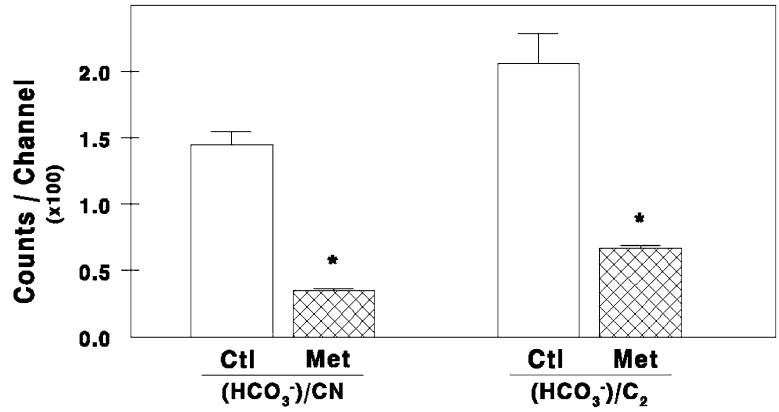

Fig. 22.16 Ratio of bicarbonate (HCO_3^-) to the carbon nitrogen bond (CN) and bicarbonate to carbon (C_2) in the mid-cortex of neonatal mouse femurs after drinking only distilled water (Ctl) or water with 1.5 % NH_4Cl (Met) for 7 days. Values are expressed as mean plus the upper 95 % confidence limit. Compared to Ctl, there was a significant fall in the ratios of HCO_3^-/CN and HCO_3^-/C_2 after acid treatment. *$p<0.05$ vs. Ctl (data from [134])

However, oral ammonium chloride led to a fall in the ratios of phosphate to carbon and phosphate to the carbon nitrogen bond (Fig. 22.15). Additionally there was a marked decrease in the ratio of bicarbonate to carbon and bicarbonate to the carbone nitrogen bond with acidosis (Fig. 22.16). Thus it appears that both bicarbonate and phosphate are used as buffers to mitigate the increase in hydrogen ion concentration during in vivo metabolic acidosis.

22.3.7 Role of Pco_2 vs. $[HCO_3^-]$

Clinically, a decrease in blood pH may be due to either a reduction in bicarbonate concentration ($[HCO_3^-]$, metabolic acidosis) or to an increase in the partial pressure of carbon dioxide (Pco_2, respiratory acidosis). In mammals, metabolic acidosis induces a far greater increase in urine calcium

excretion than respiratory acidosis [135–138] and this increase occurs without an alteration in intestinal calcium absorption, indicating that the additional urinary calcium is derived from the bone mineral [11–13, 42].

Most in vivo and in vitro studies have utilized hydrochloric acid or ammonium chloride to decrease bicarbonate as a model of metabolic acidosis. In vitro the type of acidosis appears to be critical in determining the magnitude of net calcium flux and proton buffering by bone. There are clear distinctions between the effects of metabolic (decrease bicarbonate) and respiratory (increased partial pressure of carbon dioxide) acidosis on cultured bone [1, 2, 9, 20, 22, 32, 70, 71, 73, 74, 89, 94, 139]. In acute studies there was a greater net calcium efflux during culture in decreased bicarbonate medium than during culture in isohydric acidosis produced by an increase in the partial pressure of carbon dioxide [22]. The decreased net calcium efflux during respiratory, compared to metabolic, acidosis is due to decreased unidirectional calcium efflux from the mineral coupled to deposition of medium calcium on the bone surface during hypercapnia [71]. There was decreased bone carbonate in response to metabolic, but not respiratory, acidosis [32]. These results suggest that over this short time period acidosis affects the physicochemical driving forces for mineral formation and dissolution [23, 26, 69, 73, 81]. During metabolic acidosis the decreased bicarbonate favors the dissolution, while during respiratory acidosis the increased partial pressure of carbon dioxide and bicarbonate favors the deposition of carbonated apatite. Indeed there is no net proton influx into bone during respiratory acidosis [22]. Extending these studies to compensated metabolic and respiratory acidosis we found that at a constant pH, whether physiologically neutral or acidic, net calcium efflux from bone is dependent on bicarbonate concentration; the lower the medium bicarbonate the greater the calcium efflux from bone [73].

During more chronic incubations there is cell-mediated net calcium efflux from bone during models of metabolic, but not respiratory, acidosis [70, 74]. A number of studies have shown that metabolic acidosis stimulates osteoclastic resorption [70, 72, 97, 140–142]. Respiratory acidosis does not alter osteoclastic β-glucuronidase release or osteoblastic collagen synthesis or alkaline phosphatase activity as does metabolic acidosis [74]. Medium PGE_2 levels and net calcium efflux from bone were increased with metabolic, but not respiratory, acidosis [76]. Respiratory acidosis does not appreciably alter the surface ion composition of bone [89], as does metabolic acidosis [25, 26, 30, 31, 89, 132].

22.3.8 Relationship Between Calcium Release and Hydrogen Ion Buffering

During acute metabolic acidosis a reduction in pH causes both bone calcium release and proton buffering by bone. Were all buffering the result of mineral dissolution there should be a 1:1 ratio of protons buffered to calcium released in the case of calcium carbonate, 5:3 for apatite and 1:1 for brushite [143, 144]. However, with cultured calvariae the ratio was found to be 16–21 to 1 indicating that proton buffering could not simply be due to mineral dissolution [24]. That calcium release is only one component of proton buffering by bone is demonstrated by the microprobe studies which show substantial sodium and potassium exchange for protons [25, 26, 30, 31, 89] and loss of bone phosphate and bicarbonate with acidosis [132].

In vivo studies have shown that metabolic acidosis induces changes in the mid-cortical bone mineral [134], which are consistent with its purported role as a proton buffer. There is a fall in mineral sodium, potassium, carbonate, and phosphate. Each will buffer protons and lead to an increase in systemic pH toward the physiologic normal. This apparent protective function of bone will come, in part, at the expense of its mineral stores. Future studies will be necessary to determine if the proton buffering properties of bone are described by a dynamic equilibrium: protonation of phosphate and

carbonate and release of sodium and potassium during acidosis coupled to deprotonation and uptake of sodium and potassium during alkalosis. This attractive hypothetical mechanism has clear survival advantage for mammals [2, 11].

22.4 Conclusion

Thus, through a variety of mechanisms bone appears to decrease the magnitude of fall in serum [HCO_3^-] and blood pH during metabolic acidosis in conjunction with an increase in net calcium efflux (Fig. 22.17) [2, 11, 9]. The acidosis may be mild and secondary to the consumption of food rich in acid precursors. Initially there is physicochemical sodium for hydrogen and potassium for hydrogen exchange on the mineral surface in conjunction with dissolution of carbonate and release of bone calcium. This is followed by stimulation of cell-mediated osteoclastic resorption and inhibition of osteoblastic collagen deposition. The increased resorption releases calcium and the buffers carbonate and phosphate. The fall in bone formation prevents calcium uptake and blocks the hydrogen ion release that accompanies bone mineral formation.

With adequate renal function mild metabolic acidosis leads to an increase in urine calcium excretion, evidence for bone mineral dissolution and resorption, with buffering of the additional hydrogen ions. However, as we age renal function slowly deteriorates decreasing the ability of the kidney to excrete the daily acid load (Fig. 22.18). Exchange of hydrogen ions for bone sodium and potassium and release of carbonate and phosphate all help to restore the decrease in pH. Hydrogen ions are exchanged for bone sodium and potassium. Osteoclastic bone resorption is further stimulated and osteoblastic bone formation is further suppressed. Bone mineralization continues to decrease setting the stage for osteoporosis and fracture.

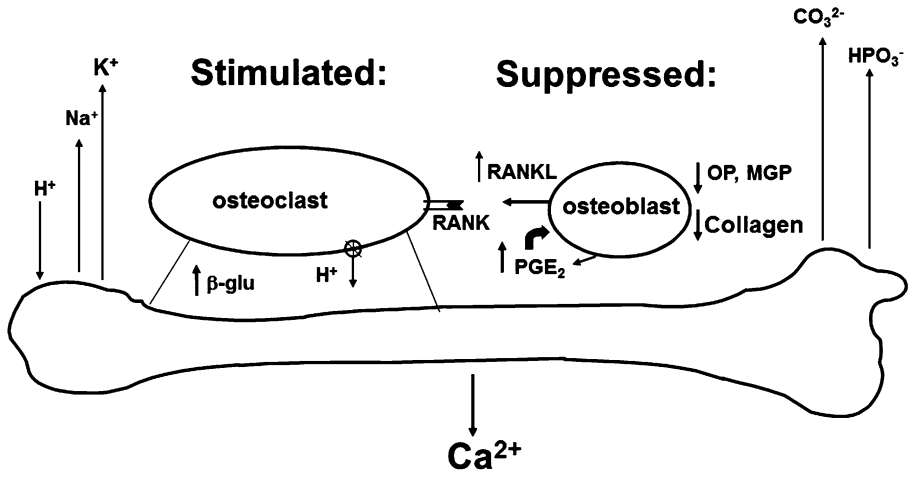

Fig. 22.17 Schematic of effects of metabolic acidosis on bone. Details as in text. *β-glu* beta glucuronidase, *OP* osteopontin, *MGP* matrix gla protein. Modified from [1, 2, 9]

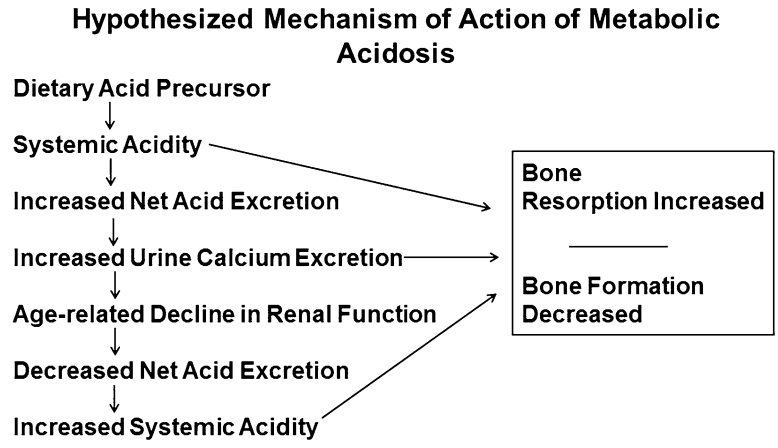

Fig. 22.18 Hypothesis for the mechanisms leading to dietary acid-induced increased bone resorption and decreased bone formation. Detail as in text. Modified from [1]

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Part IV
Minerals

Chapter 23
Quantitative Clinical Nutrition Approaches to the Study of Calcium and Bone Metabolism

Connie M. Weaver, Meryl Wastney, and Lisa A. Spence

Key Points

- Understanding the role of nutrients, food components, and diet in bone health is important as a means available to individuals to build and maintain peak bone mass within their genetic potential.
- Approaches to study the relationship between diet and bone, include epidemiology, randomized controlled trials, or metabolic balance and kinetic studies.
- In this chapter, we will review methodologies that allow quantitative nutrition effects on bone to be determined in small populations due to the resources needed.
- Metabolic balance studies can offer a highly controlled independent variable, that is, diet. When used in a crossover design, the effect of one dietary change on net calcium retention can be quantitated.
- Kinetic studies provide additional information over balance studies alone, and analysis of tracers offers greater precision.
- When balance studies are used in conjunction with isotopic tracers, parameters of calcium metabolism can be studied including absorption, endogenous secretion, excretion, bone formation rates, and bone resorption rates.
- As 99 % of the body's calcium resides in the skeleton, to study calcium metabolism is to study bone metabolism.

Keywords Quantitative clinical nutrition • Calcium • Bone metabolism • Calcium balance • Calcium isotopes • Calcium balance determination • Bone turnover • Fractional absorption of calcium • Urinary calcium excretion • Children • Adults • Calcium kinetics • Balance studies • Calcium isotopic tracers • Mathematic modeling • Calcium absorption

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23.1 Introduction

23.1.1 Why Use Quantitative Clinical Nutrition Approaches?

Understanding the role of nutrients, food components, and diet in bone health is important as a means available to individuals to build and maintain peak bone mass within their genetic potential. There are several approaches an investigator can take to study the relationship between diet and bone, including epidemiology, randomized controlled trials, or metabolic balance and kinetic studies. Epidemiology is conducive to the study of large numbers of subjects, a distinct advantage for power to find an interesting relationship. The outcome measures can be quite precise for studying diet and bone health, primarily bone mineral density and incidence of fracture. However, the assessment of nutrient intake or even dietary patterns is quite poor, owing to an accumulation of errors and intrasubject variation associated with subject recall, inability to estimate portion size accurately, variability of food composition, and availability of this information [1]. Moreover, the number of confounders is nearly infinite. The quantitative effect of any one nutrient on some aspect of bone is nearly impossible to determine, as the relationship is dwarfed by other factors such as body weight and age of the subject. Nevertheless, this approach allows hypotheses to be generated. The randomized controlled trial improves the ability to determine the effect of the intervention nutrient or food on the outcome measure of interest, but it is far from quantitative as the background diet is as difficult to assess as for epidemiological studies and compliance with the intervention is unsupervised, and therefore uncertain.

In this chapter, we will review methodologies that allow quantitative nutrition effects on bone to be determined. They are resource intensive, and thus, not feasible to use in large population studies. The necessarily small study group cannot represent the entire population, which is a limitation of this approach. Metabolic balance studies can offer a highly controlled independent variable, that is, diet. When used in a crossover design, the effect of one dietary change on net calcium retention can be quantitated.

Kinetic studies provide additional information over balance studies alone, and analysis of tracers offers greater precision. When balance studies are used in conjunction with isotopic tracers, parameters of calcium metabolism can be studied including absorption, endogenous secretion, excretion, bone formation rates, and bone resorption rates. As 99 % of the body's calcium resides in the skeleton, to study calcium metabolism is to study bone metabolism.

23.2 Metabolic Balance Studies

23.2.1 Application of Balance Studies

Metabolic balance studies have been used at least since the 1920s [2]. A thorough accounting of calcium balance methodology was reported in 1945 by a group of authors that included Fuller Albright, for whom the highest award given by the American Society for Bone and Mineral Research was named [3].

Balance studies calculate net retention as intake minus excretion. Balance studies are sufficiently sensitive, when rigorously controlled, to distinguish differences when large effects are expected, as exists when comparing pubertal growth vs. adults [4], lactating state vs. nonlactating state [5], racial differences [6], the effects of skeletal unloading [7], and some diet effects such as calcium intake [8]. The treatment differences in these examples exceed 200 mg calcium retention per day. Power calculations show that sample sizes of 5–6 subjects per group are sufficient to find significant differences of

Fig. 23.1 Maximal calcium retention curve by calcium intake. (a) Reference curve used to determine calcium requirements for adolescents. (b) Curve shifted left by a change such as decreasing salt intake. (c) Curve shifted to the right by a change such as increasing dietary salt. Curve (a) was developed from data from Jackman et al. [10]

this magnitude at an α of 0.05 with 80 % power even though the variances were large. The ability to determine smaller effects of diet depends on the magnitude of the effect and the specific population. We have been able to show treatment effects on calcium retention of 40 mg/day with 10–15 adolescent subjects in crossover studies. However, for treatment differences in calcium retention of approx. 40 mg/day in postmenopausal women using the variance we observed in one study [9], power calculations suggest that 180 subjects would be needed to show significance using a crossover design.

Balance studies can also be used to determine calcium requirements, as the response of calcium retention to calcium intake reaches a plateau when calcium intake is no longer limiting maximal calcium, that is, bone retention. Useful information can be determined about the role of other dietary factors or lifestyle choices in shifting the maximal retention curve, which shifts calcium requirements higher or lower (Fig. 23.1) [10]. This application has the advantage of not putting so much weight on actual values of calcium retention. The maximal retention approach seeks the intake where a plateau occurs rather than an absolute retention. The errors associated with balance are not equally distributed. Errors associated with incomplete consumption of the diet or collection of urine and fecal excretion and failure to measure other losses including dermal are often-cited limitations of this method. Also, analytical procedures typically have a coefficient of variation of $\geq$5 %. Some guidance for minimizing these errors is discussed in subsequent sections of this chapter.

23.2.2 Conducting Balance Studies

Metabolic balance studies involve feeding a controlled diet, collecting excreta, and measuring calcium input and output. Intake cannot be estimated from food composition tables. All foods and beverages containing calcium need to be prepared by weighing ingredients to the nearest 0.1 g. Prepared commercial foods can be used if their composition is homogeneous. Duplicate collections of all of the foods and beverages consumed in a 24-h period are analyzed for calcium and other constituents that influence calcium balance, including protein, phosphorus, fiber, and electrolytes. Diets should be designed to be constant in these constituents throughout the study period. Foods, beverages, and oral health care products that contain calcium, including tap water, inhibitors of calcium absorption such as tea, which contains oxalate, or hypercalciuric ingredients such as salt cannot be allowed ad libitum.

If the metabolic study is not conducted in all subjects simultaneously, but rather as a rolling enrollment, it may not be practical to analyze a duplicate sample of each day. In that case, dietary composites representing each cycle day from a dietary intervention should be prepared in intervals throughout a study period to track the variability that occurs over time and the variability between the daily diets. Dietary composites should be measured for calcium and those nutrients that could potentially affect calcium metabolism, that is, protein, sodium, potassium, and phosphorus. Dietary homogenates representing each day of the menu cycle, that is, 7 days for a 7-day menu cycle, should be freeze-dried

and aliquoted in triplicate for all nutrient analysis. Variation among these triplicate samples is an indication of homogeneity in the sample and analytical precision. Replicate analysis of dietary composites prepared over the entire study period demonstrates variation due to variability in food items, dietary preparation, and laboratory analysis. Analysis across cycle menus represents daily variability within the diet.

Analysis of dietary composites from a metabolic balance study in our laboratory that used a 7-day menu cycle over a 19-month period demonstrated 3 % variation in calcium from triplicate analysis of dietary samples. The variation in calcium from the replicate analysis of dietary composites, which were collected at quarterly intervals over the 19-month study period, was 5 %. Daily variation in calcium across the 7-day cycle menus was 6 %. Protein varied by 8 % in both replicate analysis and across cycle menus. Phosphorus, potassium, and sodium varied by 9, 13, and 18, respectively in replicate analysis and by 15, 16, and 16, across cycle menus. The diets were designed with the aid of the computer program, Nutritionist IV. The most careful attention was paid to the calcium and protein content of the diets to test the study hypothesis. Thus, laboratory analysis revealed less variation in the daily analysis of the diets for calcium and protein compared to the daily variation in the other nutrients measured.

Urine and feces are collected in acid-washed containers for later analysis of total calcium by atomic absorption spectrometry or inductively coupled plasma. A 1-day lag is used when calculating intake minus fecal excretion to account for the approx. 19-h transit time in the gut. Menstrual losses of calcium can generally be ignored. Dermal losses are often ignored but can be measured by extracting preacid-washed clothing worn for 24 h in addition to whole-body wash-down procedures before and after the collection. Using this method we have determined dermal losses of approx. 52 mg/day in adolescents. Dermal losses determined by patches over estimated calcium losses by almost eightfold [11]. Dermal losses in adults have been estimated to be 60 mg/day by the difference between whole-body retention of ^{47}Ca and excretion in urine and feces [12].

To determine the effect of a variable on calcium retention within a population, the best approach is the use of a crossover design in the same subjects, to minimize such confounding effects that are constant within an individual such as hormonal status, gastrointestinal and kidney function, mucosal mass, transit time, and vitamin D status. Randomized-order assignments of treatment can minimize seasonal effects of vitamin D status. Nevertheless, the presence or absence of an order effect should be tested statistically. Subjects can also be pretreated with vitamin D supplements and continued throughout the study period if they have hypovitaminosis D.

The length of the run-in period needed for a subject to adapt to the study diet and the length of the balance period once steady state is reached must be carefully considered. Misinterpretations have occurred when subjects have been switched from high- to low-calcium intake periods without an appropriate adaptation period, as the higher-calcium intakes spill into the feces during the lower-calcium period for several days. When a nonabsorbable fecal marker such as polyethylene glycol (PEG) is given at every meal, the fecal calcium:PEG ratio can be used to determine when steady state is achieved. We have found that the Ca:PEG ratio becomes constant after 6 days in adolescents and most adults (Table 23.1, adapted from ref. [13] plus unpublished data). Similarly, when adult black and white women were switched from a diet containing 2,000 mg/days for 3 week to 300 mg/day for 8 weeks, whole-body retention of ^{47}Ca varied from week 1 to week 2, but not from week 2 to week 8 [14]. Thus, determining balance during the run-in period can give useful information about when steady state is achieved in calcium balance or another dietary constituent being tested for its effect on calcium retention. Subjects cannot adapt to a low calcium intake to become “in balance,” as the homeostatic control mechanisms is inefficient. Malm [15] studied prisoners for up to 2 years and found continued negative balances.

Balance periods should be sufficiently long to evaluate trends over multiple periods. Some investigators make collections in several day pools and monitor multiple periods. We typically make collections in 24-h periods for 2–3 weeks after a 1-week adaptation period. Calculating daily balances for

Table 23.1 Fecal calcium: peg ratios (mg/mg) during a 3-week balance period*

Group	Calcium intake (mg/day)	Week 1	Week 2	Week 3
Adolescent girls				
	800	0.25 ± 0.13^a	0.21 ± 0.06^b	0.19 ± 0.04^b
	1,300	0.82 ± 2.20^a	0.32 ± 0.06^b	0.38 ± 0.72^b
	1,800	0.53 ± 0.08^a	0.48 ± 0.09^b	0.48 ± 0.07^b
Adults				
	1,300	1.49 ± 5.20^a	0.36 ± 0.09^b	0.36 ± 0.08^b

*Different letter superscripts within rows indicate means are significantly different for each level of calcium intake at $p<0.05$. Data from ref. [13] and unpublished data

multiple periods allows an error term to be determined so that differences from zero balance can be determined for each individual. In pubertal children, balance periods should not exceed rapid hormonal shifts, which can outweigh the influence of diet on calcium retention [13].

23.2.3 Monitoring Compliance

Methods to assess compliance of urine and fecal collections and to adjust for discrete 24-h periods are helpful in reducing variation in balance data and in interpreting the quality of data. However, errors can be made in measuring compliance markers, so that corrected data may be less accurate than uncorrected data for any given day. Thus, all components of any calculation should be carefully inspected. Especially troublesome is the apparent overcorrection of fecal calcium using a marker to adjust for low compliance.

Adjustment of urine is usually made with creatinine. Subjects excrete a rather constant level of creatinine proportional to lean body mass. The mean daily creatinine excretion of a subject over the study period can be used to adjust each day to a more precise 24-h period, as it is difficult to completely empty one's bladder at precise regular time periods, especially for children. Twenty-four-hour pools with creatinine values less than 11 mg/kg should be discarded.

Daily fecal calcium output is highly variable despite constant conditions due to variable gut transit times, which does not follow a continuum of discrete periods [16]. A number of nonabsorbable fecal markers have been employed to evaluate compliance, transit time, and to convert individual stools collected at irregular intervals to daily fecal calcium output. We use PEG 4000 as a continuously administered, nonabsorbable marker as developed by Wilkinson [17], who demonstrated clearly a reduction in daily variation by correcting stool samples by recovery of this marker. Capsules are prepared containing PEG weighed to the nearest milligram and consumed at each meal throughout the study. The ratio of Ca:PEG in each 24-h pool is multiplied by the amount of PEG consumed during 24-h to determine daily fecal calcium. This marker is superior for water-soluble dietary constituents such as calcium to previously used markers which more closely follows the insoluble pool such as Cr_2O_3 and barium sulfate, although recovery of all three markers was 98–100 % [17].

Adjusting fecal calcium as described above supposedly corrects for incomplete stool collections. However, Eastell et al. [18] reported a PEG recovery of only 81 % of that compared to 95 % with ^{51}Cr in the same experiment. Therefore, we suspect that adjusting fecal calcium with PEG may overcorrect, which becomes worse with decreasing compliance. To examine this issue, we used data from one of our studies in which we calculated calcium balance using the PEG adjustment. Each group was studied three times, and we computed residuals for calcium balance by subtracting the group mean

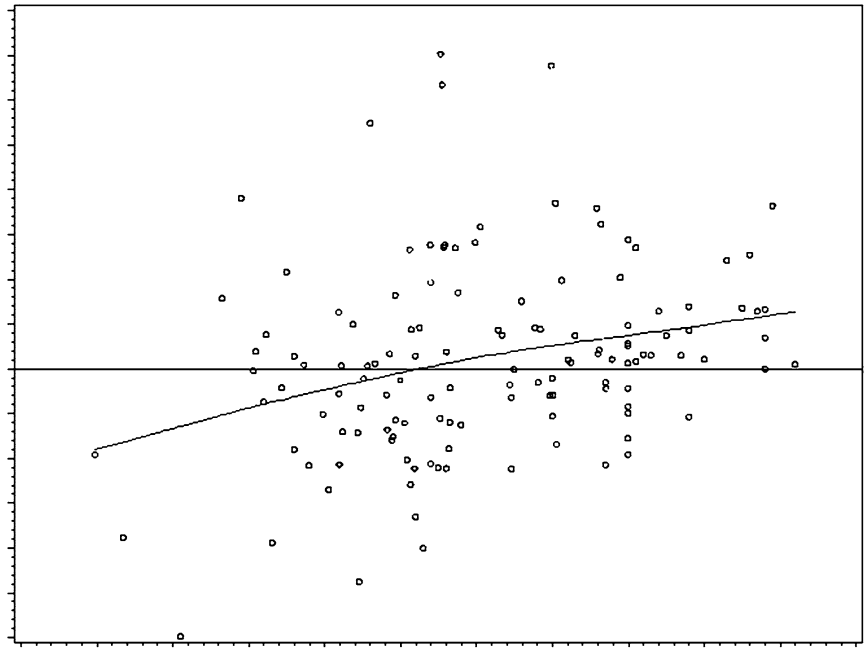

Fig. 23.2 Calcium balance residuals vs. percent PEG recovery in one study of postmenopausal women

from each individual observation. A plot of these residuals vs. PEG is given in Fig. 23.2. The plot includes a centerline at zero (the mean of the residuals) as well as a smooth fit to the data. There appears to be a positive association between the PEG value and the residual. This means that observations with low values of PEG tend to be associated with balance values that are low relative to the group mean and, similarly, high PEG values are associated with balance values that are high relative to the group mean. This association is consistent with a scenario in which the PEG overcorrects the fecal calcium values: when the PEG is low, the corrected fecal values are too high, and therefore the balance values are too low. More research is needed to understand this issue.

23.2.4 Feasibility of Free-Living Subjects vs. Metabolic Ward

In metabolic studies with free-living subjects or subjects housed in metabolic wards, consumption compliancy and excreta collection compliancy can influence the study results. Classical balance studies have typically been conducted in metabolic wards, where subjects' activities are monitored, particularly, food consumption and excreta collection. Once individuals are allowed to participate in balance studies as free-living individuals, monitoring activities becomes difficult. Food consumption and excreta collection cannot be monitored in free-living individuals as in a metabolic ward. In studies with free-living subjects, all foods and beverages are provided along with instructions to consume each item. When subjects do not consume all food and beverage items due to various reasons, they are instructed to return uneaten portions of the food or beverage. These items are then analyzed in the laboratory for calcium content and calcium balance is corrected based on the calcium content of the uneaten food or beverage.

In a metabolic study with postmenopausal women [9], our laboratory analysis demonstrated a consistent daily creatinine output and average PEG recovery rate of approx. 80 %. These indicators of

collection compliancy were consistent with results seen in adult subjects participating in in-house metabolic studies [4, 19]. Collection compliancy remains an obstacle in metabolic studies whether subjects are free-living or maintained in monitored environments. Success depends on committed subjects in either environment. Some populations, such as children, undoubtedly require a supervised environment to be successful. The effect of compliancy on treatment effect can be determined by examining the *F* statistic when data are evaluated by using various cutoffs for percent PEG recovery as inclusion criteria.

23.3 Tracer Studies

23.3.1 Application of Tracer Studies

Isotopic tracer data are less variable than balance data. Thus, although fractional absorption determined by tracer studies is similar to net calcium absorption determined by balance studies [20], more subtle treatment differences can be discriminated with isotopic tracer studies. Tracers are required for kinetic studies. Kinetic studies involve the study of movement of calcium using a calcium isotope from one compartment to another, rates of transfer, and body pool sizes. Depending on the route of administration, the number of tracers used, the samples collected, and data analysis, the amount of information gained over balance studies alone can be considerable. Calcium tracer experiments offer insights into the bone microenvironment and transfer at the blood–bone interface. Because most of the calcium in bone is not exchangeable with tracers in short-term experiments, total body pool size cannot be measured, but processes of bone turnover can be measured with tracers. Calcium clearance rates can be used to determine metabolic bone disease. Pitfalls of tracer studies occur if isotopes fail to mix adequately [21]. Compartments do not generally equate to anatomically distinct entities and their contents may not be able to be interpreted without further biochemical or physiological investigation.

23.3.2 Available Calcium Isotopes

A list of isotopic tracers of calcium appears in Table 23.2. Useful radiotracers of calcium are ^{47}Ca and ^{45}Ca. ^{47}Ca is a γ-emitter, and therefore can be used for whole-body counting in studies of calcium retention in animals or humans in facilities where animal or human γ counters are available. Its short

Table 23.2 Calcium isotopic tracers

		Radioisotopes			Stable isotopes
			Maximum radiation energies		
Atomic number	Symbol and mass number	Half-life	*β* (Mev)	*E* (Mev)	Naturalabundance (%)
20	^{41}Ca	10^5 years	–	–	10^{-15}
	^{42}Ca	–	–	–	0.646
	^{43}Ca	–	–	–	0.135
	^{44}Ca	–	–	–	2.083
	^{45}Ca	164 days	0.255	–	–
	^{46}Ca	–	–	–	0.0033
	^{47}Ca	4.53 days	1.98	1.29	–
	^{48}Ca	–	–	–	0.18

There are many more nonradioactive (stable) isotopes of calcium than radioisotopes

half-life limits the length of the experiment and is the reason for its scarcity and relatively high expense. However, whole-body retention using ^{47}Ca is attractive, as neither compliance nor failure to collect dermal losses are issues as occur with balance studies [22]. A limitation of whole-body counting is that mechanisms cannot be investigated because the tissue perturbed, that is, gut, kidney, or bone, cannot be inferred from whole-body retention curves. As a β-emitter, ^{45}Ca is measured in a liquid scintillation counter and is appropriate for biological fluids or samples that can be converted to fluids. Although ^{47}Ca can also be measured in biological fluids, the lower costs and longer half-life typically make ^{45}Ca the preferred radioisotope for tracer studies. Precision of analysis with radioisotopes depend on the counting rate, but samples can be counted to 1–2 % precision.

There are many more nonradioactive (stable) isotopes of calcium than radioisotopes. These isotopes of heavier mass than ^{40}Ca, which represents almost 97 % of calcium in nature, are measured as isotopic ratios by mass spectroscopy. The methods of choice currently are high-resolution, inductively coupled plasma, mass spectrometry (HR-ICPMS) [23], and thermal ionization mass spectrometry (TIMS) [24]. The former has the advantage of greater sample throughput and the latter has the advantage of greater precision (1–2 vs. <0.1–0.2 %). Stable isotopic tracers have the advantage of not exposing subjects to radioactivity and not having to time experiments around a short half-life. They have the disadvantage of being more expensive to purchase and analyze. Use of calcium stable isotopes for clinical studies of calcium metabolism was first proposed in 1983 [25].

The long-lived radioisotope, ^{41}Ca, can be used in such small doses (≤100 nCi) that it can be considered to be radiologically benign. A single dose of this size labels the skeleton for life, which poses a lifetime radiation exposure of less than 2 μrem. The benefits of this tracer are that the tracer can be monitored for long experiments, in contrast to the upper limit of approx. 2 weeks with other isotopes. Urinary appearance of ^{41}Ca after 100 days from dosing, when the ^{41}Ca can be considered coming from the skeleton, provides a direct, sensitive measure of bone calcium loss. Changes in bone loss can be accurately measured following an intervention. The disadvantage of this approach is that ^{41}Ca is measured with an accelerator mass spectrometer (AMS), which is not available in most research centers. There are two in the United States, one at Purdue University and one at Lawrence Livermore National Laboratory. Opportunities with AMS in nutrition have been reviewed [26].

23.3.3 Kinetic Studies

23.3.3.1 Conducting Kinetic Studies

A comprehensive kinetic study will be described first to present the model and nomenclature. In subsequent sections, individual components of calcium metabolism will be discussed with comments on simplifying experimental designs. The most complete model for calcium metabolism can be developed when subjects participate in a metabolic balance study and have achieved steady state before isotopes are administered. Isotopes are administered orally and intraveneously in doses that should not perturb the normal movement of calcium, that is, less than 10 % of the circulating calcium pool. The actual dose administered depends on the precision of the detection method and the length of time the tracers are to be followed, typically limited to 2 weeks. If different isotopes are administered orally and intravenously, the isotopes can be administered almost simultaneously. This dual-isotope procedure was described by De Grazia et al. [27]. Oral isotopes take longer to enter the plasma pool than intravenous doses, so we give the oral isotope 1 h prior to giving the intravenous isotope. The two isotopes track identically after 20 h [28]. Activity of radioisotopes or stable isotope ratio measurements are made on the urine and fecal samples collected for the balance study in addition to total calcium. In addition, plasma or serum samples are collected periodically for isotope measurements. Measurements can also be made on saliva [29]. With kinetic studies, the more data collected, the

better the model will be. However, there are ethical limits on the volume of blood that can be taken over the study period. More measurements are needed early postadministration, when turnover is more rapid. For subject comfort, frequent collection of blood during the first few hours postadministration is often done through a catheter. Stable isotopes are administered intravenously over several minutes through a catheter and blood collected serially through a separate catheter to avoid contamination.

23.3.3.2 Mathematical Modeling

Mathematical modeling is the expression of metabolism in terms of equations. Some of the reasons for analyzing data by modeling are to test an hypothesis against data, integrate information from different parts of a system (e.g., absorption and bone deposition), measure attributes of a system that are not measurable directly, calculate a parameter of interest, investigate processes that cannot be studied directly, and to identify changes in metabolism between two treatments.

There are many approaches to modeling. For example, models may be descriptive or mechanistic. Descriptive models tend to be simple and parameter values are arbitrary. Mechanistic models tend to be complex, parameters relate to actual physiological processes of a system, and they can be used for prediction. The approach chosen relates to the questions being addressed by the study [30]. A number of modeling packages are available specifically for modeling biological systems, and some of these have been described by Wastney et al. [30].

Compartmental models are useful as movement between pools represent known physiological processes, such as absorption, endogenous excretion, and bone deposition. The number of compartments defined from a study can vary based on the sampling frequency and period of the study, but generally three compartments describe the exchange of calcium with serum [31] (Fig. 23.3). The general

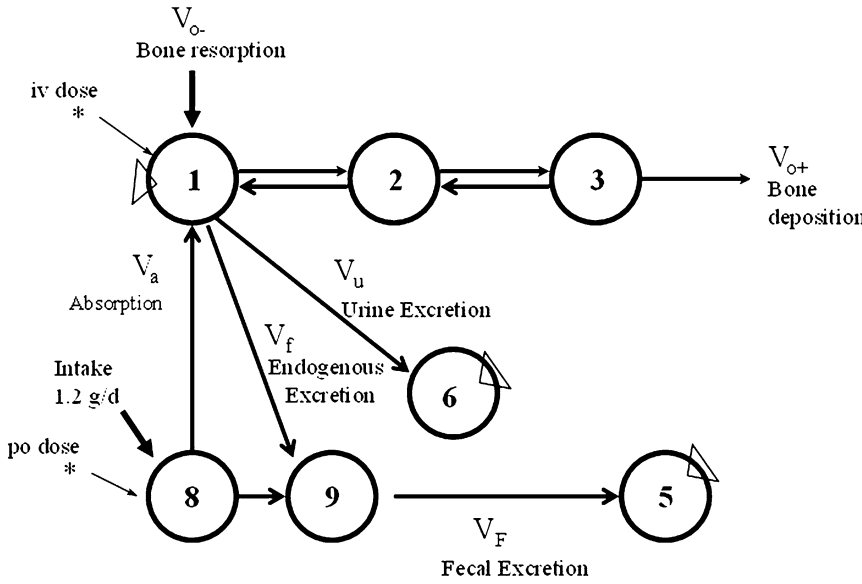

Fig. 23.3 Model for calcium metabolism. *Circles* represent compartments, *numbers in circles* represent compartment number, *thin arrows* represent movement between compartments, *thick arrows* represent entry of calcium via the diet or bone resorption (Vo_). *Asterisks* indicate entry of tracer and *triangles* identify sample compartments. Compartment 1 contains blood, compartment 2 soft tissue, and compartment 3 exchangeable calcium on bone. (Copyright Wastney, with permission; adapted from ref. [31])

development of a compartment model has been described [30], and some of the challenges in fitting a model to calcium data from human studies have been detailed [32]. These include the need to simulate multiple tracers per subject, tracer levels in multiple tissues, carryover between studies, and multiple studies, that is, identifying kinetic differences between populations or changes between treatments.

In addition to compartmental approaches, noncompartmental models have been used (e.g., power functions), and results with both approaches have been compared [21, 33]. Weiss et al. [34] proposed the use of a non-Markovian model as a new generalized compartment model for calcium kinetics. It differs only in the interpretation of the pathways from other three-compartment models.

In fitting any model to data, assumptions made are important for the interpretation of the results. In compartmental modeling these include (1) the system is in steady state (i.e., pool sizes do not change during the study), (2) the tracer does not perturb the system, (3) the sampling period covers both rapid and slow pools, and (4) if the system has been perturbed, a new steady state has been reached.

In addition to the underlying assumptions of a model, an important criterion of modeling is how well the model fits the data. This is determined by lack of consistent deviations between observed and calculated values, low errors associated with fitted parameters, and low correlations between fitted parameters.

23.4 Calcium Metabolic Parameters

23.4.1 Absorption

V_a can be determined from balance and kinetic studies as described above (see model Fig. 23.3). There are many simpler experimental designs that can be used when absorption is the primary metabolism parameter of interest. Eastell et al. [35] reported a 1-day method in which oral and intravenous isotopes were administered with all three meals and the ratios determined in the urine. Nevertheless, subjects were adapted to the diets for 7 days prior to administration of the isotope. The method predicted well calcium absorption by balance.

Fractional calcium absorption from a fixed load is useful for determining intrinsic absorptive capacity or for determining bioavailability of calcium sources. There are many study designs that have been used to determine fractional calcium absorption. Most do not adapt subjects to a controlled diet. When absorption is calculated from unabsorbed tracer appearing in the stools, the diet might be controlled long enough to encompass the transit time of the tracer [36]. When tracer appearance in blood or urine is used to monitor calcium fractional absorption, often the tracer is given at breakfast following an overnight fast. Typically, the diet is not controlled except for the breakfast when blood is collected or for just 1 day when urine is collected [37]. A 24-h urine collection may be sufficient, but when a response delay is expected as occurs in the presence of nondigestible fiber [38], urine might need to be collected for several days. Ideally, oral and intravenous tracers are given and the ratio determined in the blood or urine. This is the most accurate of the simpler methods, aside from whole-body counting, as the oral isotope labels the dietary calcium and its absorption and the intravenous isotope measures the calcium removal from blood. However, a single oral dose may be sufficient. A single 5-h blood draw following an oral dose has been demonstrated to correlate highly with the double-isotope tracer technique [39, 40]. Good agreement has also been reported between the double-radioisotope method and the fecal recovery method from a single isotope [27]. Also, the double stable isotope method and whole-body retention of ^{47}Ca were highly correlated [41]. By comparing results from kinetic analysis and modeling, Lee et al. showed that a statistical model could be used to predict absorption from a single blood sample after oral isotope administration in adolescents [42] and young women [43]. Various equations were developed using blood or urine samples collected at different times post dose to allow different protocols to be developed. Also different equations were developed using different parameters as a proxy for rate of bone turnover as appropriate to the study population.

Fermetable fibers or pre-biotics are being studied for their ability to improve mineral absorption from the lower gut. Using dual stable isotopes and 48 h urine collections, absorption was calculated in teen girls while they on two levels of soluble fiber [44] by using population values of calcium distribution and a compartmental model [31]. Because absorption was delayed, the traditional time points (<24 h) showed no effect. Only after 24 h did differences in calcium absorption appear consistent with lower gut absorption. This study was done in free living children on self-selected diets except for the calcium absorption test at the end of the 3-week intervention. The long intervention allowed sufficient time for the intestine to adapt to the fibers, but steady state on a fixed level of calcium intake was not achieved. Steady state requires one week retention and bone turnover [10], but not for calcium absorption measures.

When determining intrinsic absorption capacity, important considerations are the size of the calcium load and the chemical form of calcium to be administered. As fractional absorption is inversely related to load, all comparisons should be made using the same load. Frequently, loads of between 100 and 300 mg calcium are tested. Some choose the load equivalent to one-third of the daily intake. When fractional absorption is compared across experiments, it is better to include a common source as a reference. Radioisotopes typically are purchased as $CaCl_2$. This soluble isotope can be mixed with milk or juice for consumption or converted to another salt. It is not recommended to give pure $CaCl_2$, as it is a stomach irritant. Alternatively, a capsule of a pre-weighed calcium salt containing the tracer can serve as the oral dose. This is common with stable isotopes of calcium that are purchased as calcium carbonate.

Bioavailability studies are undertaken to determine relative calcium absorption from a calcium-containing food, beverage, or supplement compared to a reference, typically milk or calcium carbonate. Use of a crossover design to compare two or more sources adjusted to the same calcium load eliminates variance associated with factors endogenous to the subject, as described earlier under balance studies. A 5 % difference in fractional calcium absorption can usually be detected with 10–15 subjects using a crossover design.

The method chosen to incorporate an isotope into the food being tested for bioavailability deserves thoughtful consideration. Intrinsic labeling techniques, which incorporate isotopes during growth of plants or animals as previously described [45] or during the synthesis of a supplement [46], attempts to prepare the label in the same form as endogenous calcium. Extrinsic labeling of calcium sources is simpler and frequently, but not always, allows a good approximation of calcium absorption from intrinsically labeled sources [47]. This approach involves premixing a soluble form of the calcium isotope with the food to be tested prior to consumption and assumes the tracer has adequately exchanged with endogenous calcium.

23.4.2 Urinary Excretion

The major route of obligatory calcium loss is through the urine. Urinary calcium can derive from diet or bone. Thus, it can reflect absorption or bone resorption. Tracers can help distinguish the source of urinary calcium. Typically, urinary calcium is expressed as a 24-h excretion rate.

23.4.3 Endogenous Excretion

Endogenous fecal excretion is absorbed calcium that has been reexcreted into the gut. Determination of endogenous secretions in 191 perimenopausal women varied inversely with fraction of calcium absorbed and directly with calcium intake [48]. The mean was 102 ± 25 mg/day; thus, variance is

25 % of the mean. With this amount of variation and dependency on exogenous factors, balances calculated using fecal calcium estimated as unabsorbed calcium from absorption measurements and corrected for estimates of endogenous secretion from the literature without performing stool collections, as proposed by some [49], can give quite different results. These "tracer-assisted" calculated balances reduce variation arising from variation in fecal calcium. However, in a recent metabolic study of postmenopausal women in our laboratory, balances calculated in this way overestimated observed balances by an average of approx. 130 mg/day.

Another method employed to determine endogenous excretion is fecal appearance of tracer administered intravenously. However, we observed twice as much intravenous tracer excretion by adult women compared to teen girls, but endogenous excretion rate (calculated by complete kinetic analysis) was shown not to differ between girls and women [31]. Fecal intravenous tracer levels were higher in women because serum levels were higher. Serum levels were higher because bone deposition was lower compared to the teens, meaning that tracer remained in serum longer. This shows that tracer approaches that do not utilize data from several tissue sites may lead to erroneous results.

23.4.4 Bone Turnover

Rates of changes in calcium metabolism and bone turnover can be determined with calcium tracer kinetics in rather short studies (days to weeks) compared to changes in bone properties such as bone mineral density (months to years). Bone formation rates and bone resorption rates can be determined as Vo_+ and Vo_- using kinetic modeling (Fig. 23.3). These values are expressed as 24-h rates. To examine the influence of length of study on the value calculated for Vo_+, we fitted various serum profiles of an adolescent subject who participated in a 3-week balance study reported previously [31]. Data were fitted assuming we had 7, 14, and 21 days of blood samples following administration of oral and intravenous stable isotopes (Fig. 23.4). Model fitting produced different curves if only 7 days of data were available compared to 14 days of data, but the curve did not change appreciably with an additional data point at 21 days. This resulted in considerable differences in calculated Vo_+ and Vo_- if data were available for 2 weeks or more compared to only 1 week (Table 23.3). The shorter the study, the greater the overestimate of Vo_+ and Vo_-. Assuming 21 days resulted in accurate values for Vo_+ and Vo_-, 7-days data overestimated Vo_+ by 46.6 % and Vo_- by 57.1 %, whereas 14 days overestimated Vo_+ and Vo_- by only 1.7 %. Similarly, the error overestimate of Vo_+ in adults with decreasing length of study compared to 20 days was 3 % for 10 days, 16 % for 4 days, and 24 % for 1 day [50]. In contrast, 7 days were sufficient to accurately determine calcium absorption and urinary calcium (Table 23.3). Calcium retention determined as $Vo_+ - Vo_-$ was greatly underestimated by only 7 days of data compared to 14 days or more. Note that this applies to the use of kinetic studies to calculate balance. Balance estimated by difference between calcium intake and calcium excretion is not time dependent.

A variety of biochemical markers of bone turnover have been used to estimate bone formation and bone resorption rates from serum and urine samples. They are convenient and conducive for monitoring clinical interventions. However, these methods are not specific for either calcium or bone. They are reported in units reflecting enzyme activities of osteoblasts or collagen breakdown products. Attempts have been made to develop regression equations to transpose biochemical marker values into bone formation and bone resorption rates expressed as mass of calcium per day [51–53]. This enables quantitative changes in bone turnover to be calculated from qualitative changes. Although biochemical markers and Vo_+ are highly correlated, the variance of biomarkers is greater than for kinetic parameters of bone turnover [51]. Thus, in a small study population, differences in bone formation or resorption rates due to age [51] or calcium intake [8] are not reflected by differences in biochemical markers of bone turnover. In contrast, calcium supplementation did result in reduced

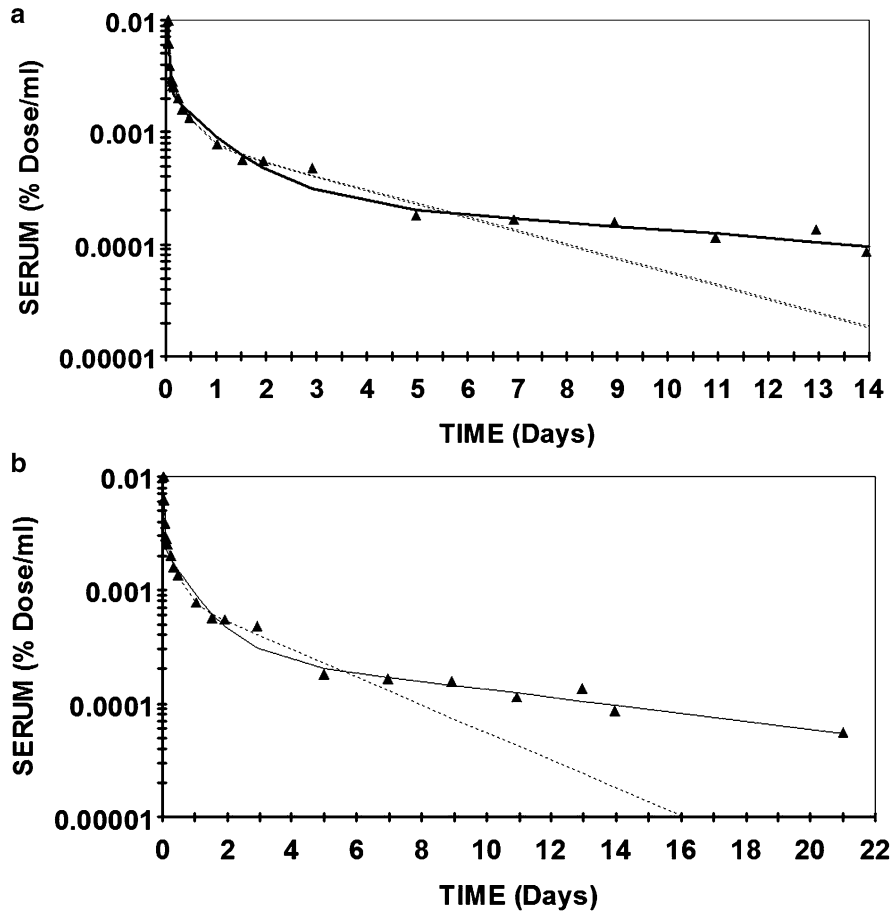

Fig. 23.4 The effects of length of study on serum disappearance curves of iv stable calcium isotopic tracer in an adolescent girl. *Symbols* are observed data, *lines* are values calculated by the model shown in Fig. 23.2. Model fit to data from 14 days (*solid line*) vs. 7 days (*dotted line*) (**a**), and model fit to 21 days (*solid line*) vs. 7 days (*dotted line*) (**b**). (Copyright Wastney, with permission)

Table 23.3 Results of 7, 14, and 21 days study in teen girl (1,300 mg Ca/day intake)

	7 days	14 days	21 days
L (0,3) fract/day	0.355	0.090	0.085
Absorption (%)	52	49	49
Vo_+ (mg/day)	2,282	1,583	1,557
Vo_- (mg/day)	2,273	1,472	1,447
Balance (mg/day)	8	110	110
V_u (mg/day)	113	113	113

hydroxyproline:creatinine values, a marker of bone resorption, in 14 postmenopausal women in fasting urine samples [54]. The variance of biomarkers of bone turnover is reduced in 24-h urine samples compared to fasting urine samples.

Bone resorption rates of change can be monitored sensitively by monitoring urinary ^{41}Ca output from prelabeled skeleton. Using urine samples collected for up to 1,808 days after i.v. injection of ^{41}Ca in a population of postmenopausal women, a compartmental model was developed that included calcium in a faster (labeled "trabecular") and a slower (labeled "cortical") turning over compartments,

considered to be bone [55]. The model was used to calculate rates of bone turnover, and to predict changes in urinary ^{41}Ca:Ca ratios following perturbations of calcium metabolism.

It was predicted that any change in calcium metabolism that resulted in an increase in bone mass would result in a decrease in urinary ^{41}Ca:Ca ratio. Conversely, increases in ^{41}Ca:Ca ratio in urine were associated with decreases in bone mass. The qualitative change in ratio was related to an absolute change in calcium in bone. In addition the model was used to show that interventions at different times after dosing gave the same response in ^{41}Ca:Ca ratio, how small a change in bone turnover could be detected and how long a recovery period is required after an intervention, for bone balance to return to the pre-intervention level. These insights can be used to aid the design and interpretation of studies using ^{41}Ca in humans.

23.4.5 Using ^{41}Ca to Compare Efficacy of Interventions to Improve Bone Calcium Retention

There is a great need to compare interventions and treatments, combination therapies, effective doses, effective length of treatments, and consequences of drug holidays. Current Food and Drug Administration guidelines for approval of treatments rely on up to 4-year RCTs of bone mineral density. After a person is equilibrated with ^{41}Ca, urinary or serum appearance of ^{41}Ca:Ca can be used to quickly and sensitivity evaluate effectiveness of treatment. The suppression of urinary ^{41}Ca:Ca by alendronate, an osteoporosis treatment therapy is shown clearly and abruptly in Fig. 23.5 [56]. Fifty days intervention was sufficient to show the main effect of the drug. For interventions that do not have a carryover effect, such as most nutritional interventions, a 50 day recovery period is adequate to return the urinary ^{41}Ca:Ca ratio to baseline and another treatment can be tested. Thus, for a person equilibrated to ^{41}Ca, as many as seven interventions can be tested in 2 years. The sensitivity of AMS to measure ^{41}Ca:Ca allows measurements for more than a decade following a single dose. The precision of this approach is an order of magnitude greater than for biochemical markers of bone turnover

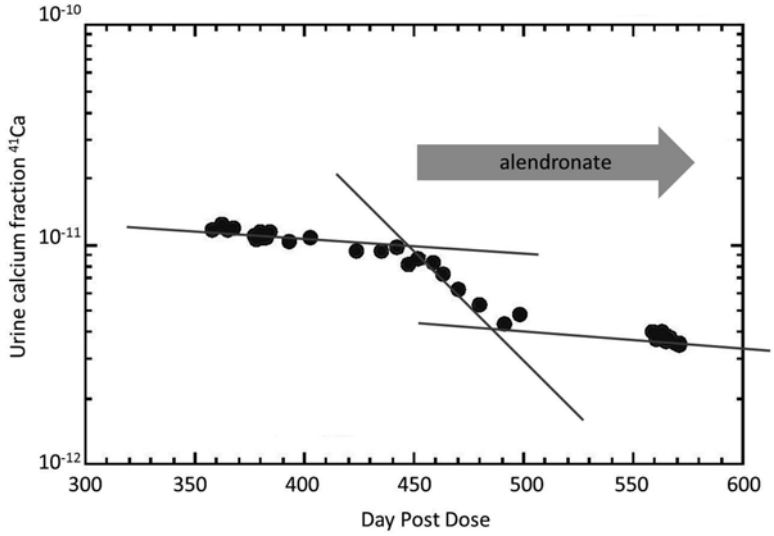

Fig. 23.5 ^{41}Ca release accurately reflects inhibition in bone loss. The subject in this figure was a postmenopausal woman treated with alendronate. (Adapted from ref. [56])

and specific for bone mineral. These attributes together with the crossover design gives adequate power in about a dozen participants. We have used this approach to compare estrogen, a bisphosphonate, and dietary supplements for ameliorating bone loss [57].

Use of ^{41}Ca to compared interventions can be used to optimize interventions for RCTs. It is also possible to personalize treatments if a person is administered ^{41}Ca and urine or serum samples were used to assess response to lifestyle interventions and addition of drugs as necessary to block bone loss.

23.5 Conclusions

Quantitative nutrition studies such as the balance and kinetic studies described here are seldom undertaken because of the resources required. They typically are limited to rather small sample sizes and can be criticized for lack of generalizability to the larger public. However, when properly conducted, they contribute much information about the quantitative relationship between a nutrient or diet to bone health. For example, such studies have identified differences between races that may not have been detected in population studies. Furthermore, they provide insights on the point of metabolism affected by a dietary or nondietary intervention, that is, the gut, kidney, or bone. Development of applications of ^{41}Ca led to the ability to assess pool size and turnover rates from bone that is more slowly turning over. It also has opened the possibility for rapid screening and comparisons of many interactions to improve bone calcium retention.

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Chapter 24
Sodium, Potassium, Phosphorus, and Magnesium

Robert P. Heaney

Key Points

- Sodium, potassium, phosphorus, and magnesium affect the calcium economy and bone status.
- The contemporary Western diet is generally thought to contain more sodium than our hunter–gatherer ancestors would have consumed, and substantially less potassium.
- Increases in the filtered load of either sodium or calcium leads to increased clearance of both ions.
- Urine calcium rises by from 1.0 mmol (40 mg) for every 100 mmol (2,300 mg) sodium ingested.
- Sodium intake accounts for most of the obligatory urinary loss of calcium from the body if calcium intake is low then bone mass will be impacted.
- Potassium is important to bone health because of its effects on the processes that maintain calcium homeostasis, particularly urinary calcium conservation and excretion.
- Foods high in potassium generally have an alkaline ash characteristic, including fruits and green and root vegetables which should be increased in the diet.
- A diet containing an amount of protein adequate for health will also contain adequate phosphorus.
- The RDA in the U.S. for phosphorus is 700 mg (23 mmol)/day for adults and median intakes for adults are above that level at all ages.
- Low food phosphorus intake and large calcium supplement doses along with osteoporosis treatments may lead to phosphorus deficiency.
- The richest dietary source of magnesium is legumes, followed by grains and root and green vegetables.
- More than 70 % of the adult population in the U.S. falls below recommended intakes of magnesium, but it is unclear whether this shortfall has skeletal consequences.
- Outside of certain special therapeutic or disease situations, the usually encountered variations in intakes of sodium, potassium, phosphorus, and magnesium are without major skeletal consequences.

Keywords Sodium • Potassium • Phosphorus • Magnesium • Western diet • Hunter–gatherer • Alkaline

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24.1 Introduction

Bone health is not a mononutrient issue. While predominant attention has been given to calcium in recent years (with vitamin D getting honorable mention), other nutrients are also known to affect the calcium economy and bone status, even if they are less commonly factored into dietary recommendations for the prevention of osteoporosis or the support of anti-osteoporosis therapy. In this chapter I shall deal with sodium, potassium, phosphorus, and magnesium, and will overlap with Chap. 23 in matters of the acid/alkaline ash characteristic of the diet. Taken together these four minerals make up about 6 % of the dry, fat-free mass of the human body. Table 24.1 presents their contributions individually, together with that of chloride and calcium—the former because chloride accompanies sodium, both in extracellular fluid and in the diet, and the latter (which is the subject of other chapters in this book) for comparative purposes.

Principal among the ways these nutrients influence bone is the effect that several of them have on obligatory urinary calcium loss. In Nordin's series, urine calcium accounts for ~40 % of the variability in calcium balance [2], and in my own, ~50 %. Thus nutrients affecting urinary calcium loss could in theory have a profound influence on bone maintenance or age-related bone loss. At the same time it must be noted that the effects of sodium and potassium on the handling of renal calcium, for example, are much more firmly established than are their putative consequences for bone status. This may be because bony outcomes are harder to study in relation to intakes of these minerals. But it may also be that compensatory mechanisms are, to a greater or lesser extent, offsetting their renal effects and hence preventing or reducing skeletal consequences.

It may be helpful to note, at the outset, that contemporary intakes of sodium and potassium, particularly, are different from those to which human physiology is adapted by evolution. Diets of primitive populations today are sometimes judged to be very low in sodium (2–30 mmol/day) and relatively very high in potassium (150–250 mmol/day). However, contemporary primitive populations may not be reflective of early humans. Salt licks and brine pools are ubiquitous in many environments and could have contributed substantially to ancestral sodium intakes. In any case, the contemporary Western diet is generally thought to contain more sodium than our hunter–gatherer ancestors would have consumed, and substantially less potassium.

24.2 Sodium

The best studied of these four nutrients is sodium, the effects of which are nicely summarized in several review papers [3–5]. As long ago as 1937 Aub et al. observed that sodium chloride increased urine calcium [6], and in 1961 Walser showed that sodium and calcium competed for the same

Table 24.1 Mineral composition of the adult human body[a]

	Content[b]	Percent[c]
Sodium	80 (1.84)	0.66
Potassium	69 (2.69)	0.96
Phosphorus	387 (12)	4.29
Magnesium	19.6 (0.47)	0.17
Chloride	50 (1.78)	0.63
Calcium	560 (22.4)	8.00

[a]Based on Documenta Geigy [1]
[b]mmol (g)/kg fat-free mass
[c]Based on dry fat-free mass

reabsorption mechanism in the proximal renal tubule [7]. This means that an increase in the filtered load of either sodium or calcium leads to increased clearance of both ions, thereby establishing the mechanistic basis by which a sodium load produces calciuria. A possible role for sodium intake in the pathogenesis of osteoporosis was first emphasized by Goulding who, in a series of animal and human experiments, showed that sodium intake could affect bone mass in animals, and that the effect required a functioning parathyroid apparatus [8–12].

Numerous studies have found statistically significant positive correlations between 24-h urine sodium excretion and 24-h urine calcium [6, 9–11, 13–22]. Taken together, the available studies indicate that urine calcium rises by from 0.5 to 1.5 mmol (20–60 mg) for every 100 mmol (2,300 mg) sodium ingested [5]. Most reviewers have used the midpoint of that range (i.e., 1.0 mmol/100 mmol) to characterize the effect.

Given the fact that contemporary sodium intakes average about 150 mmol/day [ranging between 100 and 200 mmol/day (i.e., 2,300–4,600 mg/day)], it follows that approximately 1.0–2.0 mmol (40–80 mg) of the 24-h total urine calcium excretion is being pulled out of the body by sodium. Ho et al. [21] concluded that sodium intake was the principal determinant of urine calcium in Hong Kong Chinese, and Matkovic et al. [22] came to a similar conclusion for pubertal girls in the U.S. Itoh and Suyama [13], in a study of nearly 900 Japanese adults in whom sodium intakes tend to be much higher than in Europe or North America, found a positive correlation between sodium intake and urine calcium in both sexes, and across all age groups, even after adjusting for weight and for dietary intakes of protein, phosphorus, and calcium.

Thus, on prevailing diets, sodium intake accounts for most of the obligatory urinary loss of calcium from the body. Clearly, if absorbed calcium is less than the amount needed to offset this loss (in addition to what is needed to cover cutaneous and digestive juice losses), then bone mass must suffer.

It would be expected that increased urinary loss following a sodium load would produce a fall in extracellular fluid calcium ion concentration, and this has been described in some studies [16, 23]. Such a fall would also produce a rise in parathyroid hormone, with a consequent increase in synthesis of $1,25(OH)_2D_3$, and ultimately in calcium absorption efficiency. Breslau et al. demonstrated that a change in urine calcium evoked the predicted change in PTH, $1,25(OH)_2D_3$, and calcium absorption efficiency, at least in premenopausal women [16, 18]. But they failed to observe changes in absorption in a small study involving postmenopausal women. These findings suggested, at least qualitatively, that premenopausal women could handle contemporary sodium intakes with less skeletal impact than postmenopausal women and, by implication, that high sodium intakes may be contributing to postmenopausal osteoporosis.

At least two groups of investigators have found that calcium absorption efficiency varies directly with induced calciuria whether from a sodium load [16, 23] or from the calcium-sparing effect of thiazides [24]. Breslau et al. [16] reported that the change, while occurring in normal subjects, did not occur in two patients with surgical hypoparathyroidism, consistent with Goulding's findings in rats [8]. By contrast, Meyer et al. [23] did find an increase in calcium absorption efficiency in two patients with hypoparathyroidism following a sodium load, suggesting that the response was mediated by some mechanism other than increased PTH secretion. While the discrepancy between these studies cannot be resolved with available data, it does appear reasonably certain that, other effects aside, a substantial salt load leads to increased PTH secretion with all of its usual consequences [increased $1,25(OH)_2D_3$ synthesis, increased calcium absorption, increased bone resorption, and improved renal tubular reabsorption of calcium]. But other mechanisms may be operative as well.

Additionally, several groups of investigators have shown that bone remodeling, as measured by various remodeling biomarkers, varied directly with sodium intake [9, 11, 19, 25] and that sodium restriction reduced excretion of resorption biomarkers. This finding is consistent with the effect of sodium loads on PTH secretion. Often this remodeling effect has been taken to indicate that sodium increases bone loss, although this does not necessarily follow, and there are few reports of a direct connection between sodium intake and subnormal bone status in humans at typical sodium intakes.

There is at least one case report of probably salt-associated osteoporosis [26]. A 50-year-old postmenopausal woman with adequate hormone replacement therapy had high turnover osteoporosis with vertebral compression fractures and urine calcium in excess of 7.5 mmol/day (300 mg). She was observed to be using table salt, covertly, from a paper bag, in quantities so large as to make the food on her plate white, and she reported having done so for the previous 20 years. Reduction in salt intake reduced her urinary calcium loss to below 2.5 mmol (100 mg)/day.

Whether clinically significant bone loss actually occurs at more typical salt intakes has been the subject of very few studies. Greendale et al. found no association between sodium intake from diet records and bone status 15 years later [27]. Sodium intakes in their subjects averaged about 150 mmol (3,450 mg)/day in men and 112 mmol (2,576 mg)/day in women. However, estimates of sodium intake from diet records correlate poorly with actual sodium intake, and thus this negative finding cannot absolve sodium intake in this connection. Accurate estimates of sodium intake require measurement of 24-h urine sodium, preferably over a several day interval. This need probably explains the virtual absence of epidemiological studies showing an association of sodium intake with bone mass.

Dawson-Hughes et al. [14], in a 4-year prospective study, found a highly significant correlation between sodium intake (as measured by urinary sodium) and urinary calcium excretion in healthy elderly men and women, but no correlation of sodium intake with bone mineral density at any site, in either sex. This failure to find skeletal differences is suggestive of some degree of intestinal absorptive compensation for the sodium-induced calciuria. Sodium intakes in their study averaged 156 mmol (3,600 mg)/day in men, and 118 mmol (2,700 mg)/day in women.

The principal human study linking high salt intake to bone loss was by Devine et al. [28], who showed that, in postmenopausal women, change in bone mineral density at the total hip site over a two-year period was inversely related to sodium intake estimated from urine sodium content. But they found no such effect at the spine, femoral neck, intertrochanteric region, or radius. From multiple regression models these investigators calculated that halving the sodium intake of their subjects would have obliterated hip bone loss. But in the same model, doubling of calcium intake (i.e., raising it into the currently recommended range) would have produced approximately the same beneficial effect.

Summarizing the data available up to 2000, Burger et al. [29] concluded that a sodium-osteoporosis link was still conjectural.

Comment. Based on the multiple regression model of Devine et al. [28], one might conclude that contemporary sodium intakes elevate the calcium requirement—at least for bone status. However, the two strategies suggested by the Devine model are not equivalent. High calcium intakes confer numerous nonskeletal health benefits [30], while the benefits of low sodium intakes, although widely touted, are at best problematic [31, 32]. Furthermore, from the standpoint of feasibility, higher calcium intakes are much easier to achieve and sustain than are reductions in sodium intake of the magnitude required to offset sodium's effect on obligatory urinary calcium excretion [33].

Finally, one must note that, even if sodium's effect on bone mass is normally compensated for by adaptive increases in calcium absorption (or by high calcium intakes), any accompanying increase in bone remodeling may constitute a risk factor for fracture [34]. Hence, choosing one or the other of the options offered by the Devine model would seem to be more prudent than doing neither.

For the most part, when the papers cited in this connection speak of "sodium," what is meant is "sodium *chloride*," i.e. table salt, the form in which about 90 % of contemporary sodium intakes are ingested. The accompanying anion is usually ignored. This is probably a mistake. Berkelhammer et al. [35] showed clearly, in patients receiving total parenteral nutrition (TPN), that substituting acetate for chloride in TPN solutions reduced urine calcium losses dramatically. In oral feeding studies, Lutz [36] showed that substituting sodium bicarbonate for sodium chloride promptly reduced urine calcium. Similarly, sodium bicarbonate loads do not induce an increase in urine calcium, unlike sodium chloride [36, 37]. Thus, clearly the anion is important, at least for the understanding of what

is happening. Nevertheless it remains true that contemporary diet sodium is overwhelmingly in the form of sodium chloride, and as such is usually hypercalciuric in its effect. Even this statement, however, is not absolute, as the next section will show.

24.3 Potassium

Potassium is a largely intracellular cation. Bone mineral does not contain appreciable quantities of potassium—only that small amount which is trapped when calcium phosphate is precipitated out of an extracellular fluid phase that inevitably contains some potassium. There are no recognized abnormalities of bone or bone cellular function associated with values of serum potassium in the range of concentrations typically encountered.

Probably the principal importance of potassium lies in its effects on the processes that maintain calcium homeostasis, particularly urinary calcium conservation and excretion. Low potassium diets are known to increase urinary calcium loss and high potassium diets to reduce it [38–41]. Such observations, by themselves, could mean simply that diet potassium is a marker for other food constituents responsible for the effect. This, in fact, is partly correct (see below). But pure potassium salts—typically the bicarbonate or citrate salts—exhibit the same inverse relationship to urine calcium, pointing to a role specifically for potassium itself.

Perhaps most striking of potassium's effects is the fact that potassium (as the citrate) completely blocks the calciuria of a large sodium chloride load (Fig. 24.1) [42]. It is believed that both the potassium cation and the bicarbonate anion (to which citrate is metabolized) function in the distal renal tubule facilitating reabsorption of the extra calcium not reclaimed in the proximal tubule because of competition with sodium for the transport mechanism. Figure 24.2 illustrates, schematically, the differing effects on urine calcium of various sodium and potassium salts.

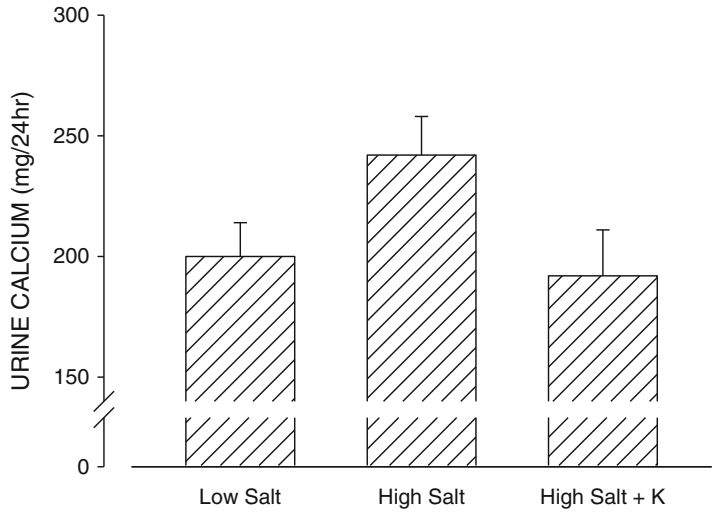

Fig. 24.1 Effect of a high salt load, with and without supplemental potassium citrate, on 24-h urine calcium excretion in postmenopausal women. The low salt regimen provided 87 mmol (5 g) salt/day and the high salt, 225 mmol (13.2 g)/d. The potassium supplement provided 90 mmol (29.2 g) potassium citrate/d. $N=26$ for each of the treatment groups. The rise in urine calcium on the high salt regimen was highly statistically significant ($P<0.005$). Plotted from the data of Sellmeyer et al. [42] (Copyright Robert P. Heaney, 2003. Used with permission)

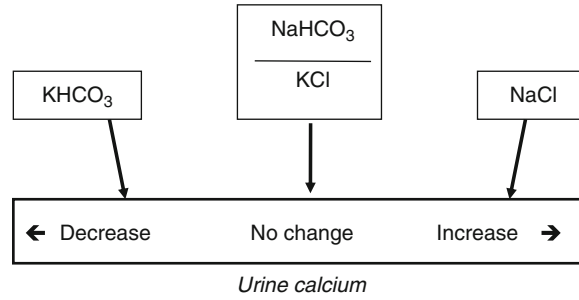

Fig. 24.2 Effects of various sodium and potassium salts on urine calcium (Copyright Robert P. Heaney, 2003. Used with permission)

However, just as the undoubted effects of sodium on urine calcium have not yet been unambiguously shown to have corresponding effects on bone (see prior section), so, therefore, amelioration of those effects by potassium has not been clearly shown to confer a skeletal benefit, although, in short-term metabolic experiments, potassium bicarbonate does produce a positive calcium balance shift [41, 43]. However, this probably is not the case under steady state conditions. Rafferty et al. [44] showed that, while urine calcium was inversely related to dietary potassium intake, intestinal calcium absorption was also inversely related to diet potassium, and the two effects approximately canceled one another.

There are, however, limited data in regard to the association of bone status and dietary potassium intake. New et al. [45, 46], for example, in observational studies, showed a significant inverse relationship between potassium intake and bone mineral density (BMD) at both hip and spine. Once again, it is not certain that the effect is due solely to the higher potassium content or whether potassium instead is a marker for other food constituents.

Potassium is ubiquitous in the diet, but is found most abundantly in green and root vegetables, followed closely by fruits, then by legumes and milk (or yogurt). A diet high in potassium will usually be high in fruits and vegetables. In addition to their potassium content, such foods have an alkaline/ash characteristic and thus, effectively, the anion associated with potassium in such foods will be bicarbonate. By contrast, wheat, rice, corn, and other cereal grains have very low potassium contents and generally exhibit an acid ash characteristic (because of their high content of sulfur-containing amino acids). Thus, in brief, foods high in potassium generally have an alkaline ash characteristic, and alkaline ash foods will generally be good sources of potassium.

New et al. [47, 48] have also shown a significant inverse relationship in free-living subjects between net endogenous acid production (NEAP) from ingested foods and lumbar spine bone mineral density. The effect was small (less than 2.5 % difference in BMD between upper and lower quartiles of NEAP), but consistent with studies by others showing a calciuric effect of food-based acid production [38, 39]. New has also shown significantly higher excretion of bone remodeling biomarkers at the highest quartile of NEAP, and she also reports a small, but significant difference in NEAP between postmenopausal women with and without fracture.

Given the generally higher potassium content of vegetarian diets, it may be useful to contrast bone status data in individuals with vegan and omnivore diets, which represent quasi-extremes of food intake patterns. Studies using contemporary bone assessment technologies [49–51] indicate that, in general, not only do the vegans not have denser bones, but they tend actually to have somewhat lower BMD, despite the fact that they have higher potassium intakes than do omnivores. In a meta-analysis published in 2009, Ho-Pham et al. found a significantly lower BMD in individuals on vegetarian or vegan diets [52], though the difference was small and judged not clinically important.

While the vegetable intake of vegans will usually be higher than that of omnivores, their intake of cereal grain products will usually be higher as well. Cereals, as already noted, are very poor sources of potassium and generally produce an acid-ash residue as well (i.e., high NEAP). Hence any switch between vegan and omnivore diets involves trade-offs. Clearly the mere substitution of vegetable for

animal protein sources does not seem to confer a skeletal advantage, and may, in fact, do the opposite. One may note, in passing, that the primitive human diet was omnivorous.

Comment. Given the abundant mechanistic evidence of beneficial effects on the calcium economy of high potassium foods and the absence of evidence to support a sometimes presumed dichotomy between animal and vegetable protein sources, the most prudent recommendation for total health (and possibly skeletal health as well) would seem to substitute fruits and green and root vegetables for the high energy, low nutrient density foods (so-called junk foods) common in the Western diet. This, by itself, would move contemporary intakes of potassium toward the paleolithic norm and would decrease NEAP as well, thereby helping to restore the environmental context to which human physiology is adapted.

24.4 Phosphorus

Bone mineral is generally characterized as an imperfect hydroxyapatite, i.e., a calcium phosphate salt with a Ca:P molar ratio approximating 1.7:1. The phosphate anion actually makes up more than half the mass of bone mineral. Like the calcium cation, the phosphate of bone mineral is derived from the blood flowing past a bone mineralizing site. While the initiation of the apatite crystal nucleus requires osteoblast work, subsequent crystal growth is purely passive, involving diffusion of the constituent ions down a concentration gradient from the solution phase in blood to the solid phase in bone. At average adult concentrations of calcium and phosphorus, blood is approximately twice saturated with respect to hydroxyapatite, and hence it contains a sufficient quantity of both ions to sustain usual levels of bone formation. However, at the mineralizing site, concentrations in the extracellular fluid around the osteoblast are lower than in the general circulation because calcium and phosphorus are being pulled into bone. In fact, adult serum concentrations may not suffice during growth, when a great deal of mineral is being transferred into the skeleton. In essentially all growth situations—animal and human—serum inorganic phosphorus (P_i) is 2–3× higher than in human adults. Growth hormone, which raises the renal phosphorus threshold and thereby helps to sustain higher serum P_i concentrations, is a part of the explanation.

Phosphorus is widely distributed in natural food sources and, because of its incorporation into the structure and machinery of most cellular tissues, protein and phosphorus tend to go together in the diet. As a result, a diet containing an amount of protein adequate for health will also contain adequate phosphorus.

Unlike calcium, for which net absorption is low (10–15 % of intake), net absorption of phosphorus is much more efficient, ranging from 50 to 70 % from typical diets. The distinction between net and gross absorption is important because of the fact that there is a considerable amount of both calcium and phosphorus entering the digestive stream from endogenous sources. Much of this is in the form of digestive secretions, but for phosphorus particularly, shed mucosal cells contribute importantly. Total digestive juice phosphorus has been estimated to be on the order of 8 mmol (250 mg)/day [53], much of which is absorbed along with food phosphorus. Fecal phosphorus of endogenous origin has not been frequently measured, but available data suggest that it is on the order of 2–3 mmol (60–90 mg)/day.

For individuals with normal or even moderately impaired renal function, the kidneys are able to handle the relatively large amount of absorbed phosphorus without difficulty, and with only minor elevation of the serum P_i. In Nordin's calculations [54] for normal renal functioning, serum P_i rose by less than one-third over a tripling of phosphorus intake (This is one of the reasons why high phosphorus intakes are probably not much of a problem for individuals with adequate renal function.).

Serum P_i concentrations substantially above the normal range increase the risk of extraskeletal calcification. However, at physiological pH, and without an apatite crystal nucleus, calcium and phosphorus would come out of solution as $CaHPO_4 \cdot H_2O$, and for this solid phase, serum is only half saturated.

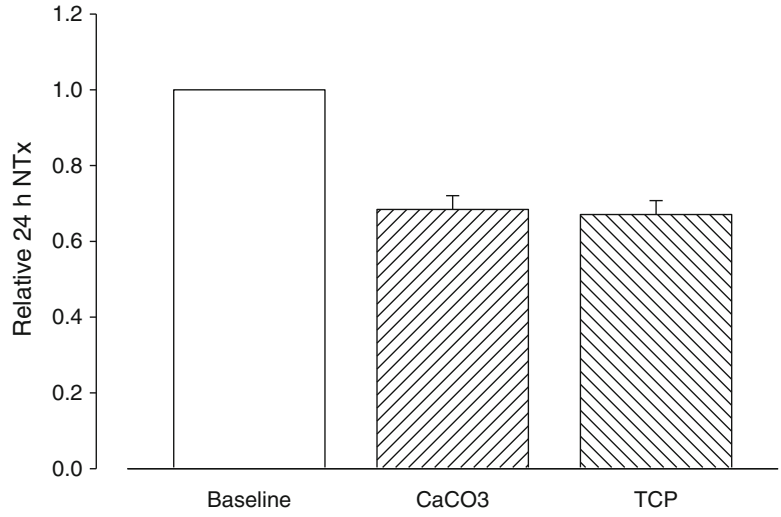

Fig. 24.3 24-h urine NTx excretion in 28 postmenopausal women under conditions of low calcium intake ("Baseline") and then following 1 week each of supplementation with calcium at 45 mmol (1,800 mg)/d, either as calcium carbonate or as tricalcium phosphate (TCP). The latter provided, in addition to its calcium, 30 mmol (930 mg) supplemental phosphorus daily and resulted in an increase over baseline intake of 150 %. All values are expressed relative to each woman's baseline excretion. NTx excretion was decreased by 32 and 33 %, respectively ($P<0.001$ relative to baseline). There was no difference in effect between the two calcium salts (Copyright Robert P. Heaney, 2003. Used with permission)

This elegant arrangement means that prevailing serum concentrations of calcium and P_i are indefinitely stable in the absence of an apatite crystal nucleus, but the same concentrations provide abundant mineral to support crystal growth at suitably nucleated sites. The risk of extraskeletal calcification occurs mainly when the serum Ca×P product rises to or past saturation. Preventing this result is a principal reason for controlling phosphorus absorption in patients with end-stage renal disease.

Also, it is sometimes argued, high phosphorus intakes lead to increased PTH secretion, an outcome presumed to be bad for bone. High phosphorus intakes have even been proposed as contributing to the pathogenesis of osteoporosis [55]. However, when these issues have been directly examined [56, 57], it has generally been found that phosphorus supplements *decrease* bone turnover markers, rather than increasing them, an effect probably due to interference by ambient phosphate with osteoclast response to PTH [58]. Figure 24.3 shows the results of one such experiment, in which 24-h urinary N-telopeptide excretion after one week of supplementation with tricalcium phosphate was contrasted with that following calcium carbonate. As Fig. 24.3 shows clearly, remodeling suppression, produced by the calcium in each source, was the same for both salts. Moreover, as is generally recognized, phosphorus supplements lower, rather than raise, urine calcium loss, and increased phosphorus intake does not lead to negative calcium balance [59]—as would be expected if phosphorus were somehow adversely affecting bone mass.

While there are some animal data indicating that very high phosphorus intakes can produce bone disease [60], there are no corresponding data for humans [61]. Moreover, it must be noted that laboratory animal chows have much higher phosphorus densities than do human diets [61], and diets as high in phosphorus as produced in the animal models are essentially never encountered in humans. Thus, in the remainder of this section I shall ignore the high end of the distribution of phosphorus intakes, and will focus instead on the possible importance of low phosphorus intakes, first in relation to osteomalacia and second in the context of anti-osteoporosis therapy.

As noted, the bone forming cell (the osteoblast) is particularly sensitive to ambient P_i concentrations, mainly because the mineralizing process it has induced in the osteoid deposited beneath it

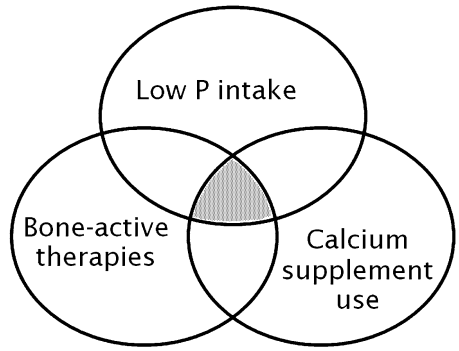

Fig. 24.4 Venn diagram illustrating the set of patients with osteoporosis most likely to exhibit effective phosphorus deficiency (i.e., the intersection of the three sets). For these purposes, "low P intake" refers to intakes below 70 % of the RDA; "calcium supplement use" refers to the carbonate or citrate salts (principally); and "bone active therapies" refers to both anti-resorptives and anabolics, but particularly the latter. The sizes of the sets and of their intersections are not intended to be quantitatively accurate (Copyright Robert P. Heaney, 2003. Used with permission)

depletes its microenvironment of phosphate. This is the reason why essentially all of the osteomalacias exhibit not only impaired mineralization but impaired osteoblast function as well. So long as serum P_i concentrations are above 1.0 mmol (3.1 mg/dayL)/L working osteoblasts and bone mineralizing sites in a typical mature adult will "see" adequate quantities of phosphorus to support their activities. (Although the reference lower limit of normal for serum phosphorus extends down to as low as 0.7 mmol (2.2 mg/dL)/L, effects of concentrations at the lower end of the "normal" range on osteoblast and osteoclast function have not been well studied. Hence the *functional* lower limit of normal has yet to be rigorously defined.) But hypophosphatemia, by whatever definition, is uncommon in older adults; fully two-thirds of patients in our osteoporosis clinic have serum P_i concentrations above 1.2 mmol (3.6 mg/dL)/L.

It is important to stress, before the analyses that follow, that a patient who does not experience a fall in serum phosphorus to suboptimal levels, is not experiencing effective phosphorus deficiency, no matter what else may be happening in the operation of the calcium and phosphorus economies.

Hypophosphatemia in adults is almost never caused by low dietary phosphorus intakes and is essentially always a reflection of nondietary metabolic disorders, ranging from nutritional vitamin D deficiency to tumor-induced osteomalacia and X-linked hypophosphatemia. Whatever the cause, hypophosphatemia results in impairment of osteoblast function and in failure adequately to mineralize newly deposited bone matrix. With the exception of certain heavy metal intoxications, hypophosphatemia is the ultimate pathogenesis of all osteomalacias, whatever their ultimate cause. Hypophosphatemia has important nonskeletal effects [61] as well which are beyond the scope of this chapter.

The RDA in the U.S. for phosphorus is 700 mg (23 mmol)/day for adults [62], and median intakes for adults are above that level at all ages [63]. Hence, unlike with calcium (and assuming the correctness of the RDA), there is no evidence of prevailing phosphorus deficiency. However, the low tail of the distribution of phosphorus intakes reveals a different picture [63]. Five percent of all adult women ingest less than 70 % of the RDA on any given day; 10 % of women over age 60, and 15 % of women aged 80 or older also have such low intakes. As already hinted, low phosphorus intakes mean insufficient intakes of protein and calcium as well, and they thus reflect a more global situation of malnutrition. Many of these individuals with low phosphorus intakes will have osteoporosis as well, and will be recipients of current generation anti-osteoporosis therapies, essentially all of which now include recommendations for supplemental calcium. The potential for phosphorus deficiency increases precisely in the context of individuals with low phosphorus intakes, who are taking calcium supplements, and who are receiving anti-osteoporosis therapies (Fig. 24.4).

Although calcium supplements are widely recognized (and employed in nephrology) to bind food phosphorus, the field of osteoporosis has largely ignored that fact [64]. In individuals with already low intakes, the effect of calcium supplementation will be a reduction in available phosphorus, which could, potentially, induce hypophosphatemia. The extent of absorptive interference will depend upon the relative quantities of calcium and phosphorus ingested and on the timing of their ingestion. Heaney and Nordin [64] showed that, for mixed food intake, each 12.5 mmol of calcium (500 mg) reduces phosphorus absorption by ~5.4 mmol (332 mg). The binding observed by these investigators was considerably lower than what would be predicted from the chemistry involved, and probably reflects variability in timing of the calcium and phosphorus-containing foods in their subjects' diets. As would be expected, given the fact that the mechanism is chemical complexation of the two species, calcium not ingested at the same time as phosphorus will produce less interference with phosphorus absorption [65].

Using the binding relationship defined above, it can be calculated that calcium supplement intake amounting to 1,500 mg (37.5 mmol) Ca/day as the carbonate (or citrate), and ingested with meals (in accordance with usual instruction), will bind ~16 mmol (~500 mg) phosphorus, i.e., essentially all of the ingested phosphorus in individuals with intakes below 70 % of the RDA. Given the ability of the kidney to reabsorb essentially all of the filtered phosphorus at low filtered loads, even this total binding would probably not lead to negative phosphorus balance or to hypophosphatemia in most mature adults, and would likely not adversely affect ordinary bone repair and maintenance. The potential for deficiency arises principally in the context of anti-osteoporosis therapy. Anti-resorptive agents produce as much as a 5 % bone gain in the first year of therapy, followed by slow gain thereafter at a rate of between 0.5 and 1.0 %/year [66]. Anabolic agents increase bone mass, at least at the axial skeleton, by as much as 15 %/year [67, 68]. It is not clear that the associated positive phosphorus balance could develop or be sustained if most or all of the diet phosphorus were to be rendered unavailable.

The steady-state slow bone gain of anti-resorptive therapy poses the smaller challenge. A gain of 0.5–1.0 %/year translates to a positive calcium balance of 0.25–0.5 mmol (10–20 mg)/day, and a positive phosphorus balance of 0.15–0.3 mmol (4.5–9 mg)/day. Given the likelihood that not all food phosphorus would be co-ingested with a calcium supplement, it is unlikely that binding would be complete; thus sufficient phosphorus would probably be absorbed to sustain the amount of bone gain plausible with anti-resorptive treatment.

By contrast, the steady-state bone gain produced by the anabolic agents is a full order of magnitude greater. A gain of 15 %/y translates to a positive calcium balance of ~7.7 mmol (308 mg)/day and to a phosphorus balance of ~5.0 mmol (155 mg)/day. With complete or near complete binding of food phosphorus in individuals on low phosphorus intakes, such a high positive balance would not be possible. Moreover, teriparatide, the only directly anabolic agent approved for osteoporosis treatment in the U.S., directly lowers the renal phosphorus threshold, thus lowering serum P_i concentration in its own right, apart from any effect of net movement of P_i out of serum and into bone.

It must be stressed that this analysis is largely theoretical. The calculations above are for something approaching the largest bone gain possible with each class of treatments—unlikely at a total skeletal level. Further, no data exist showing impairment of treatment response in patients with low phosphorus intakes (as would be predicted from the foregoing analysis). But at the same time it must be noted that a phosphate effect has not actually been looked for. Subjects entered into clinical trials typically exhibit a healthy volunteer effect and are thus unlikely to show the prevalence of low phosphorus intakes found in a true population sample. Furthermore, calcium supplement doses in most published trials have probably been suboptimal (and hence food phosphorus binding correspondingly incomplete). Finally, phosphorus intake would not have been assessed in trials of anti-osteoporosis agents. Hence to the extent phosphorus deficiency may have been present in their treatment groups, or contributed to the variability of their response, the effect of such deficiency would necessarily have gone unrecognized.

In brief, beyond the certainty of phosphorus binding by nonphosphate calcium supplements (such as the carbonate or citrate salts), there are very few facts on either side of the question.

Actually, the question itself probably never would have arisen had it not been for advances in the treatment of osteoporosis which now make possible bone gains rivaling in magnitude those experienced during the adolescent growth spurt.

Comment. It should be noted that the combination of low food phosphorus intake and large calcium supplement doses constitutes a distinctly unnatural situation. Typical high calcium diets from food sources exhibit a Ca:P molar ratio in the range of 0.8:1–1.2:1, while the treatment context just analyzed has a Ca:P ratio of about 2.5:1, something virtually never found in nature.

Several courses of action present themselves at this stage of our ignorance. Perhaps most obvious is to use a calcium phosphate supplement, instead of the carbonate or citrate salts, in patients treated with potent anabolic agents. Another, of course, is to assess total nutritional status in our patients, and to correct *all* insufficiencies. This option, while preferable, is unlikely to occur. Typically patients with osteoporosis will be placed on pharmacotherapy and given a calcium supplement recommendation without regard to their home diets and nutritional status. While this is unfortunate, it is also the reality.

24.5 Magnesium

Although 50–60 % of total body magnesium is found in bone, functionally magnesium is largely an intracellular cation, and signs of its deficiency include system-wide cellular dysfunctions. In the field of calcium and bone, severe magnesium deficiency, with hypomagnesemia, results in refractory hypocalcemia, due to both impaired parathyroid gland response to hypocalcemia and impaired bone cell response to parathyroid hormone. In brief, the entire calcium homeostatic apparatus is disabled. But there is little evidence (see below) that this kind of dysfunction occurs at levels of magnesium nutrition associated with normal serum magnesium levels or typical dietary magnesium intakes.

Magnesium is also considered to condition bone mineral crystal solubility, largely by substitution of magnesium for calcium in surface positions of the hydroxyapatite lattice. While this effect may help optimize the processes that control fluxes of calcium into and out of bone, at the same time, this magnesium effect is largely passive, and will be a function of ambient magnesium levels in the extracellular fluid. While serum magnesium is generally considered an unreliable indicator of magnesium nutritional status, still it may be the only relevant factor with respect to this effect on bone mineral solubility.

The RDA for magnesium is ~13 mmol (320 mg)/day in women and 17.5 mmol (420 mg)/day in men [62]. More than 70 % of the adult population in the U.S. falls below these recommended intakes [63]. It is unclear whether this shortfall has skeletal consequences. Rude [69] has reviewed the evidence relating magnesium intake to bone status. In general observational studies of the association of magnesium intake and bone mass or age-related bone loss have produced mixed results—some showing a weak interrelationship, but most showing no apparent association. Similarly, magnesium supplementation trials have produced mixed results—some showing small skeletal effects; others, nothing at all.

There is a widespread misconception in the general public that magnesium is necessary for calcium absorption, or at least for calcium to exert its proper efficacy. This is the reason given for the popularity of combined calcium-magnesium supplements, such as Dolomite. If there is any factual basis for this belief, it must lie in the effects of magnesium in animals or humans with severe magnesium deficiency in whom, as already noted, calcium regulation is crippled. But, at typical magnesium intakes and prevailing levels of serum magnesium, there is abundant evidence showing that no such relationship exists. Spencer et al. [70] more than doubled magnesium intake in normal volunteers and found no effect on calcium absorption, using rigorous absorption methodology.

Even more to the point, the large body of literature showing an effect of calcium on bone accrual during growth, and the reduction of age-related bone loss and fragility fractures in the elderly, consists of studies all of which were done without magnesium supplementation. Both the Chapuy [71] and the

Dawson-Hughes [72] trials produced dramatic fracture reductions (40 % and 55 %, respectively) using calcium and vitamin D supplementation alone without extra magnesium. It might be argued that giving supplemental magnesium would have improved these results still further, but that is pure speculation, without a base in evidence (except possibly in patients with celiac sprue, in whom magnesium supplementation may confer a skeletal benefit [73]).

However, it has recently become clear that celiac sprue may often be asymptomatic and that, therefore, some individuals with ordinary osteoporosis may have malabsorption contributing to the pathogenesis of their condition. Serum magnesium measurement will not usually detect the associated magnesium deficiency. The magnesium-tolerance test [74] is a much more sensitive measure of magnesium deficiency but it not likely to be done in most patients. For that reason, low dose magnesium supplementation needs to be considered in all patients with osteoporosis.

There is, finally, one instance in which moderately low magnesium status may influence bone. Sahota et al. [75] observed that, while patients with low vitamin D status typically have elevated serum parathyroid hormone (PTH) values, many did not. Seeking an explanation, these investigators evaluated magnesium status, using the magnesium-tolerance test [76]. In brief, they found that, despite normal serum magnesium values, those D-deficient patients with low PTH values had positive magnesium-tolerance test results. To confirm the role of magnesium in this phenomenon, they supplemented their patients with magnesium and observed the sought-for rise in PTH concentration. The ultimate clinical significance of these findings is uncertain, but these results are powerful examples of the interplay of nutrients and of the difficulty of investigating those interactions.

Comment. The richest dietary source of magnesium is legumes. Grains and root and green vegetables also have high magnesium densities, but their content of magnesium per serving is lower than in legumes. As a consequence, just as a diet without dairy products will fall short of the calcium intake recommendation, so a diet without legumes will tend to fall short of the magnesium RDA. To the extent that currently recommended magnesium intakes may reflect the paleolithic norm, our hunter–gatherer forebears would have gotten their magnesium principally from roots, greens, and nuts.

24.6 Summary

The health of bone is dependent upon total nutrition, including adequate intakes not only of calcium and vitamin D, but of potassium, phosphorus, magnesium, and trace metals as well. Furthermore, bone health may be compromised by excessive sodium intakes. Bone health is manifested in two principal ways—bone massiveness (expressed as structural strength) and bone cell function (expressed as bone growth and repair). Acute nutrient deficiencies may significantly impair bone cell function without appreciably affecting bone strength, at least immediately. Hence such deficiencies tend to be silent. Conversely, excess of sodium chloride or deficiencies of potassium and phosphorus, which can impair calcium conservation or bone mineralization, when they have any effect at all, alter bone mass, although slowly. Hence their effects, if any, are hard to detect and often delayed in onset. Outside of certain special therapeutic or disease situations, the usually encountered variations in intakes of sodium, potassium, phosphorus, and magnesium are without major skeletal consequences.

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Chapter 25
Assessing Nutritional Requirements for Preterm Infants

Ian J. Griffin

Key Points

- The role of the trace minerals in bone health is much less well developed than for the macrominerals.
- In many cases, studies have used animal models, which are difficult to extrapolate to humans.
- In others, the relationship between serum levels of minerals and markers of bone health or assessment of bone mineral density are described along with their limitations.
- Any relationships are confounded by the other lifestyle and socioeconomic factors that may cause such differences in dietary intakes.
- In addition, low-quality diets may be deficient in more than one nutrient, making it extremely difficult to ascribe the change to any single nutrient.
- There are very few well-designed intervention studies in humans that address the importance of trace and ultratrace minerals in human bone metabolism.
- Strontium, has some good-quality data (i.e. randomized controlled studies) suggesting that high-risk adults may benefit from strontium supplementation.

Keywords Nutritional requirements for preterm infants • Macrominerals • Copper deficiency • Zinc • Boron • Strontium • Silicon • Trace minerals • Skeletal health • Animal studies

25.1 Introduction

Although the macrominerals calcium, phosphorus, and magnesium are of primary concern in bone health, other minerals, including trace minerals can also play an important role. In this chapter, the role of some of these will be considered. In general, the data supporting and defining the role of the trace minerals in bone health is much less well developed than for the macrominerals. In many cases, studies have used animal models, which are difficult to extrapolate to humans. In others, the relationship between serum levels of minerals and markers of bone health or assessment of bone mineral density are described. These are difficult to interpret, and even if a correlation between low serum copper and low bone mineral density (for example) is demonstrated this does not mean that additional

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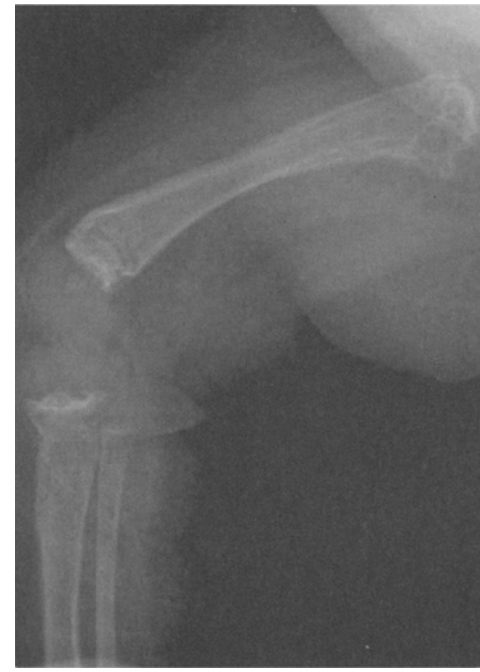

Fig. 25.1 Radiograph of the lower limb of a former preterm infant demonstrating changes of severe copper deficiency

dietary copper would improve bone mineral density. Such relationships are confounded by the other lifestyle and socioeconomic factors that may cause such differences in dietary intakes. In addition, low-quality diets may be deficient in more than one nutrient, making it extremely difficult to ascribe the change to any single nutrient.

There are very few well-designed intervention studies in humans that address the importance of trace and ultratrace minerals in human bone metabolism. The one exception appears to be strontium, where there is increasing good-quality data (i.e. randomized controlled studies) suggesting that high-risk adults may benefit from strontium supplementation.

25.2 Copper

Copper is an essential element in human nutrition and is required by many enzymes, including lysyl oxidase, which is responsible for cross-linking of collagen and elastin [1]. The prototypical disease of copper deficiency is Menkes' kinky hair syndrome.

25.2.1 Copper Deficiency

Menkes' kinky hair syndrome is a congenital cause of copper deficiency resulting from impaired copper absorption, which can present with skeletal changes resembling scurvy, fractures, or delayed bone age. Acquired copper deficiency has also been reported in humans, most commonly in premature or low-birth-weight (LBW) infants who had low enteral or parenterally copper intake [2, 3] or children on prolonged copper-free parenteral nutrition [4]. In premature infants the symptoms of copper deficiency may include osteopenia, fractures, or other bony changes [3, 5].

Figure 25.1 shows an extreme example of copper deficiency-induced bone disease in a former preterm infant who had been on prolonged copper-free parenteral nutrition (PN). This infant had been born extremely prematurely and developed complications including necrotizing enterocolitis—a severe, potentially fatal gastrointestinal infection. This led to the development of widespread gut necrosis requiring multiple surgeries, and ultimately to severe short-gut syndrome. Because of this, establishment of enteral feeds was extremely difficult, and he required prolonged PN, and developed cholestatic liver disease (PNAC, PN-associated cholestasis). Copper is excreted in the bile, and therefore may not always be used in infants with cholestasis due to the possibility that it may worsen liver failure. In this infant, copper had been completely removed from his TPN for a prolonged period of time. After several months on copper-free TPN and minimal enteral copper intake, a routine chest X-ray revealed radiological changes consistent with copper deficiency. A low serum copper level and low ceruloplasmin concentration and characteristic changes in long bone films confirmed the diagnosis of acquired copper deficiency. Radiological features suggestive of copper deficiency include osteopenia, metaphyseal cupping and flaring, spurs and fractures, and retarded bone age [6].

25.2.2 Animal Studies

Several studies have shown that although calcium content may not change, copper-deficient experimental animals have decreased bone strength [7, 8], The cause of this is believed to be the reduced activity of lysyl oxidase, the copper metalloenzyme that is responsible for formation of collagen cross-links. Copper deficiency has been shown to cause decreased collagen cross-linking and this is accompanied by decreased bone strength in chicks [9]. Furthermore, the reduction in bone strength was reversed by chemical induction of cross-links in vitro, suggesting that the decrease in bone strength results from decreased cross-linking [9]. Long-term copper deficiency may also reduce ostrogenesis and reduce osteoclast activity [10].

In ovarectomized rats, copper deficiency increases bone loss [11], whereas copper supplementation may reduce it [12]. However, a similar effect is seen with manganese, with no additional benefit coming from copper supplementation [13].

25.2.3 Human Studies

Although frank copper deficiency clearly has adverse effects of bone health, the importance of mild deficiency or poor copper intakes is much less clear. It has been hypothesized that suboptimal copper nutrition may be a major cause of osteoporosis in Western societies [14], although good evidence for this is lacking. One epidemiological study has described a relationship between copper intake (and indeed iron and zinc intake) and forearm bone mineral content in premenopausal women [15]. Another, has shown that in frail elderly men, higher serum zinc concentrations, relative to zinc concentrations, were associated with *reduced* femoral neck BMD [16]

Two small, randomized studies have examined the effect of copper intake on markers on bone health. In one study, 11 males aged 20–59 years were studied sequentially on diets containing low (0.7 mg/day), medium (1.6 mg/day), and high (6.0 mg/day) copper intakes for 8 weeks each. When these subjects were switched from the medium to low copper intake there was a significant increase in urinary markers of bone resorption, and a significant decrease when they were switched from the low to high copper intake [17]. A further study by the same investigators [18] considered 24 adults, 22–46 years, who were studied three times—following 6 weeks of treatment with 3 mg/day copper sulfate, 3 mg/day copper-glycine chelate, and 6 mg/day copper-glycine chelator.

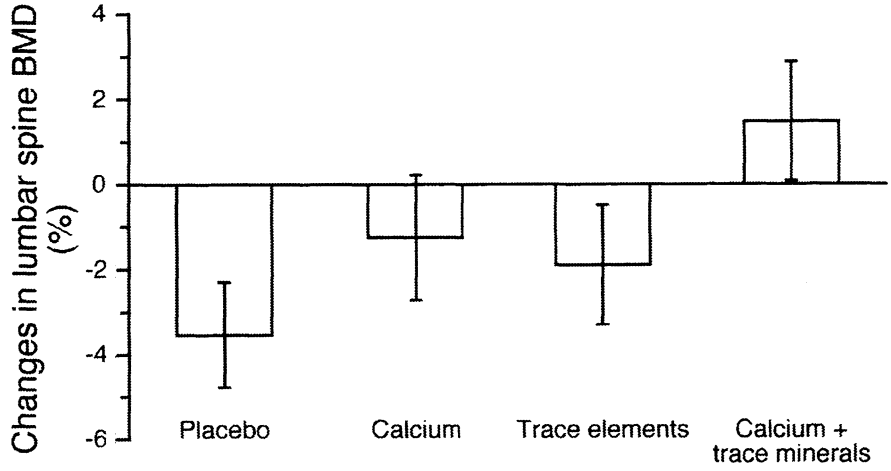

Fig. 25.2 Changes in percent bone mineral density (BMD) from the study of Strause et al. (data extracted from ref. [20]). Mean changes over the 2-year study period are shown for the four treatment groups. Error bars represent ±1 standard error of the mean

There were no differences in serum osteocalcin (a marker of bone formation), or the urinary pyridinoline: creatinine ratio or the urinary deoxypyridinoline:creatine ratio (markers of bone resorption). It is worth noting, however, that markers of copper status did not change among the three treatments [18]. A similar study by Cashman et al. [19] compared changes in markers of bone turnover and resorption after 4 weeks treatment with 3 mg/day copper, 6 mg/day copper, and placebo in 16 healthy females, 20–28 years old. Copper treatment significantly increased the markers of copper status, serum copper, and erythrocyte superoxide dismutase. However, no differences were noted in markers of bone formation (serum osteocalcin) or bone resorption (urinary pyridinoline: creatinine ratio or urinary deoxypyridinoline: creatinine ratio).

Strause and coworkers reported the only long-term interventional study. They studied 59 postmenopausal women randomized to one of four treatments for 2 years [20]. They were given either a placebo, calcium (1,000 mg/day), trace elements alone (15 mg/day zinc, 5 mg/day manganese, and 2.5 mg/day copper), or both calcium and trace elements. After 2 years of treatment lumbar spine bone mineral density fell in all the groups except those receiving both calcium and trace elements (Fig. 25.2). The only significant difference was between the placebo group and the group receiving both calcium and trace elements. The calcium-only and the trace element only groups were intermediate between the placebo and calcium-plus-trace element group (Fig. 25.2).

25.2.4 Conclusions

Although overt copper deficiency has serious effects of the skeleton, the role of milder forms of copper deficiency remains unclear. Although it has been hypothesized that suboptimal copper intake may be an etiological factor in human osteoporosis, direct evidence for this is lacking. Indeed, the few interventional trials that have looked at the effect of copper supplementation on bone health have been small, of generally short duration and they have assessed proxy markers of bone formation and resorption [17, 18] rather than bone density. One study, however, does suggest a benefit to addition of magnesium, copper, and zinc to calcium to reduce postmenopausal bone loss. Whether this benefit is attributable to copper, zinc, or manganese is unclear. Clearly, there is much to learn about the role of

copper in bone health, especially in populations without clinically apparent copper deficiency. What is urgently needed are large-scale, long-term, randomized studies of copper supplementation on bone density or fracture risk. In the absence of such studies the role of copper as an etiological factor in osteoporosis will remain unclear.

25.3 Zinc

Zinc is a component of more than 200 enzymes, and overt zinc deficiency is well characterized. The principal clinical features are diarrhea, dermatitis, alopecia, delayed sexual maturation, and decreased taste acuity [21]. Bony changes do not typically feature as symptoms of zinc deficiency; however, even the earliest reports of human zinc deficiency recognized that short stature was a relatively consistent feature [22].

25.3.1 Observational Human Studies

The possible role of zinc as a cause of osteoporosis has increased as several studies have shown that humans with osteoporosis have reduced plasma zinc concentrations [23, 24] and increased urinary zinc excretion [25, 26]. The latter, however, may be a result of increased bone loss, as approximately one-third of total bone zinc is found in bone [21]. However, a number of studies have suggested that lower zinc intakes are associated with lower bone mineral content [15], lower bone mineral density [27], and may be a risk factor for subsequent fractures [28].

25.3.2 Animal Studies

Data from cell culture, tissue culture, and animal studies have identified a large number of potential beneficial effects of zinc on bone formation and mineralization [29]. Zinc may stimulate osteoblasts proliferation, stimulate bone protein formation [30, 31], increase transcription factors involved in pre-osteoblast differentiation, decrease bone resorption, and reduce osteoclast differentiation [29]. Zinc may increase bone protein content [32], DNA content [32], and insulin-like growth factor-1, and transforming growth factor-β production [33, 34], which may be important for fracture healing.

In rats, experimental zinc deficiency can lead to low-turnover osteopenia [35] and worsen experimental diabetic osteoporosis [36]. Conversely, zinc supplementation may ameliorate the bone loss that accompanies skeletal unloading in rats [37], and increase serum and bone alkaline phosphatase content in mice [38] and rats [39]. Increasing zinc intake can lead to dose-dependent increases in bone strength [40]. However, on a low-calcium diet it had the opposite effect, worsening bone strength and elasticity [41]. One confounding factor may be the anorexia that accompanies zinc deficiency. Zinc-deficient rats have lower femur weights than pair-fed or ad lib-fed controls, but bone volume was similarly reduced in zinc-deficient and pair-fed controls, compared to ad lib-fed controls [42]. In ovarectomized rats, zinc supplementation restored normal bone morphology (improved bone area, perimeter, and max diameter in the tibia and femur), and restored bone zinc and copper levels [43].

In higher animals, zinc deficiency leads to poor bone growth in pigs [44]. In rhesus monkeys made marginally zinc deficient from conception to 3 years of age, bone maturation was delayed. Although bone mineralization was reduced at 6 months of age, by 3 years of age it had largely returned to normal [45].

25.3.3 Human Studies

Given the confusion of the animal and basic sciences literature, what is needed is well-designed, large-scale intervention studies in humans. However, there is a paucity of these. In a study of calcium supplementation, Freudenheim et al. [46] showed that subjects with higher dietary zinc intake have reduced losses in radial bone mineral density. However, the benefit was seen only in those subjects in the placebo limb of the trial, not in those who received calcium supplementation.

The study of Stause et al. [20] is discussed above, although the design of that study does not allow an assessment of whether copper, zinc, or manganese was responsible for the benefits observed. Peretz et al. [47] examined the effect of 12 weeks of zinc supplementation in healthy men. They demonstrated a significant increase in serum alkaline phosphatase in the zinc-treated group, but not in controls. There was no effect of urinary and C-terminal collagen peptide (a measure of bone resorption).

A recent randomized controlled trial in women (51–70 years old) compared the effect of combined zinc (12 mg/day) and copper (2 mg/day) supplementation in women already receiving calcium and vitamin D supplementation [48]. Both groups demonstrated a fall in BMD during the study, but the rate of decline was higher in the group supplemented with zinc and copper [48]. In a secondary analysis, the main determinant of the between-group differences appeared to be zinc intake [48]. In women whose dietary zinc intake was less than 8 mg/day, zinc supplementation was beneficial [48]. But in those whose dietary zinc intake was greater than 8 mg/day, addition zinc and copper supplementation worsened BMD [48].

25.3.4 Conclusions

Profound zinc deficiency appears to lead to reduced bone growth and maturation, probably through an effect on protein synthesis. There is very limited evidence (based on a trial of combined zinc and copper supplementation), that zinc may be beneficial is *some* women [48]. Those most likely to benefit appear to be those with lower dietary zinc intakes [48]. However, the detrimental effect in women with higher zinc intakes is worrisome, and further studies using zinc supplements alone are needed before clear guidance can be given.

25.4 Boron

The role of boron in human nutrition remains uncertain. Boron has been hypothesized to enhance bone mineral balance, although its mechanism of action is uncertain.

25.4.1 Animal Data

A study in ovariectomized rats showed that a combination of boron and estrogen (17-β-estradiol) increased apparent absorption of calcium, phosphorus, and magnesium. This effect was not seen for boron alone or for estrogen alone. No benefit was seen for boron in combination with parathyroid hormone [49]. This study is consistent with previous data in several animal species indicating an increase in mineral balance with supplemental boron [50]. Rats gain more bone mass in response to exercise when provided with boron compared to those without boron [51].

25.4.2 Human Studies

Few human studies have evaluated the role of boron in bone mineral metabolism. In one, providing boron to 12 postmenopausal women, who had been maintained on a low-boron diet for about 4 months, decreased the urinary excretion of calcium and magnesium. A lowered urinary phosphorous excretion was seen in those with a low-magnesium diet. In that study and in one in adults, it has been suggested that boron may act by increasing serum 17-β-estradiol [50].

Two recent studies have reported nutritional interventions that include boron. In the first, women age 40y or aged were recruited to three different nutritional supplements sequentially. The plans all contained about 750 mg calcium, but variable amounts of vitamin D (plan 1 = 1,000 IU, plan 2 = 800 IU, plan 3 = 1,600 IU) [52]. Two of the plans (#2 and #3) contained 780 mg/day strontium, and one (plan #3) contained 3 mg/day boron [52]. After 6 m, the women with the best compliance had higher increases in BMD if they were on plan 3 [52]. It is impossible to say what, if anything, drove this between group difference as the plans varied in several nutrients, particularly in vitamin D [52]. A similar study by the same authors [53] also showed benefits to the nutritional plan containing 3 mg/day boron. However, once again, this was confounded by the higher vitamin D level of the boron containing plan (1,600 IU vs. 800 IU) [53].

Finally, in a randomized trial of supplementation with dried plums (prunes) or dried apples, in women receiving calcium and vitamin D supplementation, the group consuming dried prunes had significantly higher ulnar and spine BMD than those receiving dried apples [54]. Prunes are one of the best dietary sources of boron, but it is not possible to say whether these differences were due to the difference in boron intake.

25.4.3 Conclusion

Although some data support a role for boron on bone health, especially in postmenopausal women, substantial further research, including well designed controlled trials, is needed to clarify the role for this nutrient as well as its physiological mechanisms of action. The quality of the currently reported trials of boron supplementation (or supplementation with boron-rich foods) is inadequate to guide any recommendation.

25.5 Strontium

Strontium has been proposed as effective in enhancing bone health. Stable strontium has been widely used as a marker for assessing calcium absorption, as it appears to be absorbed via similar pathways and share physical properties, including having its absorption stimulated by vitamin D [55].

25.5.1 Animal Studies

Several studies have evaluated the effects of strontium (as strontium ranelate) on bone formation and resorption [56]. These studies have demonstrated a positive effect on bone formation in growing rats as well as prevention of bone resorption in ovariectomized rats [56, 57] The mechanism of action is unknown, but the similarities of calcium and strontium suggest that it may be directly implicated in

physically strengthening bone as well as having hormonal effects. Of significant interest is that bone strontium levels are closely correlated with plasma strontium, a relationship not seen with calcium [58]. A recent study in mice confirmed a significant increase in trabecular bone mass strontium with long-term strontium ranelate treatment [59].

25.5.2 Human Studies

Results of two relatively large randomized controlled trials of strontium supplementation are now available: the SOTI trial [60] and the TROPOS trial [61, 62]. Both trials examined the effect of strontium ranelate (2 g/day) given for 3 years, in subjects who were already receiving calcium and vitamin D supplementation.

In the SOTI trial, 1,649 postmenopausal women with osteoporosis and history of vertebral fracture where randomized to 2 g/day strontium ranelate or placebo. Over the 3 years treatment period, BMD increased in the strontium group, but fell in placebo group [60]. After 3 years, the difference in BMD was 14.4 % at the lumbar spine, 8.4 % at the hip, both favoring strontium treatment [60]. More importantly, not only was BMD improved, the risk of fractures was reduced by 41 % in the strontium group compared to the placebo group [60].

The TROPOS trial was of similar design [61]. A total of 5,091 postmenopausal women were randomized to 2 g/day strontium ranelate or placebo [61]. As in the SOTI trial, BMD increased in the strontium-treated subjects but fell in the placebo-treated subjects [61]. At 3 years, femoral neck BMD was 8.2 % higher in the strontium group than the placebo group [61], very similar to the 8.4 % difference seen in SOTI trial [60]. After 3 years of treatment, strontium lead to significant reductions in the risk of nonvertebral and major fragility fractures. In subgroup with highest risk (most similar to the SOTI population [60]) it reduced risk of hip and vertebral fractures [61]. Slightly over half of the TROPOS population were followed-up at 5 years [62], when strontium reduced nonvertebral fractures by 15 %, reduced hip fracture by 43 %, and reduced vertebral fractures by 24 % [62]. Both the SOTI and TROPOS studies recruited only women. However, one small multicenter study suggests that strontium (2 g/day, as strontium ranelate) has similar effects on BMD in men as it did previously in women [63]. Lumbar spine BMD, femoral neck BMD, and hip BMD were all higher in men receiving strontium than those receiving placebo [63].

Data from the SOTI and TROPOS trials suggests that strontium is well tolerated [60–62], and this also appears to be true after 10 years of follow-up [64]. In one observation study of 1,200 subjects with a mean follow-up of 32 m, strontium seemed well tolerated and compliance with therapy was good [65].

At present, strontium ranelate is supported by expert panels [64], and licensed in the EU, but not in the US [66]

25.5.3 Conclusions

These animal and human studies suggest that relatively large doses of strontium are beneficial in decreasing bone resorption, enhancing bone mineralization, and reducing fractures. They suggest that benefits are maintained for at least 10 years. No toxicity or significant adverse effects have been reported with this therapy, although as this therapy becomes more widespread, ongoing surveillance and follow-up are needed.

25.6 Silicon

Silicon has been suggested as an important trace mineral necessary for bone development, but few specific data are available. Rico and coworkers found that ovariectomized rats that were provided silicon had a lower rate of bone loss [55]. The beneficial effect of silicon on bone health in ovariectomized rats may be limited to those with inadequate calcium intake [67], although data is contradictory [68]. A very small retrospective study suggested a benefit to silicon in bone density in osteoporotic adults [69].

Data from the Framlington offspring cohort has demonstrated a significant positive association between silicon intake and hip BMD in men, and premenopausal women [70]. But no such relationship was seen for postmenopausal women [70]. In men, beer is a significant source of silicon intake and the authors suggest that this may explain the previous reports of a positive association between alcohol intake and bone health [70].

This is supported by a more recent study showing that silicon intake is associated with improved bone mineralization, but only in estrogen-replete women (i.e. premenopausal or postmenopausal women on hormone replacement therapy) [71].

25.7 Other Trace and Ultratrace Minerals

Fluoride may have a role in bone mineralization in rodents [72]. Data in humans in contradictory, and the exact dose may be critical [73].

Early studies examining relatively high fluoride intakes (≥50 mg/day) showed that fluoride supplements significantly increased BMD [74, 75] particularly in cancellous bone [74]. Despite this change in BMD, the rate of fractures was not reduced by fluoride supplementation [74, 75] and side-effects were more common in the fluoride-treated individuals [74, 75].

Three studies have examined low dose fluoride supplementation (≤20 mg/day) using a variety of continuous [76–78] or intermittent (3 m on, 1 m off) [77] dosing schedules, with inconsistent results. One study has shown that 20 mg/day fluoride increased BMD and reduced vertebral fractures over 4 years from 10 to 2.4 % [76], while another suggests that doses of 2.5–10 mg/day have no effect on either BMD or markers of bone turnover [78]. Finally, Ringe et al. compared daily dosing of 20 mg/day fluoride as monofluorophosphate, intermittent dosing of monofluorophosphate (3 m on, 1 m off) or placebo [77]. Both fluoride dosing schedules lead to improved BMD, and the intermittent schedule was better tolerated [77].

Numerous other minerals have been proposed to have either an enhancing or harmful effect on bone (e.g., aluminum). Among these is manganese, although evidence for an effect is very minimal [79]. Because these are uncommonly deficient in diets and are difficult to assess in isolation from other minerals, it has been difficult to obtain solid information regarding their role, and therapeutic use should be considered only in the context of controlled trials.

Conclusion: There are only limited data on the role of trace minerals in bone health. Although overt copper deficiency has serious effects of the skeleton, the role of milder forms of copper deficiency remains unclear. Profound zinc deficiency appears to lead to reduced bone growth and maturation, probably through an effect on protein synthesis. There is very limited evidence that zinc may be beneficial is *some* women probably those with lower dietary zinc intakes. However, the detrimental effect in women with higher zinc intakes is of concern. Although some data support a role for boron on bone health, especially in postmenopausal women, substantial further research, including well

designed controlled trials, is needed to clarify the role for this nutrient as well as its physiological mechanisms of action. These animal and human studies suggest that relatively large doses of strontium are beneficial in decreasing bone resorption, enhancing bone mineralization, and reducing fractures. Silicon may play a role in bone health but data are limited.

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Part V
Fat-Soluble Vitamins/Micronutrients

Chapter 26
Vitamin A and Bone Health

Peter Burckhardt

Key Points

- The main sources of Vitamin A are preformed vitamin A (retinol or retinyl esters) from animal food, fortified foods and pharmaceutical supplements.
- Precursors as beta-carotene and other Vitamin A forming carotenoids from green plants, carrots and some fruits.
- Studies evaluating the association between serum retinol level or retinol intake and skeletal health in humans showed inconsistent results.
- It is difficult to get an accurate assessment of vitamin A intake and serum retinol level is an unstable marker and a poor indicator of vitamin A status.
- The proposed safety limit of 3,000 mcg retinol (10,000 IU) is not based on the hip fracture risk.
- Very high and low intakes of vitamin A are associated with a decreased BMD.
- In the elderly, the balance which ensures a sufficient retinol intake and simultaneously protects against excessive supplementation, is very delicate.

Keywords Vitamin A • Retinol • Isotretinoin • Deficiency • Hypervitaminosis • Supplementation • Bone loss • Fracture risk

26.1 Introduction

Vitamins are presumably not produced by the human organism although they are vital for its health. They were discovered by their efficacy in healing the detrimental consequences of deficiency states. They are taken as supplements by large proportions of the population, especially the elderly persons, and used as drugs in pharmacological doses for their presumed health effects beyond the doses known for the correction of a given deficiency, and for the fear of latent states of deficiency. Because of the lack of dose finding studies and the insufficient knowledge of toxicity, they are often consumed in inappropriately high amounts. The unconsidered overuse of vitamins led to the hazardous discovery of adverse effects, which were later documented by appropriate investigations. By consequence, the recommendations for an adequate vitamin intake moved from the "Recommended Daily Allowances,"

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which prevent deficiency states in the majority of the population, to the definition of safety limits and to "Estimated Safe and Adequate Daily Dietary Intakes," rather confusing terms for the consumer [1].

This chapter analyses the effect of vitamin A and of its overuse on bone health, in order to emphasize the importance of adequate doses.

The term "Vitamin A" is used for a group of essential, fat-soluble, unsaturated 20 carbon cyclic alcohol compounds, the most important ones being retinol and provitamin A (beta-carotene). The main sources of Vitamin A are preformed vitamin A (retinol or retinyl esters) from animal food, especially liver and fish oil, from fortified foods and pharmaceutical supplements, and precursors as beta-carotene and other Vitamin A forming carotenoids from green plants, carrots and some fruits. Globally, preformed vitamin A provides 30 % of all dietary vitamin A activity [2]. In the intestine, retinyl esters are hydrolyzed to retinol, and carotenoids are cleaved to retinol. Retinol is readily absorbed and reesterified to long-chain fatty acids in the epithelial cells, which then are incorporated into chylomicra and transported to the liver, where they are stored mainly as retinyl palmitate. Transport and intracellular distribution occur by the retinol binding protein.

Vitamin A is necessary for growth, visual health, reproduction, and for the immune system.

Vitamin A deficiency is a worldwide concern, especially in developing countries, where the main sources are plant carotenoids, which are poorly absorbed. It is characterized by increased mortality of neonates, xerophtalmia, night blindness, increased susceptibility to infections, and by negative effects on bone, leading among others to cessation of growth. Vitamin A deficiency is also associated to an increased cancer risk, because beta-carotene is a dietary anti-oxidant [3]. Intakes of beta-carotene-rich vegetables, vitamin A, and carotenoids were inversely associated with lung cancer risk [4]. But this relationship to cancer risk is still debated [2]. Vitamin D intake varies considerably by region, socioeconomic class, and age. The populace, particularly young children, is largely dependent on the consumption of provitamin A carotenoids (primarily beta-carotene) in vegetables and fruit. The bioavailability of dietary sources of b-carotene is low, and the food supply of Asia and sub-Saharan Africa can cover only half of the vitamin A required per capita [2]. In the year 2011, globally, over two billion people were considered at risk for vitamin A, iodine, and/or iron deficiency [5]. On the other side, inadequately high vitamin A intake affects, among others, bone and bone metabolism, as known since almost 70 years [6].

26.1.1 Animal and In Vitro Studies on the Bone Effect of Vitamin A

26.1.1.1 Vitamin A Deficiency

Vitamin A deficiency definitely has a negative impact on bone development in animals. In pregnant rats it induces congenital spinal deformities in the postnatal rats. It is suggested that vertebral birth defects may be caused by a defect in the retinoid acid signaling pathway during somatogenesis [7], which goes along with the observation that defects in organogenesis occur at a high penetrance in case of late embryonic vitamin A deficiency [8]. Negative effects were also observed in adult mice, where vitamin A deficiency delays bone healing in association with reduced BMP2 expression [9].

26.1.1.2 Hypervitaminosis

In vitro studies showed in summary that vitamin A (retinol) inhibits collagen synthesis [10], that retinoic acid directly stimulates osteoclastic bone resorption [11], whereas other studies showed that retinol and retinoic acid have an inhibitory effect on osteoblastic cell proliferation in vitro [12]. As a short, and not referenced overview it can be mentioned, that osteoblasts and osteoclasts express

nuclear receptors for retinoic acid, that high doses of vitamin A increased osteoclast numbers and reduced osteoid surfaces in rats, that vitamin A—and in some experiments also retinoic acid - inhibited bone collagen synthesis, and that despite stimulation of collagenase synthesis in osteoblasts, formation of osteoclasts and osteoclastic bone resorption were enhanced.

In vivo *studies* showed that Vitamin A is the only molecule known to induce spontaneous fractures in laboratory animals, as already reported in the 1940s. Research on vitamin A toxicity has been carried out on acute effects mainly, with various forms of vitamin A given parenterally, and for this reason the results cannot be extrapolated to humans. In general, vitamin A toxicity was reported to lead to accelerated bone resorption and to fractures [13], hypercalcemia and various bone anomalities. Other studies showed that high vitamin A intake in rats increased BMD although trabecular area was decreased.

The negative effects of vitamin A intoxication are already present in utero and in neonatal animals, with a detrimental effect on bone growth in the fetus of pregnant rats. As summarized by Ahmadieh et al. on the basis of partially older references [14], excess feeding with vitamin A or synthetic retinoids was associated with poor bone growth and radiolucency, accelerated bone remodeling with consequent loss of bone mineral content (BMC), and increased rate of spontaneous fractures in different species of animals. In the long bone of adult rodents treated with retinoids bone diameter shrank, due to subperiosteal bone resorption [15]. Moreover, treatment of rats for 15–20 weeks with either all-trans retinoic acid or 13-*cis* retinoic acid reduced BMC, BMD, bone diameter, and cortical thickness of the femur, and increased the incidence of spontaneous fractures as compared to controls [16].

More recent studies added further evidence: Perinatal exposure to vitamin A differentially regulates chondrocyte growth and the expression of matrix metalloprotein genes in the femur of neonatal rats [17]. Excess retinol impairs rapidly the endosteal/marrow blood flow which leads to hypoxia and pathological endosteal mineralization [18]. On the other side, retinoic acid also inhibits nuclear factor of activated T cells c1 (NFATc1)) expression and osteoclast differentiation [19]. It increases proliferation of human osteoclast progenitors and inhibits RANK-stimulated osteoclast differentiation by suppressing RANK [20]. This is in agreement with the previous observation that retinoids inhibit differentiation of hematopoietic osteoclast progenitors [21]. But it was also reported that retinoids stimulate periosteal bone resorption by enhancing the protein RANKL [22]. Some contradictions in these observations are explained be the fact that the effects of vitamin A seem to be area specific with reduced activity in cortical bone but increased activity in the endosteal/marrow compartment [23]. In addition to this partially confusing picture, cellular retinol-binding protein 1 (CRBP-1) also has a role, since it regulates osteogenenesis and adipogenesis of mesenchymal stem cells [24].

A new light was shed on the bone effect of vitamin A with the investigations of the interactions with vitamin D. In aged rats, a model closer to human osteoporosis than young and growing animals, a high vitamin A intake had negative effects in conjunction with a low calcium intake [25]. Embryonic skeletal hypoplasia was partially explained by the suppression of retinoic acid-receptors resulting from vitamin A deficiency in maternal rats with chronic vitamin D deficiency [26]. Later investigations pointed to an interference with the utilization of vitamin D and its hydroxylased metabolites. Vitamin A antagonised the ability of vitamin D to maintain normal plasma calcium levels in the rat [27], resulting in higher vitamin D requirements. On the other side, it has a protective effect against hypervitaminosis D [28, 29], specifically against the bone resorption induced by 1.25 $(OH)_2$ vitamin D_3 in vitro [30]. There are nuclear receptors of vitamin A in the bone of rats, and vitamin A influences gene expression [31] in conjunction with Vitamin D. The receptors of both vitamins bind to the target genes as heterodimers, with 1,25$(OH)_2D_3$ activating the vitamin D receptor-Retinoid X receptor complex [32]. Too much vitamin A increases the formation of retinol X receptor–retinoic acid receptor complexes, thereby reducing the availability of retinol X receptor [33] and decreasing the efficacy of vitamin D. It also causes rickets in normally replete animals [29]. This interaction has been demonstrated also in man by the observation that retinyl palmitate decreased plasma calcium and diminished the Calcium response to 1,25 $(OH)_2$ vitamin D_3 [34].

26.1.2 Inadequately High Vitamin A Intake in Humans

Chronic toxicity of vitamin A results from the ingestion of high amounts of preformed vitamin A for months or years. A daily intake of 25,000 IU (7,500 mcg) for 6 years or 100,000 IU (30,000 mcg) for 6 months is considered toxic, but there is wide inter-individual variability for the lowest intake required to elicit toxicity [28, 35, 36].

Individual tolerances to different amounts of vitamin A ingested on a chronic basis have not been adequately studied. The role that genetics may play in this regard is unknown. Toxicity from provitamin A, the major nutritional source, is largely impossible. The absorption of beta-carotene [37] and its transformation into vitamin A seems to be sufficiently controlled. For the same reason, supplementation with beta-carotenes does not increase serum retinol concentrations [38]. In contrast, absorption and hepatic storage of preformed vitamin A occur very efficiently until a pathologic condition develops.

Vitamin A and its precursors are quantified in micrograms, Retinol Activity Equivalents (RAE or RE in mcg), and International Units (IU). 1 IU is 0.1 mcg RE when the origin is plants, and 0.2 mcg RE when the origin is animal. 1 mcg Retinol is obviously 1 mcg RE. 1 mcg RE corresponds to 3.3 IU.

The recommended intake for postmenopausal women is 700 mcg/day (IOM), resp. 2,310 IU Retinol Equivalents (NOF). The upper limit is set at 3,000 mcg (IOM), resp. 10,000 IU RE [39]. The respective amounts for men are 900 mcg/day and 3,000 IU [40, 41].

In developed countries, where preformed Vitamin A is the major form of supplements, intakes often exceed these recommendations [40–42]. In the US an average intake of vitamin A of about 2,300 mcg was reported [41]. Recent data from the 2007–2008 NHANES survey showed a medium vitamin A dietary intake (all vitamin A compounds, e.g., alpha carotene, beta carotene, and cryptoxanthin converted into vitamin A) of 551 mcg/day, and with food plus supplements of 1,205 mcg. In 10 % of the population the intake was above 2,370 mcg [43]. In another American study the vitamin A intake of postmenopausal women was clearly above the RDA at 1,400 mcg RE, with >75 % coming from preformed vitamin A, and the retinol intake of nearly one-third of the women exceeded 1,500 mcg/day [44]. In 20 % of American women of a recent WHI report the intake of Vitamin A was above 7,508 mcg RE/day and that of Retinol above 1,426 mcg/day [45]. Many subjects take a daily dose of even 7,500 mcg, resp. 25,000 IU [40, 41]. In Norway the intake was reported to be 1,500–2,000 mcg RE [46] with intakes up to 17,000 IU per day [36].

This overuse is the consequence of the frequent use of vitamin supplements. Herbals or supplements are consumed by 14–46 % of the U.S. population [47]. Nearly half of U.S. adults aged 20–69 have taken at least one dietary supplement in the month before the test [43]. Over one third of women physicians in the U.S. and of postmenopausal women take vitamin supplements regularly [48, 49]. Indeed, Vitamin A preparations are easily available [50]. Therefore, in some countries considerable proportions of the population exceed the safety limit of 3,000 mcg/day. Indeed, in developed countries, the increasing interest and availability of fortified foods and supplements resulted in a large percentage of the population with preformed vitamin A intakes higher than recommended. Observational studies suggest that more than 75 % of people may be routinely ingesting more than the recommended dietary allowance (RDA) for vitamin A, much of it as preformed vitamin A [51].

High vitamin A intake can be toxic in humans. Intakes of 15,000 mcg (resp. 25,000–50,000 IU) per day taken over several months [13] or an acute ingestion of more than 100,000 IU [52] lead to adverse effects, such as birth defects, various bone anomalies, and liver toxicity, effects that are rarely observed at lower doses [1]. Because vitamin A can be toxic, due to its long half-life (large storage capacity of the liver), the Food and Nutrition Board of the U.S. set the safety limit at 3,000 mcg per day retinol (10,000 IU/day, as already mentioned [53, 54], but this limit may still not provide an adequate margin of safety [13].

26.1.3 Fish Oil

Fish oil is rich in vitamin A and D. A large Norwegian study showed that women reporting no cod liver oil intake in childhood had statistically significantly higher BMD than those who reported any ingestion of cod liver oil [55]. This was explained by the very high content of Vitamin A in cod liver oil at the time. The vitamin A content of commercial cod liver oil was recently reduced by 75 % in Norway [59]. In general, the vitamin A content of fish oil depends mainly on the fish species. An analysis of 22 fish oil preparations commercialized in Germany showed high a vitamin A content only in a few brands [56]. In one the levels were so high that an intake of 3–4 times over the recommended dose would be dangerous. Therefore, the uncontrolled consumption of fish oil preparations might hardly lead to intakes beyond the safety limits, but added to a relatively vitamin A rich food and vitamin supplements, it can be a crucial contribution to the total intake.

26.1.3.1 Assessment of Vitamin A Intake

Vitamin A can be measured either by assessing intake, which is difficult, or by measuring the plasma levels. Intake is assessed by dietary records, food frequency questionnaires, and records of supplement intake, and has to report the total vitamin A intake, the sum of all sources of vitamin A and its metabolites, or the different sources separately. Most studies calculated the total vitamin A intake in IU or mcg. The more accurate but cumbersome approach of the separate assessment of retinol and of provitamin A carotenoids, mainly beta-carotene is rarely applied to larger studies. The 24 h recall is also unfit for evaluating the average intake over a longer period because of the daily variation of vitamin A intake due to the unequal distribution of vitamin A in food—at least when applied several times [40]. It has even been stated that more than 365 days would be required to estimate the long-term intake of vitamin A [57]. In some studies the large number of individuals examined compensated for this statistical requirement.

Measurements of plasma levels can include retinol, which correlates with the consumption of meat, fish, oil, and alcohol, or beta-carotene, which reflects the intake of green vegetables [58]. The normal range of plasma retinol is very wide, which almost excludes the interpretation of an individual value in search of an insufficient or a high intake. Retinyl-esters are markers of excess intake [59, 60]. Their level increase when the intake exceeds either the capacity of liver storage, or that of retinol-binding protein production. An increased ratio of retinyl-esters over the sum of retinyl-esters plus protein bound retinol is a marker of excess intake. The vitamin A status can also be assessed in humans by measuring the dilution after injection of a labelled vitamin A, a technique that was not used in relation to bone health [61]. The measurement of retinol binding protein evaluates the nutritional state and not specifically the vitamin A intake of an individual, and was found—only for this reason—to be low in osteoporosis [62].

The range of serum retinol concentrations under normal conditions is 1–3 mol/L [63]. Retinyl esters in serum are normally below 0.2 mol/L in the fasting state [60], but they increase significantly after a large intake of vitamin A, such as a vitamin A–rich meal or vitamin A supplements. Elevated levels in the fasting state have been used as markers for chronic hypervitaminosis A in humans and monkeys [64].

26.1.4 Effect of Vitamin A Intake on Bone Health; Human Data (See Table 26.1)

The first reports on chronic adverse effects of Vitamin A were isolated case reports of children and adults, published from the 1960s into the 1990s [65]. They described effects on the skeleton and on

Table 26.1 Studies on the association between vitamin A intake and bone health

Author year	Ref	Population	Assessments[a]	Bone measurements	Sign. negat. association
Cross-sectional studies					
Yano (1985)	70	1,208 men >60 years 912 women >50 years	Vit. A intake e	BMC by SPA radius, ulna, calcaneus	None (men) Women: positive correlation with forearm BMC
Sowers (1985)	71	324 PM women	Vit. A intake suppl. and/or diet e	BMC by SPA mid-radius	None
Sowers (1990)	72	246 women 55–80 years	Vit. A intake suppl.and/or diet e Serum retinol	BMC by SPA radius Fractures	None None
Freudenheim (1986)	37	81 women 35–65 years	Vit. A intake suppl. + diet e	BMC by SPA radius, ulna, humerus	None
Melhus (1998)	78	175 women 40–74 years 47 fractures/873 controls	Vit. A and beta-Carotene intake a	BMD at any site Hip fract	BMD reduced at all sites for retinol intake >1,500 mcg/day vs ≤500 mcg/day Increased fx risk; OR 2.1
Sigurdsson (2001)	76	232 women 70 years	Vit. A intake a	BMD at any site	None
Ballew (2001)	73	5,790 men and women, 20→80 years	Serum etinyl esters	BMD at any site	None
Rejnmark (2004)	74	1,869 women ± 55 years After 5 years: 1,142 women	Retinol or beta-carotene intake g	BMD LS, FN	None
Wolf (2005)	77	11,068 women WHI 50–79 (±63) years	Retinol or total vit. A intake = retinol + pro-vit. A-carotenoids +/− suppl. f	BMD at any site + total body	None
Penniston (2006)	68	30 women with osteoporosis + 29 controls 49–82 years	Serum retinol and retinyl-esters	BMD LS, TF by DXA	None[b]
Follow-up studies					
Freudenheim (1986)	37	80 women, 35–65 years Follow-up 3 years	Vit. A intake d	Loss in BMC forearm by SPA	Sign. only ulna in post MP women
Houtkooper (1995)	82	66 women, 28–39 years Follow up 1.5 years	Vit. A and Caroten intake g	BMD by DXA	*Slowing* of the total body BMD loss
Promislow (2002)	84	570 women, 388 men, 55–92 years Follow-up 4 years	Vit. A intake f	BMD by DXA	Sign
Michaelsson (2003)	85	2,322 men, 49–51 years Follow-up 30 years	Serum retinol Serum beta-carotene	Hip fractures	Sign. RR 2.47 in highest retinol group
Rejnmark (2004)	74	1,694 women 45–58 years Follow-up 5 years and: 163 fractures and 978 controls	Retinol or beta-carotene intake g	BMD by DXA Fractures	None None

Barker (2005)	75	Women 79–81 years 312 fractures and 934 controls 3.7 years follow-up	Serum retinol, retinyl palmitate, and beta-carotene	BMD tot.hip Fractures	None[c] None
Caire-Jevera (2009)	81	75,747 women WHI 49–77 years 3 years	Vit. A intake Retinol intake e	Hip fract Total fract	None But modest increase of total fract. in the group with low Vit.D intake
Feskanich (2002)	83	72,337 woman of NHS, 34–77 years	Vit. A intake b	Hip fractures	RR for hip fract. 1.48 when intake >3 mg versus <1.25 mg
			Beta-caroten intake b		None
Interventional trials			Intervention:		
Johansson (2001)	86	9 men, 21–41 years 1 day	Retinol-palmitate	Response to $1,25(OH)_2$ Vit D_3	Interaction
Kawahara (2002)	87	80 men, 18–58 years 6 weeks	Retinol-palmitate	Bone markers	None

BMD at any site : lumbar spine + all conventional sites of proximal femur

[a]Assessments: In addition to the registration of supplement intake: (a) 1-week dietary record, four times; (b) FFQ, type of fat/oil; (c) FFQ including retinol, beta-carotene; (d) structured 24 h record, 72 times/3 years; (e) 24 h recall, eventually numerous, eventually structured, (f) self-report with semi-quantitative FFQ; (g) 4- or 7 days food record, controlled, ev. multiple

[b]Trend for the association of serum retinyl esters as percentage of total vitamin A with osteoporosis ($p < 0.070$) after adjustment for BMI and triacylglycerols

[c]High serum retinol tends to predict BMD benefit ($p < 0.002$)

bone metabolism, such as hypercalcemia, retardation or arrest of growth, bone pain, hyperostosis, accelerated bone loss, etc. [40]. These observations were usually made with extreme doses of 100,000 IU per day or more. The question if long-term intake of high doses of vitamin A have a lasting negative effect on bone has been answered by many studies, with different results.

26.1.4.1 Cross-Sectional Studies

A series of cross-sectional studies which correlated Vitamin A intake with BMC, showed no such negative association. These studies used single photon absorptiometry (SPA) to measure bone density (i.e. BMC) on the forearm, which mostly concerns cortical bone [36, 49, 66, 67]. Trabecular bone, the bone tissue which is most sensitive to changes in bone metabolism, is mainly present in the ultradistal part of the radius (as in vertebral bodies), but this site was rarely included in the studies. Another reason why these studies might have missed an effect of vitamin A on bone, is the relatively small number of subjects included.

When the Vitamin A intake was assessed by measurements of Vitamin A or its metabolites in serum, only the current intake was captured, which is not representative for the long-term intake. This explains the absence of any significant association with BMD or fractures [49, 64], even when the number of subjects was high [65, 68], or even when the study was otherwise perfectly well designed as a controlled follow-up study or a nested case control study with a large number of patients [69].

More recent cross-sectional studies used DXA for evaluating BMD, but again could not show any association with Vitamin A intake, probably again because of the relatively small number of subjects [70]. In the WHI study [71], the number of subjects studies was very high, but the subjects were probably too young. Studies not focused on elderly subjects, where the impact of vitamin A is easier to demonstrate, might have less chance to detect any influence of vitamin A on bone. In addition, the pitfalls in evaluating the nutritional intake of vitamin A, as discussed above, also add to the difficulties in performing conclusive studies.

However, a Swedish study [72] showed a negative influence of high Retinol intake on BMD and on hip fracture risk in two cross-sectional studies and in a nested case control analysis of a 5 year follow-up, despite the fact that neither the age of the women, nor the number of subjects were higher than in other studies. BMD was reduced at all sites by 6–14 % and the fracture risk doubled (OR 2.1) for a retinol intake of >1.5 mg/day compared to an intake of ≤0.5 mg/day. These significant results were explained by the especially high vitamin A intake in Sweden, where low-fat dairies and breakfast cereals are fortified with vitamin A. The total vitamin A intake exceeded the limit recommended by the WHO by more than three times.

Very high intakes probably concern only a small subgroup of the population [73], but since older adults have a diminished capacity to clear high levels of ingested retinol, it was hypothesized that excess retinol intake may explain the high incidence of osteoporosis in northern Europe [74].

26.1.4.2 Follow-Up Studies

Follow-up studies, which by design are of higher statistical power than cross-sectional studies, also could hardly demonstrate significant correlations between the loss of BMD and the vitamin A intake or fracture incidence. A Danish study showed no significant associations [68], but others showed a vitamin A effect in special conditions. In one study the loss in BMC was associated with high vitamin A intake only at the ulna, but not at the radius, and only in postmenopausal women [36]. The huge WHI study found only a modest association in the sub-group with low vitamin D intake [45]. A study on 312 fracture cases, 79–81 years old, and 934 controls showed even a tendency for higher BMD to be predicted by increased serum retinol [69], while serum retinol was not significantly associated with

fracture risk. In another, although not conclusive and early study, the loss in total body BMD was slowed with high vitamin A intake [42].

The significant results come from three follow-up studies. The Nurses Health Study [75], a large longitudinal study, assessed the vitamin A intake of 72,337 postmenopausal women and found a significant correlation with hip fracture incidence. The highest quintile of vitamin A intake taking more than 3 mg Retinol Equivalents showed a significantly increased fracture risk (RR 1.48), compared to the lowest quintile, except for women on HRT. The RR of hip fracture was 1.89 for Retinol intake, while beta-carotene intake had no significant influence [75]. The impact of vitamin A taken with food on the hip fracture risk was significant, while that with supplements was at the limit of significance. Considering that the population was relatively young for hip fractures (34–77 years), it is probable that an evaluation of the same population some years later will yield a significant relationship with the consumption of vitamin supplements.

The second significant results come from a 4 years follow-up study in elderly men and women (mean age 71 years), where a high retinol intake was associated with BMD and BMD loss at any site. Supplement users were the most afflicted with a 0.23 % greater annual loss at the femoral neck. Total intake was more important than the source of the vitamin A and its metabolites. In both sexes, increasing retinol became negatively associated with skeletal health at intakes not far beyond the recommended daily allowance [76].

The third significant data set comes also from a large follow-up study, where 2,322 Swedish men aged 49–91 years were followed for 30 years. Their relative risk of hip fracture was 2.47 in the group with the highest serum retinol level (75.62 mg/dL) as compared with median quintile, and the RR for any fracture was 1.64 [77].

Interventional studies are scare, since they should be long-term. None measured bone density changes over a long period. The acute trial in nine healthy men confirmed the animal data of an interaction with vitamin D [34] and a controlled 6 weeks trial, where 25,000 IU (7,576 mcg) retinol palmitate were given to adult men, could not detect any effect on bone markers [78].

Taken together, these studies are not conclusive. Many studies applied inappropriate methods. Problems include: patients that are too young, especially for evaluating hip fracture risk; too small numbers of subjects for detecting a subtle association at the edge of significance; the use of SPA at the forearm with insufficient measurement of trabecular bone; serum measurement of other metabolites than Retinol; difficulties in capturing all confounding factors such as nutritional status and intake of multivitamin supplements; or simply examining populations with a modest level of vitamin A intake. This summarizes the difficulties that are encountered clinical nutrition research. However there remains some evidence that very high intake of vitamin A is linked to an increased risk of osteoporosis and hip fractures.

26.1.4.3 Adverse Effects on Bone of Treatment with Synthetic Retinoids

The pharmacological use of synthetic retinoids, such as isotretinoin against acne, and etretinate against psoriasis, are often accompanied by adverse side effects, which illustrates the relatively narrow therapeutic window of these substances. Observational reports link various rheumatologic complications to the use of retinoids [79]. These include bone pain, premature closure of epiphyses, development of osteophytes, calcification of ligaments, etc. [80], and radiographic changes of bone [81]. The main question, if long-term treatments lead to decreased bone mineral density (BMD) and to osteoporosis, remains unanswered. Short-term treatments with isotretinoin did not affect BMD [82]. Etetrinate caused bone loss, while Isotretinoin had again no effect [83, 84]. But in another study Isotretinoin did reduce bone markers, but only for 1–2 weeks [85], and treatment of acne with isotretinoin for 3 months produced significant changes in Calcium metabolism, showing an effect on vitamin D metabolism [86]. In another study, Isotretinoin reduced BMD, but significantly only at the Wards

triangle, which is meaningless, while the measurements of relevant areas of interest did not reveal any significant changes [35]. Children treated with 13-*cis*-retinoic acid for neuroblastoma had growth problems [87]. But not all studies showed bone effects [88]. Especially a large controlled cross-sectional study, which included 124,000 fracture cases and 373,000 controls showed no association between treatments with Isotretinoin or Acitretin and fracture incidence. However, the subjects were relatively young, 43 years in average [89].

These observations, and several others, illustrate the presence of adverse effects of synthetic retinoids on the musculo-skeletal system, but they cannot serve as proof for a negative effect of vitamin A on bone mass; they just point to a probable impact of vitamin A on bone metabolism and bone health.

26.2 Conclusion

Human hypervitaminosis A clearly involves the skeleton, as early reviews already have revealed [13]. However, studies evaluating the association between serum retinol level or retinol intake and skeletal health in humans showed inconsistent results, and this is still the conclusion of recent and excellent reviews [14, 90]. This inconsistency may be related to the difficulty in obtaining an accurate assessment of vitamin A intake, and to the use of serum retinol level, which is an unstable marker and a poor indicator of vitamin A status. There is some evidence that high vitamin A intake accelerates bone loss and increases fracture risk. This association, which is still uncertain, seems to concern the very high retinol intake of a minority and not the usual intake experienced by most persons [40]. Although, it also has to be reminded, that the usual levels of intake are often close or beyond the approximate safety limit, and that the proposed safety limit of 3,000 mcg retinol (10,000 IU) is not based on the hip fracture risk. Skeleton-related adverse effects of vitamin A are sometimes observed and only when supplement intakes reach 25,000 IU [1]. Vitamin A seems to have a relatively narrow optimal dosage window with low and high intakes associated with negative effects, especially in elderly persons [91]. In one of the mentioned studies [76], regression analyses, adjusted for standard osteoporosis covariates, showed indeed an inversed U-shaped association of retinol intake with BMD [76]. Very high and low intakes went along with a decreased BMD, suggesting that in the elderly the balance which ensures a sufficient retinol intake and simultaneously protects against excessive supplementation, is very delicate.

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Chapter 27
Vitamin D

Michael F. Holick

Key Points

- Vitamin D, the sunshine vitamin, is well recognized as being important for the development and maintenance of bone health throughout life.
- The major source of vitamin D for children and adults is from sun exposure. Solar ultraviolet B radiation converts 7-dehydrocholesterol to previtamin D_3 which in turn thermally isomerizes to vitamin D_3.
- Vitamin D_3 from the skin enters the circulation and along with vitamin D_2 and vitamin D_3 coming from dietary sources travels to the liver and is converted to the major circulating form 25-hydroxyvitamin D [25(OH)D].
- 25(OH)D enters the circulation and travels to the kidneys where it is converted to its active form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. 1,25$(OH)_2$D interacts with its vitamin D receptor (VDR) in the intestine resulting in an increase in intestinal calcium absorption. In the skeleton it increases the number of osteoclasts to mobilize calcium from the skeleton when necessary.
- Vitamin D deficiency, defined as a 25-hydroxyvitamin D <20 ng/mL and vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/mL and sufficiency as >30 ng/mL.
- Vitamin D toxicity is usually not observed until 25(OH)D levels are >200 ng/mL.
- Essentially every tissue and cell in the body has a VDR and many cells including macrophages have the ability to convert 25(OH)D to 1,25$(OH)_2$D.
- Epidemiologic and association studies have suggested that vitamin D deficiency increases risk for many acute and chronic illnesses including autoimmune diseases such as multiple sclerosis and type 1 diabetes, cardiovascular disease, several cancers, type 2 diabetes, infectious diseases and neurocognitive dysfunction.
- The Endocrine Society's practice guidelines recommends children 1 year and older receive 600–1,000 IU daily and adults 1,500–2,000 IU daily with the caveat that obese people require 2–3 times more.

Keywords Vitamin D • Sunlight • 25-Hydroxyvitamin D • 1,25-Dihydroxyvitamin D • Rickets • Osteoporosis • Osteomalacia • Cancer • Autoimmune diseases • Infectious diseases

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27.1 Introduction

Vitamin D, the sunshine vitamin, is well recognized as being important for the development and maintenance of bone health throughout life. The major source of vitamin D for children and adults is from sun exposure. Solar ultraviolet B radiation converts 7-dehydrocholesterol to previtamin D_3 which in turn thermally isomerizes to vitamin D_3. Once formed it enters the circulation and along with vitamin D_2 and vitamin D_3 coming from dietary sources travels to the liver and is converted to the major circulating form 25-hydroxyvitamin D [25(OH)D]. 25(OH)D enters the circulation and travels to the kidneys where it is converted to its active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. 1,25(OH)2D interacts with its vitamin D receptor (VDR) in the intestine resulting in an increase in intestinal calcium absorption. In the skeleton it increases the number of osteoclasts to mobilize calcium from the skeleton when necessary. Vitamin D deficiency, defined as a 25-hydroxyvitamin D <20 ng/mL, is one of the most common medical disorders worldwide. Strategies using sensible sun exposure with the app dminder.info along with vitamin D supplementation are discussed in detail. Vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/mL and sufficiency as >30 ng/mL. Vitamin D toxicity is usually not observed until 25(OH)D levels are >200 ng/mL. Essentially every tissue and cell in the body has a VDR and many cells including macrophages have the ability to convert 25(OH)D to $1,25(OH)_2D$. Epidemiologic and association studies have suggested that vitamin D deficiency increases risk for many acute and chronic illnesses including autoimmune diseases such as multiple sclerosis and type 1 diabetes, cardiovascular disease, several cancers, type 2 diabetes, infectious diseases and neurocognitive dysfunction. Because vitamin D toxicity is an extremely rare occurrence based on the totality of evidence to date about the many health benefits of vitamin D it is reasonable to encourage sensible sun exposure in combination with vitamin D supplementation. The Endocrine Society's practice guidelines recommends children 1 year and older receive 600–1,000 IU daily and adults 1,500–2,000 IU daily with the caveat that obese people require 2–3 times more.

27.2 Evolution of Vitamin D

Although it is not certain when vitamin D became critically important for calcium metabolism and bone health for our early ancestors, there is evidence that some of the earliest phytoplankton life forms were photosynthesizing vitamin D more than 750 million years ago [1–3]. Life evolved in a fertile soup that contained all of the organic and inorganic compounds necessary for life to evolve. One of the key elements that early life forms used was calcium for regulation of many metabolic processes. As invertebrates and vertebrates evolved, they took advantage of the high calcium content of their ocean environment (approx. 400 mmol) and used it as a major component for their exo- and endoskeletons, respectively. When vertebrate life forms ventured onto land, the calcium on which they became dependent was plentiful in the soils, but they had no mechanism to extract it. Plants, however, extracted the precious calcium out of the soils and distributed it throughout their structures. Thus, calcium was harvested by vertebrates from the soil indirectly by the ingestion of these plants. To utilize the dietary calcium there was a need for a mechanism to recognize the calcium status of the organism and to regulate the efficiency of intestinal calcium absorption depending on the organism's calcium needs. It is likely that vitamin D played a crucial role in early vertebrate development by regulating intestinal calcium absorption and calcium metabolism [1–3].

27.3 Vitamin D Metabolism and Action on the Intestine

Once vitamin D is made in the skin, it enters the circulation. Vitamin D (vitamin D represents either vitamin D_2 or vitamin D_3) from the diet is incorporated in chylomicrons and absorbed into the lymphatic system, where it eventually is deposited into the venous circulation. Both dietary and skin sources of vitamin D are bound in the circulation to a vitamin D-binding protein (DBP) [4]. Some of the lipophylic vitamin D in the circulation is deposited in the body fat, while most of it is directed to the liver [5–7]. Once it enters hepatocytes, it is metabolized by the vitamin D-25-hydroxylase (CYP27A) and transformed to 25-hydroxyvitamin D [25(OH)D] [6, 7]. 25(OH)D leaves the hepatocyte and enters the circulation and is once again bound to the DBP. 25(OH)D is the major circulating form of vitamin D and, as a result, is used to determine the vitamin D status of both children and adults. The 25(OH)D–DBP complex is recognized by megalin that is located in the plasma membrane of the renal tubular cells. Megalin facilitates the endocytic transport of the 25(OH)D–DBP complex into the renal cell [8]. 25(OH)D is then released and enters the mitochondria, where the cytochrome P450-25-hydroxyvitamin D-1-hydroxylase (CYP27B1; 1-OHase) converts it to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] [3, 6, 7] (Fig. 27.1). The renal 1-OHase is upregulated by hypocalcemia and hypophosphatemia. Parathyroid hormone (PTH) is a potent stimulator of the renal 1-OHase whereas fibroblast growth factor 23 (FGF23) produced by osteocytes and osteoblasts inhibits its activity (Fig. 27.1). During pregnancy and lactation, estrogen and prolactin are also thought to play a role in upregulating the 1-OHase [6, 7].

$1,25(OH)_2D$ is considered to be the biologically active form of vitamin D. It binds to its specific nuclear vitamin D receptor (VDR), which in turn binds with the retinoic acid X receptor (RXR) to form a herterodimeric complex. This complex interacts with specific sequences in the promoter region of vitamin D-responsive genes, known as vitamin D-responsive element (VDRE) [3, 7, 9, 10]. The binding of the VDR-$1,25(OH)_2D$-RXR complex to the VDRE initiates the binding of several transcriptional factors that ultimately results in either an increased or decreased expression of vitamin D-responsive genes [9–11].

$1,25(OH)_2D$ is recognized by the VDR in the small intestine, resulting in an increase in the expression of the epithelial calcium channel on the mucosal surface of the intestinal absorptive cell [2, 3, 7]. In addition, there is an increase in the expression of the calcium-binding $protein_{9K}$ (calbindin), calcium-dependent ATPase, and several other brush border proteins [2, 3, 7, 10, 12]. The ultimate result is that $1,25(OH)_2D$ enhances the efficiency of intestinal calcium absorption from a baseline of approx. 10–15 to 30–40 %. Most of the dietary calcium is absorbed in the duodenum and to a lesser extent in the jejunum and ileum.

Once $1,25(OH)_2D$ carries out its function in the small intestine, it then induces the expression of the 25-hydroxyvitamin D-24-hydroxylase (CYP-24). This results in the initiation of a cascade of metabolic steps that culminates in the cleavage of the side chain between carbons 23 and 24 to yield the water-soluble, biologically inactive excretory product, calcitroic acid [3, 7, 10].

27.4 Vitamin D Action on Bone Calcium Mobilization

Although vitamin D is associated with bone health, the principal physiological function of vitamin D is to support the serum calcium within a physiologically acceptable range in order to maintain neuromuscular and cardiac function and a multitude of other metabolic activities [2]. Thus, when dietary calcium is inadequate to satisfy the body's requirement for calcium, this results in vitamin D becoming a catabolic hormone that mobilizes calcium stores from the skeleton.

$1,25(OH)_2D$ increases the removal of calcium from the skeleton by increasing osteoclastic activity. It was originally believed that $1,25(OH)_2D$ interacted with specific nuclear receptors in preosteoclasts

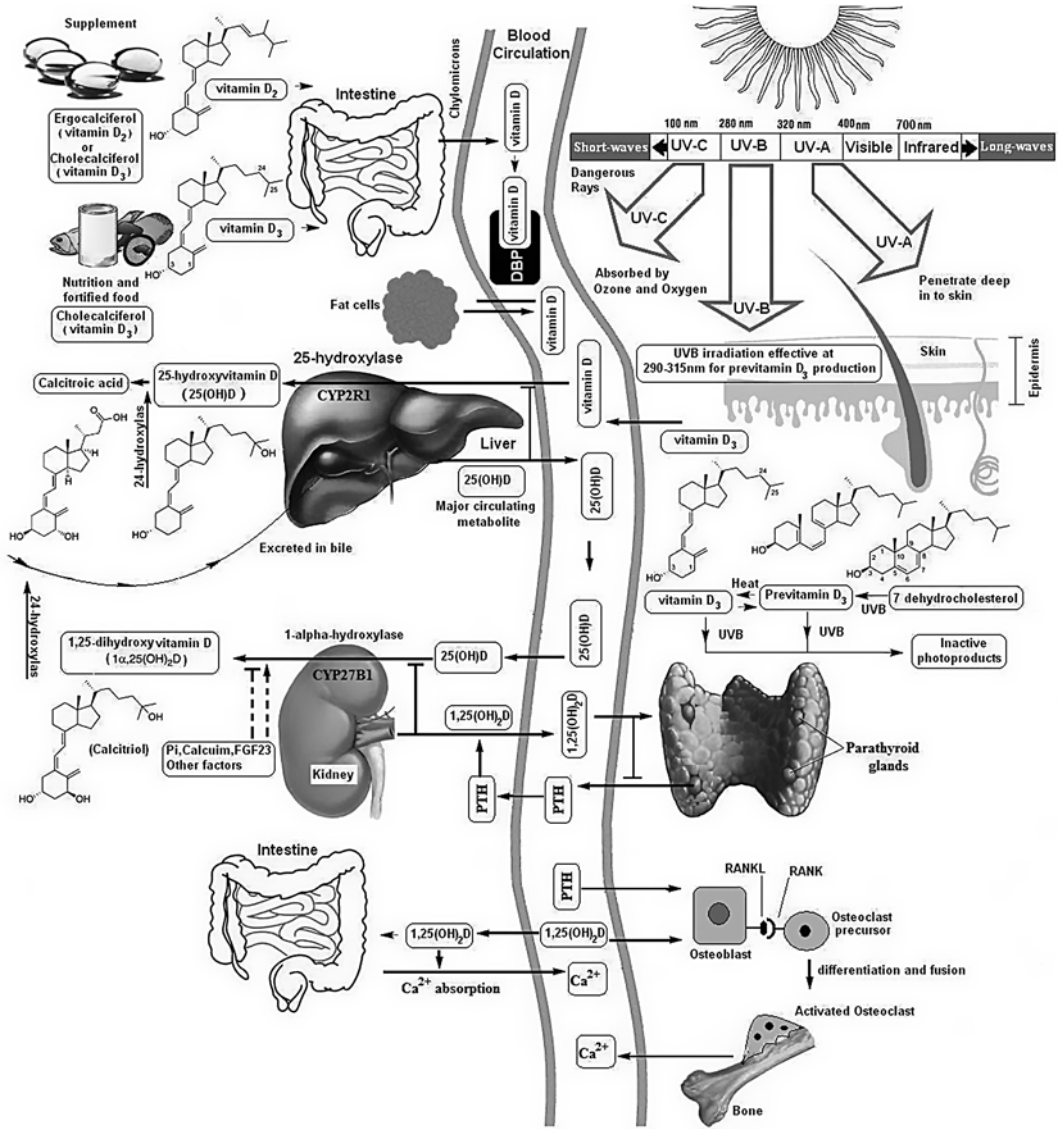


Fig. 27.1 Schematic representation of the synthesis and metabolism of vitamin D for regulating calcium, phosphorus, and bone metabolism. During exposure to sunlight, 7-dehydrocholesterol in the skin is converted to previtamin D_3. Previtamin D_3 immediately converts by a heat-dependent process to vitamin D_3. Excessive exposure to sunlight degrades previtamin D_3 and vitamin D_3 into inactive photoproducts. Vitamin D_2 and vitamin D_3 from dietary sources are incorporated into chylomicrons, transported by the lymphatic system into the venous circulation. Vitamin D (D represents D_2 or D_3) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein, which transports it to the liver, where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D (25(OH)D). This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status (although most reference laboratories report the normal range to be 20–100 ng/mL, the preferred healthful range is 30–60 ng/mL). It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Serum phosphorus, calcium fibroblast growth factors (FGF-23), and other factors can either increase or decrease the renal production of $1,25(OH)_2D$. $1,25(OH)_2D$ feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands. $1,25(OH)_2D$ increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize $1,25(OH)_2D$ to the water-soluble, biologically inactive calcitroic acid, which is excreted in the bile. $1,25(OH)_2D$ enhances intestinal calcium absorption in the small

to initiate the formation of mature osteoclasts. We now recognize that $1,25(OH)_2D$ initiates the mobilization of preosteoclasts through its interaction with its VDR in osteoblasts. The osteoblast serves as the master cell for regulating bone metabolism. $1,25(OH)_2D$ interacts with the VDR in mature osteoblasts and induces the expression of RANKL (receptor for RANKL) on its plasma membrane surface [3, 7, 13–15]. The precursor monocytic osteoclasts have a membrane receptor for RANKL, known as RANK (receptor activator NFκB). It is the intimate interaction of the preosteoclast's RANK with the osteoblast's RANKL that ultimately signals the preosteoclast to become a mature bone-resorbing multinucleated osteoclast (Fig. 27.1). Thus, in calcium-deficient states $1,25(OH)_2D$ production is enhanced and in turn mobilizes an army of osteoclasts that resorb bone-releasing precious calcium stores into the circulation to maintain ionized calcium levels in the normal range.

27.5 Vitamin D and Bone Mineralization

$1,25(OH)_2D$ interacts with osteoblasts, not only to increase the expression of RANKL, but also to enhance the expression of osteocalcin, alkaline phosphatase, and osteopontin [6, 7, 10, 16, 17]. Despite all of these biological functions in the osteoblast, there is no evidence that $1,25(OH)_2D$ is essential for the ossification process of the collagen matrix [18–20]. This is based on the observation that severely vitamin D-deficient rats that either received a high-calcium and high-phosphorus-with-lactose diet or received calcium intravenously had bones that had no evidence of rickets or other pathology (Fig. 27.2) [19]. This has also been confirmed in rachitic patients with a VDR defect known as $1,25(OH)_2D$-resistant rickets (vitamin D-dependent rickets type 2) and who received an infusion of calcium, resulting in the healing of their rickets [20].

27.6 Dietary Sources of Vitamin D

There are very few foods that naturally contain vitamin D. These foods include oily fish including makerel, eel, and salmon, cod liver oil, sun- and UV-exposed mushrooms, and egg yolks (Table 27.1).

Steenbock [21] recognized the importance of promoting antirachitic activity in foods by irradiating them with ultraviolet radiation. He suggested irradiation of milk that was fortified with ergosterol (provitamin D_2) as a mechanism to provide children with their vitamin D requirement. This recommendation was embraced by the United States, Canada, and Europe, and this simple food fortification program essentially eradicated rickets by 1940.

In the 1930s, the fortification of milk with vitamin D was a novelty and many companies became interested in fortifying their products with vitamin D. This included, among others, Bond bread, Rickter's hot dogs, and Twang soda. Schlitz Brewery cleverly marketed their beer as containing the sunshine vitamin D (Fig. 27.3). In Europe, custards, milk, and other foods were fortified with vitamin D [22].

In the late 1930s, the US Food and Drug Administration forbade any nutritional claims for alcoholic beverages, and vitamin D fortification of beer was halted. In Europe in the 1950s there were

Fig. 27.1 (continued) intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9K (calcium binding protein; CaBP). $1,25(OH)_2D$ is recognized by its receptor in osteoblasts, causing an increase in the expression of the receptor activator of the NFκB ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL, which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton. UVB, ultraviolet B. Reproduced with permission from Holick, copyright 2012

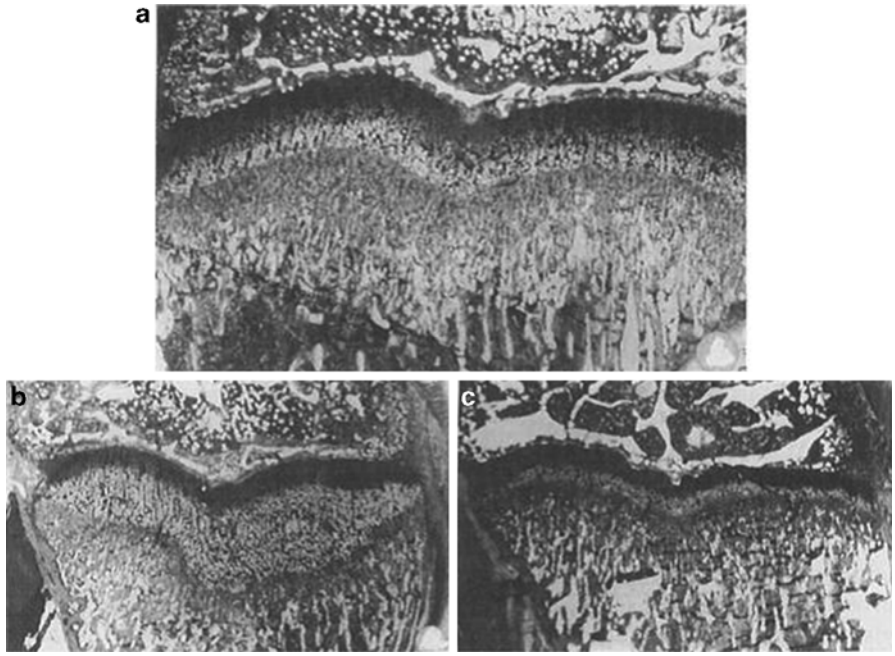


Fig. 27.2 Epiphyseal plates of tibias from rats that were fed (**a**) a vitamin D-deficient diet and supplemented with 125 ng (5 IU) of vitamin D_3 orally five times a week, (**b**) a vitamin D-deficient diet containing 3 % calcium and 0.65 % phosphorus, and (**c**) a vitamin D-deficient diet with 20 % lactose, 4 % calcium, and 1 % phosphorus. Note the wide and disorganized hypertrophic zone in the vitamin D-deficient rat's tibial epiphyseal (**b**) fed high calcium and normal phosphorus diet compared with normal tibial epiphyseal plates from the rats that were either vitamin D repleted (**a**) or maintained on normal serum calcium and phosphorus by being on a high-calcium lactose, high-phosphorus diet (**c**). (Reproduced with permission from ref. [17]

several outbreaks of vitamin D intoxication, that is, hypercalcemia in children, which caused great alarm [23]. This resulted in most European countries forbidding the fortification of any food product with vitamin D.

Based on reports that these infants who were hypercalcemic also suffered from mental retardation, heart problems and had altered facial features is consistent with these children suffering from the rare genetic disorder Williams syndrome. Children with this genetic disorder have elfin faces, heart problems, mild mental retardation and have a hypersensitivity to vitamin D which can cause hypercalcemia. Recently Finland and Sweden have lifted restrictions on the fortification of milk with vitamin D [7].

In the United States, milk, orange juice, some breads, cereals, and yogurts are fortified with vitamin D. There is 100 IU (2.5 μg) of vitamin D in 8 oz of milk. In most European countries, margarine and some cereals are fortified with vitamin D (Table 27.1).

The reason milk was the vehicle for the vitamin D supplementation program was that children drank milk and they were at risk for developing rickets. However, with the awareness that vitamin D deficiency is an epidemic in both young, middle-aged, and older adults, there is a need for other dietary sources of vitamin D other than milk. Tangpricha et al. [24] observed that the fat content in milk does not influence vitamin D bioavailability. They also demonstrated that vitamin D added to orange juice was bioavailable for young and middle-aged adults. Thus, the recent introduction of vitamin D-fortified orange juice and other juice products heralded a new era in the vitamin D fortification process and should have a significant impact on vitamin D status of children and adults who consume these products.

Table 27.1 Sources of vitamin D_2 and vitamin D_3

Source	Vitamin D content
Natural sources	
Cod liver oil	About 400–1,000 IU/teaspoon vitamin D_3
Salmon, fresh wild caught	About 600–1,000 IU/3.5 oz vitamin D_3
Salmon, fresh farmed	About 100–250 IU/3.5 oz vitamin D_3, vitamin D_2
Salmon, canned	About 300–600 IU/3.5 oz vitamin D_3
Sardines, canned	About 300 IU/3.5 oz vitamin D_3
Mackerel, canned	About 250 IU/3.5 oz vitamin D_3
Tuna, canned	About 236 IU/3.5 oz vitamin D_3
Shiitake mushrooms, fresh	About 100 IU/3.5 oz vitamin D_2
Shiitake mushrooms, sun-dried	About 1,600 IU/3.5 oz vitamin D_2
Egg yolk	About 20 IU/yolk vitamin D_3 or D_2
Sunlight/UVB radiation	About 20,000 IU equivalent to exposure to 1 minimal erythemal dose (MED) in a bathing suit. Thus, exposure of arms and legs to 0.5 MED is equivalent to ingesting about 3,000 IU vitamin D_3
Fortified foods	
Fortified milk	100 IU/8 oz, usually vitamin D_3
Fortified orange juice	100 IU/8 oz vitamin D_3
Infant formulas	100 IU/8 oz vitamin D_3
Fortified yogurts	100 IU/8 oz, usually vitamin D_3
Fortified butter	56 IU/3.5 oz, usually vitamin D_3
Fortified margarine	429 IU/3.5 oz, usually vitamin D_3
Fortified cheeses	100 IU/3 oz, usually vitamin D_3
Fortified breakfast cereals	About 100 IU/serving, usually vitamin D_3
Pharmaceutical sources in the United States	
Vitamin D_2 (ergocalciferol)	50,000 IU/capsule
Drisdol (vitamin D_2) liquid	8,000 IU/cc
Supplemental sources	
Multivitamin	400, 500, 1,000 IU vitamin D_3 or vitamin D_2
Vitamin D_3	400, 800, 1,000, 2,000, 5,000, 10,000, and 50,000 IU

IU = 25 ng

27.7 Vitamin D from Sunlight Exposure

Because very few foods contain vitamin D, most children and adults receive their vitamin D requirement from exposure to sunlight. During sunlight exposure, the solar ultraviolet B photons (UVB; with energies 290–315 nm) penetrate into the epidermis and are absorbed by 7-dehydrocholesterol (provitamin D_3) that resides in the plasma membrane of the epidermal cells [3, 15, 25]. This absorption results in a rearrangement of the double bonds that causes the B ring to open to form previtamin D_3 (Fig. 27.1). Previtamin D_3 exists in two conformeric forms, the *s-cis*, *s-cis* (czc) and its more thermodynamically stable counterpart the *s-trans*, *s-cis* (tzc) conformer (Fig. 27.4). It is only the czc conformer that can undergo rearrangement of its double bonds to form vitamin D_3. In order for the skin to efficiently convert previtamin D_3 to vitamin D_3, the previtamin D_3 is made in the plasma membrane and is locked into the czc conformation, which then can rapidly isomerize to vitamin D_3 [26, 27]. Once formed, this molecule no longer is sterically compatible to reside in the cell's plasma membrane and is released into the extracellular space, where it is picked up in the dermal capillary bed and bound to the DBP (Fig. 27.1).

Unlike vitamin D that is absorbed in the small intestine into the chylomicron fraction, where no more than two-thirds of it is bound to DBP, essentially 100 % of the vitamin D_3 that comes from the

Fig. 27.3 In 1932–1936, Schlitz fortified their beer with vitamin D to market it as a unique nutrient-enriched product. However, in 1937 the FDA forbid any nutrient claims for alcoholic beverages and vitamin D was removed from beer

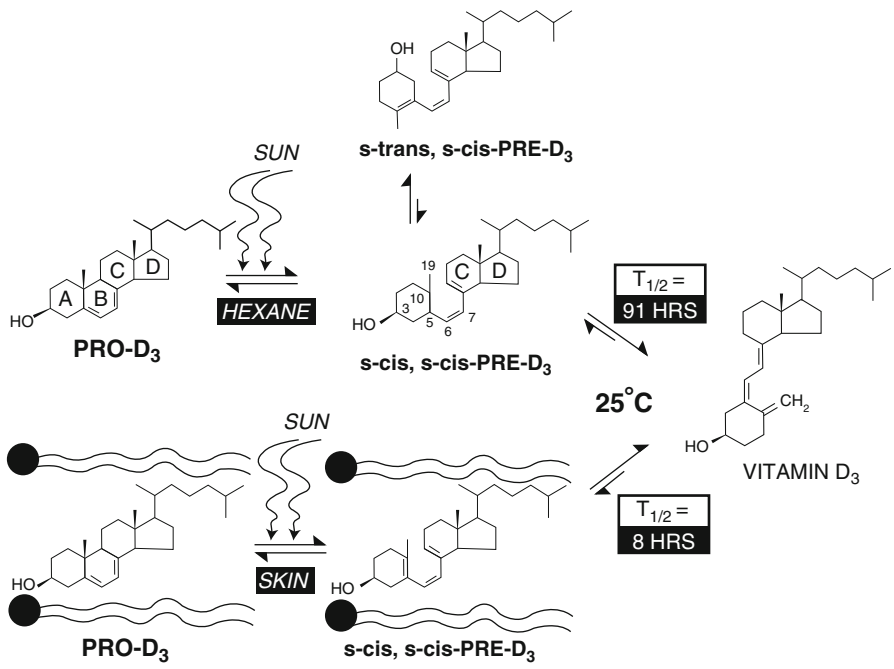


Fig. 27.4 Photolysis of provitamin D_3 (pro-D_3) into previtamin D_3 (pre-D_3) and its thermal isomerization of vitamin D_3 in hexane and in lizard skin. In hexane pro-D_3 is photolyzed to *s-cis*,*s-cis*-pre-D_3. Once formed, this energetically unstable conformation undergoes a conformational change to the *s-trans*,*s-cis*-pre-D_3. Only the *s-cis*,*s-cis*-pre-D_3 can undergo thermal isomerization to vitamin D_3. The *s-cis*,*s-cis* conformer of pre-D_3 is stabilized in the phospholipid bilayer by hydrophilic interactions between the 3β-hydroxyl group and the polar head of the lipids, as well as by the van der Waals interactions between the steroid ring and side-chain structure and the hydrophobic tail of the lipids. These interactions significantly decrease the conversion of the *s-cis*,*s-cis* conformer to the *s-trans*,*s-cis* conformer, thereby facilitating the thermal isomerization of *s-cis*,s-*cis*-pre-D_3 to vitamin D_3. (Reproduced with permission from ref. [26])

skin and enters into the venous circulation is bound to the DBP [28]. This gives the cutaneous vitamin D_3 a more prolonged half-life in the circulation and thus provides an advantage for obtaining vitamin D from exposure of the skin to the sun.

27.8 Factors That Influence the Cutaneous Production of Vitamin D_3

Since the vitamin D_3 synthetic process is dependent on the number of UVB photons that enters into the epidermis, anything that interferes with the number of photons reaching the Earth's surface and ultimately penetrating into the viable epidermis results in an alteration in the production of vitamin D_3 in the skin.

During exposure to sunlight, the UVB photons enter into the skin and initiate the photochemistry necessary for producing previtamin D_3. The UVB photons also signal melanocytes to increase the production of melanin. Melanin acts as a natural sunscreen and is efficiently packaged into melanosomes that migrate upward to the upper layers of the epidermis, where they efficiently absorb UVB and ultraviolet A (321–400 nm) radiation. An increase in skin pigmentation is inversely related to the number of UVB photons that can penetrate into the epidermis and dermis. Thus, the efficiency in utilizing UVB photons to produce vitamin D_3 in the skin is inversely related to the amount of skin pigmentation. This effect can be quite dramatic. A person with deep skin pigmentation of African

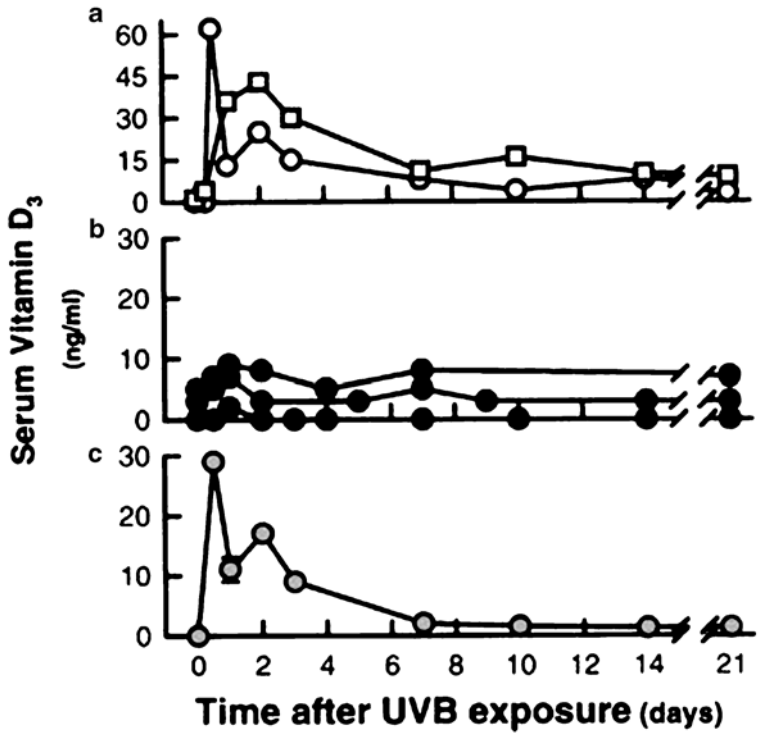


Fig. 27.5 Change in serum concentrations of vitamin D in two lightly pigmented white (skin type 2) (**a**) and three heavily pigmented black subjects (skin type 5) (**b**) after total-body exposure to 54 mJ/cm^2 of UVB radiation. (**c**) Serial change in circulation vitamin D after reexposure of one black subject in **b** to a 320-mJ/cm^2 dose of UVB radiation. (Reproduced with permission from ref. [29]

origin (skin type 5), who is exposed to the same amount of sunlight as a person with minimum skin pigmentation of Celtic or Scandinavian origin (skin type 2), will produce no more than 5–10 % of that produced in the lighter-skinned individual [3, 15, 29] (Fig. 27.5).

Sunscreens are heavily promoted for the prevention of skin cancer and wrinkles. Sunscreens, like melanin, efficiently absorb UVB radiation when applied topically to the skin. As a result, there is a marked diminishment in the penetration of UVB photons into the epidermis. The proper use of a sunscreen (2 mg sunscreen/cm^2 skin surface, i.e., about 1 oz or 25 % of a 4-oz bottle applied to all sun exposed skin of a person wearing a bathing suit) with an SPF of 8 reduces the production of previtamin D_3 by more than 95 % [30] (Fig. 27.6a). Clothing absorbs 100 % of the incident UVB radiation, and thus no vitamin D_3 is made in the skin covered by clothing [31]. This is the reason why women who wear veils and cover all sun-exposed skin with clothing when outside are often vitamin D deficient [32, 33]. Glass also absorbs all UVB photons. Therefore, exposure of the skin from sunlight that has passed through glass will not promote vitamin D_3 synthesis in the skin [34].

Aging causes a decrease in the amount of 7-dehydrocholesterol in the epidermis [7, 34]. Elders exposed to the same amount of sunlight as a young adult will produce approx. 25 % of the amount of previtamin D_3, compared to a young adult [34] (Fig. 27.6b).

The angle by which sunlight penetrates the Earth's atmosphere also dramatically influences the production of previtamin D_3 in the skin. This angle, known as the zenith angle, is related to season, time of day, and latitude. There is a direct relationship with increase in latitude and in the zenith angle of the sun. The higher the zenith angle, the longer is the path length that solar UVB photons have to travel through the ozone layer, which efficiently absorbs most of these vitamin D_3-producing photons.

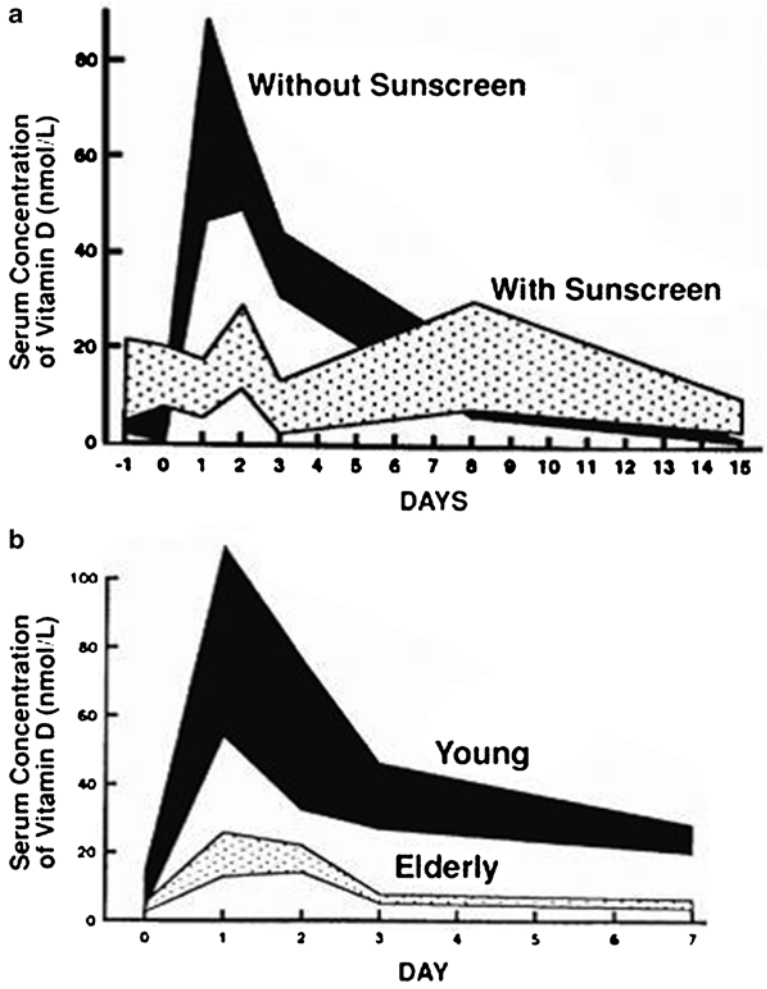


Fig. 27.6 (**a**) Circulating concentrations of vitamin D after a single exposure to 1 minimal erythemal dose of simulated sunlight with either a sunscreen, with a sun protection factor of (SPF-8) 8, or a topical placebo cream. (**b**) Circulating concentrations of vitamin D in response to a wholebody exposure to 1 minimal erythemal dose in healthy young and elderly subjects. (Reproduced with permission from ref. [34]

Typically, in the summer no more than about 0.1 % of the solar UVB photons that hit the outer stratosphere reach the Earth's surface. The lowest zenith angle, which permits more UVB photons to penetrate to the Earth's surface, occurs at around noontime and in the middle of the summer at the Equator.

During the winter (i.e., November–February) above and below 35° latitude, the zenith angle is so oblique that essentially all of the UVB photons are absorbed by the stratospheric ozone layer. As a result, very little, if any, previtamin D_3 can be produced in human skin. At very high latitudes, such as Bergen, Norway, and Edmonton, Canada, little, if any, previtamin D_3 is produced between the months of October and March. Figure 27.7 shows how latitude, season, and time of day dramatically influence the production of previtamin D_3 in the skin [35].

There has been a lot of confusion about the mixed message of avoiding all direct sunlight because of skin cancer risk and the need for some sensible sun exposure to provide children and adults with their vitamin D requirement. Sensible sun exposure which means to never be exposed to an amount of

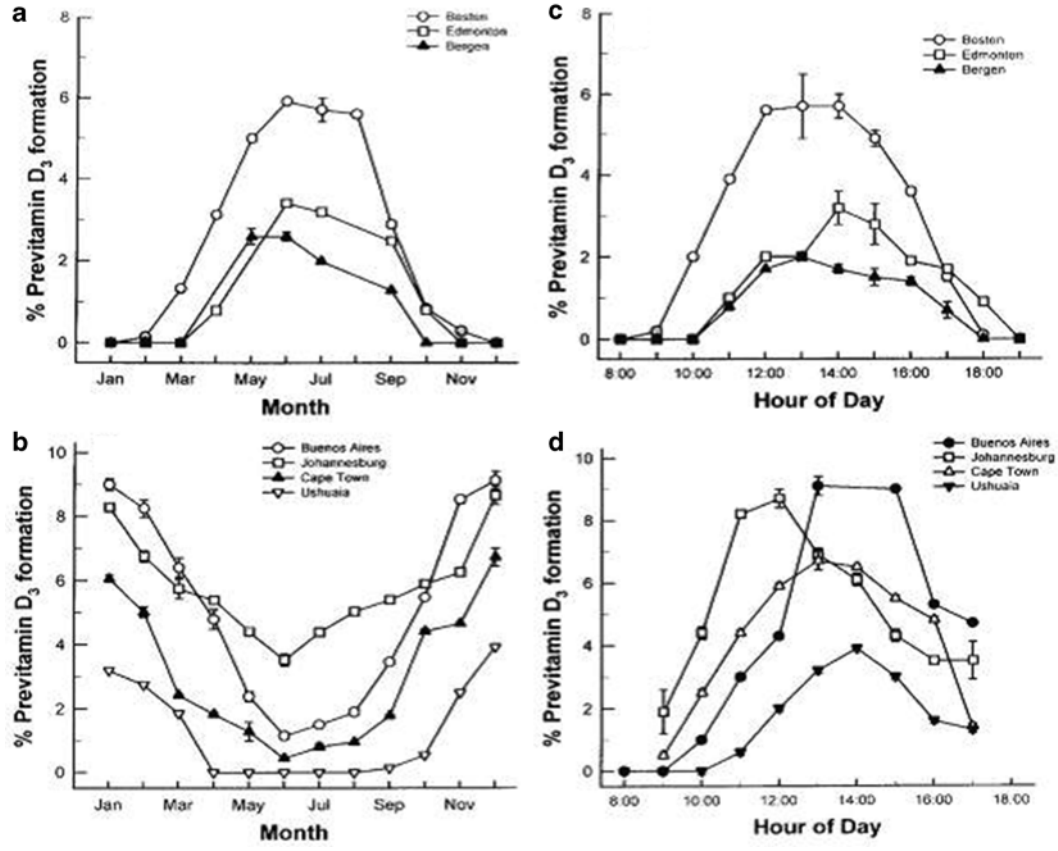


Fig. 27.7 Influence of season, time of day, and latitude on the synthesis of previtamin D_3 in Northern (**a** and **c**) and Southern Hemispheres (**b** and **d**). The hour indicated in **c** and **d** is the end of the 1-h exposure time. (Reproduced with permission from ref. [35]

sunlight that would cause a sunburn, which is the major cause for both skin cancer and skin damage, can provide children and adults with their vitamin D requirement. The recommendation is to always protect the face which is most sun damaged from direct sun exposure. However exposure of other body parts such as arms legs abdomen and back to about 50 % of the time that it would take to get a light pinkness 24 h later (known as one Minimal Erythemal Dose) can be a good source for vitamin D production. To overcome the vagaries of the variables associated with sun-induced vitamin D synthesis including time a day, cloud cover, season, latitude and skin type a free app has been developed that provides the user not only with useful information for how much vitamin D is being produced but also alerts the user when they are at risk for over exposure to sunlight. It is available at dminder.info.

27.9 Consequences of Vitamin D Deficiency on Musculoskeletal Health

Chronic vitamin deficiency in infants and young children causes the bonedeforming disease commonly known as rickets. Vitamin D deficiency disrupts chondrocyte maturation and inhibits the normal mineralization of the growth plates. This causes a widening of the epiphyseal plates that is commonly seen at the ends of the long bones in rachitic children, as well as bulging of the costo-chondral

Fig. 27.8 Typical presentation of two children with rickets. The child in the middle is normal; the children on either side have severe muscle weakness and bone deformities including bowed legs (*right*) or knock knees (*left*)

junctions that results in what is known as the rachitic rosary. The skeleton is also poorly mineralized, due to the low calcium × phosphate product. This poor mineralization makes the skeleton less rigid, and when the rachitic child begins to stand, gravity causes either inward or outward bowing of the long bones in the lower extremities, resulting in bowed legs or knocked knees, respectively (Fig. 27.8).

In adults after the epiphyseal plates have been fused, the skeletal abnormalities resulting from vitamin D deficiency are more subtle. Vitamin D deficiency results in a decrease in efficiency of intestinal calcium absorption. This causes a decrease in the serum ionized calcium, which is immediately recognized by the calcium sensor in the parathyroid glands [36]. This results in an increase in the expression and production of PTH. PTH, in turn, has three options to maintain serum calcium levels within a physiologically acceptable range. It can increase the efficiency of the renal tubules, especially the distal convoluted tubules, to increase the reabsorption of calcium from the ultrafiltrate. It also stimulates the kidney to produce more $1,25(OH)_2D$, which in turn increases intestinal calcium absorption (Fig. 27.1). If these actions are not adequate to maintain the serum calcium levels, then PTH will stimulate the expression of RANKL in osteoblasts to mobilize preosteoclasts to become mature bone-resorbing osteoclasts by a mechanism similar to $1,25(OH)_2D$ [13, 37] (Fig. 27.1). Thus, an increase in osteoclastic activity results in the destruction of the matrix and release of calcium into the extracellular space. The net effect is to increase the porosity of the skeleton, thereby causing a decrease in bone mineral density and precipitating or exacerbating osteoporosis.

A more subtle, but important, effect of PTH on skeletal health is its effect on phosphorus metabolism in the kidney. PTH causes an increase in the urinary excretion of phosphorus. Although subtle in nature, the low-normal or low serum phosphorus is inadequate to maintain a supersaturated level of calcium × phosphorus product, resulting in a mineralization defect of the newly laid-down osteoid by osteoblasts. Histologically this appears as widened osteoid seams (Fig. 27.9) and is known as osteomalacia. Because osteoid has no mineral component, it provides little, if any, structural support to the skeleton and increases risk of fracture [38–41]. In addition, the lack of calcium hydroxyapatite

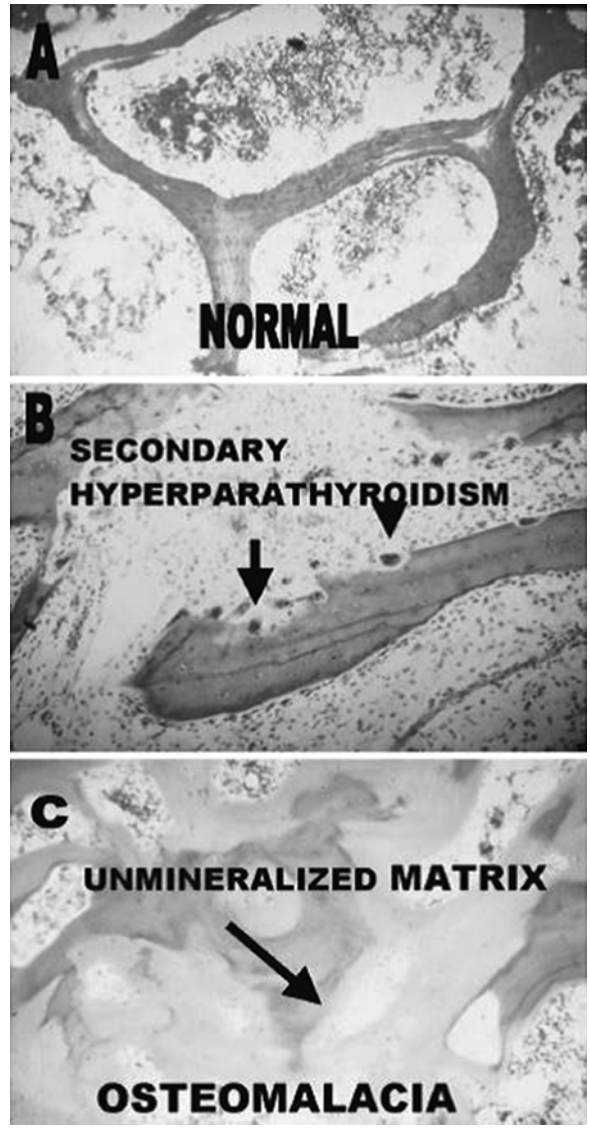


Fig. 27.9 Bone histology demonstrating (**a**) normal mineralized trabecular bone, (**b**) increased osteoclastic bone resorption due to secondary hyperparathyroidism, and (**c**) osteomalacia with widened unmineralized osteoid light gray areas. (Reproduced with permission from ref. [15]

deposition in newly laiddown osteoid results in no increase in bone mineral density. It is not possible to detect either by standard X-rays or bone densitometry the difference between osteoporosis, that is, holes in the skeleton, vs. osteomalacia, which is simply a collagen matrix without mineral [42, 43].

Unlike osteoporosis, which is a silent disease until a fracture occurs, osteomalacia is often associated with bone discomfort. Patients often complain of an aching in their skeleton that is unexplained. This can be detected on physical exam by palpating the sternum with minimum pressure of the thumb or forefinger on the sternum or on the anterior tibia. The patient often complains of discomfort with minimum to moderate applied pressure. Although the exact cause for this pain is not known, its possible that the collagen-rich osteoid that is laid down on the periosteal surface of the skeleton becomes hydrated similar to gelatin in Jell-O and causes an outward pressure on the periostial covering that is innervated with sensory pain receptors [44].

Patients with osteomalacia often complain of muscle aches and muscle weakness. There is mounting evidence that vitamin D deficiency results in muscle weakness and increases sway, which can result in increase in falling, thereby increasing risk of skeletal fractures [33, 45, 46].

Patients often complain to their physicians about nonspecific bone aches, muscle aches, and discomfort. Often after a thorough workup, including a sedimentation rate, rheumatoid factor, and even a bone scan, the physician will inform the patient that no specific cause has been found and often these patients are given the diagnosis of fibromyalgia. It has been estimated that upwards of 40–80 % of patients complaining of nonspecific bone pain and muscle aches and weakness are suffering not from fibromyalgia, but from chronic vitamin D deficiency [33, 44].

27.10 Prevalence of Vitamin D Deficiency in Children and Adults

It is both surprising and alarming that vitamin D deficiency continues to plague both children and adults [7, 38–55] (Fig. 27.10).

Infants who receive their total nutrition from breast feeding are at high risk of vitamin D deficiency because human milk contains very little, if any, vitamin D to satisfy their requirement [53]. This is especially true for infants of color, because their mothers are often vitamin D deficient as well and provide no vitamin D nutrition in breast milk [52, 54]. Even in Caucasian and African American women who had a mean intake of 457 IU/day, the concentrations of vitamin D and 25(OH)D in their milk was 12.6 IU/L and 37.6 IU/L, respectively [7, 53, 54]. It has been estimated that human milk contains no more than about 15 IU of vitamin D in 8 oz.

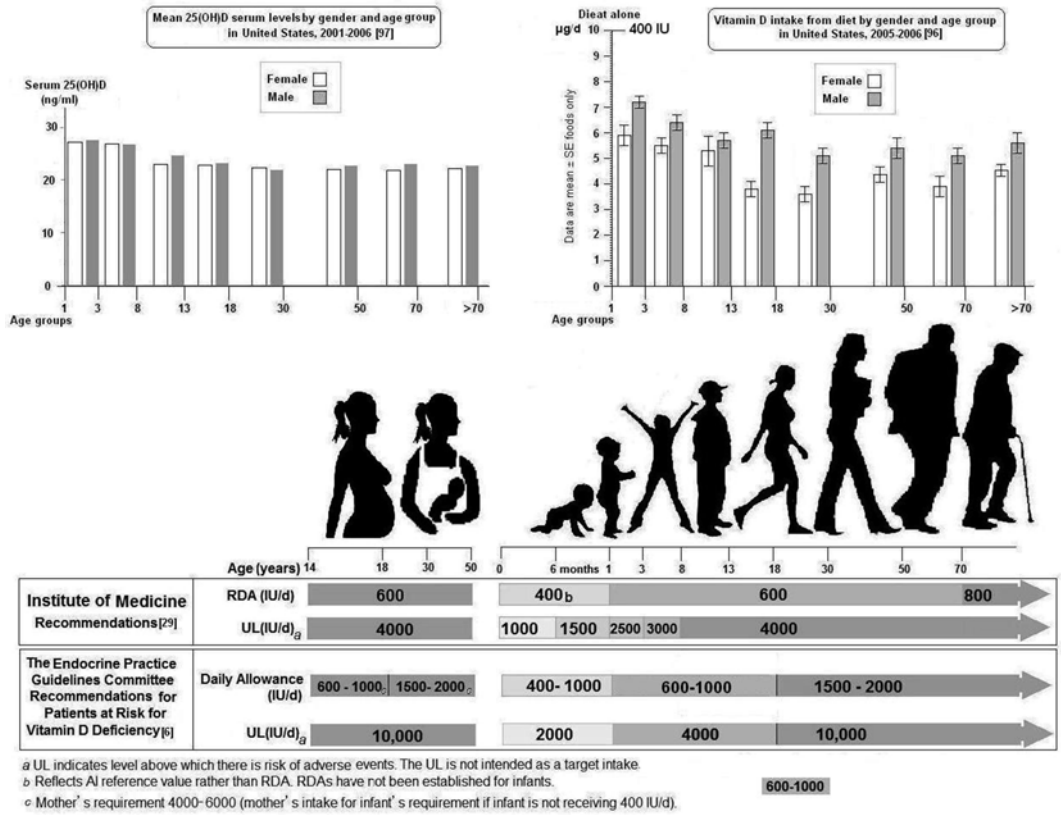


Fig. 27.10 Vitamin D intakes recommended by the IOM and the Endocrine Practice Guidelines Committee. Holick copyright 2012; reproduced with permission

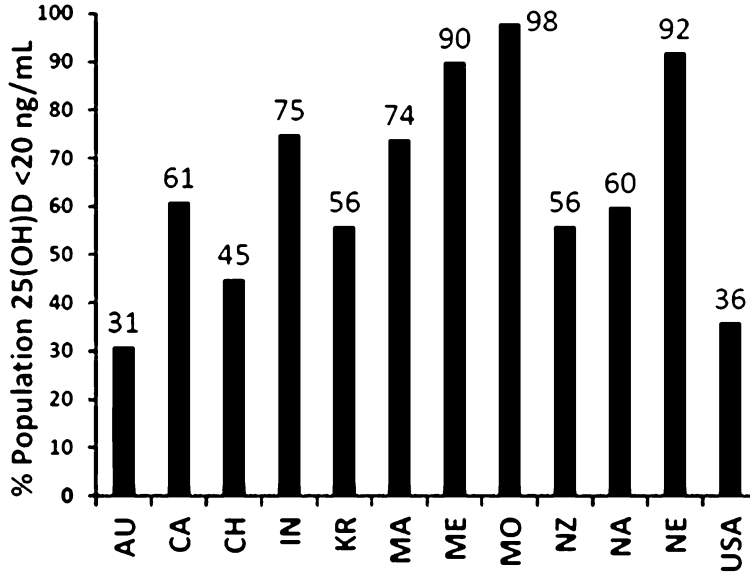


Fig. 27.11 Reported incidence of vitamin D deficiency defined as a 25-hydroxyvitamin D <20 ng/mL around the globe including Australia (AU), Canada (CA), China (CH), India (IN), Korea (KR), Malaysia (MA), Middle East (ME), Mongolia (MO), New Zealand (NZ), North Africa (NA), Northern Europe (NE), United States (USA). Holick copyright 2012; reproduced with permission

Children who are active and outdoors are at little risk of vitamin D deficiency as long as there is a short period of time when they wear no sun protection, such as clothing or sunscreen, on face, arms, and legs.

It has been recognized for more than three decades that globally children and adults are at high risk of developing vitamin D deficiency [7] (Fig. 27.11). Vitamin D deficiency is extremely common in older adults in Europe because essentially no foods are fortified with vitamin D. In the United States and Canada, vitamin D deficiency is also more common than expected [49, 50]. Gloth et al. [47] reported 54 % of community dwellers and 38 % of nursing home residents in the Baltimore area were severely vitamin D deficient [25(OH)D < 10 ng/mL]. Numerous studies have reported that between 25 % and more than 60 % of adults aged 50+ years were vitamin D deficient. In Boston, we observed in independently living elders (83 ± 8 years; 50 white, 14 Hispanic, and 5 African American subjects) in August of 1997 30, 43, and 84 % of white, Hispanic, and black elders were vitamin D deficient [15]. Inpatients are especially at high risk of vitamin D deficiency [55]. It was reported that 57 % of middle-aged and older adults were vitamin D deficient. Sixty percent of the patients consumed less than the recommended adequate intake of vitamin D, and 37 % who had intakes above the recommended daily allowance were found to be vitamin D deficient [7].

It would be expected that young and middle-aged active adults would not be at risk of vitamin D deficiency. However, they have several risk factors for vitamin D deficiency, including long hours of work indoors with little exposure to sunlight, and they are also more likely to wear sun protection on all sun-exposed areas because of their worry about increased risk of skin cancer and wrinkles. As a result, when exposed to sunlight they make little vitamin D_3 in their skin. In Boston, we observed 32 % of medical students and young doctors, aged 18–29 years, were vitamin D deficient [51]. Fifteen percent had secondary hyperparathyroidism, and 4 % of the students and residents remained vitamin D deficient at the end of the summer.

27.11 Causes of Vitamin D Deficiency

The major cause of vitamin D deficiency is that it is not appreciated that very few foods naturally contain vitamin D and that most (80–100 %) of our vitamin D requirement comes from casual exposure to sunlight [7, 15, 56] (Fig. 27.12). Even though oily fish contain vitamin D, it is highly variable depending on what season they were caught and whether they were farm raised and what their vitamin D intake was from their diet. Furthermore, it would require that a person eat oily fish at least 2–3 times a week. To satisfy the vitamin D requirement by drinking milk, would require ingesting six and eight glasses a day for children and adults up to the age of 70, and adults aged 70+ years, respectively [53].

Intestinal malabsorption syndromes, especially of the small intestine where vitamin D is absorbed, can lead to severe vitamin D deficiency [15, 57, 58] (Fig. 27.13). Patients with end-stage hepatic failure not only are unable to produce an adequate amount of 25(OH)D, but often suffer from fat malabsorption and are unable to absorb dietary vitamin D. Patients who are on total parenteral nutrition often suffer from a severe metabolic bone disease that is characteristic of vitamin D deficiency osteomalacia. However, the inclusion of 400 IU of vitamin D in the total parenteral nutrition solution does not protect the patient from vitamin D deficiency bone disease [59, 60].

The principal cause of vitamin D deficiency is lack of adequate exposure to sunlight. The skin has a large capacity to produce vitamin D_3. Exposure of an adult in a bathing suit to simulated sunlight that mimicked the amount of time that would be 1 minimal erythemal dose (1 MED), that is, cause a minimum pinkness to the skin, resulted in an increase in blood levels of vitamin D_3 comparable to ingesting between 10,000 and 25,000 IU of vitamin D [15] (Fig. 27.14). Although aging substantially reduces the amount of 7-dehydrocholesterol in the skin, it still has an adequate capacity to make vitamin D [15, 61–63] (Fig. 27.15).

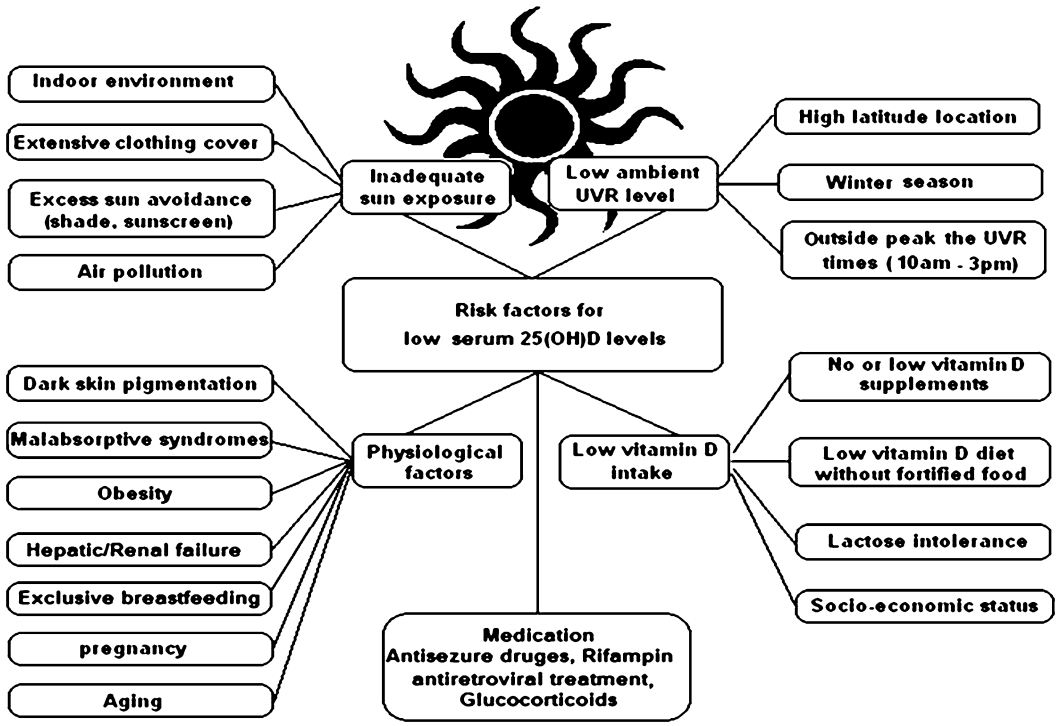


Fig. 27.12 Risk factors of low vitamin D status. Holick copyright 2012; reproduced with permission

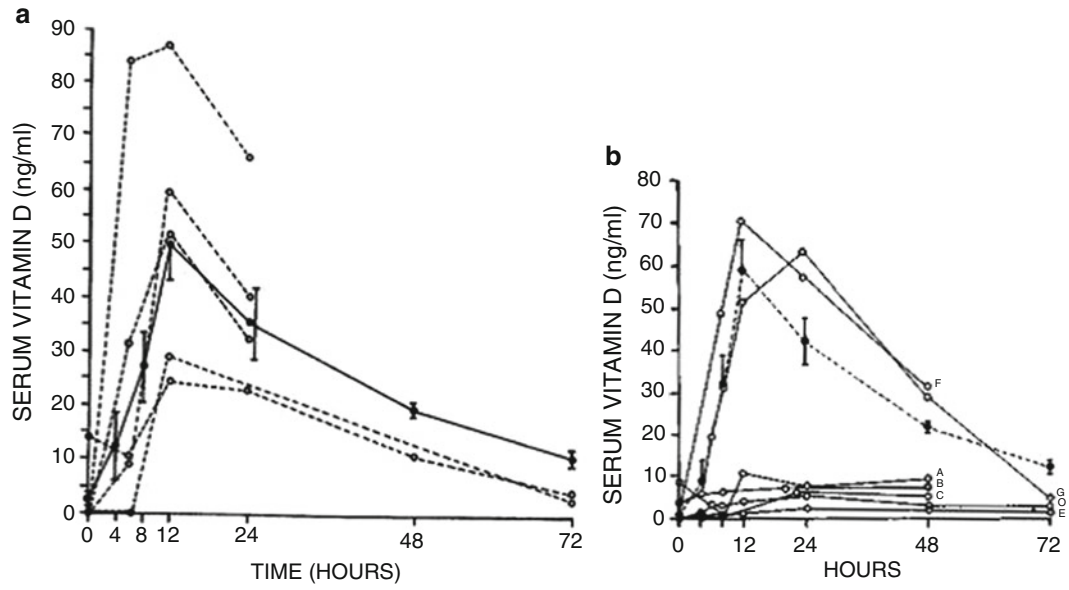


Fig. 27.13 (**a**) Serum vitamin D concentrations in seven patients with intestinal fat malabsorption syndromes after a single oral dose of 50,000 IU (1.25 mg) of vitamin D_2. For comparison, the means and standard errors of vitamin D concentrations measured in seven normal control subjects after a similar dose are indicated by the *filled circles* and *dotted lines*. Note that two patients, one with Crohn's ileocolitis (patient F) and one with ulcerative colitis (patient G), had essentially normal absorption *curves*. Five patients, however, absorbed very little, if any, vitamin D_2. (**b**) Vitamin D absorption in young (*filled circles*) and elderly (*open circles*) adults. Each subject received an oral dose of 50,000 IU of vitamin D_2 and at various times blood determinations were made for circulating concentrations of vitamin D. (Reproduced with permission from ref. [57])

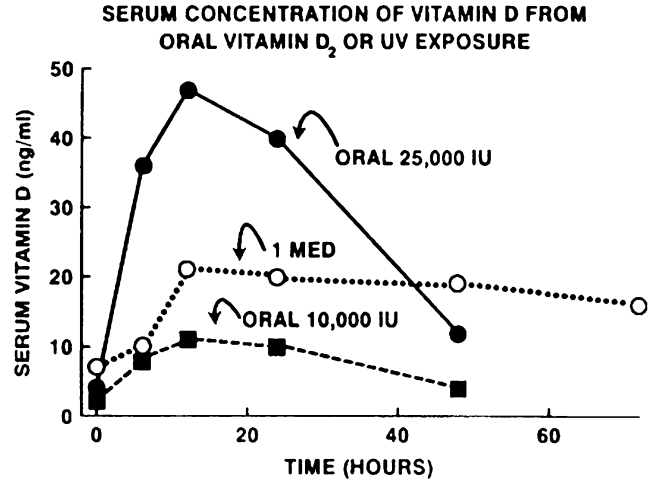


Fig. 27.14 Comparison of serum vitamin D levels after a whole-body exposure to 1 MED (minimal erythemal dose) of simulated sunlight compared with a single oral dose of either 10,000 or 25,000 IU of vitamin D_2. (Reproduced with permission, Holick copyright 2002)

Patients with obesity often complain of bone aches, muscle aches, and weakness, which exacerbates their inability to be active and their obesity. It is recognized that obesity is associated with vitamin D deficiency [5, 64]. This is due to the fact that body fat acts as a sink for vitamin D. Thus, whether vitamin D is produced in the skin or ingested in the diet, a majority of it is deposited in an almost irreversible manner into the body fat and is not bioavailable to the body (Fig. 27.16).

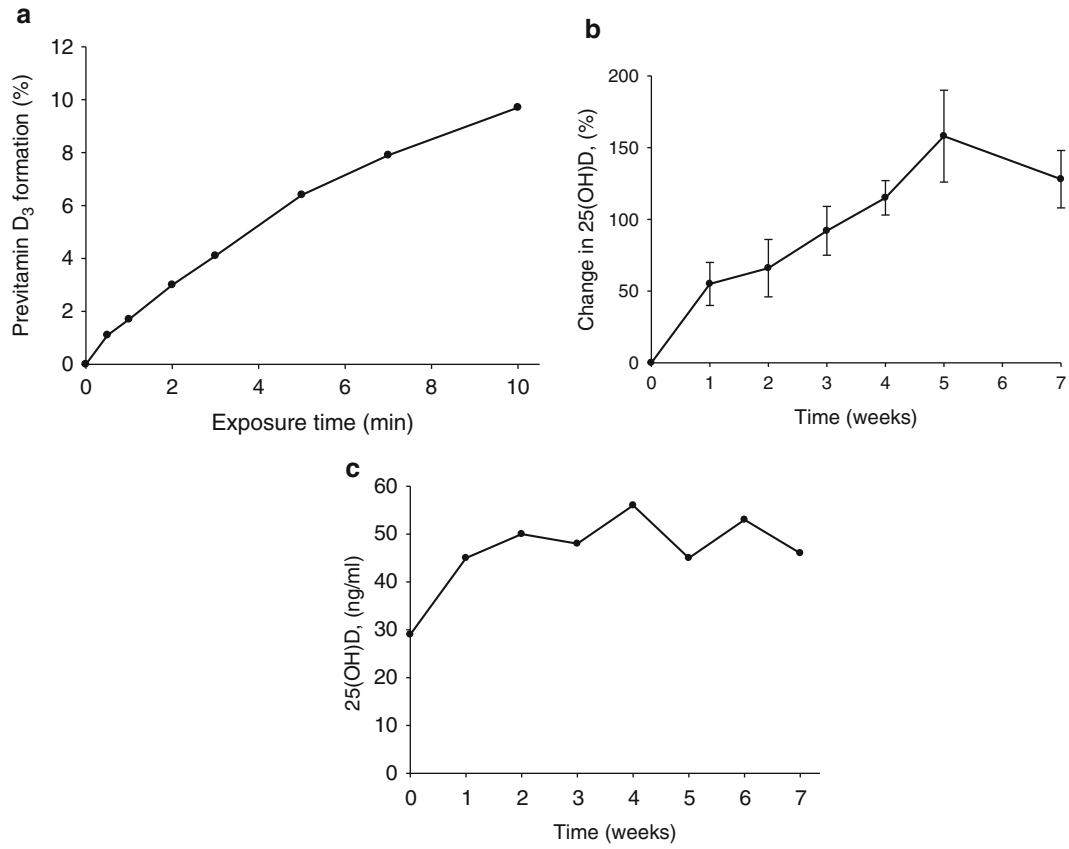


Fig. 27.15 (**a**) Ampoules containing 7-dehydrocholesterol were placed in a tanning bed at various times and conversion of 7-dehydrocholesterol to previtamin D_3 was measured by high performance liquid chromatography. (**b**) Healthy adults were exposed to 0.75 MED in a tanning bed three times a week for 7 weeks. Circulating concentrations of 25(OH)D were determined at baseline and once a week thereafter. (**c**) A 76-year-old healthy male was exposed to tanning bed radiation equivalent to 0.75 MED three times a week for 7 weeks. His circulating concentrations of 25(OH)D were obtained at weekly intervals. Reproduced with permission Holick, M.F., Chen, T.C., Sauter, E.R. Vitamin D and Skin Physiology: A D-Lightful Story. J Bone Miner Res. 2007. 22(S2):V28-V33

27.12 Diagnosis of Vitamin D Deficiency

Often physicians assume that the most sensitive indicator to detect vitamin D deficiency is to observe a below-normal serum calcium value. Unfortunately, as explained previously, the body is vigilant to maintain the serum calcium within the normal range in order to maintain most bodily functions. As a result, a person with vitamin D deficiency develops secondary hyperparathyroidism and maintains serum calcium within the normal range until most available calcium is depleted from the skeleton. The secondary hyperparathyroidism results in mild to moderate hypophosphatemia. However, this is also difficult to detect, especially if the patient's blood is taken in a nonfasting state. Serum phosphorus levels are influenced by dietary phosphorus intake, sugar intake, and by acidosis and alkalosis [6].

With the exception of observing widened epiphyseal plates and Looser's pseudo-fractures in the long bones, it is not possible to detect vitamin D deficiency by X-rays.

The only method to determine vitamin D deficiency is to measure the blood level of the major circulating form of vitamin D, 25(OH)D. Although $1,25(OH)_2D$ is the biologically active form of

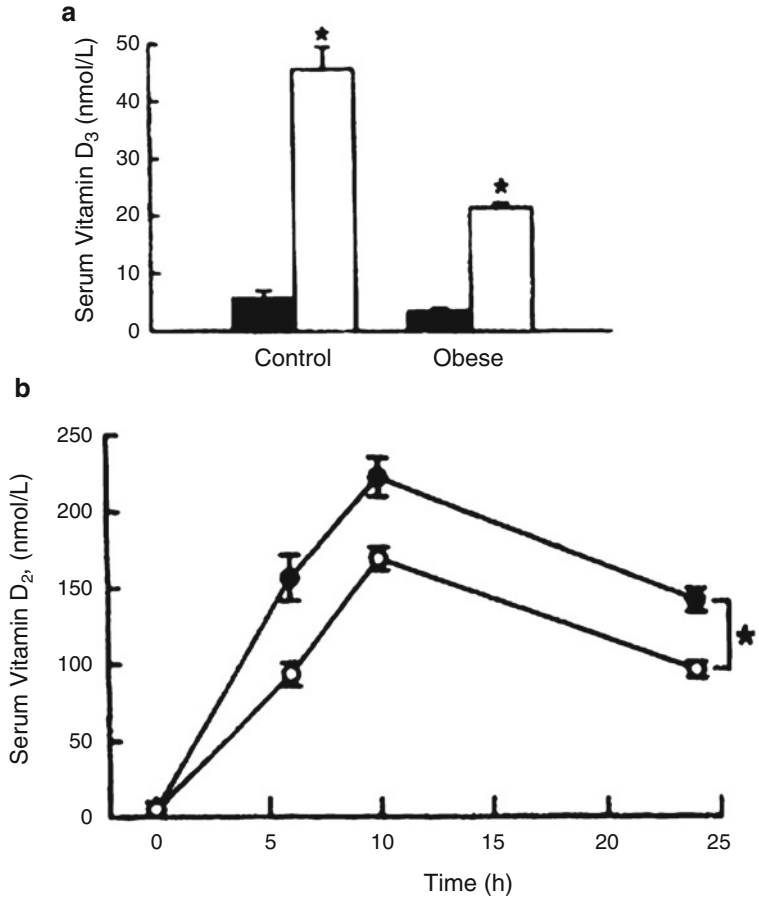


Fig. 27.16 (**a**) Mean (±SEM) serum vitamin D_3, concentrations before (*filled square*) and 24 h after (*open square*) whole-body irradiation (27 mJ/cm^2) with UVB radiation. The response of the obese subjects was attenuated when compared with that of the control group. There was a significant time-by-group interaction, $p = 0.003$. *Significantly different from before values ($p < 0.05$). (**b**) Mean (±SEM) serum vitamin D_2 concentrations in the control (*filled circle*) and obese (*open circle*) groups before and after 25 h after oral intake of vitamin D_2 (50,000 IU, 1.25 mg). Vitamin D_2 rose rapidly until ~10 h after intake and then declined slightly thereafter. *Significant time and group effects by ANOVA ($p < 0.05$) but no significant time-by-group interaction. The difference in peak concentrations between the obese and nonobese control subjects was not significant. (Reproduced with permission from ref. [5])

vitamin D and would appear to be the ideal marker for vitamin D deficiency, it is not. There are several reasons for this. The circulating concentration of $1,25(OH)_2D$ is 1,000th the concentration of 25(OH)D (pg vs. ng/mL). The half-life for $1,25(OH)_2D$ is only 4–6 h, compared to 2 weeks for 25(OH)D [15]. Finally, as a person becomes vitamin D deficient and develops secondary hyperparathyroidism, the kidney's 1-OHase produces more $1,25(OH)_2D$ [3, 6, 7, 15]. Thus, when a patient is vitamin D insufficient there is often a normal or even elevated blood level of $1,25(OH)_2D$ [7, 15, 56]. The measurement of $1,25(OH)_2D$ as a gauge of vitamin D status is not only useless, but often misleads physicians into thinking their patient is vitamin D sufficient since the $1,25(OH)_2D$ levels can be normal.

27.13 Vitamin D Requirement: Adequate Intake vs. Healthy Intake

In 2010, the Institute of Medicine announced the new Recommended Daily Allowances (RDA) for vitamin D (1 μg = 40 IU) for children and adults aged 0–1, 1–70, and 71+ years to be 400, 600, and 800 IU/day, respectively (Fig. 27.10) [53]. In 2011 the Endocrine Society issued its guidelines for the treatment and prevention of vitamin D deficiency. They recommended that children in the first year of life should be receiving 400–1,000 IU daily and children 1 year and older should be receiving 600–1,000 IU daily. For all adults it was recommended that they receive 1,500–2,000 IU daily. They also recommended for those who are obese, i.e. BMI >30, require at least 2–3 times more vitamin D to both treat and prevent vitamin D deficiency [65]. Several investigators have reported on the effect of vitamin D intake on circulating concentrations of 25(OH)D. Vieth et al. [66] gave healthy adults (41 ± 9 years) 4,000 IU of vitamin D a day for 2–5 months and did not observe any untoward toxicity. Their 25(OH)D levels during the winter increased from 10.2 ± 4 to 24.1 ± 4 ng/mL. Barger-Lux et al. [67] evaluated a dose response of vitamin D and 25(OH)D intake in healthy males for 4 and 8 weeks, respectively. The groups of adults treated with 1,000, 10,000, or 50,000 IU of vitamin D_3/day for 8 weeks demonstrated increases in their serum vitamin D_3 levels of 5.0, 52.6, and 300.2 ng/mL, respectively. In the same groups, the 25(OH)D increased by 11.6, 58.4, and 257.2 ng/mL, respectively. Male adults who received 10, 20, or 50 μg of 25(OH)D_3/day for 4 weeks demonstrated increases of 25(OH)D by 11.6, 58.4, and 257.2 ng/mL, respectively. None of the men demonstrated any significant change in either their calcium or 1,25$(OH)_2$D levels. In a follow-up study, Heaney et al. [68] gave 67 men who were in general good health either 0, 25, 125, or 250 μg of vitamin D_3 for approx. 20 weeks during the winter. They observed serum 25(OH)D levels increased in direct proportion to dose with a slope of approximately 0.28 ng/mL for each additional 1 μg of vitamin D_3 ingested. The calculated oral input required to sustain serum 25(OH)D concentrations present in the men during autumn was 12.5 μg/day. The total amount from all sources (supplement, food, tissue stores) needed to sustain the starting 25(OH)D level was estimated at 96 μg (approx. 3,800 IU/day). They concluded that healthy men used between 3,000 and 5,000 IU vitamin D_3/day to meet greater than 80 % of their winter vitamin D requirement that was provided by cutaneous production of vitamin D_3 during the previous spring, summer, and fall (Fig. 27.17).

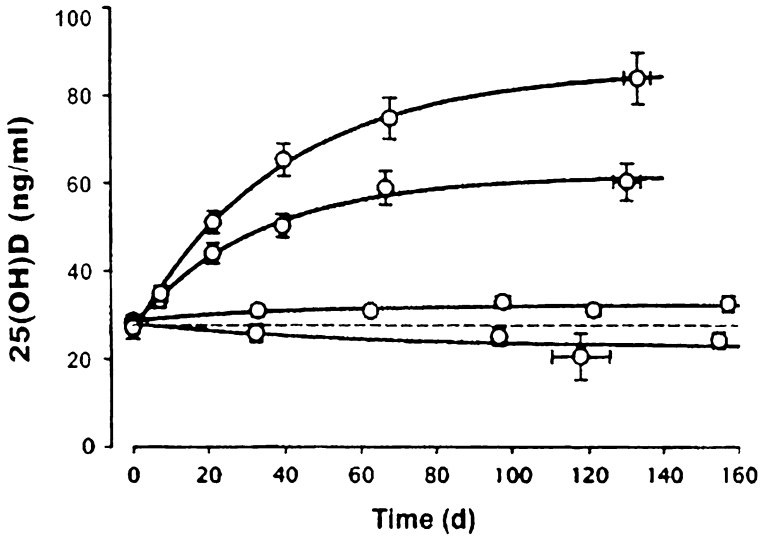


Fig. 27.17 Time course of serum 25-hydroxyvitamin D_3 [25(OH)D] concentration for the four dose groups. The points represent the mean values, and error bars are 1 SEM. The curves are fitted to the mean 25(OH)D_3 values for each dosage group. The curves, from the lowest upward, are for 0.25, 125, and 250 μg vitamin D_3 (labeled dose)/day. The horizontal dashed line reflects zero change from baseline. (Reproduced with permission from ref. [68])

Tangpricha et al. observed that healthy young and middle-aged female and male adults who ingested 1,000 IU of vitamin D/day for 3 months increased their blood levels of 25(OH)D from 15 ± 3 to 38 ± 8 ng/mL after 2 months. Continued intake of 1,000 IU of vitamin D/day did not increase blood levels of 25(OH)D above 40 ng/mL.

27.14 Interpreting Serum 25(OH)D Levels

The normal blood level of 25(OH)D varies from different laboratories, but generally is in the range of 20–100 ng/mL [15, 65]. A normal range for an assay is typically obtained by collecting blood from hundreds of healthy volunteers and then determining the blood level of the analyte and using the mean ± 2 SD as the normal range. Because most healthy volunteers are likely to be vitamin D deficient or insufficient a different strategy has been used to define vitamin D deficiency. This became obvious when Malabanan et. al. [48] reported that healthy adults who received 50,000 IU of vitamin D_2 once a week for 8 weeks along with calcium supplementation had a more than 30 % decline in their PTH values when there blood levels of 25(OH)D were between 11 and 19 ng/mL. No significant change was observed in adults whose blood levels of 25(OH)D were at least 20 ng/mL. The IOM used this data and other observations and concluded that for maximum bone health the 25(OH)D level should be >20 ng/mL. Thus vitamin D deficiency was defined as a 25(OH)D <20 ng/mL [53]. The Endocrine Society also concluded that vitamin D deficiency should be defined as a 25(OH)D <20 ng/mL. They further recommended that vitamin D insufficiency should be defined as a 25(OH)D of 21–29 ng/mL and that vitamin D sufficiency was defined as a 25(OH)D of 30–100 ng/mL with the preferred range of 40–60 ng/mL [65]. These recommendations were based on observations relating serum 25(OH)D with PTH levels. Many studies reported that PTH levels declined and plateaued when a serum 25(OH)D was between 30–40 ng/mL [69]. A study of 675 otherwise healthy German adults who died in an accident had a blood level of 25(OH)D determined and related to bone biopsy that was evaluated for evidence of unmineralized matrix, i.e. osteomalacia, a hallmark of vitamin D deficiency bone disease. The authors concluded that to guarantee no evidence of vitamin D deficiency bone disease that a blood level of 25(OH)D should be at least 30 ng/mL [70].

The upper range of normal by most assays is 80–100 ng/mL. However, this upper normal range is more an estimate than based on any reports of toxicity. Indeed, lifeguards routinely have blood levels of 25(OH)D of 100 ng/mL with no untoward consequences. Heaney et al. [68] observed blood levels of 25(OH)D_3 of 100 ng/mL without any untoward side effects or hypercalcemia. Based on reports of vitamin D intoxication, that is, associated with hypercalcemia, and suppressed PTH levels, 25(OH)D need to be at least 200 ng/mL (Fig. 27.18) [65, 71–74].

Thus, based on the available literature today, it has been suggested that in the absence of any exposure to sunlight, the vitamin D requirement for children and adults is at least 600–1,000 IU and 1,500–2,000 IUof vitamin D/day (Fig. 27.12) [65]. Furthermore, a 25(OH)D level of between 40 and 60 ng/mL should be considered as a healthy range for 25(OH)D [65].

27.15 Treatment for Vitamin D Deficiency

The best method to treat vitamin D deficiency is to give pharmacological doses of vitamin D. This can be accomplished by giving an oral dose of 50,000 IU of vitamin D once a week for 8 weeks [48]. To prevent recurrence of vitamin D deficiency a maintenance dose of 50,000 IU of vitamin D every 2 weeks which is equivalent to ingesting approximately 3,000 IU of vitamin D daily is effective in normal weight adults in maintaining their blood levels of 25(OH)D in the range of 40–60 ng/mL.

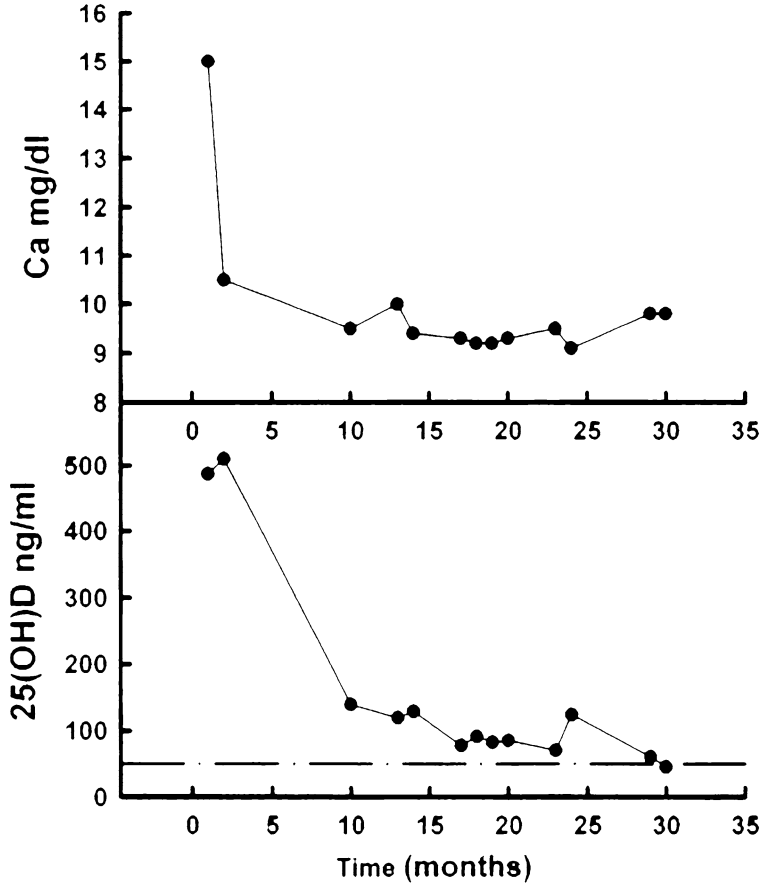
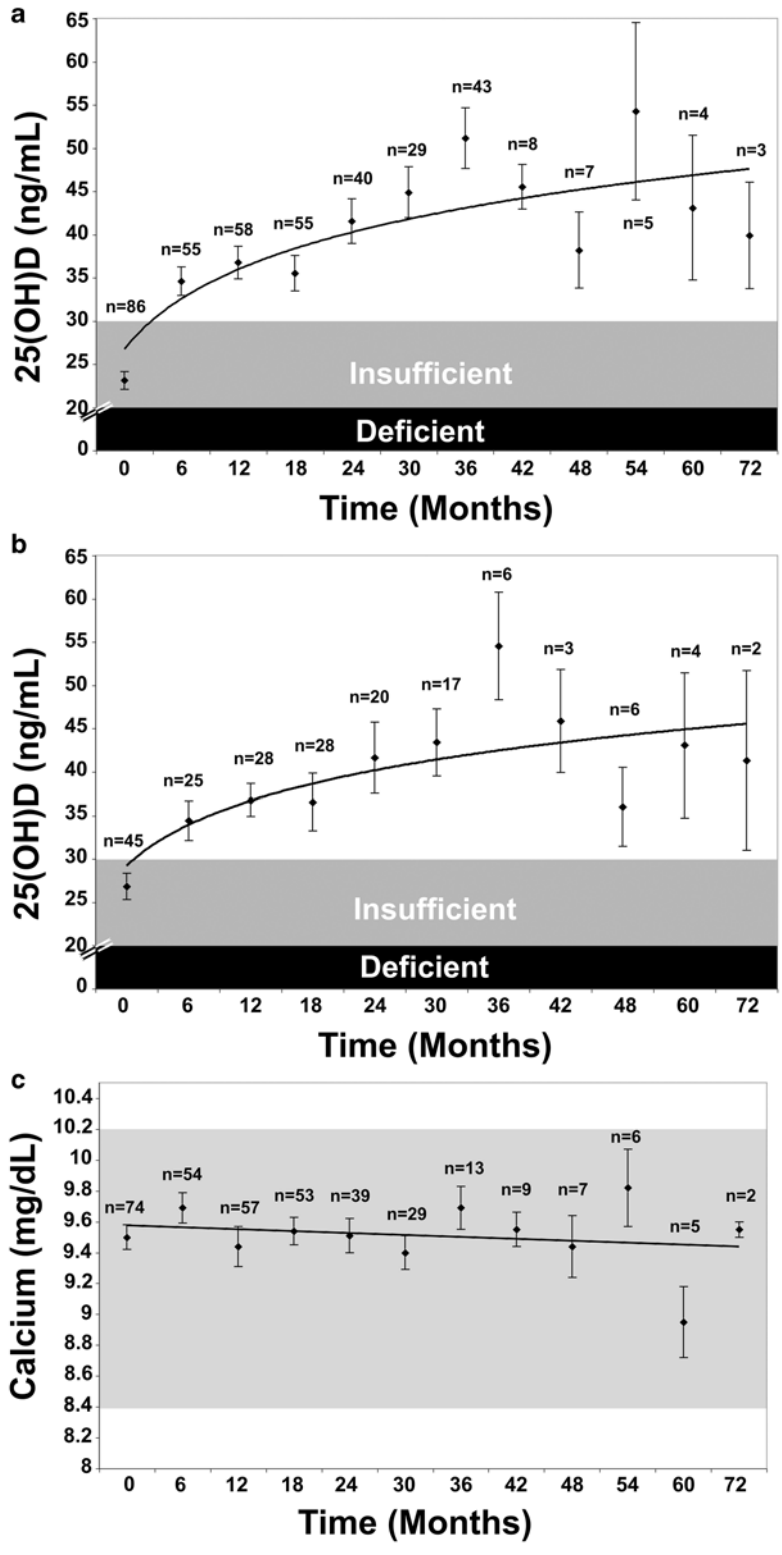


Fig. 27.18 Serum calcium level (*upper panel*) and 25(OH)D level (*lower panel*) in a patient who had vitamin D intoxication after ingestion of an over-the-counter vitamin D supplement that contained as much as one million units of vitamin D_3 in a teaspoon. The patient stopped all vitamin D intake and wore sunscreen before going outside after his hospitalization (month 0). The *dotted line* (*lower panel*) represents the upper limit for the 25(OH)D assay that was 46.7 ng/mL. (Reproduced with permission from ref. [71]

This strategy has been effective for at least 6 years without any toxicity (Fig. 27.19) [75, 76]. Alternatively, intramuscular injection of up to 500,000 IU of vitamin D has been demonstrated to prevent vitamin D deficiency in elderly nursing home residents when given twice a year [77]. However, the intramuscular preparation has been ineffective in many patients in raising blood levels of 25(OH) D when given intramuscularly. This may be a bioavailability problem. In addition, a relatively large volume of oil in which the vitamin D is dissolved when given intramuscularly can be quite uncomfortable, which again is a good reason to give a pharmacological doses of vitamin D orally to correct vitamin D deficiency. Aging does not alter vitamin D absorption [15].

An alternative and an inexpensive method to treat vitamin D deficiency is to encourage patients to be exposed to some sensible sunlight. The amount depends on the person's skin sensitivity, time of day, season of the year, and latitude. For example, for an adult in Boston with a skin type 2, who would get a sunburn after being outside for 30 min at noontime in July, the recommendation is exposure to approx. 30–50 % of that time or 9–15 min 2–3 times a week. Always protect the face because of increased risk of wrinkles or skin damage. Exposure of arms, legs, back, abdomen and legs when possible and using the app dminder.info can provide guidance for sensible sun exposure. No sunscreen or


a
25(OH)D (ng/mL)
65
60
55
50
45
40
35
30
25
20
0
n=86
n=55
n=58
n=55
n=40
n=29
n=43
n=8
n=7
n=5
n=4
n=3
Insufficient
Deficient
0 6 12 18 24 30 36 42 48 54 60 72
Time (Months)
b
25(OH)D (ng/mL)
65
60
55
50
45
40
35
30
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n=45
n=25
n=28
n=28
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n=17
n=6
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Insufficient
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0 6 12 18 24 30 36 42 48 60 72
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n=74
n=54
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n=53
n=39
n=29
n=13
n=9
n=7
n=6
n=5
n=2
0 6 12 18 24 30 36 42 48 54 60 72
Time (Months)

sun protection should be used for this brief period of time. However, if the person wishes to stay outdoors for a longer period of time, then use of a sunscreen with an SPF of at least 30 and sun protection with clothing is recommended. For those with marked increased skin pigmentation, the time outside could be as much as 30–60 min, again depending on the person's skin sensitivity, time of day, season of the year, and latitude [3, 7, 10, 15, 78].

Patients with severe intestinal malabsorption syndrome and who are on total parenteral nutrition can obtain their vitamin D requirement from sun exposure. However, if they cannot go outside or the season will not permit them to make any vitamin D in their skin, then the use of a UVB radiation source, either a home device or a tanning bed at a tanning salon, would be appropriate. In one patient who had only 2 ft of small intestine left, Koutia et al. [79] reported that exposure to 0.75 MED of tanning bed radiation three times a week markedly increased blood levels of 25(OH)D by 700 % and decreased PTH values into the normal range (Fig. 27.20). In addition, the patient, who suffered from severe bone pain and muscle aches and weakness, had complete relief of her symptoms.

Chuck et al. [62] also have demonstrated that the use of subliminal UVB lighting in an activity room in a nursing home was the most effective means to sustain 25(OH)D levels within the normal range and was far superior to taking a multivitamin that contained 400 IU of vitamin D a day (Fig. 27.21).

27.16 Nonskeletal Consequences of Vitamin D Deficiency

As early as 1941 it was reported that people who lived in higher latitudes were at higher risk of dying of cancer [80]. A multitude of epidemiological studies have now confirmed this early observation [81–89]. There is firm evidence that people living at higher latitudes are at higher risk of developing and dying of breast, colon, ovarian, and prostate cancers [81–87]. Indeed, mortality rates in both men and women are related to their exposure to sunlight [85] (Fig. 27.22).

There is also a latitudinal association with increased risk of developing hypertension and multiple sclerosis [88, 89].

It is now recognized that most tissues and cells possess a VDR. The exact function of $1,25(OH)_2D$ in tissues, such as the brain, breast, prostate, skin, β-islet cells in the pancreas, monocytes, and activated T- and B-lymphocytes, is not fully understood. However, it is known that $1,25(OH)_2D$ is extremely effective in downregulating cellular growth in cells that possess a VDR. Indeed, the potent antiproliferative activity of $1,25(OH)_2D$ has been taken advantage of by the development of activated vitamin D analogs for the treatment of the hyperproliferative disorder psoriasis [90].

Fig. 27.19 (**a**) Mean serum 25-hydroxyvitamin D [25(OH)D] levels in all patients: Includes patients treated with 50,000 IU vitamin D_2 every 2 weeks (maintenance therapy, $N=81$), including those patients with vitamin D insufficiency who were initially treated with 8 weeks of 50,000 IU vitamin D_2 weekly prior to maintenance therapy ($N=39$). Error bars represent standard error of the mean, mean result over 5 years shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. When mean 25(OH)D in each 6-month group was compared to mean initial 25(OH)D, $p<0.001$ up until month 43; $p<0.001$ when all remaining values after month 43 were compared to mean initial 25(OH)D. (**b**) Mean serum 25(OH)D levels in patients receiving maintenance therapy only: Levels for 37 patients who were vitamin D insufficient (25[OH]D levels <30 ng/mL) and five patients who were vitamin D sufficient (25[OH]D levels ≥30 ng/ml) who were treated with maintenance therapy of 50,000 IU vitamin D_2 every 2 weeks. Error bars represent standard error of the mean, mean result over 5 years shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. When mean 25(OH)D in each 6-month group were compared to mean initial 25(OH)D, $p<0.001$ up until month 37; $p<0.001$ when all remaining values after month 43 were compared to mean initial 25(OH)D. (**c**) Serum calcium levels: Results for all 81 patients who were treated with 50,000 IU of vitamin D_2. Error bars represent standard error of the mean. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. Normal serum calcium: 8.5–10.2 mg/dL. (Reproduced with permission from ref. [75])

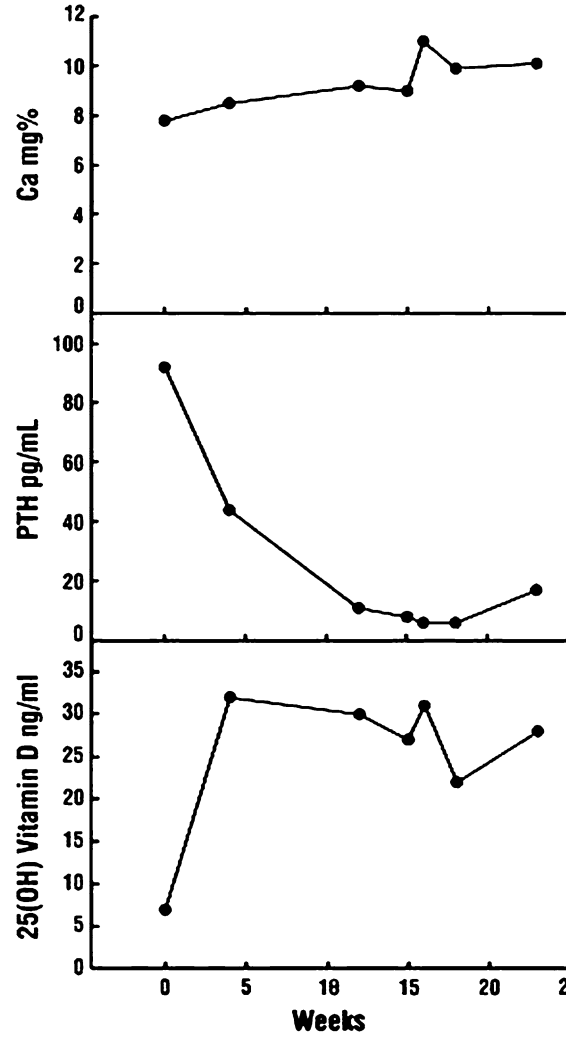

Fig. 27.20 Serum 25(OH)D, PTH, and calcium levels in a patient with Crohn's disease who had whole-body UVB exposure for 10 min, three times in a week for 6 months. (Reproduced with permission from ref. [77])

It is recognized that the β-islet cells have a VDR and that $1,25(OH)_2D$ modulates insulin production and secretion [3, 7, 10]. $1,25(OH)_2D$ also modulates the immune system by regulating the activity of both activated T- and B-lymphocytes and activated macrophages [7, 10, 15, 91, 92]. This may be the explanation for why Hyponnen et al. [93] observed that children treated with at least 2,000 IU of vitamin D a day reduced their risk of developing type 1 diabetes by 80 %. This was similar to what was observed when NOD mice, which invariably develop type 1 diabetes, received $1,25(OH)_2D_3$ [91, 92]*:* they showed an 80 % reduction in developing the disease.

The kidney is an endocrine organ for producing $1,25(OH)_2D$ for regulating calcium metabolism. Recently, it was recognized that $1,25(OH)_2D$ also downregulates the production of renin in the kidney [94]. This may be the explanation for why vitamin D deficiency is associated with hypertension and increased risk of coronary artery disease and congestive heart failure [44, 95–99]. Krause et al. [97] reported that exposure of hypertensive adults to a tanning bed that emitted UVB radiation raised the blood levels of 25(OH)D by more than 100 % and controlled their hypertension. A similar group of hypertensive adults exposed to a similar tanning bed for 3 months that emitted UVA but no UVB radiation not only did not increase their blood levels of 25(OH)D, but also had no effect on their hypertension (Fig. 27.23).

Fig. 27.21 Exposure of nursing home residents to ultraviolet-B lamps that were installed near the ceiling in the day room. This was found to be the most effective method of maintaining serum 25(OH)D levels in these residents. (Reproduced with permission from ref. [62]

27.17 Vitamin D and the Cancer Connection

Although in the 1990s there were several reports that some of the most common cancers occurred in people living at higher latitudes and that colon cancer and prostate cancer rates were significantly reduced in individuals with higher circulating levels of 25(OH)D, it was difficult to understand how increased exposure to sunlight could impact on decreasing risk of common cancers.

The reason for this is that it was well known that any significant increase in vitamin D intake or exposure to sunlight did not raise blood levels of $1,25(OH)_2D$. Thus, it was difficult to understand how increasing one's 25(OH)D levels would be able to regulate cellular growth and prevent some cancers, since circulating levels of $1,25(OH)_2D$, the antiproliferative hormone, were not increased. The mystery was solved when it was observed that prostate cells and prostate cancer cells expressed a functional 1-OHase similar to what was observed in the skin [3, 15, 99–101]. Since this initial observation, it is now recognized that normal colon tissue and colon cancer, breast and breast cancer cells, as well as variety of other cell types have the enzymatic machinery to convert 25(OH)D directly to $1,25(OH)_2D$ [3, 15, 16, 102–104]. Thus, it appears that when 25(OH)D levels are adequate, probably above 30 ng/mL, it acts a substrate for the extra renal 1-OHase in these tissues. The local production of $1,25(OH)_2D$ may be necessary to maintain and regulate genes responsible for cellular growth and to prevent the cells from becoming autonomous, that is, carcinogenic. It has been suggested that once it carries out its function, it induces the 25-hydroxyvitamin D-24-hydroxylase, which in turn catabolizes $1,25(OH)_2D$ to the inactive water-soluble calcitroic acid (Fig. 27.1).

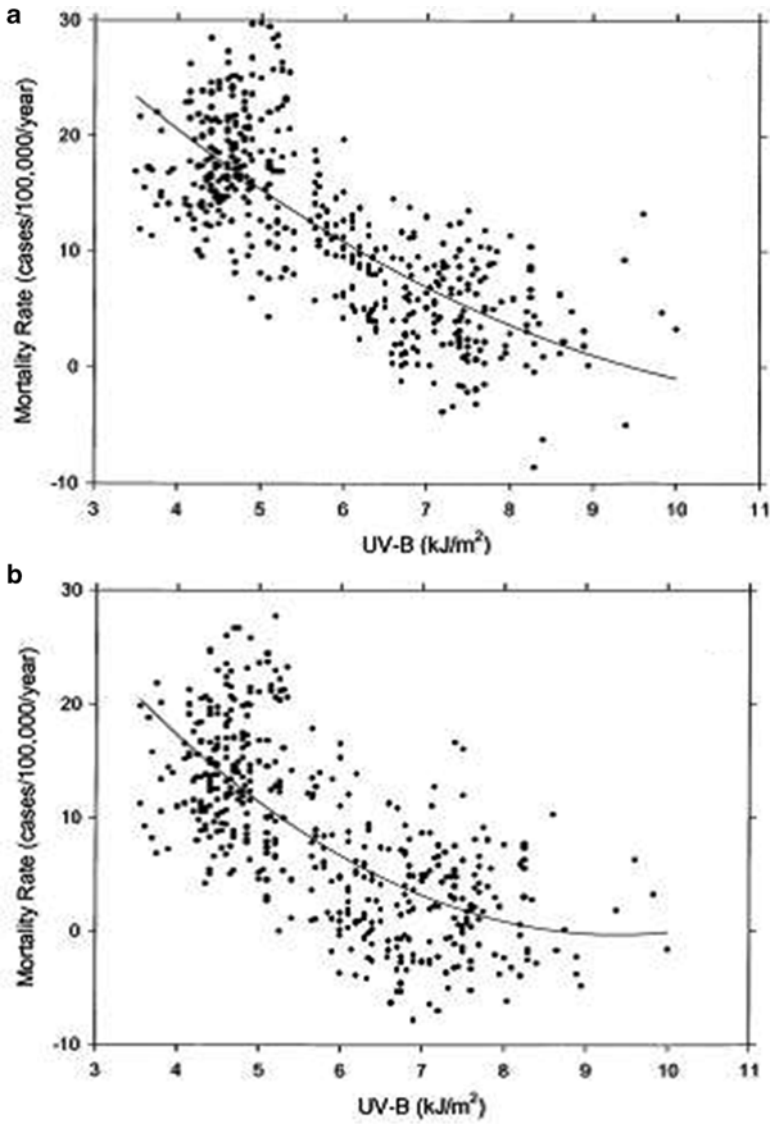

Fig. 27.22 (**a**) Premature mortality due to cancer, white females, vs. total ozone mapping spectrometer (TOMS), July 1992, DNA-weighed UV-B. (**b**) Premature mortality due to cancer with insufficient UV-B, white males, U.S., 1970–1994, vs. July 1992 DNA-weighted UV-B radiation. (Reproduced with permission from ref. [85])

27.18 Conclusion

Vitamin D deficiency is extremely common and needs to be recognized. Vitamin D deficiency in children and teenagers can result in poor bone health and the inability to attain the genetically predetermined peak bone mass. In young, middle-aged, and older adults, vitamin D deficiency causes osteomalacia and can precipitate and exacerbate osteoporosis. In addition, many of the symptoms associated with vitamin D deficiency mimic fibromyalgia, and as a result, many patients go undiagnosed.

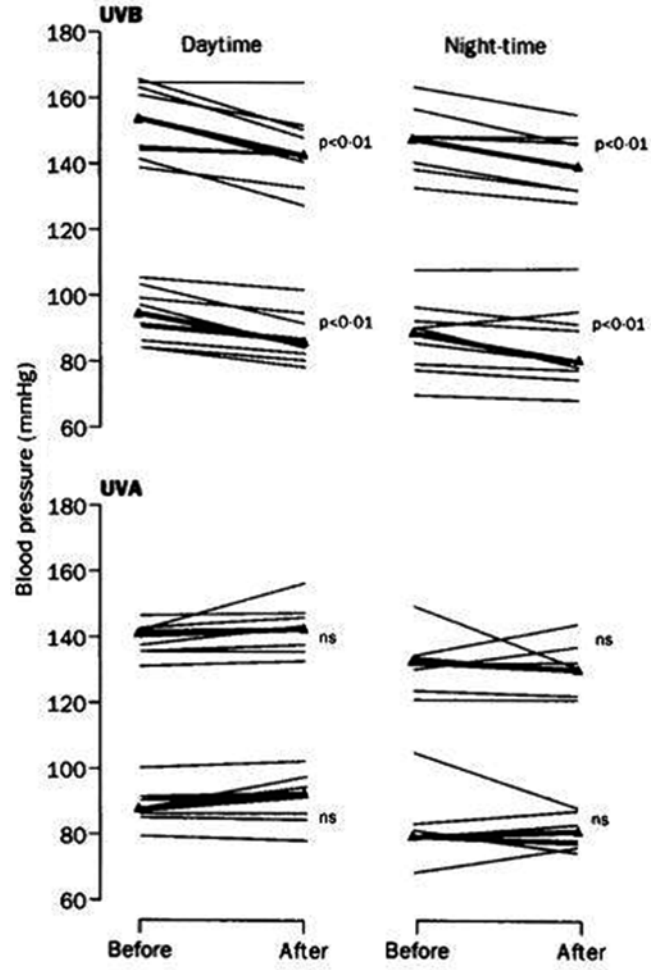

Fig. 27.23 Effect of UV-B and UV-A irradiation on ambulatory daytime and night-time blood pressure in hypertensive adults. *ns* nonsignificant. *Thick line* = mean. (Reproduced with permission from ref. [97]

Vitamin D deficiency, however, may have extremely important health consequences that heretofore have not been fully appreciated. Maintenance of an adequate 25(OH)D level of at least 20 ng/mL and preferably 40–60 ng/mL throughout life may help reduce the risk of developing many chronic diseases, including type 1 diabetes, hypertension, multiple sclerosis, infectious diseases, and cancers of the breast, prostate, colon, and ovary. Vitamin D deficiency has also important health implications for pregnant women and their newborns [7] (Fig. 27.24).

Thus, there needs to be a reawakening about the appreciation of maintaining a healthy vitamin D status throughout life. The best method to determine vitamin D adequacy is to measure 25(OH) D. Similar to evaluating patients for their blood pressure and blood lipid profile on their yearly exam, they should also be evaluated with a 25(OH)D to measure their vitamin D status. This will ensure vitamin D health and mitigate the consequences of vitamin D deficiency.

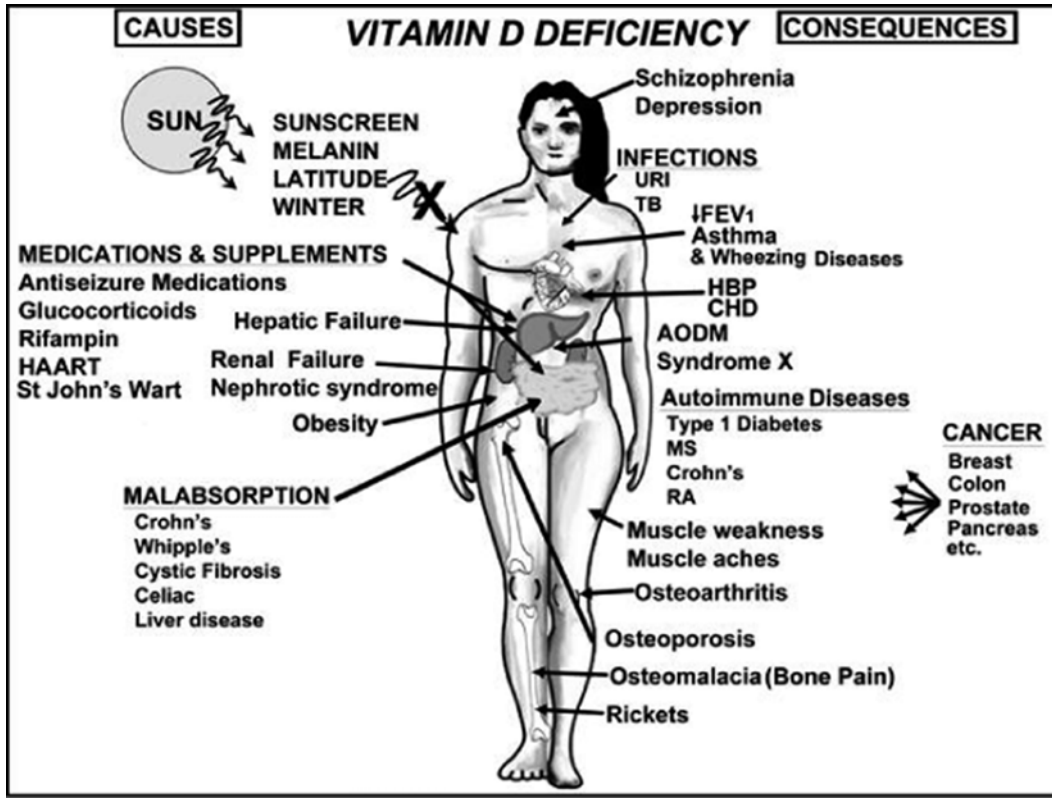

Fig. 27.24 A schematic representation of the major causes for vitamin D deficiency and potential health consequences. Holick copyright 2010. Reproduced with permission

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Chapter 28
Vitamin D Utilization in Subhuman Primates

John S. Adams, Hong Chen, Rene F. Chun, Thomas S. Lisse, Alejandro Garcia, and Martin Hewison

Man with all his noble qualities…with his god-like intellect which has penetrated into the movements and constitution of the solar system…still bears in his bodily frame, the indelible stamp of his lowly origin.

Charles Robert Darwin, in the Descent of Man

Key Points

- Nearly 30 years ago we began to investigate an outbreak of rachitic bone disease in adolescent New World primates residing at the Los Angeles Zoo.
- Our investigation of this "experiment of nature" and that of an adolescent human female with a similar phenotype led us to the discovery of a novel means for relative resistance to vitamin D in primates, including man.
- We coined this resistance-causing protein the vitamin D response element-binding protein or VDRE-BP for its ability to compete *in trans* with the liganded vitamin D receptor (VDR) for its cognate response elements.

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- VDRE-BP is now identified as a nucleic acid-binding protein(s) in the heterogeneous nuclear ribonucleoprotein C (hnRNPC) family.
- The purpose of this review is to examine the role of the VDRE-BP and other associated intracellular proteins that regulate the expression of vitamin D-controlled genes in nonhuman and human primates.

Keywords Vitamin D • Resistance • 1,25-Dihydroxyvitamin D • Monkeys • Ribonucleoprotein • Primate evolution • Steroid hormone • New World monkeys • Vitamin D response element

28.1 Early Primate Evolution

The three major primate infraorders, platyrrhines or New World primates, catarrhines or Old World primates, and lemurs, evolved independently of one another [1] (Fig. 28.1a) in the Eocene period, 50–100 million years ago owing to rupture of the great southern hemispheric landmass, Pangea. These tectonic events resulted in the American land mass and Madagascar moving away from Africa. Because this continental separation occurred early in the process of primate evolution, primordial primates in these three infraorders, were trapped in what we now know as South America, Africa, and Madagascar, respectively. Compared to Old World primates, including man, which have populated virtually every land mass on our planet over the course of last 50 million years, New World primates have remained confined to Central and South America, ±20° north and south of the equator. As such and in comparison to terrestrial Old World primate like gorillas, New World primates have evolved to be smaller in stature; a characteristic well suited to their lifestyle as plant-eating, arboreal sunbathers, residing in the canopy of the periequatorial rain forests of the Americas.

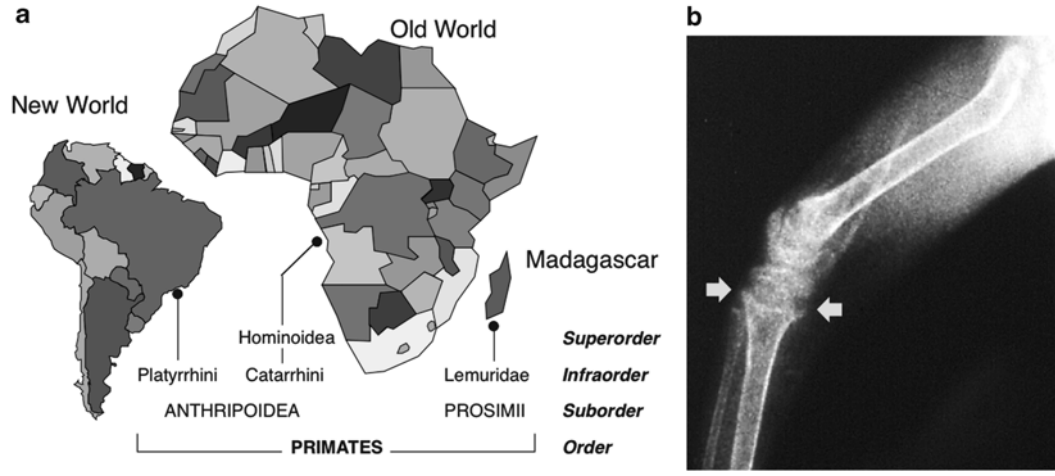

Fig. 28.1 New World primate evolution and rachitic bone lesion. Panel (**a**) describes in geographic terms the independent evolution of the three primate suborders, Platyrrhini, Catarrhini, and Lemuridae, in South America (the New World), Africa (the Old World), and Madagascar, respectively. Panel (**b**) displays the characteristic "cupping" and "fraying" of the tibial metaphysis (*arrows*) in a rachitic New World primate resident of the Los Angeles Zoo

28.2 Skeletal Disease in Captive New World Primates

The appearance of generalized metabolic bone disease in captive primates has been recognized for the last 150 years [2]. The disease, which has yet to be well studied from a histopathological standpoint, carries the clinical and radiological stigmata of rickets in adolescent primates and osteomalacia in adults, especially females [3] (Fig. 28.1b). Compared to Old World primates reared in captivity, New World primates are particularly susceptible to the disease. The disorder affects primarily young, growing animals and results in muscle weakness, skeletal fragility, and in many instances death of the affected individual. Rachitic bone disease of this sort has long presented a problem to veterinarians caring for captive platyrrhines, particularly in North American and European zoos [4, 5], because death of preadolescent and adolescent primates prior to sexual maturity severely limits on-site breeding programs.

Because the disease was reported to be ameliorated by either the oral administration of vitamin D_3 in large doses or by ultraviolet B (UVB) irradiation of affected primates, it was presumed to be caused by vitamin D deficiency [4]. The frequent occurrence of rickets and osteomalacia in New World primates was also ascribed to the relative inability of platyrrhines, compared to Old World primates including man, to effectively employ vitamin D_2 in their diet [6]; a similar observation had been made for chickens [7]. Realizing that in order to be active as a hormone vitamin D must first be converted to the pro-hormone 25-hydroxyvitamin D [25(OH)D] and then to the hormone 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (Fig. 28.2) and using an assay technology that does not discriminate between 25-hydroxylated vitamin D_2 and vitamin D_3 metabolites, Marx and colleagues [8] determined that 25(OH)D levels were two- to threefold higher when platyrrhines were dosed with supplemental vitamin D_3 than with vitamin D_2. These data suggested that 25-hydroxylation of vitamin D substrate in New World primates was much more effective when vitamin D_3 was employed as substrate. However, in the same study, two species of Old World primates demonstrated similar discrimination against vitamin D_2 in favor of vitamin D_3 to promote significantly more 25(OH)D produced.

The above results seemed to indicate that all subhuman primates, whether from Old or New World genera, were relatively resistant to vitamin D_2 in terms of its ability to engender an increase in total serum levels of 25(OH)D. This led Hay and colleagues [9] to suggest that New World primates may transport $25(OH)D_2$ and $25(OH)D_3$ in the serum by means that are dissimilar from those encountered

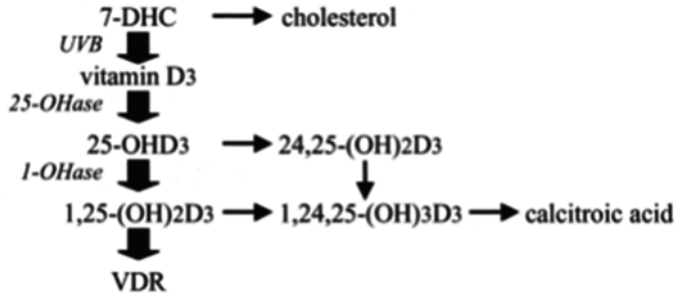

Fig. 28.2 Scheme of vitamin D synthesis and metabolism in New World primates. The *bold arrows* describe the means by which high 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] levels are achieved and maintained in New World primates. Ultraviolet B photon (UVB) exposure is increased in the natural habitat of New World primates, the canopy of the equatorial rain forests of Central and South America. Increased cutaneous vitamin D_3 synthesis results in increased production, via one of the many hepatic vitamin D-25-hydroxylases (*25-OHase*), of 25-hydroxyvitamin D_3 [$25(OH)D_3$]. Elevated $1,25(OH)_2D_3$ levels are achieved by increased synthesis of the hormone via the CYP27B1-hydroxylase (*1-OHase*) as well as by diminished catabolism to scheme $24,25(OH)_2D_3$ via the CYP24A1. In this way $1,25(OH)_2D_3$ becomes available to the VDR in relatively large quantities to compensate for the hormone-resistant state characteristic of New World primates

in Old World primate species. The Hay hypothesis was subsequently disproven by Bouillon et al. [10], who showed that the serum vitamin D-binding protein (DBP) was the major carrier of 25(OH)D_2 and 25(OH)D_3 in the serum of both New and Old World primates. It has been subsequently shown that 25(OH)D_2 does have shorter half-life in serum than does 25(OH)D_3, regardless of species examined, owing to a relative reduction in the affinity of DBP for vitamin D_2 metabolites [10].

Other possible explanations for the greatly increased susceptibility of New World compared to Old World primates to develop vitamin D-deficient skeletal disease is that New World primates in captivity somehow failed to convert the pre-pro-hormone vitamin D to the pro-hormone 25(OH)D effectively (see Fig. 28.2) and/or free-living New World primates employ a dietary source of vitamin D that is distinct from what is normally provided to the same species in captivity. The former has been shown not to be the case; there are now several reports of free-living New World primates possessing serum concentrations of 25(OH)D that approximate those encountered in captive primates of the same genera given supplemental dietary vitamin D or exposed to supplemental UVB [11]. The latter possibility is likely. It is now recognized that many genera of New World primates consume fungi in the wild [12–15]. When exposed to UVB, fungi are the richest natural source of vitamin D_2 on the planet [16].

28.3 Vitamin D and Steroid Hormone Resistance in New World Primates

The question of why platyrrhines were more susceptible to vitamin D deficiency than were catarrhines began to be answered with the detection of extraordinarily high circulating levels of the active vitamin D metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in New World primates [17, 18]. These data pointed to the fact that New World primates were resistant to the active vitamin D hormone. Beginning in 1985, our research team was asked to investigate an outbreak of rickets among New World primate species at the Los Angeles Zoo. The index case in our original studies was a preadolescent female New World primate of the Emperor tamarin species. When investigated radiographically (Fig. 28.1b), this tamarin and those like her displayed classical rickets complete with growth retardation, metaphyseal cupping, and fraying characteristic of rickets. In order to investigate this rachitic syndrome, blood and urine was collected from involved monkeys as well as from control, nonrachitic New and Old World primates. Compared to Old World primates and as shown in Fig. 28.3a, that comparison yielded a biochemical phenotype that was most remarkable for an elevated serum 1,25$(OH)_2$D level in rachitic New World primates [18]. In fact, with the exception of nocturnal primates in the genus *Aotus*, New World primates in all other genera had vitamin D hormone levels ranging up to two orders of magnitude higher than that observed in Old World primates, including man [19–21].

In the initial analysis New World primates affected with rickets were those with the lowest 1,25$(OH)_2$D levels, while their healthy counterparts were those with the highest serum 1,25$(OH)_2$D levels. These data were interpreted to mean that most New World primate genera were naturally resistant to the vitamin D hormone, and that the resistant state could be compensated by maintenance of high 1,25$(OH)_2$D levels. If this was true, then an increase in the serum 1,25$(OH)_2$D concentration in affected primates should result in biochemical compensation for the resistant state and resolution of their rachitic bone disease. When rachitic New World primates were exposed to artificial sunlight of 6 months duration in their enclosures, both substrate serum 25(OH)D and product 1,25$(OH)_2$D levels rose dramatically, resulting in cure of rickets [22] (Fig. 28.3b). New World primates are periequitorial sunbathers for a reason. As depicted by the oversized arrows in a simplified scheme of vitamin D synthesis and metabolism (Fig. 28.3), New World primates require a lot of cutaneous vitamin D synthesis in order to push their 25(OH)D and 1,25$(OH)_2$D levels high enough to interact effectively with the vitamin D receptor (VDR). The question remained as to why these primates are resistant to all but the highest levels of the vitamin D hormone.

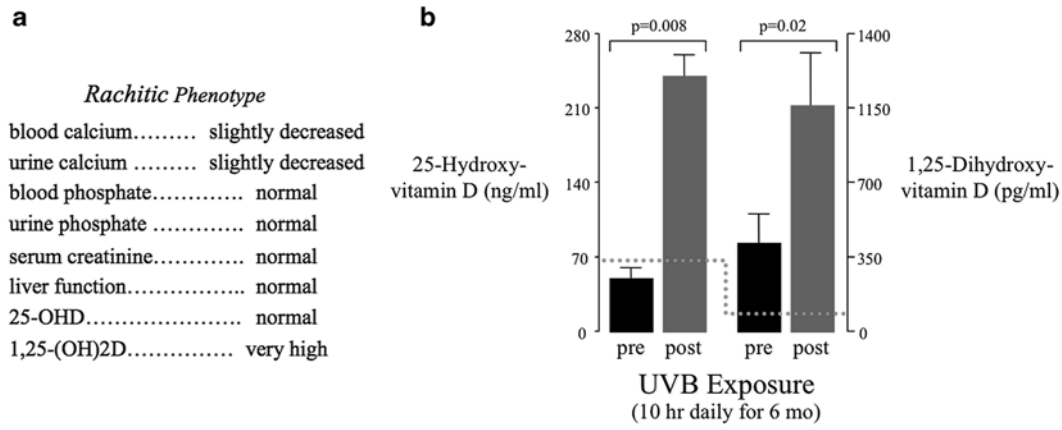

Fig. 28.3 Biochemical phenotype of rachitic New World primates. Panel (**a**) demonstrates biochemical indices of bone health in New World primate suffering from rickets compared to developmental age- and sex-matched nonrachitic Old World primates. The outstanding characteristic is a 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] level 2–3 orders of magnitude greater than that observed in Old World primates, including man. Panel B shows the mean 25-hydroxyvitamin D (*left*) and 1,25-dihydroxyvitamin D levels (*right*) in seven different rachitic New World primates before (pre) and after (post) exposure to 6 months of artificial sunlight in their enclosures. The upper limits of the normal human Old World primate range is described by the *dotted line*. Both substrate and product rose significantly with light therapy and resulted in cure of rickets. (Data from ref. [22])

The concept of generalized steroid hormone resistance in New World primates was first revealed by Brown et al. in 1970 [23]. These investigators discovered greatly elevated serum cortisol levels in platyrrhini compared to catarrhini. Despite biochemical evidence of resistance to glucocorticoids, platyrrhines affected with high cortisol levels showed no sign of glucocorticoid deficiency or toxicity at the level of the target organ; glucose homeostasis, electrolyte balance, blood pressure, and life expectancy were all similar to that observed in Old World primates [24]. These data indicated that glucocorticoid resistance in New World primates was physiologically compensated by increased synthesis of the hormone. Increased production of the hormone was achieved by lack of feedback inhibition of pituitary adrenocorticotropic hormone (ACTH) production [25], adrenal (zona fasiculata) hypertrophy [26], and increased enzymatic synthesis and decreased catabolism of cortisol [27, 28]. A relative increase in the availability of glucocorticoid to target tissues was also proposed to occur in New World primates [29] and to participate in the counter-response to cortisol resistance. What is the proximate cause of glucocorticoid resistance in New World primates? Early studies from the group of Lipsett and Loriaux [24, 30, 31] suggested that resistance was caused by expression of a glucocorticoid receptor (GR) in New World primates with a lower affinity for cortisol. Most recently, relative overexpression of FKBP51, the FK506-binding immunophilin that normally interacts with the heat shock protein 90 (hsp90)-GR complex, was postulated to be the cause of lowered affinity of the New World primate GR for its cognate ligand [32, 33] and by sequestration of the GR in the cytoplasm of the cell [34, 35]. It remains to be determined whether constitutive overexpression of the New World primate FKBP51 in Old World primate cells will squelch GR-directed transactivation in host cells.

New World primates are also resistant to steroid hormones produced by the ovary [25]. Until recently, it was considered that estrogen and progesterone resistance resulted from a diminishment of the estrogen receptor (ER) and progesterone receptor (PR) population in target tissues. A similar mechanism was proposed for vitamin D resistance [36]. As will be discussed below, it now appears that the steroid hormone and vitamin D hormone receptor complement of New World primates is not functionally distinct from that of their Old World primate counterparts. What is different is the relative

overexpression in New World primate cells of at least two distinct families of intracellular proteins, the heterogeneous nuclear ribonucleoproteins (hnRNPs) and the heat-shock proteins 70 (hsp70), which conspire to legislate, by receptor-independent means, the degree of steroid/sterol hormone resistance among the various New World primate species.

28.4 Investigating the Biochemical Nature of Vitamin D Resistance in New World Primates

In order to answer the above question, cultured fibroblasts and immortalized cell lines from both resistant and hormone-responsive New and Old World primates were used to track, step by step, the path taken by the vitamin D hormone from the serum vitamin D-binding protein (DBP) in the blood in route to the nucleus and transactivation of hormone-responsive genes [19–22, 37–42] (Fig. 28.4). It was determined that the movement of hormone from DBP across the cell membrane and through the cell cytoplasm and nuclear membrane in New World primate cells was indistinguishable from that observed in Old World primate cells.

It was also determined that the ability of the New World primate VDR to bind to $1,25(OH)_2D_3$ or $1,25(OH)_2D_2$ and induce receptor dimerization with the retinoid X receptor (RXR) was normal. In fact, when removed from the intranuclear environment and in distinction to previous reports [36], the VDR in New World primates was similar to the Old World primate VDR in all biochemical and functional respects [40]. What was not the same in New World primate cells (*see* Fig. 28.4b) was the reduced ability of VDR-RXR complex to bind to its cognate *cis* element and transactivate gene expression. In addition to this failure was the apparent buildup of hormone in the cytoplasm of the New World primate cell.

In order to elucidate nuclear receptor events in New World primate cells, the nuclei of New World primate cells were isolated and extracted. In addition to the VDR–RXR, it was determined that these extracts contained a second protein that was bound by a consensus vitamin D response element

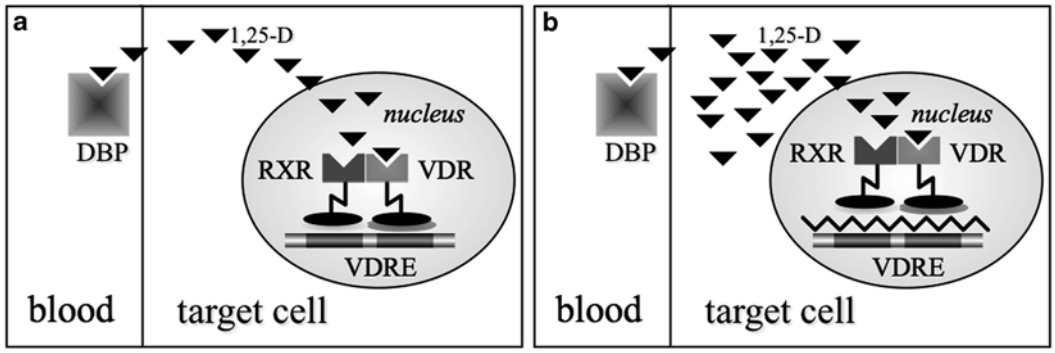

Fig. 28.4 Pathway of hormone 1,25-dihydroxyvitamin D (1,25-D) from the blood to nucleus of the target cell in the vitamin D-resistant New World primate. In Old World primate cells (Panel **a**) the vitamin D hormone (*dark triangles*) moves normally from the circulating vitamin D-binding protein (DBP), through the cell membrane and cytoplasm, and onto the vitamin D receptor (VDR) paired with the retinoid X receptor (RXR) forming a heterodimeric complex in the cell nucleus. This heterodimeric complex then interacts with its cognate cis element, the vitamin D response element (VDRE), and initiates transcription of vitamin D-regulated genes. Panel (**b**) demonstrates similar events as they occur in New World primate cells. The *jagged line* at the VDRE represents a relative inability of the heterodimeric receptor complex to engage the cis element. This and the accumulation of hormone in the cell cytoplasm represent salient disparities in hormone handling and action in the New World and Old World primate cells

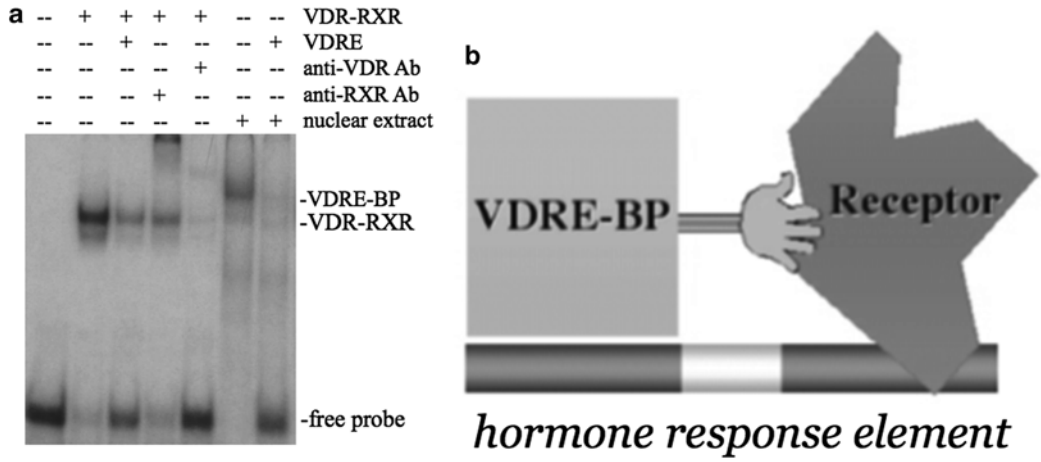

Fig. 28.5 Evidence for the dominant-negative action of the New World primate vitamin D response element-binding protein (VDRE-BP). Panel (**a**) shows an electromobility shift assay using consensus vitamin D response element as probe, showing the presence of a second trans-binding protein, in addition to the vitamin D receptor (VDR)–retinoid X receptor (RXR), in nuclear extracts of vitamin D-resistant New World primate cells. Addition of excess unlabeled VDRE is shown in *lanes 3* and *7*. Addition of anti-RXRalpha antibody (*lane 4*) supershifts, while addition of either anti-VDR antibody (*lane 5*) or New World primate nuclear extract containing the VDRE-BP (*lane 6*) competes away probe-VDR-RXR binding; these data are reprinted with permission of the authors from ref. [43]. Panel (**b**) is a cartoon representing the proposed competition for binding to the VDRE between the VDRE–BP and vitamin D receptor (VDR)

(VDRE). This protein was coined the vitamin D response element-binding protein (VDRE-BP) [43]. In electromobility shift assay (EMSA) using the VDRE as probe (Fig. 28.5a), Old World primate cell extract contained only the VDR–RXR bound to the VDRE probe, while the New World primate extract contained two probe-reactive bands, one compatible with the VDR–RXR and a second, more pronounced VDRE–BP–VDRE band. This VDRE–BP–VDRE binding reaction was specific, as the VDRE–BP was competed away from VDRE probe by the addition of excess unlabeled VDRE. These data suggested that VDRE–BP might function as a dominant-negative inhibitor of receptor-response element binding by competing *in trans* with receptor, "knocking it off" the VDRE (Fig. 28.5b). That was the case. When recombinant human VDR and RXR were permitted to interact in EMSA with increasing amounts of nuclear extract from either vitamin D-resistant cells containing a VDRE–BP or from normal vitamin D-responsive cells, the addition of more control extract acted only to amplify the VDR–RXR–retarded probe on the gel. By contrast, increasing amounts of the hormone-resistant extract competed away VDR–RXR–probe binding in favor VDRE–BP–probe binding.

28.5 Response Element-Binding Proteins

The VDRE–BPs in New World primates have been identified [44, 45]. Previously considered to be only single-strand mRNA-binding proteins [46] the VDRE-BPs are members of the heterogeneous nuclear ribonucleoprotein C (hnRNP C) family, hnRNP *C1* and *hnRNPC2* [47], that acts by binding to nucleic acid substrates as a tetramer ($C1_3C2_1$) [48]. However, as pointed out (*see* Fig. 28.5a), VDRE–BPs can also bind specifically to double-strand DNA. In fact, it is by virtue of their ability to bind DNA that they can be distinguished from traditional corepressor proteins [49]. When overexpressed, they can effectively squelch VDR-directed transactivation. This ability to squelch transactivation is shown in Fig. 28.6. Depicted is VDRE-directed reporter activity in four different subclones

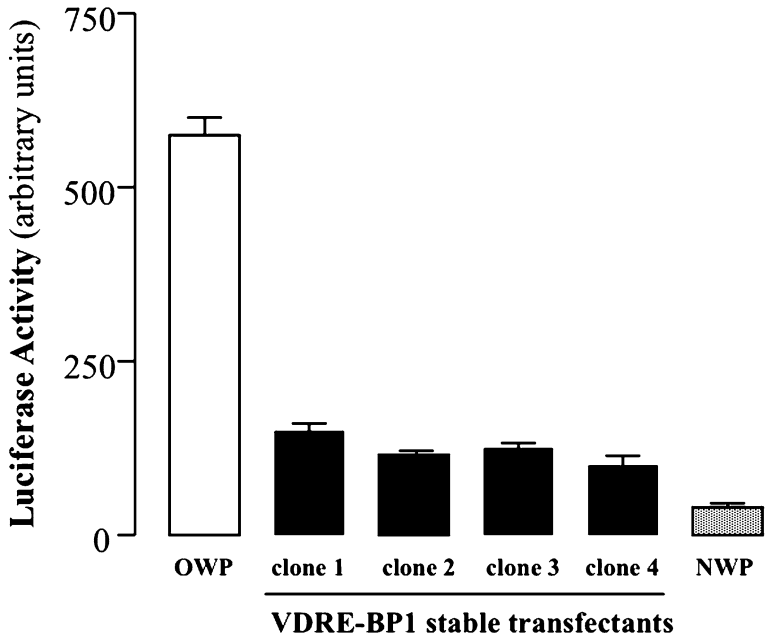

Fig. 28.6 Vitamin D response element-binding protein (VDRE-BP) squelched transactivation. Shown is significant squelching ($p<0.001$) of VDR–RXR-directed, VDRE–reporter-driven transactivation in four different clones of Old World primate (OWP) cells after stable transfection with the New World primate VDRE–BP; reporter activity in untransfected New World primate (NWP) cells is shown for comparison. (Data from ref. [43])

of wild-type Old World primate cells stably overexpressing the New World primate VDRE–BP as well as New World primate cells that are naturally hormone-resistant. In all instances, stable overexpression of VDRE–BP squelched VDRE-directed luciferase activity substantially compared to the untransfected, wild-type host cell to levels observed in hormone-resistant New World primate cells that naturally overexpress the protein. These data provide strong confirmatory evidence that when naturally overexpressed in vivo, VDRE–BP is the cause of vitamin D resistance in these monkeys.

As previously noted New World primates appear to be resistant to a host of steroid/sterol hormones, including estrogen, androgen, progesterone, glucocorticoids, and mineralocorticoids, not just vitamin D. We have isolated and characterized the functional equivalent of the VDRE-BP for estrogens. This estrogen response element-binding protein (ERE-BP) is also an hnRNP, hnRNPD [48, 50–53].

28.6 Intracellular Vitamin D-Binding Proteins

On the way to the discovery of the VDRE–BPs in New World primate cells, it was also observed that these cells were extraordinarily efficient at accumulating 25-hydroxylated vitamin D metabolites in the cytoplasmic space (*see* Fig. 28.4b). Accumulation here was the result of expression of a second set of resistance-associated proteins. These intracellular vitamin D-binding proteins [54, 55], or IDBPs as they have come to be called, exhibit both high capacity and high affinity for 25-hydroxylated vitamin D metabolites. In fact, among all of the vitamin D metabolites that have been tested, IDBP purified from vitamin D-resistant New World primate cells binds 25(OH)D_3 and 25(OH)D_2 best [21, 55]; in a competitive displacement assay using radioinert 25(OH)D_3 as competitor and [^{3}H]25(OH)D_3 as labeled ligand, the concentration of metabolite required to achieve half-maximal displacement of

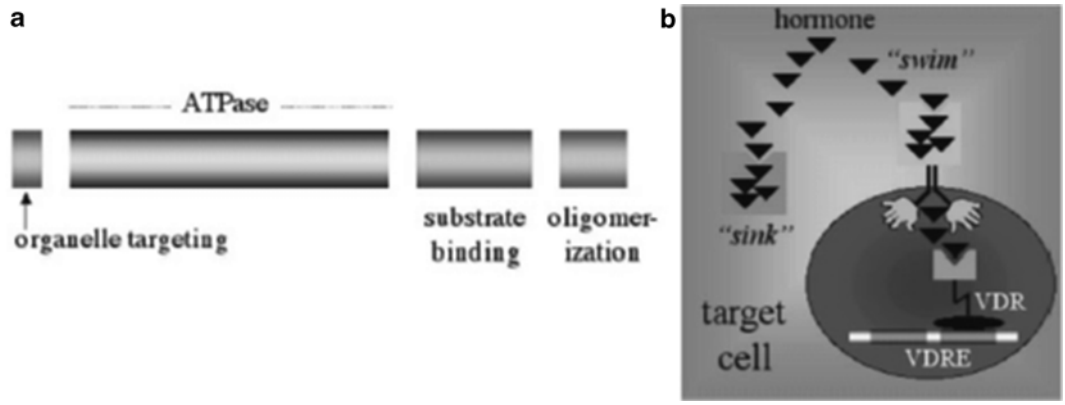

Fig. 28.7 The hsp-70-related intracellular vitamin D-binding proteins and their proposed function in vitamin D-resistant New World primate cells. Panel (**a**) shows the general domain structure of these hsp-70-like proteins. They all contain an ATP-binding ATPase domain ahead of two protein–protein interaction domains. Some also harbor an N-terminal organelle-targeting domain. Two countervailing hypotheses for the function of these proteins were considered (Panel **b**). One hypothesis held that these IDBPs were "sink" molecules that worked in cooperation with the response element-binding proteins to exert vitamin D resistance by disallowing access of the hormone to the vitamin receptor (VDR) and the nucleus of the cell. The opposing hypothesis held that these were "swim" molecules, promoting the delivery of ligand to the vitamin D receptor, improving the ability of the VDR to dimerize and bind to the vitamin D response element (VDRE–BP), antagonizing the actions of the vitamin D response element-binding protein that is overexpressed in New World primate cells

labeled hormone (EC_{50}) < 1 nM. Although normally present in Old World primate, including human cells, these proteins can be overexpressed some 50-fold in New World primate cells. They are highly homologous to proteins in the human (Old World primate) heat-shock protein-70 family [56]. The first three members of this family cloned and characterized by our laboratory, IDBP-1, -2, and -3, bear a high degree of sequence identity with constitutively expressed human heat-shock protein-70, heat-shock-inducible heat-shock protein-70, and mitochondrial-targeted grp-75, respectively. The general domain structure of the IDBPs [56] is shown in Fig. 28.7a. They all contain an ATP-binding-ATPase domain ahead of a protein–protein interaction domain. Some, such as IDBP-3, also harbor an N-terminal organelle-targeting domain. The vitamin D ligand (substrate)-binding domain is in the middle of the molecule [57].

What are these IDBPs doing inside the hormone-resistant New World primate cell? Two countervailing hypotheses were considered to explain the function of these proteins (Fig. 28.7b). One hypothesis held that these IDBPs were "sink" molecules that worked in cooperation with the VDRE–BP in the nucleus to exert vitamin D resistance by disallowing access of the hormone to the VDR and the nucleus of the cell. The opposing hypothesis held that these were "swim" molecules that actually promoted the delivery of ligand to the vitamin D receptor, improving the ability of the VDR to dimerize and bind to DNA, antagonizing the actions of the VDRE–BP that was overexpressed in New World primate cells. In order to determine which of these hypotheses was correct, we stably overexpressed the most abundant of these IDBPs, IDBP-1, or hsc70, in wild-type Old World primate cells and demonstrated that IDBP-1 imparted protransactivating potential [57]; the endogenous transcriptional activity of three different $1,25(OH)_2D$-responsive genes, the vitamin CYP24-hydroxylase, osteopontin, and osteocalcin genes, in Old World primate wild-type cells was markedly enhanced (Fig. 28.8a). It was concluded from these studies, at least for the function of transactivation, that IDBP-1 was a "swim" molecule for the vitamin D hormone, promoting delivery of ligand to the VDR.

Considering the facts that New World primates are required to maintain very high serum levels of $1,25(OH)_2D$ in order to avert rickets (*see* Figs. 28.2 and 28.3), it was also hypothesized that the IDBPs, which are known to bind 25(OH)D even better than $1,25(OH)_2D$, will also promote the synthesis of the active vitamin D metabolite via promotion of the CYP27B-1-hydroxylase.

Evidence that this is the case is provided in Fig. 28.8b. When human kidney cells expressing the CYP27B-1-hydroxylase gene were stably transfected with IDBP-1 and incubated with substrate $25(OH)D_3$, $1,25(OH)_2D_3$ production went up 4-8-fold compared to untransfected wild-type cells [58]. This increase in specific 25(OH)D-1-hydroxylase activity occurred independent of a change in expression of the CYP27B-1-hydroxylase gene [58]. In fact, data [58] now strongly indicate that this increase in hormone production is the result of the ability of IDBPs to promote the delivery of substrate 25(OH)D to the inner mitochondrial membrane and the CYP27B-1-hydroxylase stabled there.

28.7 A New Model for Intracellular Vitamin D Trafficking

Dogma has held that sterol/steroid hormones such as vitamin D, by nature of their lipid solubility, move through the plasma membrane of the target cell and "ping-pong" around the cell interior until they encounter another specific binding protein such as the CYP27B-1-hydroxylase or the VDR with which to bind. Recent results, developed from a compendium of confocal imaging studies with fluorescently labeled IDBPs and vitamin D metabolites as well as with gst-pull-down, co-immunoprecipitation, and yeast 2-hybrid binding experiments [45], indicate that the hormone does not haphazardly "ping-pong" around the cell interior. Rather, the hormone enters the cell and is distributed to specific intracellular destinations by a series of protein–protein interactions that involve the hsp family of intracellular vitamin D-binding proteins.

For example, it is now know from the work of Willnow and coworkers [59, 60] that vitamin Ds can enter some target cells (e.g., the proximal tubular epithelial cell of the kidney, the principal site of endocrine-acting $1,25(OH)_2D$ synthesis) via internalized vesicles (Fig. 28.9). The vitamin D stays bound to the serum vitamin D-binding protein (DBP), which is in turn bound by megalin and cubulin, members of the LDL superfamily of proteins. Once inside the cell there is interaction between the C-terminal domain of megalin, which protrudes into the cytoplasm, and the N-terminal domain of at least two different IDBPs, IDBP-1 and -3 [61, 62]. Upon acidification of the vesicle interior and denaturation of DBP, 25(OH)D is free to interact with the IDBP.

If one overexpresses either IDBP-1, the hsc-70 homolog, or IDBP-3, a mitochondrially-targeted hsp, and incubates transfected IDBP-overexpressing cells with a fluorescently labeled 25-hydroxylated vitamin D metabolite, one will observe a significant increase in the uptake of the labeled hormone [63].

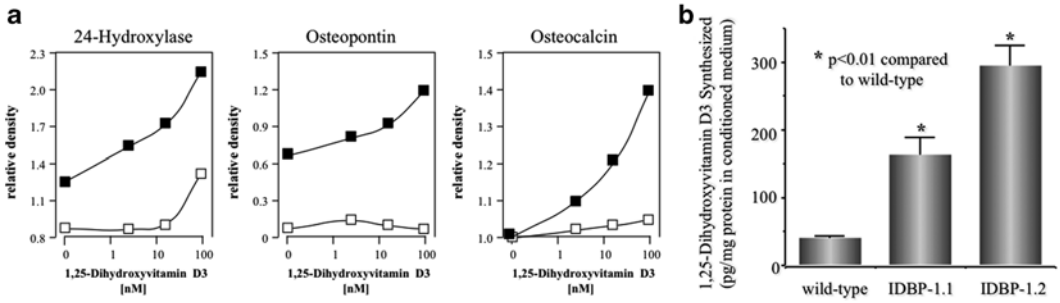

Fig. 28.8 Consequences of stable overexpression of members of the New World primate intracellular vitamin D-binding protein (IDBP) family in vitamin D-responsive Old World primate cells. Panel (**a**) depicts the 1,25-dihydroxyvitamin D concentration-dependent relative endogenous expression level, by Northern blot analysis, of three hormone-responsive genes in Old World primate cells before (*open squares*) and after (*filled squares*) stable overexpression of IDBP-1. Panel B shows the 1,25-dihydroxyvitamin D synthetic capacity of Old World primate (wild-type) before and after and stable overexpression of IDBP-1; IDBP-1.1 and -1.2 represent different clones of cells stably transfected with IDBP-1. (Data from refs. [57, 58])

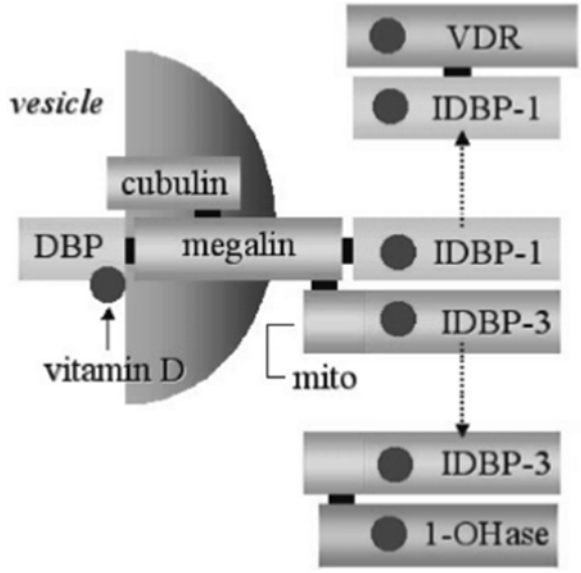

Fig. 28.9 Proposed roles for the intracellular vitamin D-binding proteins (IDBPs) in the intracellular trafficking of vitamin D metabolites. Vitamin Ds enter target cells via internalized vesicles. The vitamin D remains bound to the serum vitamin D-binding protein (DBP), which is, in turn, bound by members of the LDL superfamily of proteins, megalin and cubulin. Once inside the cell there is the potential for interaction between the C-terminal domain of megalin, which protrudes into the cytoplasm, and the N-terminal domain of at least two different IDBPs, IDBP-1 and -3. It is proposed that if the interaction is with IDBP-1, then there is transfer of the vitamin D cargo to IDBP-1. IDBP-1 can undergo protein–protein interaction with the vitamin D receptor (VDR), resulting in delivery of its vitamin D cargo to the receptor and promotion of transactivation. On the other hand, if the megalin interaction is with IDBP-3, which possesses an amino-terminal mitochondrial targeting sequence (mito), then there exists the potential for the IDBP-3 and its cargo to enter mitochondrial. A protein–protein interaction between the CYP27B-1-hydroxylase and IDBP-3 can result in transfer of the vitamin D cargo to the enzyme for metabolism

Moreover, if the protein–protein interaction is between megalin and IDBP-1 and the ligand is $1,25(OH)_2D_3$, then the ultimate destination for that hormone and its chaperone is the unliganded VDR [45] residing in the perinuclear region of the cell. If, on the other hand, megalin interacts with IDBP-3, which contains an N-terminal targeting sequence for the inner mitochondrial membrane, then the ultimate destination for the hormone is the mitochondria. Confirmation of a protein–protein interaction between a substrate-carrying IDBP molecule and a target enzyme, the CYP27B-1-hydroxylase, has been accomplished with gst "pull-down" assays using the carboxy-terminal domain of the CYP27B-1-hydroxylase as bait [61]; this is the portion of the CYP27B-1-hydroxylase that is exposed to the intermembrane space of the mitochondria. Employing this substrate-accessible part of the enzyme to capture CYP27B-1-hydroxylase-interacting proteins, it has recently been shown that the grp-75-like IDBP-3, but not the hsc-like IDBP-1, interacts with the 25(OH)D-1-hydroxylase (Chun, unpublished).

28.8 Conclusion

In summary, it is proposed that these hsp-mediated-chaperoning events are normally active in man but overly active in hormone-resistant New World primates, where they function to compensate for the VDRE-BP (hnRNP C1/C2)-directed vitamin D-resistant state by augmenting receptor function and increasing vitamin D hormone synthesis. We anticipate that further analysis of these events will more

clearly define the chaperone mediated vitamin D trafficking circuits that contribute to a determination of the action and metabolism of vitamin D metabolites in target cells harboring the VDR and/or vitamin D hydroxylases.

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Chapter 29
Vitamin K's Role in Age-Related Bone Loss: A Critical Review

M. Kyla Shea and Sarah L. Booth

Key Points

- Vitamin K is a group of structurally similar vitamers that all function as an enzymatic cofactor in the conversion of specific glutamic acid (Glu) residues to gamma (γ)-carboxyglutamic acid (Gla) residues in certain proteins.
- The common feature of these proteins is that the Gla residues are essential for binding calcium. While the most commonly known vitamin K-dependent (VKD) proteins function in coagulation, several VKD proteins are present in extra-hepatic tissue, including bone.
- There are two naturally occurring form of vitamin K that share a common chemical structure (a 2-methyl-1,4-napthoquinone (Fig. 29.1)) and are capable of carboxylating VKD proteins.
- The primary dietary form, phylloquinone (vitamin K_1), which has a phytyl group at the 3-position, is found in green leafy vegetables and vegetable oils. Phylloquinone contributes up to 60 % of total dietary vitamin K intakes.
- Menaquinones, collectively known as vitamin K_2, differ structurally from phylloquinone by their 3′-substituted unsaturated multiprenyl group side chain. Menaquinone-4 (MK-4) is primarily found in poultry and pork products because a synthetic precursor to MK-4, menadione (vitamin K_3) is abundant in animal feed.
- Physiologically, phylloquinone can also be converted to MK-4. While phylloquinone is the predominate form of vitamin K in circulation and in bone, MK-4 concentrations are higher than phylloquinone in other extrahepatic tissues. Unlike other menaquinones, MK-4 is not formed from bacterial synthesis.
- Therefore the common usage of the term, vitamin K_2, to include all menaquinones is misleading as there are different origins and potential functions within this large group of vitamers. Longer-chain menaquinones [menaquinone-7 (MK-7)–menaquinone-10] originate from bacterial synthesis, and are primarily found in fermented dairy products and fermented plant-based foods.
- Natto is a fermented soy food traditionally eaten in Japan and is rich in MK-7. However, menaquinones generally contribute less to total vitamin K intakes of Western diets than phylloquinone.
- The role of long-chain menaquinones to human health is complicated by the fact that they are also synthesized by bacteria in the lower intestine. Not all intestinal bacteria synthesize menaquinones and the intestinally synthesized menaquinones are not well-absorbed, so their contribution to vitamin K nutritional status is uncertain.

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29.1 Abbreviations

BMC	Bone mineral content
BMD	Bone mineral density
Ca	Calcium
cOC	Carboxylated osteocalcin
D_3	Vitamin D_3 (cholecalciferol)
DXA	Dual X-ray absorptiometry
ELISA	Enzyme-linked immunoassay
FFQ	Food frequency questionnaire
Mg	Magnesium
MK-4	Menaquinone-4
MK-7	Menaquinone-7
NFkB	Nuclear factor kappa B
OC	Osteocalcin
RCT	Randomized clinical trial
SXR	Steroid and xenobiotic nuclear receptor
ucOC	Undercarboxylated osteocalcin
VKD	Vitamin K-dependent
Zn	Zinc

29.2 Introduction

Vitamin K is a group of structurally similar vitamers that all function as an enzymatic cofactor in the conversion of specific glutamic acid (Glu) residues to gamma (γ)-carboxyglutamic acid (Gla) residues in certain proteins. The common feature of these proteins is that the Gla residues are essential for binding calcium. While the most commonly known vitamin K-dependent (VKD) proteins function in coagulation, several VKD proteins are present in extra-hepatic tissue, including bone.

There are two naturally occurring forms of vitamin K that share a common chemical structure (a 2-methyl-1,4-napthoquinone (Fig. 29.1)) and are capable of carboxylating VKD proteins. The primary dietary form, phylloquinone (vitamin K_1), which has a phytyl group at the 3-position, is found in green leafy vegetables and vegetable oils. Phylloquinone contributes up to 60 % of total dietary vitamin K intakes [1]. Menaquinones, collectively known as vitamin K_2, differ structurally from phylloquinone by their 3′-substituted unsaturated multiprenyl group side chain (Fig. 29.1). Menaquinone-4 (MK-4) is primarily found in poultry and pork products because a synthetic precursor to MK-4, menadione (vitamin K_3) is abundant in animal feed [2]. Physiologically, phylloquinone can also be converted to MK-4 [3, 4]. While phylloquinone is the predominate form of vitamin K in circulation and in bone, MK-4 concentrations are higher than phylloquinone in other extrahepatic tissues [5]. Unlike other menaquinones, MK-4 is not formed from bacterial synthesis. Therefore the common usage of the term, vitamin K_2, to include all menaquinones is misleading as there are different origins and potential functions within this large group of vitamers. Longer-chain menaquinones [menaquinone-7 (MK-7)–menaquinone-10] originate from bacterial synthesis, and are primarily found in fermented

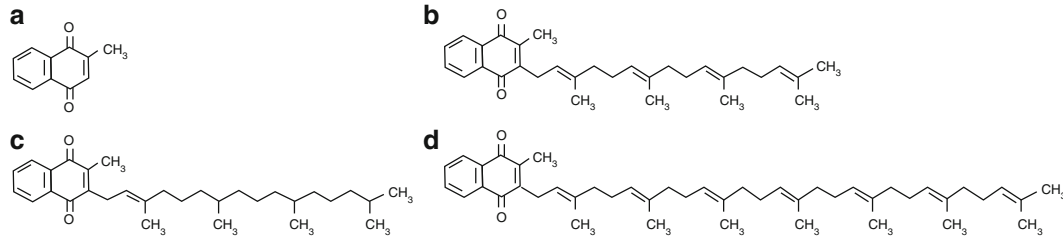

Fig. 29.1 Common structures of vitamin K: (**a**) Menadione, a synthetic form of vitamin K used in animal feed, and the intermediate form in the conversion of phylloquinone to MK-4 [2]; (**b**) MK-4 is formed by tissue-specific conversion from phylloquinone or menadione [3, 4]; (**c**) PK is the primary dietary source, and is present in highest concentrations in green leafy vegetables and certain plant oils [1], and (**d**) MK-7 is primarily the product of fermentation using *Bacillus natto*, and is present in a traditional Japanese soybean-based product called *natto* and is commercially available as a dietary supplement [85]

dairy products and fermented plant-based foods. *Natto*, for example, is a fermented soy food traditionally eaten in Japan and is rich in MK-7. However, menaquinones generally contribute less to total vitamin K intakes of Western diets than phylloquinone. The role of long-chain menaquinones to human health is complicated by the fact that they are also synthesized by bacteria in the lower intestine. Not all intestinal bacteria synthesize menaquinones and the intestinally synthesized menaquinones are not well-absorbed [6], so their contribution to vitamin K nutritional status is uncertain [2, 7]. For the purpose of this review, we will focus on vitamin K intakes from food and/or supplements, and will assume that circulating vitamin K concentrations are derived primarily from intakes.

29.2.1 Recommended Intakes

The current dietary recommendations for vitamin K in North America are based on median intakes estimated using national surveys in healthy adults [8], and are set at 90 μg/day and 120 μg/day for women and men respectively. Dietary recommendations for vitamin K vary widely globally, as reviewed elsewhere [9]. Nutritional survey data indicate habitual intakes are much lower than recommended in North America and Europe [10–12], but are reported to be higher in Asian countries [13]. The only defined clinical outcome linked to vitamin K deficiency is prolonged clotting time, which is not sensitive to dietary manipulations. It is not certain whether recommended vitamin K intakes are sufficient to maintain vitamin K status of extra-hepatic tissues, including bone.

29.3 Role of Vitamin K in Bone

29.3.1 Presence of VKD Protein in Bone

Several VKD proteins are present in bone, including osteocalcin (OC, also known as bone gla protein), matrix gla protein, and protein S [14–16]. OC is one of the most abundant noncollaganeous proteins in bone and is the most extensively studied VKD bone protein. The carboxylated form of OC (cOC, also known as glaOC) can bind calcium and is thought to function in hydroxyapatite maturation [17]. While the majority of OC is found in bone matrix, small amounts are also detectable in circulation. OC is secreted from osteoblasts and circulating OC is considered a biomarker of bone formation

because the OC released in circulation correlates with bone formation [18, 19]. Because total OC does not reflect vitamin K status [20], assays that measure only the cOC and undercarboxylated OC (ucOC, also known as gluOC) fractions are now available [21, 22]. UcOC can be measured directly by ELISA [21] or indirectly by hydroxyapatite binding assay coupled with an immunoassay [17]. The absolute concentration of ucOC positively correlates with total OC the more total OC there is, the more absolute ucOC the more total OC there may potentially be. To minimize confounding and isolate the vitamin K effect from the bone formation effect, ucOC should be expressed as a percentage of total OC (%ucOC; when measured using a hydroxyapatite binding assay) or as a ratio to the carboxylated OC when measured directly [21]. Based on hydroxyapatite binding assays, it is estimated that up to 40 % of OC in circulation is not completely carboxylated [20, 23]. Dietary depletion/repletion studies demonstrate that when vitamin K intakes are low, %ucOC is elevated and that intakes of approximately 1 mg/day are required to maximally carboxylate OC [24, 25].

29.3.2 Alternative Roles of Vitamin K in Bone

Emerging evidence suggests that vitamin K may have roles in skeletal tissue independent of its enzymatic cofactor function. MK-4 is reported to regulate expression of genes implicated in bone homeostasis by binding to the steroid and xenobiotic nuclear receptor (SXR) [26–28]. SXR activation has also been shown to down regulate activity of nuclear factor kB (NFkB), a transcription factor which regulates expression of several inflammatory cytokine genes [29]. Interestingly, MK-4 treatment of macrophage cells suppressed expression of interleukin-1β (IL1β) and interleukin-6 (IL6), cytokines that have been implicated in bone loss [30], and this suppression was found to be via inhibition of NFkB translocation [28, 31].

29.4 Evidence for a Role of Vitamin K in Age-Related Bone Loss

Several reviews have summarized animal and human studies of vitamin K and bone health, including Chap. 27 of the first edition of *Nutrition and Bone Health* [32]. The aim of this chapter is to summarize the studies published since then, with a focus on human observational and intervention studies that evaluated the potentially protective role of vitamin K against age-related bone loss.

29.4.1 Observational Studies

As reviewed previously [32], observational studies reported phylloquinone intakes were inversely associated with fracture risk [33, 34]. In contrast, associations between phylloquinone intakes and bone mineral density (BMD) were inconsistent [34, 35]. Subsequent analyses continue to yield inconsistent findings [36–40] (Table 29.1). In a cross-sectional and longitudinal analysis of over 2,000 Caucasian Danish perimenopausal women, phylloquinone intake estimated using administered 4- or 7-day diet records was not associated with BMD or change in BMD over 5–10 years of follow-up. In a nested case–control analysis of this same cohort, phylloquinone intake was also not associated with incident fracture over that same time frame [40]. Likewise, in a prospective analysis of older community-dwelling men and women from Hong Kong, self-reported phylloquinone intake (estimated using a validated food frequency questionnaire (FFQ)) was not associated with hip or any nonvertebral fracture over 7 years of follow-up [38]. Conversely, in a longitudinal analysis of the

Table 29.1 Observational studies of vitamin K status and bone health published since 2004

Participants/design	Design	Exposure(s)	Outcome(s)	Results	Reference
Studies of vitamin K intake					
2016 peri-menopausal, Danish women, 43–58 years	Longitudinal, 5 and 10 year follow-up	Phylloquinone intake	Femoral neck and lumbar spine BMD, fracture	No association between phylloquinone intake and BMD, change in BMD or fracture risk at any time point	[40]
583 men, 768 women, mean age 59 years	Cross-sectional	Phylloquinone intake, plasma phylloquinone, %ucOC	BMD parameters measured using calcaneal ultrasound	Positive association between phylloquinone intake and calcaneal BMD in men only; no other associations detected	[39]
1,238 men, 1,569 women, 71–75 years, community-dwelling, Hordaland Health Study (Norway)	Longitudinal, 10 year follow-up	Phylloquinone and menaquinone intake	Hip fracture	Inverse association between phylloquinone intake and hip fracture risk; no association with menaquinone intake	[36]
162 men, 200 women, non-osteoporotic; mean age 67	Cross-sectional and longitudinal, 2 year follow up	Phylloquinone intake	BMD parameters measured using calcaneal ultrasound	Positive association between phylloquinone and calcaneal BMD	[37]
1,605 men, 1,339 women community-dwelling adults from Hong Kong, mean age 74 years	Longitudinal, 7 year follow-up	Phylloquinone intake	Total hip BMD, hip fracture and any nonvertebral fracture	No association between phylloquinone intake and any fracture in any analysis	[38]
Studies of vitamin K status biomarkers					
741 men, 863 women, mean age 59 years	Cross-sectional	Plasma phylloquinone, %ucOC	Femoral neck, greater trochanter and lumbar spine BMD	Men: plasma phylloquinone positively and %ucOC inversely associated with femoral neck BMD; Women: plasma phylloquinone positively associated with lumbar spins BMD only in postmenopausal not using estrogen; no association in premenopausal women or postmenopausal women taking estrogen	[57]
379 healthy women, 30–88 years, from Japan	Longitudinal; 3 year follow-up	Plasma phylloquinone, MK-4, MK-7, ucOC concentration	Femoral neck and lumbar spine BMD; incident vertebral fracture	Low plasma phylloquinone (defined as <median 2.67 nmol/L) inversely associated with threefold higher vertebral fracture risk	[56]

(continued)

Table 29.1 (continued)

Participants/design	Design	Exposure(s)	Outcome(s)	Results	Reference
387 Italian hemodialysis patients; 62 healthy age-matched controls; 37 % women, mean age 64 years	Cross-sectional	ucOC concentration, plasma phylloquinone, MK4–MK7	Presence of vertebral fracture	Low plasma phylloquinone (defined as <5 % of the healthy control distribution) associated with threefold higher odds for vertebral fracture	[55]
337 healthy women 20–80 years, from Korea	Cross-sectional	ucOC concentration	Femoral neck and lumbar spine BMD	Inverse correlation between ucOC and BMD	[64]
334 healthy women, 50–60 years from Norway	Cross-sectional	ucOC concentration and %ucOC	Femoral neck, total hip, lumbar spine, and total body BMD	ucOC concentration inversely associated with BMD at all sites; %ucOC *not* associated with BMD at any site	[63]

Hordaland Health Study in Norway, those in the lowest quartile of phylloquinone intake were more than 50 % more likely to suffer a hip fracture over 10 years of follow-up, compared to those in the highest quartile (hazard ratio (HR) = 1.57 [95 % CI 1.09–2.26]). Menaquinone intake (which summed intake of all menaquinones) in this same cohort was not associated with hip fracture [36]. A cross-sectional analysis of 365 older adults from Spain found phylloquinone intake to be positively associated with BMD estimated using calcaneal ultrasound. Among 200 of the participants followed longitudinally, those who reported increasing their phylloquinone intake had less calcaneal bone loss over 2 years [37]. While calcaneal ultrasound has been shown to predict fracture as well as dual X-ray absorptiometry (DXA) [41, 42], its specificity and sensitivity to rule out or rule in DXA-diagnosed osteoporosis are reported to be low [43], which limits the clinical relevance of these findings. Estimation of phylloquinone intake by FFQ may also be imprecise because primary food sources are dark green leafy vegetables, which are prone to high day to day variability [44]. More importantly, these food sources are generally consumed in healthier diets [45], so it is difficult to disentangle whether it or generally healthy lifestyles are associated with improved skeletal health, even if adjusted for statistically. Because food composition databases for menaquinones are incomplete, menaquinone intakes estimated using FFQs can be less precise than estimates of other nutrients, including phylloquinone. In addition, because FFQs solely reflect intake, bioavailability of phylloquinone from plant-based foods, which is highly variable, is not captured by these surveys [46, 47].

Nutritional biomarkers are thought to more accurately reflect nutritional status than dietary intakes [48] and are not prone to the limitations that self-reported measures are [49]. There is no single robust biomarker of vitamin K nutritional status. Circulating phylloquinone is thought to reflect overall status, but correlates highly with circulating triglycerides and fluctuates with recent intakes [1, 20]. In North America, phylloquinone is almost exclusively the vitamin K vitamer measured in circulation. Even in response to phylloquinone supplementation of 5 mg/day for more than 2 years, phylloquinone was the sole vitamer measured [50]. In this study, the lower limit of detection was 0.1 nmol/L(0.05 ng/mL). Using highly specific and sensitive mass spectrometry methodology with a lower limit of detection of 0.05 ng/mL, MK-4 was measured in 57 % of 396 healthy Japanese women at a mean concentrations of 0.10 ng/mL (SD <0.2) [51]. These low values agree with other measurements in postmenopausal women from Japan and the UK [52, 53]. In addition, Japanese studies have routinely reported circulating MK-7 in select cohorts, which have been attributed to dietary intakes of *natto* [54]. In a recent study from Italy, detectable amounts of multiple menaquinones were reported in a cohort of hemodialysis patients [55]. These findings are not supported by previous literature nor is there a biologically plausible explanation for the presence of MK-5 and MK-6 in circulation. To resolve this apparent inconsistency, use of validated assays for vitamin K forms that are verified in quality assurance schemes need to be applied to these samples.

In healthy Japanese women (age range 39–88 years), higher plasma phylloquinone, but not MK-4 or MK-7, was associated with lower incidence of vertebral fracture over 3 years of follow-up [56]. Because the stepwise models used in the primary analysis were only adjusted for age, circulating bone alkaline phosphatase, and lumbar spine BMD, these findings may be confounded by other risk factors for vertebral fracture. The mean circulating phylloquinone and MK-7 concentrations reported in this study (3.5 nmol/L and 10.0 nmol/L respectively) were higher than typically seen in US cohorts [57, 58].

Because OC is the primary VKD in bone, circulating ucOC has been considered a biomarker of vitamin K status of bone and used in several epidemiological studies of bone health [17]. Several earlier observational studies reported high ucOC (reflective of low vitamin K status) was associated with increased risk for hip fracture [59–62]. In a cross-sectional evaluation of over 1,600 participants of the Framingham Offspring Study (mean age 59 years) that evaluated BMD, but not fracture, lower plasma phylloquinone and higher %ucOC were associated with lower femoral neck BMD in males. In women, the association between vitamin K status and BMD differed according to menopausal status and estrogen use. In post-menopausal women not taking estrogen, lower plasma phylloquinone was associated with lower BMD at the spine. The %ucOC was inversely associated with lumbar spine

BMD but did not reach statistical significance. Among the premenopausal women and postmenopausal women using estrogen, neither measure of vitamin K status was associated with BMD at any anatomical site [57]. Absolute serum ucOC was found to be significantly inversely associated with BMD at several anatomical sites in both Korean and Norwegian women [63, 64]. However, when Emaus et al. expressed ucOC as percentage of the total OC (%ucOC), as has been recommended [17], it was no longer associated with BMD [63]. This suggests that the inverse association of ucOC with BMD was indeed tracking the inverse association of total OC and BMD highlighting the importance of adjusting for total OC in analyses of ucOC. However, because ucOC does not consistently correlate with BMD outcomes of randomized vitamin K supplementation trials [23, 50, 65, 66], its clinical relevance to bone is questionable.

29.4.2 Intervention Studies

The need for well-designed randomized clinical trials (RCTs) is reinforced by the equivocal results of the available observational studies and their inherent limitations. Several RCTs to test the effect of vitamin K supplementation on bone loss have now been completed. The trials published through 2005 were summarized by meta-analysis [67]. Studies included in this meta-analysis were at least 6 months in duration and conducted in adults ≥18 years old. Eleven of the 13 studies were completed in Japan and used a 45 mg/day dose of MK-4 manufactured by Easai (Tokyo, Japan). Because this form and dose of vitamin K is used as an osteoporotic treatment in Japan, it has been tested in more intervention studies of bone loss and fracture than other forms of vitamin K. Overall the meta-analysis found a protective effect of vitamin K on BMD (standardized mean difference favored vitamin K = 0.27 (95 % CI, 0.03–0.50), $p = 0.02$) [67]. When the seven studies that included fracture outcomes were combined analytically, MK-4 supplementation was associated with reduced risk for hip fracture [OR (95 % CI) = 0.23 (0.12–0.47)], vertebral fracture [OR (CI) = 0.40 (0.25–0.65)] and nonvertebral fracture [OR (CI) = 0.19 (0.11–0.35)]. At the time, neither of the two phylloquinone studies included in the meta-analysis analyzed fracture outcomes. After publication of the meta-analysis, Tamura and colleagues commented on the omission of a yet-to-be published large-scale MK-4 intervention conducted from 1996 to 2005 by the Japanese Ministry of Health that would have influenced the meta-analysis' results towards a null finding [68]. This randomized open-label study with blinded evaluation that was subsequently published in 2009, compared the effect of calcium supplementation alone (as either 1.2 g/day calcium L-aspartate or 3 g/day calcium phosphate) to calcium supplementation with MK-4 (45 mg/day given as three 15 mg capsules/day, manufactured by Easai, Tokyo Japan) on fractures in over 4,000 osteoporotic postmenopausal women ≥50 years old [69]. After 3 years, there was no difference in incidence of new vertebral or clinical fracture between the two groups. The only sub-group in which MK-4 appeared to be protective was against new vertebral fracture in women with five or more vertebral fractures at baseline, suggesting MK-4 could only be beneficial in those with advanced osteoporosis [69].

Results of studies of MK-4 and age-related bone loss published since the meta-analysis continue to be equivocal [65, 66, 70, 71] (Table 29.2). Knapen et al. found MK-4 (45 mg/day for 3 years) reduced femoral neck BMC and bone strength loss, but did not affect BMD in 325 postmenopausal non-osteoporotic Dutch women. This led the authors to conclude that MK-4 may be more relevant to mechanical properties of bone [66]. However, the subsequent three interventions in postmenopausal women did not find any effect of the same dose of MK-4 on BMD loss after 1 year [65, 70, 71] Furthermore, the RCT conducted at the University of Wisconsin measured indices of femoral neck strength and geometry, and the researchers did not detect any effect of 45 mg/day MK-4 on any outcome analyzed [65]. It is plausible that differences would have been detected if follow-up exceeded 1 year, but this has yet to be demonstrated.

Prior to 2004 there was one published RCT of phylloquinone supplementation on age-related bone loss, even though phylloquinone is the primary form of vitamin K in most diets. In that study of healthy Dutch postmenopausal women, Braam et al. found a vitamin D plus mineral supplement with 1 mg phylloquinone taken daily was more effective at reducing femoral neck BMD loss, but not lumbar spine BMD loss, than the vitamin D plus mineral supplement without phylloquinone [72]. During the final year of the study, the femoral neck bone loss among women in the vitamin D + mineral supplement group was equivalent to the placebo group, while it was attenuated in those who additionally received phylloquinone. Since then three randomized trials have analyzed the effect of phylloquinone supplementation on age-related bone-loss [23, 50, 73] and two additionally compared the effects of phylloquinone to menaquinone on change in BMD in older adults [65, 74] (Table 29.2.). In all of these studies the primary outcome was BMD loss at the femoral neck, and none were able to confirm the findings of the Maastricht study. It is important to consider most of these RCTs were designed to assess the efficacy of phylloquinone when vitamin D and calcium were replete, so study participants were given vitamin D_3 (320–800 IU) and calcium (500–1,000 mg) in addition to phylloquinone. Because the UK Bones and Vitamins Study followed a 2 × 2 factorial design, some women received phylloquinone without vitamin D_3 + calcium. In that study, the ultra-distal radius BMD and BMC of women who received phylloquinone + vitamin D_3 + calcium supplements increased over 2 years, compared to the groups that received calcium + vitamin D_3 or phylloquinone alone [73], suggesting a synergistic nutrient effect on bone loss at that site. However, this finding that was not replicated by the ECKO study, the only other phylloquinone trial that measured bone loss at the radius [50]. Reductions in ucOC or %ucOC were documented across studies, demonstrating all doses of phylloquinone used (ranging from 200 μg/day to 5 mg/day) were sufficient to improve carboxylation of extra-hepatic VKD proteins. That improvements in BMD did not correlate with observed reductions in ucOC, also suggests circulating ucOC is not a robust biomarker of bone health.

While low BMD is a strong predictor of increased fracture risk [75], BMD alone does not solely predict fracture [76]. The association between phylloquinone supplementation and fracture is uncertain because ECKO is the only completed trial that analyzed fracture outcomes. Two-hundred sixty-one of the ECKO participants consented to continue the intervention for an additional 2 years to determine the effect of phylloquinone supplementation on longer-term bone loss and fracture. While the longer follow-up did not reveal differences in BMD loss over 4 years, fewer women in the phylloquinone group had overall fractures over that same period (9 compared to 20 women, $p = 0.04$). Limiting the analysis to fragility fractures (sustained by four women on phylloquinone and ten women on placebo) attenuated the significance ($p = 0.11$), while limiting to osteoporotic fractures eliminated any difference between the groups ($p = 0.56$). Some have suggested vitamin K influences bone properties not reflected by BMD, such as morphometry and strength, qualities that are independently associated with fracture [66, 77]. Because the long-term extension of ECKO to examine fractures was not planned a priori, confirmation in future studies is warranted.

MK-7 has a longer circulating half-life than phylloquinone or MK-4, and some have suggested it is more important to extrahepatic tissues than other forms of vitamin K [78, 79]. MK-7 in low doses has been reported to interfere with anticoagulation therapy in persons taking those medications [80, 81], so any benefit of MK-7 will need to be balanced against safety in certain patient groups. In 2010, two MK-7 supplementation trials, one in postmenopausal women [82] and one in lung and heart transplant patients [83], reported no effect of MK-7 (180–360 μg/day) on BMD after 1 year of follow-up. Effects on bone strength outcomes were inconsistent, and no effect was observed on any outcome of the total hip. A randomized intervention of postmenopausal women conducted in Greece compared the effect of calcium (800 mg/day) + vitamin D_3 (400 IU/day) alone or in combination with phylloquinone (100 μg/day) or MK-7 (100 μg/day) on changes in lumbar spine and total body BMD [74]. Supplementation in this study was provided by way of fortified milk or yogurt. While the control group did not receive a placebo, they were advised to consume one serving each of fortified low-fat

Table 29.2 Randomized trials of vitamin K and age-related bone loss

Intervention	Study/location	Participants	Duration (years)	Main outcome(s)	Effect of vitamin K on main outcome	Effect of vitamin K on secondary outcome(s)	Reference
Meta-analysis: 45 mg/day MK-4 (11 studies) or 1 mg/day phylloquinone (2 studies)		Meta-analysis of 13 RCTs of adults ≥18 years	≥0.5	BMD loss	Protective effect	MK-4 protective against hip fracture	[67]
Studies of menaquinone-4 published since meta-analysis							
RCT 2 groups: 1. 45 mg/day MK-4 2. Placebo	University of Maastricht, Netherlands	325 postmenopausal, mean age 66 years	3	Femoral neck, total hip and lumbar spine BMD, BMC, hip bone strength (no single primary outcome specified)	No effect on BMD; protective against femoral neck, lumbar spine BMC and hip bone strength loss		[66]
Nonblind RT with blinded evaluation, 2 groups: 1. 1–3 g/day Ca 2. 1–3 g/day Ca+45 mg/day MK-4	Japan	4,378 postmenopausal women with osteoporosis, >50 years	3–4	Vertebral fracture	No effect	Protective in women with advanced osteoporosis at baseline	[69]
RCT, 2 groups: 1. 1.5 mg/day MK-4 2. Placebo	Japan	50 postmenopausal women, 50–65 years	1	Femoral neck, total body, forearm BMD (no single primary outcome specified)	Protective against forearm BMD loss; no effect at other anatomical locations		[71]
Open RCT, 2 groups: 1. 17.5 mg/week risedronate 2. 17.5 mg/week risedronate+45 mg/day MK-4	Japan	101 postmenopausal women with osteoporosis, >60 years	1	Vertebral fracture	No effect		[70]
Studies of phylloquinone							

RCT 3 groups: 1. 500 mg Ca + 320 IU D_3 + 150 mg Mg + 10 mg Zn/day 2. 1 mg phylloquinone + Ca + D_3 + Mg + Zn/day 3. Placebo	University of Maastricht, Netherlands	181 postmenopausal women 50–60 years	3	Femoral neck and lumbar spine BMD	Protective against femoral neck BMD loss; no effect at lumbar spine BMD		[72]
RCT 4 groups: 1. 200 μg/day phylloquinone 2. 400 IU/day D_3 + 1,000 mg/day Ca 3. Phylloquinone + D_3 + Ca/day 4. Placebo	UK Bones and Vitamins Study, Scotland UK	244 postmenopausal women, ≥60 years, non-osteoporotic	2	Femoral neck BMD	No effect	No effect on femoral neck BMC or on total hip, mid-distal radius BMD, BMC; protective effect of phylloquinone + D_3 + Ca on ultra-distal radius BMD, BMC	[73]
RCT 2 groups: 1. 500 μg phylloquinone + 400 IU D_3 + 600 mg Ca/day 2. 400 IU D_3 + 600 mg Ca/day	Tufts University, Boston, Massachusetts	185 men 267 postmenopausal women not taking estrogen, 60–80 years, healthy, femoral neck BMD *z*-score not >1.8 SD of the mean	3	Femoral neck BMD	No effect	No effect on femoral neck BMC or on total hip, lumbar spine, total body BMD, BMC	[23]
RCT 2 groups: 1. 5 mg/day phylloquinone + 1,500 mg/day Ca + 800 IU/day D_3 2. 1,500 mg/day Ca + 800 IU/day D_3	ECKO, University of Toronto, Canada	440 postmenopausal women with osteopenia (a lowest *T*-score between −1.0 and −2.0 at lumbar spine, total hip, or femoral neck)	2	Lumbar spine and total hip BMD	No effect	No effect on femoral neck, total body, ultra distal radius BMD; protective effect against clinical fracture in subgroup with 4-year follow-up	[50]
Studies of menaquinone-7							
RCT, 2 groups: 1. 360 μg/day MK-7 (Natto capsules) 2. Placebo	University of Tromso, Norway	334 postmenopausal women 50–60 years	1	Total hip, femoral neck, lumbar spine and total body BMD	No effect		[82]

(continued)

Table 29.2 (continued)

Intervention	Study/location	Participants	Duration (years)	Main outcome(s)	Effect of vitamin K on main outcome	Effect of vitamin K on secondary outcome(s)	Reference
RCT, 2 groups: 1. 180 μg/day MK-7 2. Placebo	University of Maastricht, Netherlands	244 post-menopausal women, 55–65 years	3	Femoral neck, total hip, and lumbar spine BMC and BMD; hip bone geometry and strength (no single primary outcome specified)	Protective against femoral neck, lumbar spine BMD, BMC and hip bone strength loss; No effect on total hip BMD, BMC loss or hip axis length		[84]
Studies of phylloquinone and menaquinone							
RCT, 3 groups 1. 1 mg/day phylloquinone + 600 mg/day Ca + 400 IU/day D_3 2. 45 mg/day MK-4 + 600 mg/day Ca + 400 IU/day D_3 3. 600 mg/day Ca + 400 IU/day D_3	University of Wisconsin	381 postmenopausal women with lumbar spine or proximal femur BMD *T*-score >−2.0 or >−1.5 with one National Osteoporosis Foundation-defined risk factor present; ≥40 %ucOC	1	Lumbar spine and proximal femur BMD	No effect	No effect on femoral neck BMC, BMD, diameter, bone strength indices	[65]
RCT 4 groups, all given milk/yogurt supplemented with: 1. 800 mg/day Ca + 400 IU/day D_3 2. 800 mg/day Ca + 400 IU/day D_3 + 100 μg/day phylloquinone 3. 800 mg/day Ca + 400 IU/day D_3 + 100 μg/day MK-7 4. Control	Greece	173 postmenopausal, non-osteoporotic women, 55–65 years	1	Lumbar spine, total hip, total body BMD (no single primary outcome specified.)	Phylloquinone and MK-7 protective against lumbar spine BMD loss; no effect on total hip, total body BMD		[74]

milk and fortified yogurt per day. After 1 year, the three intervention groups demonstrated improvement in total body BMD compared to control and the groups treated with phylloquinone or MK-7 had improved lumbar spine BMD compared to the other two groups. In a longer trial of MK-7 alone, Knapen and colleagues followed 244 healthy Dutch postmenopausal women for 3 years and found 180 μg/day MK-7 marginally, but significantly reduced femoral neck and lumbar spine BMD and BMC loss compared to placebo, in an industry-funded trial (NattoPharma ASA; Hovik Norway) [84]. Given the collective experiences with phylloquinone or MK-4 and BMD, it would be prudent to conduct multiple clinical trials in different countries to determine if MK-7 indeed has a true protective effect on age-related bone loss or if this is a spurious effect.

29.5 Conclusions

The protective role of vitamin K in age-related bone loss continues to be controversial. The results of observational analyses are inconsistent with respect to associations between vitamin K status and bone, which arguably may be related to the limitations of observational study designs and analytical methodologies employed to measure vitamin K status. Well-designed randomized trials to test the effect of vitamin K on bone-loss without the bias of observational studies have been designed and completed. The majority of these trials do not support a protective effect of phylloquinone against age-related bone loss, especially when vitamin D and calcium status are adequate. The single RCT that included fracture as an outcome had suggestive findings but it is unlikely that phylloquinone supplementation trials will be conducted that have a primary outcome of hip fracture given the prohibitively large sample size required and the lack of ancillary supporting evidence such as changes in bone turnover markers, BMD and/or bone geometry. Although a number of the initial studies of MK-4 demonstrated a benefit of therapeutic dose on bone loss [67], the results of the largest and longest MK-4 trial to date overall did not find any benefit on vertebral fracture, except in women with advanced osteoporosis [69]. Consistent evidence is lacking to support a protective effect of MK-7 on bone loss as well. While the longest trial of MK-7 supplementation reported a beneficial effect on femoral neck bone loss [84], confirmation in multiple clinical trials is needed.

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Part VI
Lifestyle Effects/Supplements

Chapter 30
Smoking, Alcohol, and Bone Health

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Key Points

- Smoking and alcohol consumption are two lifestyle factors that have important contributions to skeletal health.
- Deleterious effects of smoking on the skeleton have been recognized for several decades. Smoking adversely affects bone density and increases hip fracture risk in postmenopausal women. In men emerging evidence is suggestive for similar associations but the evidence is not conclusive.
- The evidence is inadequate to infer a causal relationship between smoking and reduced bone density before menopause in women and in younger men.
- Previously, the role of alcohol on skeletal health was not as well studied as that of smoking, and results from those studies suggested both beneficial as well as deleterious effects on the skeleton. However, recent studies on the role of alcohol on the skeleton suggest a "J"-shaped curve. Moderate ingestion of alcohol may offer some degree of benefit to the skeleton.
- Ongoing research further suggests that both ethanol and non-ethanol components of alcohol containing beverages affect skeletal heath.

Keywords Smoking • Alcohol • Bone mineral density • Fracture

30.1 Introduction

Smoking and alcohol consumption are two lifestyle factors that have important contributions to skeletal health. Deleterious effects of smoking on the skeleton have been recognized for several decades. The 2004 Surgeon General's Report on Bone Health and Osteoporosis [1] recognized smoking and heavy alcohol use as significant contributors to reduced bone mass and increased fracture risk. The 2004 Surgeon General's Report on Women and Smoking [2] concluded that smoking adversely affects bone density and increases hip fracture risk in postmenopausal women while the association in men is suggestive but not conclusive. The evidence is inadequate to infer a causal relationship between smoking and reduced bone density before menopause in women and in younger men. Recent studies

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on the role of alcohol on skeletal health suggest a "J"-shaped curve. Moderate ingestion of alcohol may offer some degree of benefit to the skeleton. Ongoing research further suggests that both ethanol and non-ethanol components of alcohol affect skeletal heath.

30.2 Smoking and Bone Health

30.2.1 Smoking Effects on the Skeleton

There are potential direct and indirect effects of smoking on skeletal health and fracture risk. Direct toxic effects of smoking on bone cells may be related to nicotine effects [3, 4] or possibly to toxic chemicals in tobacco products such as cadmium [5]. Smoking has direct effects on osteogenesis including alteration in the RANK–RANKL–OPG system [6, 7], collagen metabolism [8], and bone angiogenesis [9] (Fig. 30.1). Indirect effects of smoking on bone may result from decreased intestinal calcium absorption [10], dysregulation in sex hormone production and metabolism [11], alterations in metabolism of adrenal cortical and gonadal hormones [12–14], calciotropic hormones [15] such as 25-hydroxy-vitamin D [11, 16] and parathyroid hormone [11]. These effects may account for the generally observed decrease in markers of bone formation, such as osteocalcin, in smokers [16, 17].

Smoking may also indirectly influence bone density and the risk of fractures through reductions in body weight. Body weight tends to be lower for smokers than for nonsmokers, and this weight difference may itself lead to lower bone density and increased risk for fracture [18, 19]. Finally, smokers may be less physically active, which itself may reduce bone density [20] and increase fracture risk [21]. In several analyses involving women, weight explains part of the increased risk of low bone

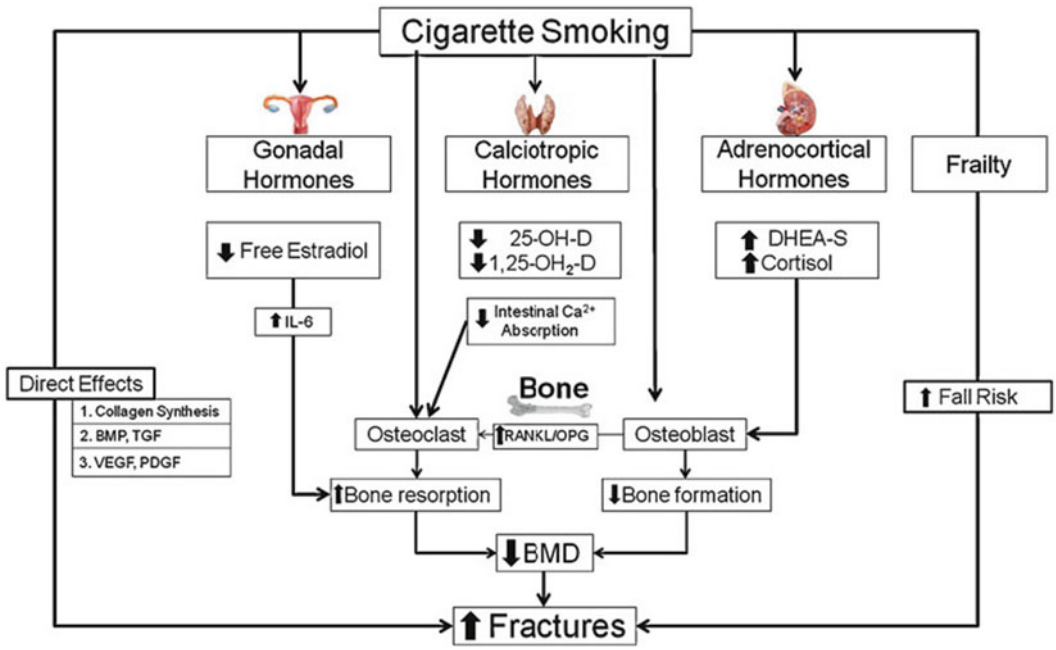

Fig. 30.1 Pathophysiologic mechanisms due to cigarette smoking that lead to decreased bone mineral density and increased fracture risk. Tobacco use increases risk through bone mineral density-dependent factors, as well as through direct effects that are independent of BMD [15]

mineral density (BMD) associated with smoking [22]; however, there are differences in BMD and fracture between smokers and nonsmokers, even after adjusting for weight differences [17, 23–25]. The lower weight in smokers compared to nonsmokers may increase the risk of fractures, such as hip fractures, through several mechanisms: reduced soft tissue mass overlying the trochanter, resulting in less energy absorption from a fall on the hip; or even reduced conversion of adrenal steroids into sex steroids in the adipose tissue. The anti-estrogenic effect of smoking may also contribute to osteoporosis in women [26, 27]. Interestingly, although estrogen appears to be a critical hormone for male skeletal health [28], smoking does not appear to attenuate the association between estradiol levels and bone density in men [29]. Finally, smoking may increase the risk of fracture through a reduction in physical performance capacity, which itself may increase the risk of falls [30].

30.2.2 Smoking and Bone Density

30.2.2.1 Skeletal Change Over the Lifespan

In adults, bone mass is dependent on the level achieved at the peak, and on losses due to aging and other factors. The skeleton grows rapidly in infancy, slows during childhood, and then accelerates during puberty, such that by age 20–30 years of age, peak skeletal mass is attained [31, 32]. Gains in BMD continue into the third decade and then BMD declines over the remaining decades of life [33, 34]. After menopause, bone loss accelerates compared with premenopausal years. These rates continue or actually increase with aging [35] and similar changes are observed in men [36, 37]. Because of these age-related patterns, smoking influences on bone density may be observed in the attainment of peak bone mass, in premenopausal women, and in men.

30.2.2.2 Smoking and Attainment of Peak Bone Mass

Data are actually somewhat limited with regard to the negative effects of smoking on the attainment of peak bone mass because less is known about the skeletal effects of smoking around the time of puberty [38–40]. A study from Belgium examined 12,446 men aged 25–45 years and reported that smoking at a young age was associated with unfavorable bone geometry and density and was associated with increased fracture prevalence, providing arguments for a disturbed acquisition of peak bone mass during puberty by smoking, possibly owing to an interaction with sex steroid action [38]. Another study of healthy military male recruits ages 16–19, reported that smoking was associated with preserved bone geometry, but worse BMD and Quantitative Ultrasound (QUS) characteristics [41].

Few data are available on the role of smoking in the attainment of peak bone mass because of the relatively rare exposure at very young ages. Some studies have been performed in premenopausal women initially suggesting that bone density does not differ between smokers and nonsmokers up to the time of menopause in women. Another study conducted in 1,061 Swedish women, all exactly 25 years of age, reported that among current smokers, negative effects were observed for BMD at the hip but not at other sites, and it was related to the amount of cigarettes smoked in a dose-dependent manner. Furthermore, young women with a long history of smoking had a higher BMI suggesting that attainment of peak bone mass is adversely associated with smoking in young women. Previous studies in young men suggested no real differences in bone density between young male smokers and nonsmokers. However, a recent study from the United Kingdom reported that smoking appeared to be detrimental to BMD and quantitative bone ultrasound measures, but not proximal femoral geometry in 723 healthy Caucasian male military recruits (age range 16–18 years) [41].

30.2.2.3 Smoking and Bone Density in Mid- and Late Life

In contrast to the results for younger persons, bone density studies performed in populations well beyond the years of peak bone mass demonstrate significant differences between smokers and non-smokers (Table 30.1). Previous data from longitudinal studies in men and women suggest there may be a causal relationship between smoking and bone loss in older women and men and that smoking cessation may slow, or partially reverse, the accelerated bone loss caused by years of smoking. However, it was unclear if there were sex differences related to smoking effect.

Recent studies examined the impact of smoking characteristics in older men and women. A Co-Twin Study of 146 female twin pairs (aged 30–65 years) by MacInnis et al. reported that a discordance of

Table 30.1 Studies of BMD and bone loss according to smoking status in women and men

Study	Sample, age (year)	Smoking status	Measurement/site	Principal finding
BMD				
Tanaka et al. [83]	325 men aged ≥50 years	10 % current smokers	BMD femoral neck	Current smokers at higher risk of developing osteoporosis (OR = 6.43).
Tamaki et al. [44]	1,576 men aged ≥65 years	17.6 % current smokers; 59.2 % former smokers	BMD lumbar spine and total hip	Longer duration of smoking years was associated with lower BMD.
Szulc et al. [46]	719 men aged 51–84 years	11.5 % current smokers; 56.3 % former smokers	BMD spine, hip, distal forearm, ultra distal radius	Compared to never smokers, current and former smokers had lower BMD at most sites.
Supervia et al. [11]	74 men and women; mean age 32.2 years	29.7 % current smokers	BMD lumbar spine, femoral neck and total femur	In men smokers had lower BMD compared to never smokers.
Muraki et al. [84]	632 women aged ≥60 years	20.0 % smokers	BMD lumbar spine	Ever-smokers had lower BMD compared to never smokers.
MacInnis et al. [42]	146 women twin pairs aged 30–65 years	Pre-menopausal women: 47 % ever smokers (8.6 mean pack-years of smoking); post-menopausal women: 32 % ever smokers (14.1 mean pack-years of smoking)	BMD spine, total hip and forearm	10 pack-years smoking related to 2.3–3.3 % lower BMD at all sites except forearm. Effect more pronounced in post-menopausal women.
Izumotani et al. [85]	686 Japanese men aged 40–59 years	Mean smoking (pack-years) among normal men: 18.9 ± 20.0; osteopenic men: 19.1 ± 21.1 and osteoporotic men: 27.7 ± 29.4	Spine BMD	Pack-years of smoking was associated with lower BMD
Forsmo et al. [86]	1,652 Norwegian pre- and post-menopausal women aged 50–59 years	Mean smoking (pack-years) was 13.9 (95 % CI: 13.1–14.7); mean number of daily cigarettes was 10.8 (95 % CI: 10.3–11.3)	BMD distal and ultradistal radius	Pack-years of smoking were associated with lower distal radius BMD but not ultradistal radius BMD. Marginally significant interaction for smoking*coffee ($P = 0.09$).

(continued)

Table 30.1 (continued)

Study	Sample, age (year)	Smoking status	Measurement/site	Principal finding
Gerdham et al. [25]	1,032 Swedish women aged 75 years	14 % current smokers; 20 % former smokers; 66 % never smokers	BMD total body, spine and hip, bone mass assessed by ultrasound of the calcaneus and phalanges	Hip and total body BMD was low in current vs. never smokers. This difference was not detected by ultrasound measurements. No difference between former vs. never smokers at any bone site.
Baheiraei et al. [47]	90 Iranian women aged ≥35 years	Current smokers 8 % (pre-menopausal women); 8 % post-menopausal women	BMD spine and femoral neck	Smoking status associated with lower BMD. Current smokers had lower BMD compared to non-smokers.
Williams et al. [87]	46 pair of monozygotic twins discordant for alcohol consumption	Current smokers vs. former smokers	BMD hip and lumbar spine	Current smoking was negatively associated with BMD.
Muraki et al. [84]	632 women aged ≥60 years	Smokers (20.6 %) vs. non-smoker	BMD lumbar spine	Smoking was negatively associated with BMD.
Kuo et al. [43]	837 Taiwanese men aged 46–64 years	30.8 % current smokers; 5.6 % former smokers	BMD spine and femoral neck	Smoking status and duration of smoking were deleterious for spine BMD. The effect was cumulative with duration and quantity.
BMD loss				
Elgán et al. [88]	152 Swedish women (aged 18–26); average follow-up 2 years	18.5 % daily smokers; 18.5 % "party" smokers; 63 % non-smokers at the follow-up time	BMD heel bone (calcaneus)	Baseline smoking associated with lower BMD at the follow-up after adjusting for baseline BMD.
Bakhireva et al. [80]	507 community-dwelling men aged 45–92 years	Current smokers aged: 45–64 years (14.6 %); 65–74 years (7.5 %); 75–92 years (2.2 %)	BMD hip and lumbar spine	Compared to former smokers, % BMD loss in current smokers was increased at the total hip and femoral neck.

BMD bone mineral density

10 pack-years of smoking was related to a 2.3–3.3 % (SE, 0.8–1.0) lower lumbar spine BMD, total hip BMD and total body BMC but not the forearm BMD, with effects more evident in postmenopausal women [42]. Studies in older Asian men have reported a 3.8 % lower lumbar spine BMD in heavy smoking (≥20 cigarette/day) [43]. The Fujiwara-kyp Osteoporosis Risk in Men (FORMEN) study reported that the negative impact of smoking on bone status is mainly associated with the number of years of smoking in older men [44]. The Male Osteoporosis Study from Hong Kong, a longitudinal study, reported that in older men, current smokers had a 2.0 % decrease in hip BMD (95 % CI: −3.8, −0.1) while past smokers had a 1.3 % decrease in hip BMD (95 % CI: −2.5, −0.2) compared to never smokers [45]. However, some studies report no bone mass differences between former and never-smokers [25, 46]. It has also been suggested that former smokers may not lose or regain BMD after cessation of smoking [46].

Certain factors such as higher body mass index [47], and higher calcium intake [48] have been reported to attenuate the smoking associations with bone. Smoking may also interfere with the treatment of osteoporosis in women using estrogen replacement therapy (ERT), as levels of estradiol are lower in smokers taking estrogen than in nonsmokers taking estrogen [49], and bone density values in women taking estrogen are lower in smokers than in nonsmokers [23].

These effects on bone density are significant in mid and late life, since for every 10-year increase in age, the bone density of smokers falls below that of nonsmokers by about 0.14 SD, or 2 % of the average bone density at the time of the menopause. Because a 1.0-SD decrease in bone density doubles the risk of fracture, and because fracture incidence increases with age, the proportion of all fractures attributable to smoking would be expected to increase as smokers continue smoking into old age. Attempts to decrease smoking as early in life as possible are likely to reduce fractures that occur in old age among smokers.

Taken together, cigarette smoking (both dosage and duration of smoking) is associated with lower BMD and increased bone loss in older men and women. Limited studies have examined if the deleterious effects of smoking on bone health may be reversible. Furthermore, smoking effects on bone may not be limited to BMD but may extend to other aspects of bone strength such as bone architecture and bone geometry, an area that has received little attention.

30.2.3 Smoking and Fracture Risk

Hip fractures, the most frequently studied fractures in relation to smoking, account for a significant proportion of the morbidity and mortality attributed to osteoporosis. Previous studies suggested that smoking appeared to increase the risk of hip fracture; however, there were fewer studies of smoking and fracture risk at other skeletal sites. Because the risk of hip fractures in smokers increases with age, and hip fracture incidence also increases with age, the proportion of hip fractures attributable to smoking increases with age.

A meta-analysis by Kanis et al. [50] included 59,232 men and women from ten prospective cohorts from across the world. This study reported that a smoking history was associated with a significantly increased risk of fracture compared with individuals with no smoking history. The highest risk was observed for hip fracture (84 % increased risk, 95 % CI: 1.52–2.22) while the risk of osteoporotic fractures considered as a group was marginally higher (29 % increased risk, 95 % CI: 1.13–1.28). The authors concluded that a history of smoking results in fracture risk that is substantially greater than that explained by the risk of lower BMD. A study by Olofsson et al. using data from the Uppsala Longitudinal Study of Adult men [51] supported these findings, and further clarified that the risk of fracture in older men depends both on recency of smoking and on the daily amount of tobacco smoked, rather than smoking duration (Table 30.2). However, Samelson et al. reported no significant associations of smoking (number of cigarettes/day compared to never smokers) and 25-year cumulative incidence of radiographic vertebral fracture in men and women [52].

Interventions aimed at helping smokers quit are likely to result in a significantly reduced number of hip fractures. Although hip fractures carry the greatest risk of mortality, morbidity, and cost, other fractures also contribute significantly to these outcomes. Further research is necessary to quantify the risk of these fractures in smokers.

Table 30.2 Studies of smoking and relative risk of fractures of the hip and other sites

Type of fracture/study	Study design	Sample	Results
Hip fracture			
Lau et al. [89]	Case–control	451 Asian men and 725 Asian women with hip fracture; aged 50 and older (mean, 72.0 for men, 73.7 for women) 1,162 healthy controls (456 men, 706 women) without hip fracture; mean age, 70.8 for men, 72.7 for women	Current smoking: Men, RR = 0.7 (95 %, CI, 0.5–1.0); women, RR = 0.5 (95 %, CI, 0.3–0.7) Former smoking: Men, RR = 2.1 (95 % CI, 1.5–2.9); women, RR = 1.4 (95 % CI, 0.9–2.0)
Baron et al. [82]	Age-matched, case–control	1,328 Swedish postmenopausal women with hip fracture; aged 50–81 years (mean, 72.5) 3,312 Swedish postmenopausal without hip fracture; mean age, 70.5	Current smokers had increased risk of fracture, OR = 1.35 (95 % CI, 1.12–1.64); Duration of smoking, particularly postmenopausal smoking was more important than the amount smoked
Du et al. [90]	Cross-sectional study	703 community-dwelling Chinese men and women (226 men, 467 women) aged ≥90 years (mean, 93.5)	Current or former smoking had no association
Porthouse et al. [91]	Prospective cohort study	703 community-dwelling English women aged ≥70 years (mean, 76.9)	Current smoking not related to fracture risk
Olofsson et al. [51]	Prospective cohort study	2,322 community-dwelling Swedish men aged 49–51 years	Current smoking (RR = 3.03; 95 % CI 1.02–3.44), former smoking (RR = 1.87; 95 % CI 1.02–3.44); ever smoking (RR = 2.12; 95 % CI 1.18–3.81) were associated with hip fracture
Jutberger et al. [92]	Prospective cohort study	3,003 men aged 69–80 years from the Swedish MrOs Study (n = 209 incident fractures over a follow-up of 3.32 years)	Current smokers had an increased risk of hip fractures (HR: 3.16, 95 % CI 1.44–6.95)
Vertebral fracture			
Jutberger et al. [92]	Prospective cohort study	3,003 men aged 69–80 years from the Swedish MrOs Study (n = 209 incident fractures over a follow-up of 3.32 years)	Current smokers had an increased risk of clinical and radiographic vertebral fractures (HR: 2.53, 95 % CI 1.37–4.65)
Klift et al. [93]	Prospective cohort study	3,001 men and women aged ≥55 years with 6.3 years of follow-up; 157 vertebral fractures (men, 44; women, 113)	Current smoking was associated with incident vertebral in women (RR = 2.1; 95 % CI 1.2–3.5)
Samelson et al. [52]	Prospective cohort study	Community-dwelling American men (252); women (452) aged 47–72 years; 92 (women) and 20 (men) new radiographic vertebral fractures occurred over 25 years follow-up	Smoking was not associated with 25-years cumulative incidence of radiographic vertebral fracture
Ankle fracture			
Valtola et al. [94]	Prospective cohort study	11,798 Finnish women aged 47–56 years with 5 years of follow-up; 194 malleolar fractures	Smoking had a dose–response effect with HR: 1.73 (95 % CI 1.11–2.71) in those smoking 1–19 cigarettes/day, and 2.94 (95 % CI 1.53–5.62) in those smoking ≥20 cigarettes/day

(continued)

Table 30.2 (continued)

Type of fracture/study	Study design	Sample	Results
Wrist fracture			
Porthouse et al. [91]	Prospective cohort study	703 community-dwelling English women aged ≥70 years (mean, 76.9)	Current smoking not related to fracture risk
Any nonvertebral fracture			
Porthouse et al. [91]	Prospective cohort study	703 community-dwelling English women aged ≥70 years (mean, 76.9)	Current smoking not related to fracture risk
Jutberger et al. [92]	Prospective cohort study	3,003 men aged 69–80 years from the Swedish MrOs Study (nonvertebral fractures defined as humerus, radius, pelvis, and hip fractures over a follow-up of 3.32 years)	Current smokers had an increased risk of nonvertebral osteoporotic fractures (HR: 2.14, 95 % CI 1.18–3.88)
Any fracture			
Jutberger et al. [92]	Prospective cohort study	3,003 men aged 69–80 years from the Swedish MrOs Study (nonvertebral fractures defined as humerus, radius, pelvis, and hip fractures over a follow-up of 3.32 years)	Current smokers had an increased risk of all new fractures (HR: 1.76, 95 % CI 1.19–2.61)

30.3 Alcohol and Bone Health

30.3.1 Alcohol Effects on the Skeleton

The mechanisms by which alcohol acts on the skeleton are poorly understood. This is due to the following factors: *First*, it is difficult to isolate the specific contribution of alcohol from other comorbidity factors known to influence bone health [53]. *Second*, it is difficult to isolate ethanol effects from other nutritional factors within alcoholic beverages, which usually differ by beverage type (for example silicon present as orthosilicic acid in beer and resveratrol in wine). *Third*, methods of assessing exposure to alcohol are inconsistent, especially in observational studies in humans. Additionally, the definition for moderate alcohol is not clear and the guidelines on acceptable intakes of alcoholic beverages is different between nations [54]. *Fourth*, the effect of alcohol on fracture outcomes is complicated, as it may be influenced by other factors such as age, drinking patterns, and alcohol effect on falls [55]. *Fifth*, ethanol-related health effects vary between populations because genetic background greatly influence the metabolism of alcohol [54].

Nevertheless, the direct effects of alcohol on bone and mineral metabolism have been described in both rats and in humans. Studies of chronic alcohol consumption in growing male and female rats have indicated that bone growth is suppressed, leading to a failure to acquire a normal peak bone mass [56]. Bone loss in adult rats fed ad libitum a liquid diet containing increasing concentrations of ethanol until receiving the appropriate percentage of total caloric intake, resulted in a dose-dependent decrease in trabecular thickness, bone turnover, and bone formation rate [57]. When equated to humans, the doses used in the adult rat experiments ranged from the low end of moderate (3 % of caloric intake) to alcoholic levels comprising 35 % of caloric intake. These findings in rats suggest that even moderate levels of alcoholic beverage consumption in humans may have the potential to reduce bone turnover and possibly to have deleterious effects on the skeleton. In rats fed ethanol over long periods, Peng et al. reported a greater risk of tibial fractures and a decrease in trabecular bone volume and bone strength [58]. Turner et al. reported that alcohol-consuming rats had decreased bone turnover after 4 months of treatment. Furthermore, an imbalance between bone formation and bone resorption at higher levels of alcohol consumption resulted in trabecular thinning [57].

In humans, alcoholics have been shown to have low BMD that is due to an inhibition of bone remodeling by a mechanism independent of the calciotropic hormones [59, 60]. Others have compared alcoholics to controls and found that serum concentrations of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 were significantly reduced among the alcoholics as compared to the controls [61, 62]. These low levels have been suggested to be the result of a deficient diet, reduced exposure to sunlight, malabsorption of vitamin D, increased biliary excretion of 25-hydroxyvitamin D metabolites, or to be the result of reduced reserves of vitamin D owing to a reduction of adipose and muscle tissue in alcoholics [63]. Laitenen and colleagues demonstrated that the low serum levels of vitamin D metabolites in non-cirrhotic alcoholics were not because of nutritional deficiency, and hypothesized that there was increased degradation of vitamin D metabolites in the liver. However, they showed that high calcium intake could counteract the vitamin D abnormalities [64]. Alcohol may also have deleterious effects on bone homeostasis through increased excretion of calcium and magnesium [65]. Consistent with the observations of reduced bone formation in alcohol-fed rats, reductions in osteoblastic activity have been observed in acute alcohol intoxication and in moderate use over 3 weeks time in humans [66, 67].

On the other hand, recent studies have also focused on non-ethanol components of alcohol beverages such as silicon and resveratrol. Recent reviews by Jugdaohsingh [68] and more recently by Price et al. [69] have provided extensive research on silicon's effect on bone and connective tissue. While the exact mechanisms are still unclear, various mechanisms were suggested in these reviews. Silicon improves bone matrix quality, facilitates bone mineralization, and plays a role in collagen synthesis and/or its stabilization as well as in the utilization (i.e. gastrointestinal uptake and metabolism) of essential elements that are required for bone and collagen synthesis. Available epidemiological data also supports silicon's role in BMD [70, 71] in humans. Research on health effects of resveratrol is limited and primarily comes from studies of animals. One study in an ovariectomized rat model showed that rats treated with resveratrol had significantly greater BMD than those not treated [72] suggesting that resveratrol could play a role in protecting against bone loss induced by estrogen deficiency. A recent study of male rats showed that trans-resveratrol supplementation (12.5 mg/kg body weight/day) appeared to preserve the skeletal system during disuse and age-related bone loss [73].

30.3.2 Alcohol and Bone Density

Most studies investigating alcohol intake and bone health suggest a "J"-shaped curve such that the inflection point is at moderate ingestion, which offers maximum protection. Increased intake beyond this level shows negative effects on the skeleton. Wosje et al. reported bone beneficial effects of moderate alcohol consumption (measured as drinking occasions/month) using the data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) [74]. This study reported that total hip BMD in men and FN-BMD in postmenopausal women were higher among those with >29 drinking occasions/month compared to abstainers. However, no associations were observed in premenopausal women or with binge drinking (Table 30.3). Results from the Cardiovascular Health Study (subgroup of 1,567 men and women with BMD measures) showed that alcohol intake (measured as drinks/week) was associated with hip BMD in a U-shaped relationship, with approximately 5 % higher BMD among participants with ≥14 drinks/week compared to abstainers [75].

Cawthon et al. examined the association of alcohol intake and problem drinking history with BMD in a cross-sectional study of 5,974 men (aged ≥65 years) [76]. Alcohol intake categories were defined as non/infrequent (<12 drinks/year, abstainers), light (≥12 drinks/year to <13 drinks/week), and moderate to heavy (≥14 drinks/week). Alcohol intake was positively associated with hip and spine BMD. Although the absolute differences in BMD levels across categories of alcohol were modest (3.5 % for total hip BMD). Men with problem drinking also had higher hip and spine BMD. The type of alcohol consumed was not ascertained.

Table 30.3 Studies of bone density and bone loss according to alcohol use

Study	Sample, age (year)	Alcohol status	Measurement/site	Principal finding
BMD				
Wosje et al. [74]	14,646 men and women aged ≥20 years	Frequency of alcohol consumed in past 1 month	BMD total hip and femoral neck	Alcohol intake was positively associated with BMD in men and postmenopausal women but not in premenopausal women. No associations with binge drinking.
Williams et al. [87]	46 pair of monozygotic twins discordant for alcohol consumption	Units per week, defined as half a pint of beer; a glass of wine or one measure of spirits	BMD hip and lumbar spine	Alcohol consumption was positively associated with BMD.
Tucker et al. [77]	1,182 men, 1,289 postmenopausal women and 248 premenopausal women aged 29–86 years	Drinks/day, defined as 356 mL beer; 118 mL wine; 42 mL liquor	BMD Spine and hip	Moderate alcohol intake was positively associated with BMD in men and postmenopausal women but not premenopausal women.
Kouda et al. [78]	1,421 Japanese men aged ≥60 years	Grams of absolute ethanol per day calculated from current alcohol intake by beverage type	BMD Spine and hip	Alcohol intake <55 g/day was associated with higher BMD and intake of ≥55 g/day was associated with lower BMD.
Cauley et al. [95]	5,995 men aged ≥65 years	Drinks/week	BMD Spine and hip	A 1 SD (approximately seven drinks/week) increase in alcohol consumption was associated with a 1 % higher hip and spine BMD.
Muraki et al. [84]	632 women aged ≥60 years	Alcohol drinker vs. nondrinker	BMD lumbar spine	Alcohol consumption was positively associated with BMD.
BMD loss				
Macdonald et al. [79]	891 women aged 45–55 years	Alcohol intake in quartiles	BMD lumbar spine and femoral neck	Modest alcohol intake was associated with less bone loss.
Bakhireva et al. [80]	507 community-dwelling men aged 45–92	Frequency of alcohol consumption (drinking ≥3 vs. ≤2 days/week)	BMD lumbar spine, total hip and femoral neck	Moderate alcohol intake was associated with less bone loss.

BMD bone mineral density

Tucker et al. further attempted to identify the different classes of alcohol in relation with BMD in older men and women (1,182 men, 1,289 postmenopausal women, and 248 premenopausal women) from the Framingham Heart Study [77]. This study sample of predominantly beer-drinking men, and predominantly wine drinking women, supported the earlier findings that moderate consumption of alcohol is associated with higher BMD in men and postmenopausal women. This protective effect peaked at one to two drinks/day for men (the benefits declined with higher intakes). However, for unclear reasons and contrary to the current guidelines for women, this protective effect peaked at a higher limit (>2 drinks/day) in women. No associations were observed in premenopausal women, perhaps due to low power. Interestingly, men with high liquor intakes (>2 drinks/day) were associated with significantly lower BMD. The authors concluded that stronger associations with beer or wine, relative to liquor, suggest that other constituents (such as silicon in beer) rather than ethanol may contribute to bone health.

Using data from the baseline survey for the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study, Kouda et al. reported a positive association between alcohol intake (g/day) and BMD as well as with bone markers (serum levels of osteocalcin and tartrate-resistant acid phosphatase 5b (TRACP5b) [78]. However, they reported an inflection point for the relation between alcohol intake and BMD as 55 g/day. Thus the range of positive association in this study was larger than the previous studies.

Only two longitudinal studies examined alcohol intakes with bone loss. One study from the Aberdeen Prospective Osteoporosis Screening Study examined the association of alcohol intake (in g/day) with recent bone loss around menopause in 891 women aged 45–44 years at the baseline and 50–59 years at the follow-up (5–7 years later) [79]. MacDonald et al. reported that participants in the highest quartile of alcohol intake (median intake of 13.6 g/day) had significantly lower bone loss (calculated as annual percentage change) at the lumbar spine bone loss compared to nonalcohol drinkers. These differences remained significant after adjustment for appropriate confounders and covariates. The other study from Rancho Bernardo in Southern California, examined 507 older men aged 45–92 years. This study reported moderate alcohol consumption of ≥3 times/week to be associated with less bone loss at femoral neck over 4 years [80].

30.3.3 Alcohol and Fracture Risk

Despite the suggestion from some of the above studies that alcohol may have beneficial effects on the skeleton, the vast majority of previous studies examining alcohol consumption and risk of fractures either showed no significant association, or in some cases, an increased risk of fracture among those men and women with high intakes of alcohol. Higher intakes may predispose to trauma-associated fracture outcomes. Finally, alcoholics appear to have low bone density and metabolic abnormalities that threaten bone health. However, recent studies suggest that moderate alcohol consumption may be protective against hip fracture risk [75] and one study on alcoholic showed that the increased lifetime prevalence of fractures among problem drinkers could be due to factors other than the acute intoxication [81].

Cawthon et al. examined the association of alcohol intake and problem drinking history with fracture risk in 5,974 men (aged ≥65 years, 256 nonvertebral fractures, and 46 hip fractures over 3.65-year follow-up) in a prospective cohort study of MrOs [76]. The authors reported no significant association between alcohol intake and risk of nonspine and hip fractures among older men (Table 30.4). There were however, weak protective trends for greater weekly alcohol intake and lower relative hazard of hip fracture. History of problem drinking, heavy drinking, or current episodic drinking were also not related to risk of nonspine or hip fractures. Similar weak inverse associations were also observed for alcohol intake and hip fracture risk from a case–control study in Swedish postmenopausal women [82].

Results from the Cardiovascular Health Study (5,865 men and women, 412 cases of hip fracture over 12 years follow-up) showed a U-shaped relationship between alcohol intake (measured as drinks/week) and hip fracture. Compared with long-term abstainers, the hip fracture risk was 22 % lower (HR: 0.78; 95 % CI: 0.61–1.00) among consumers of up to 14 drinks/week and the risk was 18 % (HR: 1.18; 95 % CI: 0.77–1.81) higher among those with ≥14 drinks/week [75]. However, it was unclear if the increased fracture risk with higher alcohol intake was mediated through an increased risk for falls or other types of trauma. Increased vertebral fracture risk in men but not women was also reported in the Framingham Study where the increased risk was observed even at lower intakes of 1–4 oz/week (~1.5–6.5 drinks/week) and at higher intakes of ≥4 oz/week (≥6.5 drinks/week) [52].

Clark et al. examined the differences in self-reported lifetime fracture prevalence in Caucasian women ($n=834$, aged 18–70 years) in treatment for alcohol abuse, in recovery and nonalcohol-dependent women [81]. Women in treatment and recovery reported more fractures during childhood and adolescence than nonalcohol-dependent women. Women with histories of alcohol dependence

Table 30.4 Studies of alcohol and relative risk of fractures of the hip and other sites

Type of fracture/study	Study design	Sample	Results
Hip fracture			
Lau et al. [89]	Case–control	451 Asian men and 725 Asian women with hip fracture; aged 50 and older (mean, 72.0 for men, 73.7 for women) 1,162 healthy controls (456 men, 706 women) without hip fracture; mean age, 70.8 for men, 72.7 for women	Occasional alcohol consumption: associated with lower risk in women, RR = 0.5 (95 %, CI, 0.3–0.9) Daily alcohol consumption (7 days/week) associated with higher risk: men, RR = 2.0 (95 % CI, 1.3–3.1); women, RR = 2.1 (95 % CI, 1.0–4.7) Number of alcoholic drinks/week ≥14; RR = 2.9 (95 %, CI, 1.2–7.1)) and years of alcohol consumption ≥25; RR = 3.0 (95 %, CI, 1.7–5.2) was associated with higher risk in men
Baron et al. [82]	Age-matched, case–control	1,328 Swedish postmenopausal women with hip fracture; aged 50–81 years (mean, 72.5) 3,312 Swedish postmenopausal without hip fracture; mean age, 70.5	Drinkers had lower risk, OR = 0.70 (95 % CI, 0.60–0.82). All types of alcoholic beverages were protective except for light beer, which showed no association
Du et al. [90]	Cross-sectional study	703 community-dwelling Chinese men and women (226 men, 467 women) aged ≥90 years (mean, 93.5)	Former alcohol consumption was associated with higher hip fracture risk (OR = 2.5; 95 % CI 1.0–5.5)
Mukamal et al. [75]	Prospective cohort study	5,865 community-dwelling American men and women aged ≥65 years; 412 fractures occurred over 12 years follow-up	U-shaped relationship with lower risk among consumers of <14 drinks/week, HR: 0.78 (95 % CI, 0.61–1.00) and higher risk among consumers of ≥14 drinks/week, HR: 1.18 (95 % CI, 0.77–1.81)
Cawthon et al. [76]	Prospective cohort study	5,974 community-dwelling American men aged ≥65 years; 46 hip fractures occurred over 3.65 years follow-up	Alcohol not significantly associated with hip fracture
Osteoporotic fracture			
Clark et al. [81]	Cross-sectional study	831 Caucasian women aged 18–70 years	Percent osteoporotic fractures: Women in treatment for alcohol abuse: 25 % vs. non-alcohol abusers: 15 % ($P < 0.01$) Women in recovery and abstainer: 25 % vs. non-alcohol abusers: 15 % ($P < 0.01$)
Vertebral fracture			
Samelson et al. [52]	Prospective cohort study	Community-dwelling American men (252); women (452) aged 47–72 years; 92 (women) and 20 (men) new vertebral fractures occurred over 25 years follow-up	Alcohol consumption (≥4 oz/week) was associated with increased 25-years cumulative incidence of vertebral fracture
Non-vertebral fracture			
Cawthon et al. [76]	Prospective cohort study	5,974 community-dwelling American men aged ≥65 years; 256 non-vertebral fractures occurred over 3.65 years follow-up	Alcohol not significantly associated with hip fracture

RR relative risk, *CI* confidence interval, *OR* odds ratio, *HR* hazard ratio

had higher lifetime prevalence of fractures, including time periods before the onset of problem drinking and following abstinence, suggesting that factors other than the acute intoxication contributed to the greater fracture prevalence.

30.4 Conclusion

Smoking and alcohol consumption are two lifestyle factors that have important contributions to skeletal health. Smoking adversely affects bone density and increases hip fracture risk in postmenopausal women. However, the association in men is not conclusive while the evidence is inadequate for premenopausal women and younger men. The role of alcohol on skeletal health can be both beneficial as well as deleterious depending upon the level of intake. Recent studies on the role of alcohol on the skeleton suggest a "J"-shaped curve and report that moderate ingestion may offer benefits to the skeleton, and both ethanol and non-ethanol components of alcohol may be involved in affecting skeletal heath.

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Chapter 31
Exercise and Bone Health

Maria A. Fiatarone Singh

Key Points

- Bone mass begins to decrease well before the menopause in women (as early as the 20s in the femur of sedentary women), and accelerates in the perimenopausal years, with continued declines into late old age.
- Similar patterns are seen in men, without the acceleration related to loss of ovarian function seen in women.
- As with losses of muscle mass and strength (sarcopenia), many genetic, lifestyle, nutritional, disease and medication-related factors enter into the prediction of bone density at a given age.
- It is important for health care professionals to understand the rationale and current recommendations for the use of exercise in the prevention and treatment of osteoporosis and osteoporotic fracture, and to place it in context with the other available strategies for this syndrome. The optimal use of exercise in this syndrome is dependent upon the prescription and adoption of a sustained, adequate dose of an evidence-based modality of exercise/physical activity in the target populations, while minimizing the risk of side effects.
- The phase of the lifecycle is of particular relevance to bone health, as the goal of exercise for fracture prevention shifts dramatically over the course of the lifespan; from an emphasis on achievement of peak bone mass in childhood and adolescence, to the preservation of bone and muscle strength and mass in middle age, to the optimization of gait and balance, muscle strength, frailty, undernutrition, neuropsychological function, and polypharmacy in old age.

Keywords Exercise • Resistance training • Impact exercise • Physical activity • Bone health • Hip fracture • Bone mineral density • Young athletes • Children • Exercise intervention • Optimizing peak bone mass • Premenopausal women • Postmenopausal women

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31.1 Introduction

Bone mass begins to decrease well before the menopause in women (as early as the 20s in the femur of sedentary women), and accelerates in the perimenopausal years, with continued declines into late old age [1]. Similar patterns are seen in men, without the acceleration related to loss of ovarian function seen in women [2]. As with losses of muscle mass and strength (sarcopenia [3]), many genetic, lifestyle, nutritional, disease, and medication-related factors enter into the prediction of bone density at a given age [4–12]. A wealth of animal and human data provide evidence for a relationship between physical activity and bone health at all ages. Mechanical loading of the skeleton generally leads to favorable site-specific changes in bone density, morphology, or strength [13–17], whereas unloading (in the form of bed rest, immobilization, casting, spinal cord injury, or space travel) produces rapid and sometimes dramatic resorption of bone, increased biochemical markers of bone turnover, changes in morphology such as increased osteoclast surfaces, and increased susceptibility to fracture [18–24]. Among these models, spinal cord injury results in the most profound loss of skeletal mass (up to 45 % at the pelvis), limited to the weight-bearing bones of the lower extremities and lumbar spine [24].

Less extreme variations in mechanical loading patterns seen within normal populations are also associated with differences in bone morphology and strength. Comparative studies of athletic and nonathletic populations usually demonstrate significantly higher bone density in the active cohorts, ranging from 5 to 30 % higher, depending on the type, intensity, and duration of exercise training undertaken, and the characteristics of the athletes studied [25–27]. Exceptions occur with non-weight-bearing activities such as swimming/cycling, or amenorrheic or competitive distance runners [28], who appear similar to controls. Similarly, on a smaller scale, differences are often observed between habitually active and sedentary nonathletic individuals [7, 9, 29–33]. Consistent with such bone density findings, hip fracture incidence has been observed to be as much as 30–50 % lower in older adults with a history of higher levels of physical activity in daily life, compared to age-matched, less active individuals [34–40].

Given this epidemiological and experimental background, it is important for health care professionals to understand the rationale and current recommendations for the use of exercise in the prevention and treatment of osteoporosis and osteoporotic fracture, and to place it in context with the other available strategies for this syndrome. *The optimal use of exercise in this syndrome is dependent upon the prescription and adoption of a sustained, adequate dose of an evidence-based modality of exercise/physical activity in the target populations, while minimizing the risk of side effects. The phase of the lifecycle is of particular relevance to bone health, as the goal of exercise for fracture prevention shifts dramatically over the course of the lifespan; from an emphasis on achievement of peak bone mass in childhood and adolescence, to the preservation of bone and muscle strength and mass in middle age, to the optimization of gait and balance, muscle strength, frailty, undernutrition, neuropsychological function, and polypharmacy in old age.*

The approach is thus similar to the specific pharmacological management of osteoporosis, which should also be targeted to the cohort at risk. A "blanket" exercise prescription for all ages without relation to other health conditions is unlikely to succeed. Although there are still many unanswered questions with regards to the optimal role of exercise and physical activity in bone health, and in particular its ultimate efficacy for fracture prevention, studies carried out over the past two decades have considerably advanced knowledge in this field. Physical activity cannot be expected to rebuild extremely osteopenic bone to normal architecture and strength, but existing literature supports the role of early, sustained, and appropriate patterns of loading for beneficial adaptations in bone size, shape, cortical wall thickness, trabecular architecture, and ultimately resistance to fracture, as well as recovery from hip fracture. A summary of the current evidence base, as well as the author's recommendations for effective and safe implementation of physical activity in various settings is reviewed in the sections that follow.

31.2 Epidemiological Associations Between Physical Activity Patterns and Bone Health

A large number of studies have attempted to define the role of physical activity and exercise patterns across the lifespan on bone density. Most of these studies are cross-sectional comparisons of healthy pre- or postmenopausal women. Due to the fact that higher physical activity level is often correlated with better health and nutritional status, the well-designed studies have attempted to control for these other risk factors for osteopenia in their analyses, as well as to identify specific periods of life during which physical activity confers benefit. For example, Krall [33] studied 239 healthy postmenopausal women prior to their entry into a trial of vitamin D supplementation, and found that those who reported walking more than 7.5 miles per week in the previous month had higher bone density (legs, trunk, and whole body) than those who reported walking less than 1 mile per week. Current walking was correlated with walking behavior earlier in life, suggesting that the bone density observed was a reflection of life-long physical activity patterns. Furthermore, in a prospective 1-year follow-up in these women, the rate of loss of bone mineral density (BMD) in the legs (but not other sites) was inversely related to the current number of miles walked per week, pointing to the [33] site-specific nature of the physical activity effect. Conflicting or nonsignificant results from other studies of walking may be due to differences in the measurement of physical activity, differences in calcium intake, as well as small sample sizes in most studies [41]. In another analysis of lifetime physical activity habits and bone density in old age [42], Vuillemin found that sporting activity in youth was associated with lumbar spine BMD in older men and women, whereas more recent physical activity helped to preserve femoral BMD.

Fewer data are available for men, but they are generally consistent with the findings in women. Need [43] found that energy expenditure in physical activity was associated with age-corrected bone density at the femoral neck in men aged 20–83 in a dose-dependent fashion, and was associated with lumbar spine and well as all femoral sites in men under 50 years of age. When men over 50 were evaluated separately, no relationship was found, the authors suggesting that exercise may have its major role in peak BMD in men. However, other studies of older men, such as Kenny's study of 83 men (average age 75) with low testosterone levels [44], and larger studies of healthy community-dwelling cohorts [45, 46], do support an independent role for physical activity in femoral and total-body BMD among older men.

A recent large prospective cohort study is the ongoing Canadian Multicentre Osteoporosis Study (CaMos) which began following a representative sample of 9,423 men and women age 25–84 in 1995 with measures of health status, lifestyle, physical activity patterns, and BMD [47]. In cross-sectional analyses adjusted for potential confounders, men with higher self-reported physical activity volume/intensity (summarized as metabolic equivalents × minutes/day) had higher hip (but not lumbar) BMD. Unexpectedly, more active women had lower lumbar BMD, possibly due to their lower body weight. The 5-year longitudinal analyses in this cohort indicated that increasing physical activity was associated with a significant but modest increase in hip and spine BMD in men, and hip BMD in women, as well as with decreased BMI in both sexes. Given the known association of weight loss with bone loss, the observed longitudinal association of physical activity with both higher BMD *and* lower BMI is highly clinically relevant to diverse health outcomes. The physical activities reported in this study were primarily low to moderate intensity, including walking, with only small amounts of moderate/strenuous sport or vigorous work.

Physical activity has also been linked to reduced osteoporotic fracture prevalence or incidence in addition to higher bone density in older adults, although not all studies have found significant reductions in risk. In the prospective study of Paganini-Hill [46], men who were active for 1 h or more per day had a 49 % reduction in the risk of hip fracture compared to less active men. In the Study of Osteoporotic Fractures [39], women who reported walking for exercise had a significant 30 % reduction in hip fracture risk compared to women who did not walk for exercise. In more detailed analyses of this cohort [48], including types and intensities of all recreational and household activities, 9,704

women over the age of 65 were followed for 7.6 years for fracture incidence. Incidence of hip fracture was 42 % lower in the most active compared to the least active quintile. A dose–response effect was seen for both volume and intensity, with low-intensity activity conferring a 27 % risk reduction, compared to a 45 % reduction associated with moderate-vigorous activity. Multivariate adjustments indicated that the relationship of physical activity to hip fracture in this cohort was minimally confounded by health status, functional status, history of falling, smoking, calcium and alcohol intake, estrogen use, or body weight, resulting in a significant 36 % reduction in risk after adjustment. The protective effect of exercise on hip fracture was only marginally reduced by adding bone density, muscle strength, and falls to the model, suggesting that its mechanism of action is multifactorial and not completely understood. Additionally, adjusted vertebral fracture risk was reduced by 33 % in women who reported moderate-vigorous exercise, whereas wrist fracture was unrelated to physical activity volume or intensity. In another large cohort, the prospective EPIDOS study of 6,901 white women over the age of 75 followed for 3.6 years, investigators found that a low level of physical activity increased the risk for proximal humerus fracture by more than twofold [49]. The relative risk (RR) of fracture in sedentary women (RR = 2.2) was greater than that attributable to low bone density (RR = 1.4), maternal history of hip fracture (RR = 1.8), or impaired balance (RR = 1.8). The interaction of these risk factors is indicated by the fracture rate, which rose from about 5 per 1,000 women years in individuals with either bone fragility or high fall risk to 12 per 1,000 woman-years for women with both types of risk factors. Such data suggest the great potential utility of multifactorial prevention programs for osteoporotic fracture that can address *both* bone density and fall risk (sedentary behavior, sarcopenia, muscle weakness, poor balance, polypharmacy, etc.) simultaneously. Moayyeri [50] reported the relationship between physical activity and fracture risk in 14,903 participants in the European Prospective Investigation of Cancer-Norfolk Study. In women, moderate intensity activities at home and for leisure significantly reduced the risk of hip fracture by almost half (HR = 0.51, $p = 0.02$; HR = 0.55, $p = 0.03$, respectively). By contrast, in men, home activities increased any fracture risk (HR = 1.25; $p = 0.008$), while leisure activities reduced hip fracture risk, similar to women (HR = 0.58, $p < 0.001$). Walking and high-impact activities, but not floor exercises, cycling or swimming reduced fracture risk, consistent with experimental studies of bone adaptation to specific kinds of loading. Similarly, a meta-analysis of 13 such prospective cohort studies reported a significant reduction in hip fracture risk (45 % in men and 38 % in women) [51] in association with moderate-to-vigorous physical activity levels, not well explained by modest differences in BMD by activity level.

In summary, cross-sectional and prospective cohort data support a relationship between lifetime physical activity patterns and preservation of bone density into old age, as well as a protective effect for hip, humerus, and vertebral fractures. These reduced risks for fracture remain after adjustment for most major known risk factors for osteoporosis, and are not simply accounted for by alterations in bone density, muscle strength, or fall rates. Experimental evidence in animal models as well as some human data suggest that other changes in bone strength apart from BMD changes may contribute to the overall benefits of mechanical loading for skeletal integrity (e.g., increased bone volume or altered trabecular morphology) [17, 52, 53], so that evaluating BMD changes alone may underestimate the skeletal effects of loading. This foundation of epidemiological research has laid the groundwork for the many experimental trials of exercise and bone health that have been carried out in healthy and clinical populations, as reviewed in the sections that follow.

31.3 Physical Activity and Bone Health During Childhood and Adolescence

The goal of physical activity in relation to bone health in youth is to maximize peak bone mass, which is attained at various sites by age 16–26 years in most studies [54], in order to potentially reduce the burden and delay the onset of osteoporotic fracture in adults. Thus, any attempts to

influence peak bone mass must occur very early in life, and have residual benefits that span 50 years or more. It is thought that approximately 20–50 % of the variation in bone mass is due to modifiable factors such as hormonal status, physical activity patterns, and nutrition, while the remainder is explained by sex, race, hereditary, and familial factors. Although recognized as one of the most effective strategies to maximize peak bone mass, the osteogenic benefits of exercise are dependent on the stage of life and the relative risk of fracture. There is strong evidence that growing bone has a greater capacity to adapt to increased loading than mature bone [55]. Thus, childhood and adolescence may represent the optimal opportunity to use exercise to protect against osteoporosis and fragility fractures in old age, but only if these exercise-induced skeletal benefits are maintained into later life. Indeed, it has been reported that a 10 % higher peak bone mass could delay the development of osteoporosis by 13 years and reduce the risk of fracture by 50 % [56]. For this reason, there has been considerable interest in quantifying the effects of exercise on bone accrual during growth and defining the appropriate mode, intensity, frequency and duration of exercise, in addition to the precise timing of exercise (childhood or adolescence), required to optimize bone health throughout life.

31.3.1 Cross-Sectional Studies of Young Athletes

Studies of athletes are most definitive with regard to the relationship between BMD and physical activity. Loaded bones have 5–30 % higher bone density than that measured in unloaded limbs or nonathletic control subjects [28, 29, 57–59]. Although genetic factors could partially explain the differences between athletes and non-athletes, the contralateral limb studies demonstrate that the side-to-side differences are primarily the result of specific effects of mechanical loading during training, as the BMD of nondominant arms in athletes is not different from non-athletic controls [60, 61]. The kinds of activities that appear to be most robust in their effect on the skeleton are those that include:

1. High-impact, rapid, forceful loading (jumping, running, gymnastics, volleyball)
2. Changing, diverse, or novel loading angles and magnitudes of forces over time (ball sports, gymnastics)
3. Weight-bearing, high forces (dancing, weight lifting; not swimming, water polo, cycling, cross-country skiing)
4. Activities that impact directly on the bone of interest (e.g., dominant arm of tennis players)

An important clinical question that remains unanswered is whether such exercise-induced skeletal benefits attained during youth are maintained into adulthood and reduce the risk of fracture later in life. There is evidence from a study of retired female gymnasts that BMD gains acquired before puberty may be maintained for up to 20 years [62]. However, limited data in older retired athletes suggest that the effects on bone mass are largely eroded over time [63]. The evidence with regards to protection from future fragility fractures is mixed, with equivocal findings from retrospective studies in former athletes [64, 65]. It is likely that each sport affects bone density, morphology, and thereby resistance to fracture very specifically, and many factors such as length of athletic pursuit, intensity of training, nutritional habits and other lifestyle activities and health status ultimately determine fracture risk. Notably, the beneficial effects of even extreme levels of training on BMD or geometry may be offset if other practices associated with elite performance such as smoking in ballet dancers or eating disorders in gymnasts counteract even powerful osteogenic influences. This speaks to the need for broad assessment of health status and bone risk in investigations and coaching programs involving elite athletes, not dissimilar to what is required in older adults.

31.3.2 Cross-Sectional Studies of Nonathletic Children and Adolescents

As important as these studies of athletes are for understanding the potential of bone to respond to large volumes of forces of high magnitude, they do not adequately address the issue of the effectiveness of normal activities and play in the vast majority of children and adolescents who will never engage in competitive athletics. This kind of data has been gathered in a number of cross-sectional studies, which generally show that the difference in BMD between low and high activity or fitness categories in normal populations of children varies between 5 and 15 % [54]. The effect of physical activity translates to slightly less than 1 SD, or an average of 7–8 % higher BMD compared to sedentary children and adolescents. An increase in peak bone mass of this magnitude, if it were to be sustained until old age, would theoretically substantially lower the risk of osteoporotic fractures, as has been observed in some studies of lifelong physical activity patterns [48, 66]. For example, each standard deviation (10 %) decrease in femoral neck BMD may be associated with a 2.6-fold increase in the risk of fracture at this site [4].

Many of these cross-sectional studies suggest that exercise effects on peak bone mass are particularly potent when the activity is begun before the onset of puberty, and is sustained throughout the young adult years. For example, the benefit of playing tennis or squash for BMD is approximately two to four times as great if female players start at or before menarche, as opposed to after the onset of puberty [61]. Mature bone appears to be far less responsive to even vigorous and extended training than developing bone [67], suggesting that major public health impacts of physical activity recommendations for osteoporosis prevention should be directed at very young children for optimal efficacy. Detraining results in a return of BMD towards normal values of sedentary individuals [68], so that any activity undertaken should be ideally feasible and sustainable in some fashion throughout adult life.

31.3.3 Exercise Intervention Trials in Children and Adolescents

Experimental trials in children and adolescents have sought to corroborate the cross-sectional observations described above, as well as define the optimum modality, dose, duration, and intensity of mechanical loading required for robust skeletal adaptations. It is well known that the skeleton adapts to changes in mechanical loading, and that loads (strains) that are dynamic, high in magnitude, applied rapidly and in unusual or diverse loading patterns are particularly effective for stimulating an osteogenic response. In addition, relatively few loads or repetitions are needed to elicit a positive skeletal response, and separating loading exercises into discrete bouts with periods of rest appears to optimize skeletal gains. Accordingly, most of the successful intervention trials in children have incorporated a variety of dynamic and diverse weight-bearing activities, such as jumping, skipping, hopping, running, dancing, plyometrics, ball games, or step aerobics, often in the setting of modified school physical education classes. Overall, there is compelling evidence that weight-bearing moderate-to-high impact activities are effective for improving bone health in this cohort.

Randomized controlled trials in children have generally been short-term (3 months to 2 years) resulting in relatively small but potentially clinically relevant improvements in BMD of up to 5 % [69–80], as compared to the larger differences observed in cross-sectional studies of athletic children vs. sedentary peers. While it is difficult to determine from these trials which exercises are most effective, examples of positive intervention trials for hip BMD in children include simple jumping programs (e.g., 100 box jumps, three times per day for 7 months [70]; or 10 jumps, three times each school day for 8 months [72]). The timing of such interventions appears to be critical to adaptation, with pre-pubescent exercise more effective than trials conducted in later adolescence. For example, a

systematic review of controlled trials of weight-bearing activity in children under 18 years found that activity consistently resulted in positive effects on the total body, lumbar spine, and femoral neck [81]. In another meta-analysis of 22 trials of physical activity in children and adolescents [69] all nine studies of pre-pubertal cohorts reported significant skeletal benefits, as did 6/8 trials in early puberty, compared to only 2/5 in post-pubertal adolescents.

The optimal dose of impact loading required for bone at this critical stage of development is not fully known. Most successful programs have been added to normal physical education classes and implemented before or after school hours for between 3 and 50 min per session, 2–5 times per week for 3–36 months. Overall, the exercise-induced gains in BMC and BMD typically ranged from 1 to 6 % in both boys and girls, with the greatest improvement seen at the femoral neck. Improvements tend to be greatest at distal sites subjected to the highest load magnitudes and frequencies (tibia and hip), with changes being more modest as distance from site of loading increases (arm and spine).

Exercise has also been observed to improve pediatric bone strength by inducing changes in structural parameters rather than simply augmenting bone density (e.g., cortical thickness and cross-sectional moment of inertia) [70, 80]. However, a recent meta-analysis of bone strength outcomes concluded that exercise during growth enhances bone strength indices in boys only [82], and more evidence is needed on this important outcome in girls. Data on the maintenance of the osteogenic benefits of childhood exercise in adulthood are also rare, although a 20-year prospective cohort study [83] suggests that childhood fitness predicts bone mass at age 30, controlled for adult fitness level.

While sedentary screen time increases fracture risk in children [84], it should also be noted that some exercises in children (most notably contact sports participation in boys) may increase *both* bone mass and fracture risk, so there is a need to also consider the current risks as well as the future benefits associated with any physical activity. Risky activity in childhood that is not accompanied by healthful participation in osteogenic modalities of physical activity throughout adulthood is unlikely to carry a long-term favorable risk: benefit ratio with regards to osteoporotic fracture prevention.

31.3.4 Physical Activity Recommendations for Optimizing Peak Bone Mass

While acknowledging the need for a more complete experimental basis for a precise exercise prescription for skeletal health in children and young adults, some recommendations can be offered at this time. Beginning in childhood (before puberty), individuals who are able should be encouraged to engage in regular weight-bearing exercise, via a combination of lifestyle choices (such as walking to school or errands), structured sports and school-based physical education, and unstructured games (outdoor play). Participation in competitive sports, although associated with even more robust effects on bone, may be hindered by many barriers, including skill level, self-efficacy, gender bias, financial burden, lack of parental encouragement, time commitments, and travel requirements, fracture risk, and is not attractive to all children. Therefore, emphasizing the replacement of sedentary activities (TV, video games) with active outdoor play, physical education in school, and less reliance on mechanical modes of transportation (cars, elevators, escalators) is likely to have a far greater impact on public health and sustained behavioral patterns in adult life than simply encouraging more competitive team sports. If sports are chosen, those involving jumping from different angles, fast rates of loading, running, and lifting appear to have the greatest effect on bone. Games that can be chosen or modified to emphasize high-impact, rapid loading (such as jumping rope, hopping, skipping, jumping over objects or steps) should be promoted if otherwise safe to do so. It is critically important for growing children and adolescents to maintain adequate energy, protein, and calcium intake, and other health habits, as bone health is compromised in the presence of eating disorders, excess phosphate from carbonated sodas, smoking, and hormonal disturbances associated with very low levels of body fat, sometimes seen in very active young women with menstrual disturbances and/or eating disorders.

The benefits of mechanical loading may be completely undone by concomitant amenorrhea in young females in the very important period from puberty to the attainment of peak bone mass in the mid-20s [85, 86].

31.4 Physical Activity and Bone Health in Premenopausal Women

31.4.1 Overview of Experimental Trials

Although the majority of randomized controlled trials of exercise and bone health have been conducted in postmenopausal women, there have been a number of similar trials in premenopausal women conducted, as reviewed in the major meta-analyses [69, 82, 87–107] summarized in Table 31.1. Although some trials have lacked statistical power on their own to demonstrate significant treatment effects due to small sample sizes, the meta-analyses generally concur that exercise has positive effects on BMD at the lumbar spine in young women. Aerobic training, resistance training, combined aerobic and resistance and high-impact programs all increase lumbar spine BMD by about 1 % per year on average, relative to sedentary controls. The magnitude of the exercise effect is approximately equivalent to that seen with calcium supplementation, and is not substantially different at the lumbar spine than that observed in postmenopausal women (see below and Table 31.1). Although this treatment effect may appear small relative to the efficacy of pharmacological agents, it does approximately counteract the rate of loss of bone with age (−1 % per year), outside of the accelerated losses in the early postmenopausal years. Femoral neck changes have been observed in programs that combine aerobic and strength training [106] as well as in high-impact aerobic jumping/stepping exercise [108], and trochanteric changes have been significant after high-impact exercise including jumping and skipping [109], 50 jumps/6 d/wk [110], and jumping/lower extremity resistance training with a weighted vest [111].

The studies in premenopausal women have ranged from 6 to 36 months in duration, enrolling women with mean ages ranging from 16 to 44 years Unfortunately, the dropout rate has been relatively high, ranging from 0 to 68 %, with five of the eight trials reported by Wallace [105] having dropout rates greater than 25 %, for example. High dropout rates raise the issue of generalizability and feasibility of exercise programs for osteoporosis, which would presumably have to be sustained for decades to exert a meaningful influence on fracture rates in this cohort. In a more recent meta-analysis of 35 studies of adults representing 3,297 participants, dropout rates in the exercise and control groups averaged 20.9 % (95 % CI 16.7–25.9 %) and 15.9 % (11.8–21.1 %) respectively, while compliance to exercise was 76.3 % (71.7–80.3 %) [112]. Notably premenopausal women in long-term studies had significantly higher dropout rates than older women, which has significant implications for clinical translation.

Withdrawal of training results in rapid reversal of skeletal adaptation. For example, in a controlled trial of unilateral limb training, Vuori [113] trained young women 5 d/wk for 12 months at high intensity on a leg-press machine and found that BMD increased and then returned to baseline values within 3 months of detraining. A similar return to baseline values was seen after 6 months of detraining in 30- to 45-year-old women who had undergone 1 year of jumping/lower-extremity resistance training [114]. Thus, skeletal adaptation to training is rapidly eroded, even after relatively long-term exposure to high-intensity loading paradigms.

There are several other limitations to the current literature on exercise training in premenopausal women. There is significant heterogeneity in the exercise interventions used in terms of exercise modality, frequency, intensity, impact loading, duration, and specific muscles and joints targeted. This makes it extremely difficult to compare results across studies, and crude categorization into

Table 31.1 Meta-analyses of physical activity and bone density

Reference	Population	Studies included	Total number of trials; subjects	Type of exercise	Study treatment effect[a]	Significance level
Berard [88]	Healthy women >50 years without osteoporosis	RCTs and NRCTs	18 trials 1966–1996	Walking, running, physical conditioning, aerobics	Lumbar spine effect size = 0.8745	Lumbar spine <0.05 Forearm = NS Femoral neck = NS
Kelley [90]	Postmenopausal women	RCTs and NRCTs	10 trials 1975–1994; 330 subjects	Aerobic activity	Lumbar spine +2.83 %	Lumbar spine <0.05
Kelley [91]	Postmenopausal women	RCTs and NRCTs	6 studies, 1978–1995	Aerobic exercise	Hip +2.42 %	Hip <0.05
Kelley [92]	Postmenopausal women	RCTs	11 studies 1975–1995; 719 subjects	Aerobic or strength training	Any exercise +0.27 % Aerobic +1.62 % Strength +0.65 %	All sites <0.05
Wolff [106]	Pre- and Postmenopausal women	RCTs and NRCTs	25 studies, 1966–1996	Aerobic, high-impact, or strength training at least 16 week duration	*Premenopausal Aerobic + strength* Lumbar spine +0.90 % Femoral neck +0. 0 % *Postmenopausal Aerobic* Lumbar spine +0.96 % Femoral neck +0.90 % *Strength training* Lumbar spine +0.44 % Femoral neck 0.86 % *Aerobic + strength* Lumbar spine 0.79 % Femoral neck 0.89 %	All sites and modalities significant (<0.05) except for strength training in post-menopausal women
Wallace [105]	Pre- and Postmenopausal women	RCTs	32 studies, 1966–1998	Impact (aerobic or heel drops) and strength training	*Premenopausal impact* Lumbar spine +1.5 % Femoral neck +0.90 % *Strength training* Lumbar spine 1.2 % Femoral neck insufficient data *Postmenopausal impact* Lumbar spine +1.6 % Femoral neck 0.9 % *Strength training* Lumbar spine 1 % Femoral neck 1.4 %	All sites and modalities significant (<0.05) except for femoral neck in pre- menopausal women

(continued)

Table 31.1 (continued)

Reference	Population	Studies included	Total number of trials; subjects	Type of exercise	Study treatment effect[a]	Significance level
Kelly [97]	Pre- and Postmenopausal women	RCTs and NRCTs	29 studies 1966–1998, 1,123 women	Resistance training	Femur +0.38 % Lumbar spine +1.26 % Radius +2.17 %	Femur NS Lumbar spine <0.05 Radius <0.05
Kelley [98]	Postmenopausal women	RCTs and NRCTs	13 studies; 699 women	Any exercise	Lumbar spine	0.000
	Individual patient data				2 % diff between groups (Ex +1 %; Control −1 %)	
Kelley [93]	Postmenopausal women	RCTs	10 studies; 592 women	Any exercise	Femoral neck	NS ($p > 0.05$)
	Individual patient data				Ex +0.73 % Control +0.45 %	
Martyn-St. James [101]	Postmenopausal women	RCTs	14 Lumbar spine	High-intensity resistance training	Lumbar spine +0.006 g/cm^2	0.006
			11 Femoral neck		Femoral neck 0.010 g/cm^2	NS (0.11)
Hind [69]	Children and adolescents	RCTs and NRCTs	22 trials: 9 Tanner I 8 Tanner II,III 5 Tanner IV,V	Games, dance, resistance training or jumping	Tanner I: +0.9–4.9 % Tanner II,III: +1.1–5.5 % Tanner IV,V: +0.3–1.9 %	All <0.05
Martyn-St James [102]	Postmenopausal women	RCTs and NRCTs	8 trials: 8 Lumbar spine	Walking	Lumbar spine +0.007 g/cm^2	NS (0.09)
			5 Femoral neck		Femoral neck 0.014 g/cm^2	0.05 (heterogeneous)
Martyn-St James [103]	Postmenopausal women	RCTs and NRCTs		High impact only	All sites Lumbar spine +0.025 g/cm^2	NS(>0.05) 0.02 <0.001 0.005
				Jogging/walking/stair climbing	Femoral neck 0.022 g/cm^2 Lumbar spine +0.016 g/cm^2	0.03
				Impact + high-intensity PRT	Femoral neck 0.005 g/cm^2	

Martyn-St James [104]	Premenopausal women	RCTs and NRCTs	13 studies	High-impact exercises	Lumbar spine Femoral neck	NS <0.00001 0.01
				High-impact + High-intensity PRT	+0.024 g/cm^2 Lumbar spine +0.009 g/cm^2 Femoral neck +0.07 g/cm^2	0.017
Nikander [82]	Children through older adults	RCTs	10 studies	Any exercise Prepubertal boys Adolescent boys Pubertal girls Adolescent girls Premenopausal Postmenopausal	Effect Size Bone strength 0.17 0.10 −0.01 0.21 0.00 0.00	<0.05 NS NS NS NS NS
Howe [89]	Postmenopausal women (45–7 years)	RCTs	43 studies; 4,320 women	Aerobic or strengthening, or combined Any exercise Aerobic/PRT High-intensity PRT	Lumbar spine +0.85 % Trochanter +1.03 % All other sites Best for spine; Best for femoral neck	<0.05 <0.05 NS
Kelly [84]	Postmenopausal women, not regularly active	RCTs	25 studies; 1,775 women	Aerobic or resistance training	Femoral neck ES 0.288 BMD Lumbar spine ES 0.179 BMD	0.002 0.05
Marques [100]	Older adults	RCTs	19 studies; 1,577 adults	Weight bearing aerobic or PRT or balance or combined	Lumbar spine 0.011 g/cm^2 Femoral neck 0.016 g/cm^2	0.007 0.004

(continued)

Table 31.1 (continued)

Reference	Population	Studies included	Total number of trials; subjects	Type of exercise	Study treatment effect[a]	Significance level
				Weight bearing + PRT	Lumbar spine	0.028
					0.016 g/cm^2	0.038
					Lumbar spine	0.004
				Odd impact	0.039 g/cm^2	NS
					Femoral neck	
				PRT only	0.036 g/cm^2	
					All sites	
Babatunde [87]	Premenopausal women 18–50 years	RCTs	6 studies; 255 women	High-impact <30 min sessions; rest between jumps	Femoral neck	0.001
						0.04
					ES +0.64	0.79
					Trochanter	
					ES +0.36	
					Lumbar spine	
					ES +0.04	
Kelley [95]	Men > 18 years, inactive	RCTs	3 studies; 275 men	Any exercise	Femoral neck	0.04
				T'ai chi, jumping, stepping, PRT, walking with weighted vest	ES +0.583	NS (0.10)
					Lumbar spine	
					ES +0.190	
Kelley [96]	Premenopausal women, inactive	RCTs	7 studies, 521 women	Weight bearing aerobic, high-impact, PRT, or combination exercise > 24 wks.	Femoral neck	0.001
					ES +0.342	0.04
					Lumbar spine	
					ES +0.201	
Kemmler [99]	Adults >45 years	RCTs and NRCTs	11 studies; 1,424 men and women	Any exercise	All fractures	<0.05
					RR = 0.49 (0.31, 0.76)	NS
					Vertebral fracture	
					RR = 0.56 (0.30, 10.4)	
Zhang [107]	Adults with low bone mass	RCTs	7 studies	Any exercise added to Antiresorptive agent	Lumbar spine	<0.00001
					SMD +0.55	

[a]Study treatment effect is the final relative (%) or absolute difference (g/cm^2) in bone density, bone mineral content, or bone strength in the training group minus the control group or the calculated Effect Size or Standardized Mean Difference if not all studies reported outcomes in the same units. A positive figure indicates a protective effect of exercise *NS* nonsignificant ($p > 0.05$), *RCT* randomized controlled trial, *NRCT* nonrandomized controlled trial, *PRT* progressive resistance training, *ES* effect size, *SMD* standardized mean difference

"impact" and "non-impact" or "endurance" vs. "strength" training, as is done in most meta-analyses, does not sufficiently describe the type or dose of exercise received, or explain potential differences between studies. For example, some lower-intensity strength-training regimens may be insufficiently robust to be considered together with high-intensity, progressive resistance training programs, resulting in a weakening of the aggregate effect size [97]. Even studies of high-impact exercises are not uniform, as investigators have created impact in various ways (jumping, stepping, skipping, plyometrics, weighted vests), and have prescribed these movements alone [109], as part of an aerobic exercise routine [108], or as part of a resistance-training routine [111], making it very difficult to compare relative efficacy or offer firm recommendations. This heterogeneity has led some to question the validity or appropriateness of combining these studies analytically via meta-analysis, which may serve to hide important distinctions between exercise trials that could lead to improved preventive and treatment strategies [115]. This would be analogous to performing a meta-analysis of "drug treatment for osteoporosis," merging the data on calcium, vitamin D, estrogen, bisphosphonates, and selective estrogen receptor modulators into one overall effect size. Unlike some other health outcomes related to exercise, the osteogenic response to mechanical loading appears to be extremely exacting in its requirements. Even apparently minor variations, such as waiting a few more seconds between loading cycles, results in large differences in bone cell response in animal models [116].

With the above caveats in mind, some general statements may be inferred from the existing data. Resistance and aerobic training regimens appear to have relatively equal efficacy on bone in premenopausal women, although they differentially affect aerobic capacity, strength, and body composition, as would be expected [117]. It is also not clear whether multimodal exercise programs are superior to single-exercise modalities, which have the potential advantage of greater simplicity and adherence. For example, in one of the largest and best-designed studies conducted in this cohort, a combination of aerobic and resistance training over 2 years significantly improved BMD at the spine, femoral neck, trochanter, and calcaneal sites, as well as improving maximal aerobic capacity and muscle strength, compared to stretching exercise in 127 women aged 20–35 years [118]. However, the dropout rate was 50 %, and the compliance rate in the remaining 50 % averaged only 61 %. Significant bone changes were not observed until the second year of treatment, at a time when attendance was at its low point of 54 % in the remaining subjects. A final limitation is that very few of these studies have enrolled women at high risk for osteoporotic fracture or followed them long enough to assess the impact of the exercise training on fall rates or fracture incidence. It is possible that the results seen in healthy women would not be replicable in high-risk individuals. For example, Hakkinen [119] applied a program of moderate-intensity resistance training for 2 years in patients with rheumatoid arthritis, who are at elevated risk for osteopenia due to their disease, inactivity, and corticosteroid medication. Although muscle strength and disease activity improved with exercise, only small differences in femoral neck BMD (0.51 %) were noted. Whether this modest adaptation in bone was related to the clinical status of the subjects or the less intense nature of the strength-training intervention (home-based, using elastic bands) is not clear. Thus, unlike analysis of the dose–response effect in drug trials, it is difficult or impossible to define a single strategy that is supported by a strong evidence base or known to be *optimally* effective for bone at this time, as well as feasible over the long term in representative populations of young women.

31.4.2 Physical Activity Recommendations for Young Women

The recommendations offered for this age group are based on the available literature reviewed above, and represent the author's interpretation of current evidence, in the areas of modality, intensity, frequency, and dose of exercise to prescribe.

31.4.2.1 Modality

After the age of 30, the physical activity prescription for the maintenance of bone health should be viewed more comprehensively than that offered to children, as both skeletal and nonskeletal risks for osteoporotic fracture need to be considered. Peak levels of muscle and bone have already been attained, and femoral bone mass may have already started to decline. Chronic health conditions that may influence bone density or temper the exercise prescription may have started to emerge. Recreational and occupational physical activity levels have simultaneously fallen in most adults. Although weight-bearing aerobic exercise, high-impact training, and resistance training have all been shown to maintain or augment bone density in this stage of life, resistance training has the added benefit of increasing muscle mass and strength, as well as balance. In the meta-analysis by Kelley [97], for example, resistance training in premenopausal women resulted in significant changes in lean mass (+2 kg) and muscle strength (+40 %) and losses of body fat (−2 %), compared to minimal changes in the control groups. This combination of effects on body composition and muscle function is a direct antidote to age-associated changes in these domains, and offers potential benefit for many health conditions in addition to osteoporosis. Aerobic exercise does not increase muscle mass and strength, and does not improve balance, and is therefore less comprehensive in its effects on the multiple risk factors for osteoporotic fracture (see Table 31.2). Additionally, there is little evidence in young women to support the isolated use of aerobic training that does not involve high-impact forces as a means to maintain or augment femoral bone density, whereas programs that include resistance training and/or high-impact training have been shown to benefit the skeleton at this clinically vital site. Therefore, the most economical prescription with the broadest benefits for body composition and bone health as well as neuromuscular function would be resistance training as the primary exercise

Table 31.2 Multifactorial risk factor targeting for bone health

Risk factor for osteoporotic fractures	Preventive or therapeutic options
Osteopenia	Bisphosphonates, SERMS, HRT, PTH, calcitonin, strontium
	Vitamin D
	Calcium
	Resistance or aerobic training, high-impact training
Low physical activity High sedentary behavior	*Exercise or physical activity prescription; reduce sitting/screen time*
Falls	*Resistance training*
	Balance training
	Multifactorial risk factor interventions (polypharmacy, vision, lower extremity dysfunction, footwear, assistive devices, home safety, etc.)
	Hip protectors
	Evaluate and treat postural hypotension
Muscle weakness	*Resistance training*
	Vitamin D
Impaired balance	*Balance training*
	Hip protectors
Depression, antidepressant medications	*Substitute aerobic or resistance training for antidepressant medication if possible*
Protein and calorie undernutrition, weight loss	Nutritional counseling and support
	Resistance training to increase nitrogen retention and appetite
Polypharmacy	Drug review and modification as appropriate
Visual impairment	Ophthalmologic evaluation and treatment as appropriate
Smoking and excess alcohol intake	Reduce or eliminate

SERMS selective estrogen receptor modulators, *HRT* hormone replacement therapy, *PTH* parathyroid hormone

modality. Adding high-impact forces/movements may further enhance benefits for the femoral neck or trochanter, lower-extremity muscle power, and dynamic balance [108], but direct comparisons of these two modalities are limited. Rest periods between sets of weight-lifting exercise may be used to complete 10–20 jumps if feasible (depending on the presence or absence of previous injuries or osteoarthritis of the knees and hips). Such a routine incorporates resistance training and high-impact loading in one session without extending the time required, an economical prescription for busy adults.

31.4.2.2 Intensity

The physiological response in bone and muscle is proportional to the magnitude and rate of strain imposed [116], and successful programs have utilized intensities at the higher ranges in general. Therefore, moderate to high-intensity progressive resistance training and/or high-impact training is recommended as the primary intensity of planned exercise in this age group. It should be noted that high-impact programs have successfully increased trochanteric BMD by 3–4 % in young women via jumps approximately 8 cm off the ground. This kind of jump produces ground reaction forces that are three to four times body weight (thus high impact), but are feasible for non-athletic women, are infrequently associated with injuries (if there are is no underlying joint pathology), and able to be completed in 2 min per day [110].

31.4.2.3 Volume

Two or three days of weight lifting, aerobic exercise, or high-impact programs per week have been shown to augment bone density significantly compared to sedentary controls if continued for at least 1–2 years. This volume of weight-lifting training is also sufficient for the other body composition changes and improvements in muscle strength, power, and balance as well (see Table 31.3). The optimal number of loading cycles, or repetitions, needed is not known, but animal studies do not show benefits of very high volumes compared to small numbers of loading cycles. For example, classical early studies showed that 36 loading cycles in a turkey ulna is not different than 1,800 applications of the same load [17], and additional evidence in rat models confirms this finding: after 50–100 cycles of loading in a given bout, additional repetitions are largely ineffective for stimulating further osteogenic response [120]. Bassey showed that 50 jumps of 8.5 cm height, 6 d/wk over 6 months, was associated with a 2.8 % increase in trochanteric BMD compared to controls [110]. It is possible that the accelerated adaptation in bone in this study compared to other studies of high-impact exercise in young women was related to the greater number of sessions (six per week compared to three per week) typically prescribed. Overall, the clinical trials literature would support a recommendation of approximately 40–50 jumps or 24–30 repetitions of a given weight-lifting exercise per training day, and this is consistent with our current understanding of osteogenic stimuli from animal studies.

31.4.2.4 Frequency

Animal data on osteogenic adaptation also suggest that the capacity of bone cells to respond to mechanical signals is quickly saturated by repetitive loading cycles without rest periods [116]. Although comparable human data are not available and are needed to confirm these findings, turkey and rat models strongly suggest that optimal recovery periods are 10–14 s between loading cycles (repetitions), and 8 h between bouts of loading (training) [116]. Such long rest intervals between repetitions are longer than currently prescribed by most trainers, who wait only 1–2 s between

Table 31.3 Comparison of exercise recommendations for specific body composition outcomes in older adults

Exercise recommendations	Adipose tissue mass visceral deposition	Muscle mass and strength	Bone mass and density; fracture risk
Modality	Aerobic[a] or resistance training	Resistance training	Resistance training High-impact activities (e.g., jumping using weighted vest during exercise, alternating angles, velocities, heights of impact) if tolerated by joints Balance training
Frequency	3–7 d/wk	3 d/wk	3 d/wk Balance training: up to 7 d/wk
Dose	30–50 min/session	Two to three sets of 8–10 repetitions of six to eight muscle groups	Two to three sets of 8–10 repetitions of six to eight muscle groups 50 jumps per session for high impact, rest periods of 10–30 s between jumps Two to three repetitions of 5–10 different static and dynamic balance postures
Intensity	60–75 % of maximal exercise capacity (VO_2 max or maximal heart rate) or 13–14 on the Borg Scale of perceived exertion	70–80 % of maximal strength (one repetition maximum)	70–80 % of maximal capacity (one repetition maximum) as load 5–10 % of body weight in vest during jumps; jumps or steps of progressive height Practice most difficult balance posture not yet mastered

[a]Aerobic exercise should be weight-bearing modalities of exercise with high ground-reaction forces (e.g., walking, jogging, running, stepping, rather than swimming or cycling)

repetitions, but are certainly not detrimental to the muscle function outcomes, and are likely to enhance excellent adherence to form and thus minimize injury. Understanding of muscle adaptation to mechanical loading also indicates that recovery periods between bouts are necessary to maximize hypertrophy and prevent injury, and are even longer than those recommended for bone (usually 1 day off between sessions). As noted earlier, it is possible to do isolated jumping exercise as frequently as 6 d/wk and produce significant bone density changes in the femur, although this regimen did not significantly improve leg power or balance in healthy young women [110].

Thus, recommending exercise no more frequently than every other day (approximately 3 d/wk) satisfies both muscle and bone requirements, and is not overly burdensome to most individuals.

31.5 Physical Activity in Postmenopausal Women and Older Men

As reviewed in Sects. 3 and 4 above, the best way to protect an older adult from skeletal fragility in late life is to optimize peak bone mass with appropriate physical activities begun as early as possible and continued throughout life. Once this phase of the life cycle has begun, however, the requirements for fracture prevention expand beyond bone to include many other factors, most notably muscle strength and balance, as well as nutrition and neurocognitive function. This is because the older the individual, the more prevention of falls becomes the major pathway for prevention of fracture. The notable exception to this is the occurrence of vertebral fracture, which is related to both muscle strength and spinal BMD, but not to fall risk.

31.5.1 Overview of Experimental Trials

Many of the observations made in young women in the previous section are also relevant to older adults, and therefore only similarities, differences, and special considerations in the older population will be highlighted in the sections that follow. Although earlier studies suffered from small subject numbers, nonrandomized designs, short intervention periods, and other methodological flaws, more recent trials have enrolled larger populations, targeted women with osteoporosis [121] or previous osteoporotic fractures [122], and continued observations for 2–5 years [111, 123] in some cases.

31.5.2 Modality of Exercise for Bone Health in Older Women

As indicated in the meta-analyses [90, 91, 93, 94, 98, 100, 101, 115] including postmenopausal women in Table 31.1, small but significant changes at the femur, lumbar spine, and radius have been seen following aerobic training, resistance training, combined programs of aerobic and resistance training exercise, or high impact loading with resistance training. The results suggest that the magnitude of the beneficial effect of exercise on bone density in older adults is both modality and intensity dependent, and heterogeneity is evident in this literature. A consistent finding however, is that clinical trials of low-impact, low-intensity exercises, such as stretching, calisthenics, or low-intensity weight-lifting exercise in postmenopausal women have not been shown to significantly improve bone density compared with controls at any site [100].

Walking in isolation appears equivocal, reported to significantly improve BMD at the spine and hip [94] in one meta-analysis; in another to have no significant effect on BMD at the spine, but significant positive effects at femoral neck [102]. Thus, earlier recommendations suggesting that weight-bearing exercise, such as simple walking, is sufficient for optimization of bone health in older adults are not consistent with the current evidence base. It is likely, therefore, that the benefits of walking on fracture risk noted in epidemiological studies are multifactorial, rather than being attributable to higher bone density alone related to walking.

At this stage in the lifecycle, a combination of decreased anabolic hormones (estrogen, testosterone, growth hormone), increased catabolic milieu (higher leptin, cortisol, and inflammatory cytokines associated with visceral adipose tissue) [124], the emergence of musculoskeletal and other diseases, retirement, and reduced recreational activities have a major negative impact on bone as well as muscle tissue. However, the majority of studies demonstrating the efficacy of aerobic or resistive exercise on bone density have been conducted in women between 50 and 70 years of age, and it is not yet known if efficacy would be similar in older women with multiple comorbidities and more catabolism, who have usually been excluded from such trials. Both types of exercise have approximately equivalent effects on bone health in postmenopausal women of about 1–1.5 % per year between exercisers and non-exercisers in meta-analyses of well-designed trials [90, 91, 93, 94, 98, 100, 101, 115]. Meta-analyses may not distinguish sufficiently between exercise modalities, however, since intensity and adequacy of training techniques are insufficiently considered in such analyses, and low-intensity "resistance training" interventions using body weight or elastic bands may dilute the effectiveness of more appropriate physiological stimuli in high-intensity weight-lifting programs, for example.

31.5.2.1 Aerobic Exercise vs. Resistance Training in Older Women

In general, the older the individual, the more favorable resistance training appears, due to its broader benefits on muscle, bone, balance, and fall risk, relative to aerobic training (see Tables 31.2 and 31.3). If aerobic training is chosen, however, activities that are weight bearing and high impact have a greater

efficacy than non weight-bearing or low-impact aerobic activities. For example, women who walked 4 d/wk for 50 min at 75–80 % of maximum heart rate while wearing a 3.1-kg leaded belt maintained lumbar spine trabecular bone mineral density (+0.5 %) compared to a 7 % loss in sedentary controls after 1 year [125]. Despite this vigorous level of aerobic exercise involving the legs, no changes were seen in the femur. In contrast, McMurdo [126] randomized 118 older women to aerobic exercise classes 3 d/wk for 2 years and found no changes at the lumbar spine and a modest effect at the distal radius. It is possible that the lower intensity of this exercise regimen limited the skeletal response. Even longer intervention periods may be required for positive results at the femur with simple walking. In a 3-year study of self-paced brisk walking vs. upper-extremity exercise 3 d/wk in 165 women recruited from an emergency department after upper extremity fracture, Ebrahim [122] reported only a trend for less decline in femoral neck BMD in walkers (−0.25 % vs. −2.8 %, $p<0.056$), and no significant difference at the lumbar spine between groups. Notably, fall rates were *increased* in the walkers, although fracture rates were similar, pointing to the possible risks of prescribing isolated aerobic exercise without other fall-prevention measures such as concurrent balance or strength training in women with a history of fall-related fractures.

Effective resistance training regimens have usually involved high-intensity (70–80 % of peak capacity as the training load) training that is progressed continually over the course of the intervention [97, 101]. However, using a similar protocol (2 d/wk at 80 % of the one-repetition maximum), McCartney [127] reported no bone changes in 142 older men and women after 2 years of high-intensity weightlifting exercise, compared to unexpected *increases* in whole-body and lumbar spine BMD and content in sedentary controls. In some resistance-training studies, although significant increases in bone density have not been seen, changes in muscle strength correlate with local changes in BMD [128], suggesting that more robust adaptations in muscle parallel osteogenic responsiveness.

The relative efficacy of aerobic vs. resistive exercise regimens for postmenopausal women may be perhaps best assessed via studies that have directly compared various intensities of these two exercise modalities in randomized subjects. Kohrt [129] found that both aerobic activities with high ground-reaction forces (walking, jogging, stair climbing) and exercises with high joint-reaction forces (weight lifting, rowing) significantly increased BMD of the whole body, lumbar spine, and Ward's triangle, whereas only the ground-reaction group increased BMD at the femoral neck. The weight-lifting group preserved femoral neck BMD relative to controls, as has been seen in other resistance training studies [123, 130]. However, lean mass and muscle strength increased only in the weight-lifting group, leaving overall benefits of these two types of exercise for ultimate fall and fracture prevention still unresolved. Heinonen [131] compared 18 months of moderate–high-intensity (55–75 % maximal aerobic capacity) weight-bearing aerobic training to low-intensity (body weight plus 1–2 kg) strengthening calisthenics or a stretching control group. The femoral neck BMD was preserved only in the aerobic group, relative to losses over time in the other two groups ($p=0.043$), with no changes at the lumbar spine. The low intensity of the strengthening regimen employed here probably explains the results observed. By contrast, Humphries [132] compared high-intensity strength training to low-intensity walking over 2 years in 64 postmenopausal women. In this study, walking was associated with decreased lumbar spine BMD of 1.3 % at 6 months, compared to minimal changes in the weight lifters ($p=0.06$ for group effect). In contrast to the high ground-reaction forces employed in the brisk walking, jogging, and stair climbing regimens of Kohrt [129] and Heinonen [131] described above, the low ground-reaction forces associated with self-paced group walking in Humphries' study [132] were insufficient to preserve or augment BMD losses of aging. In a well-designed comparative study [123], Kerr randomized 126 postmenopausal women to 2 years of high-intensity weight-lifting exercise, moderate-intensity aerobic training (circuit training and stationary cycling), or sedentary control condition. Total hip and intertrochanteric BMD was improved only by strength training, and was significantly different than aerobic training or control groups (+3.2 % at 2 years). Thus, it is important to

consider not only the optimal modality of exercise, but also the relative intensity, as the skeletal adaptation is critically linked to the *intensity* of the loading (whether due to increased amount of weight lifted during resistance training, or higher ground-reaction forces during aerobic/jumping activities). As most comparative studies other than Kohrt's [129] and Kerr's [123] have not sought to optimize both modalities, it is still not possible to definitively choose one best modality for all bone sites. A consideration of *nonskeletal* risk factors for osteoporotic fracture (muscle weakness, poor balance, sarcopenia), however, would clearly favor high-intensity resistance training over high-intensity aerobic training [97, 129, 130]. The relative importance of these risk factors in specific individuals, as well as comorbidities that may affect their tolerance of specific exercises (see below), may guide the prescription of exercise therefore.

31.5.2.2 Intensity of Resistance Training

Within the resistance training and bone literature, there is a great deal of heterogeneity in intervention techniques utilized, as well as in skeletal adaptations observed. The predominant training factor that appears to influence effectiveness is the intensity and novelty of the load, rather than the number of repetitions, sets, or days per week, or even total duration of the program. This observation is also true for animal models of mechanical loading, in which bone is most sensitive to short periods of loading characterized by unusual strain distribution, high strain magnitudes, and rapid rate of loading [116]. For example, Sinaki [133] prescribed back extension exercises at 30 % of the 1RM and shoulder girdle exercises at low to moderate intensity and found no significant improvements in spine or femoral BMD in 96 women even after 3 years. In a well-designed randomized trial comparing two different intensities of weight-lifting exercise in postmenopausal women, Kerr [134] found that 1 year of strength training at high intensity (3 sets of 8 repetitions) significantly increased BMD at the femoral trochanter, intertrochanteric site, and Ward's triangle, as well as the ultra-distal forearm, compared to low-intensity training (3 sets of 20 repetitions), which produced no significant changes in BMD at any site except the mid-forearm. Changes in muscle strength were correlated with changes in BMD only in the high-intensity group. Interesting results have been reported by Cussler [135], in a randomized trial of 140 postmenopausal women participating in a multimodal exercise program (high-intensity resistance training, and a weight-bearing circuit of moderate impact activities including walking/jogging, skipping, hopping, stair climbing/ stepping with weighted vests). Bone density improvements at the femoral trochanter were significantly and linearly related to total weight lifted during the 12 months, as well as total weight lifted in leg press, squats, and military press exercises, but not to volume or quality of the nonresistance training components of the program. High-intensity resistance training is also more beneficial than low-intensity training for muscle strength gains and muscle hypertrophy, as well as associated gait disorders, functional impairments, and disability, making it ideal as a multiple-risk-factor intervention strategy for injurious falls in osteopenic women [130, 136–138].

31.5.2.3 High-Impact Exercise in Older Women

The theoretical utility of high-impact exercise for bone health is not matched by homogeneous experimental data in older women. This is likely due to the difficulty in implementing such exercise regimens in a cohort who are more likely to have osteoarthritis and other underlying joint abnormalities predisposing to injury. However, a number of studies have now been conducted. Bassey randomized postmenopausal women to heel drops (1.5 times body weight) or control conditions, and found no difference in BMD after 12 months, perhaps due to the smaller impact of this regimen compared to

jumping [139]. Subsequently, Bassey and colleagues also reported that the same jumping intervention (50 jumps, 6 d/wk) successfully utilized in premenopausal women did not significantly improve BMD in 123 postmenopausal women exercising for 12 months, nor in a smaller subset of 38 women after 18 months [110]. It is not clear why the jumping was ineffective, as the height of the jumps was just as great in the older women, and the rate of loading and magnitude (four times body weight) of the ground-reaction forces were even higher than in the premenopausal women, who gained 2.8 % in femoral BMD after only 6 months [110]. Shaw [140] conducted a randomized trial of lower-extremity resistance training and jumping while wearing a weighted vest in 40 postmenopausal women, which improved neuromuscular function but not bone density at 9 months. Isolated high-impact loading was not reported to be beneficial in the first meta-analysis of this modality of Martyn-St James [103], but was effective for BMD increases in the more recent review of Marques [100] at the lumbar spine and femoral neck. The meta-regression analysis of Kelley [94] included 25 trials of joint or ground reaction force exercises, and reported small but significant positive effects at both femoral neck and lumbar spine, although only 4 trials represented isolated impact training (jumping/agility training), and they were not analyzed separately. It is possible that alterations in the loading frequency or recovery period are needed, or that insufficient muscle contractile forces are generated by the older women due to underlying sarcopenia or muscle weakness at baseline which needs to be addressed first to allow activity such as jumping to promote bone health. Additional studies are clearly warranted to determine the true utility and feasibility of skeletal adaptation to high-impact loading in this cohort.

31.5.3 Exercise in Older Men

Far fewer intervention trials have been conducted in older men, despite the growing importance of osteoporosis in this cohort as well. Specific subgroups of older men at increased risk include:

1. Men with habitually low lifetime levels of physical activity [7, 43, 141]
2. Men with a history of alcohol or tobacco dependence [5]
3. Hypogonadal men [44]
4. Men on chronic corticosteroid therapy for chronic lung disease, organ transplant, immunosuppression, etc. [142, 143]
5. Men with chronic renal failure
6. Men with spinal cord injury or other neurological disease associated with mobility impairment
7. Men with protein-calorie malnutrition [36].

Osteopenia associated with corticosteroid usage appears to be completely eliminated by concurrent progressive resistance training, and should be recommended for all such patients [142]. An excellent target group for such health promotion efforts is older men with steroid-dependent chronic lung disease [144, 145], in whom pulmonary cachexia, malnutrition, tobacco use, and steroid myopathy and osteoporosis combine to produce profound wasting, osteoporotic fracture, and impaired exercise tolerance [146, 147]. Aerobic training will improve functional status in this clinical cohort, but is insufficient to address the musculoskeletal wasting [148].

In healthy older men, high-intensity resistance training has been shown to increase BMD at the lumbar spine and greater trochanter compared to controls [149] similar to results in older women. In one of the few studies of older men and women with physical frailty, Kohrt [150] compared low-intensity home-based physical therapy to supervised high-intensity resistance training over 9 months. The high-intensity weight-lifting group had significantly better BMD at the whole body and Ward's triangle compared to the low-intensity exercise group at the end of the study, again demonstrating the apparent efficacy of more intensive exercise, as has been shown in most studies of healthy pre- and postmenopausal women. A meta-analysis of joint or ground reaction force exercise in older men was

reported by Kelley in 2013 [95], who noted a moderate and statistically significant improvement at the femoral neck but not lumbar spine. There is currently insufficient evidence to specifically compare the benefits of ground or joint reaction force exercises for improving and/or maintaining femoral neck and lumbar spine BMD in men, and additional well-designed randomized controlled trials are needed.

31.6 Exercise and Osteoporotic Fracture

31.6.1 Experimental Data on Exercise and Fracture Incidence

The experimental data on fracture prevention with exercise is very scarce, as fracture is rarely even a secondary outcome in exercise trials due to their relatively small size and short duration of follow-up compared to pharmaceutical investigations. In the first such trial [151], Sinaki reported significantly reduced vertebral fracture incidence in 50 postmenopausal women randomized to 2 years of low to moderate intensity, progressive back extension exercises 10 years prior. The incidence of vertebral compression fracture was 14 fractures in 322 vertebral bodies examined (4.3 %) in the control group and 6 fractures in 378 vertebral bodies examined (1.6 %) in the exercise group [$p = 0.0290$; odds ratio (OR) = 0.56; 95 % CI, 0.30–1.04]. Howe [89] reported a meta-analysis of 43 RCTs of exercise and BMD including 4,320 participants. In these trials, the most effective type of exercise intervention for the neck of femur was high-intensity progressive resistance strength training [MD 1.03; 95 % confidence interval (CI) 0.24–1.82], and for the spine was combined weight-bearing/strength/high-impact programs (MD 3.22; 95 % CI 1.80–4.64) compared with control groups. There was no effect on numbers of fractures (OR = 0.61; 95 % CI 0.23–1.64), which were adverse events rather than primary outcomes of any trial. The final data on fracture incidence comes from the meta-analysis of Kemmier in 2013 [99], who analyzed ten controlled exercise trials that reported overall fractures and three exercise trials that reported vertebral fracture outcomes. Overall fracture number in the 754 exercisers was 36 vs. 73 fractures in the 670 controls (relative risk [RR] = 0.49; 95 % confidence interval [CI], 0.31–0.76), and vertebral fracture reduction was borderline significant (RR = 0.56; 95 % CI, 0.30–1.04).

31.6.2 Role of Exercise in Hip Fracture Treatment

Exercise (both structured and incidental) and habitual physical activity patterns contribute directly and indirectly to hip fracture etiology and recovery [152–154]. The highest risk group for hip fracture are those who have suffered a prior fragility fracture [155]. Thus, any comprehensive clinical approach to hip fracture treatment should also target continuing risk factors for the index fracture, to reduce the likelihood for recurrent injurious falls and fractures [156], which would be an index of rehabilitative failure. Notably, the common rehabilitative/orthopedic goal to return the individual to his or her pre-injury state and then discontinue treatment is *completely insufficient* in the context of hip fracture. The day before the hip fracture occurred, the patient may have been frail, sarcopenic, weak, impaired in gait and balance, undernourished, depressed, cognitively impaired, vitamin D deficient, sedentary, visually impaired, socially isolated, and lacking in self-efficacy/confidence for independent living, among other things [156, 157]. Returning the patient to that state cannot be considered an appropriate rehabilitative goal, and would be unlikely to prevent future falls and fractures. It is notable that the majority of patients (75 %) undergoing usual care for a fractured hip in a prospective cohort study did not even meet this very low target of return to their prior level of function at 12 months [156].

31.6.3 Modality of Exercise After Hip Fracture

Recent exercise trials have most often included progressive resistance training (PRT) in isolation or as the focus of treatment [157–164]. Edgren reported that 12 weeks of PRT in 78 individuals with a history of hip fracture on average 3 years earlier improved strength, physical performance as well as independence in ADLs (but not IADLs) [159], and suggested the existence of a prolonged potential for further improvement with targeted therapy after rehabilitation had ended. Sylliaas randomized 95 patients who had participated in high-intensity lower extremity PRT for twice-weekly for 12 weeks [161] to an additional 12 weeks of reduced frequency training and reported additional significant improvements in strength, gait speed and gait distance, IADLs, and health-related quality of life [160] compared to controls who did not receive continued training.

Although impairments of balance and balance confidence are prevalent and related to dysfunction in this cohort [165, 166], isolated balance training has only been reported in those at risk of falling without hip fracture [167]. Howe's review of 94 such studies in 9,821 older adults concluded that there was some, albeit weak, evidence for gait/balance/functional exercises to modestly improve balance in the short term, but defining optimal prescriptive elements was premature as there were few similarities among intervention components. There was no conclusive evidence that walking, balance platform training, or vibration exposure were effective for balance enhancement in older adults.

Theoretically, given the multiple deficits in exercise capacity prevalent in hip fracture cohorts, a multimodal exercise prescription targeted to these specific deficits would be required to optimize outcomes. Two such multimodal exercise interventions incorporating combinations of strength, balance, gait/mobility, aerobic or functional exercises after hip fracture [168, 169] have been published recently. Sipila's *ProMo* intervention [42] included standard care (written home exercises) and a year-long program including evaluation/modification of environmental hazards, guidance for safe walking, pain management, progressive home exercise (strength, balance, flexibility, walking, stretching, functional movements) and physical activity counseling. Orwig's "Exercise Plus" program [149] consisted of 12 months of home-based aerobic and strength training in 180 patients with hip fracture. This program increased activity level and modestly improved bone density compared with those in usual care; however, contrary to hypotheses, no significant changes in muscle mass, strength, fat mass, ADL independence, or physical function were observed. Thus, there is as yet no evidence that such all-inclusive prescriptions are effective compared to the trials focused on robust strength training described earlier. Notably, the lack of supervision, multiple modalities of exercise required, and home-based setting would have likely resulted in lower intensities of training, even if adherence was high, which could explain the lack of efficacy for these outcomes.

No trials of isolated aerobic exercise after hip fracture have been reported. Although moderate intensity weight-bearing aerobic exercise may have a positive influence on bone density and prevention of osteoporosis [170], effects are generally less robust than those observed after resistance training, high-impact exercise, or combinations of these elements [153]. There have also been no trials of high-impact exercises in this cohort, despite growing evidence that bone is most responsive to high- and novel-impact forces [171], and muscle power may be improved concomitantly. This is likely due to appropriate concerns about joint pain and instability, poor balance and proprioception, weak muscles and tendons, and podiatric problems in these patients, which would preclude typical high-impact activities such as jumping, jogging, and plyometrics, or increase risk of injury. However, there are subgroups of younger or less frail hip fracture patients without osteoarthritis who may derive benefit from adapted high-impact training potentially, and such trials are warranted.

31.6.4 Dose and Setting of Exercise

A recent Cochrane review of extended exercise rehabilitation provides evidence that long-term training [172] results in more robust adaptations than usual care in the outcomes of strength, balance, physical performance, and maximal gait speed, and that community-based, supervised training is associated with larger effects than home-based exercise. It is notable that in these exercise studies, even when physiological and performance outcomes improved, the effects on overall ADL and IADL independence were often minimal or absent, however. This suggests that we still do not know the optimal prescription for maximizing outcomes after hip fracture, or that exercise alone may be insufficient to address all of the factors that contribute to long-term functional independence in this cohort. In a study designed to more comprehensively address the many comorbidities potentially impeding recovery, the Hip Fracture Intervention Trial (HIPFIT) [157] randomized 124 patients with hip fracture to usual care or 12 months of supervised high-intensity progressive resistance training, static and dynamic balance training, and targeted treatment of problems (if identified) in the areas of sarcopenia, protein/calorie undernutrition, polypharmacy, vitamin D, calcium, depression, cognition, vision, self-efficacy, social isolation, and environmental hazards/fall risk reduction. HIPFIT reduced both mortality and nursing home admissions by over 80 % at 1 year, and improved ADL independence, balance, strength, self-efficacy for independence in functional activities, vitamin D level, nutritional status, depressive symptoms, and cognitive function compared to usual care controls [157]. This was the first randomized controlled trial to demonstrate long-term reductions in both mortality and nursing home admissions after hip fracture with any intervention. HIPFIT differed from previous studies in terms of the intensity of the whole body resistive exercise prescribed (80 % of the most recently determined maximal strength test, progressed continually), the large number of risk factors and comorbidities targeted and treated, and the continuing presence of the geriatrician-led multidisciplinary team throughout the year. The challenge is to further refine, evaluate, and disseminate such comprehensive treatment programs in ways that are feasible and sustainable without sacrificing the diversity and robustness of the interventions underlying its efficacy.

31.6.5 Effects of Exercise on Neuropsychological Domains Relevant to Recovery and Function

Even when physical capacities are improved by exercise training, such benefits may not translate into improved mobility, function, or quality of life. Thus, specific efforts to enhance exercise and functional self-efficacy to promote use of these rehabilitative gains may be needed in addition, to decrease perceived barriers to mobility and independence. Studies of patients after orthopedic surgery [173, 174] have identified the importance of social support, personal beliefs and attitudes, adaptive strategies and goal setting in self-efficacy and motivation to adhering to a rehabilitation program. Determination to improve mobility and function through exercise is a key feature characterizing successful rehabilitators. One trial to address this was the *ProMo* Trial [168], in which 81 hip fracture patients were randomized to standard care or a year-long home-based program including evaluation/modification of environmental hazards, guidance for safe walking, pain management, progressive multicomponent home exercise program and physical activity counseling. Unexpectedly, Portegijs reported that the secondary outcome of "perceived environmental barriers to indoor or outdoor mobility" in this trial did not improve with the experimental intervention [175]. Thus, additional research is required to define the optimal prescriptive elements for exercise post-hip fracture, including not only exercise modality, dose, and intensity, but also behavioral counseling and other psychological

interventions needed to translate improved physiological *capacity* for mobility to improved *performance* and *perception* of community mobility and other functional outcomes.

Depression has been associated with increased risk of frailty, functional dependence, and mortality in a variety of cohorts, including hip fracture. For example, moderate to severe depressive symptoms in a hip fracture cohort on admission were reported to negatively affect the recovery of walking independence and were associated with elevated risk of nursing home residence and death at 1 year after surgery [176]. Phillips also recently reported in 101 older adults admitted for hip fracture that incident depression at 6 weeks was associated with lower gait speed, balance, and ADL independence, which appeared to be mediated by an elevated cortisol: DHEAS ratio [177]. However, depression is very responsive to treatment older adults, and has been shown to be successfully treated specifically after hip fracture with targeted comprehensive care including depression management [178], or multimodal programs that incorporate high-intensity resistance training, cognitive-behavioral therapy, and antidepressant medications when required [157].

Cognitive impairment is common in hip fracture, occurring in 30–50 % of this cohort [156], and is associated with falls [179], undernutrition [180], low gait speed and muscle strength [181], poor functional recovery [182], and elevated risk of institutionalization and mortality after hip fracture [183]. Cognition is thus a critical consideration in rehabilitative approaches [184]. For example, Stenvall reported a randomized controlled trial in 64 hip fracture patients with dementia [185]. The intervention consisted of staff education, individualized care planning and rehabilitation, and proactive prevention, and treatment of postoperative complications, and at 12 months ADL performance level was improved compared to standard care. This study suggests that dementia does not preclude rehabilitation, but requires additional elements of care to optimize recovery. There is also evidence in non-hip fracture cohorts that exercise can improve cognition, even in frail individuals [186]. In the HIPFIT study, cognitive status at 1 year was improved after a multimodal intervention that included resistive exercise, vitamin D repletion, nutritional supplementation, family support and education, and minimization of polypharmacy for subjects with cognitive impairment on admission, all of which were designed to benefit cognition. Thus, screening for cognitive impairment, identifying potential contributing factors and implementing therapeutic interventions (including exercise) within tailored management approaches, is crucial for improved outcomes in this prevalent subgroup of hip fracture patients.

31.6.6 Summary of Hip Fracture Rehabilitation

Overall, reviews of RCTs show that usual care after hip fracture, which includes short periods of physical therapy/rehabilitation, simple ambulation, stretching/flexibility exercises, and low-intensity strengthening exercises, is generally suboptimal, as most patients do not return to pre-morbid function afterwards. Clinically relevant improvements in strength, balance, physical performance, self-efficacy, and other outcomes may be seen with the addition of robust, structured exercise, which has most often included resistance and gait/balance/mobility training. However, improvements in sarcopenia, mobility and long-term functional independence are variable and often absent [187] in such trials. Significant improvements in muscle mass/sarcopenia status have not been reported in recent exercise trials, even when high-intensity resistance training has been used [157], and this may reflect anabolic resistance imposed by coexisting malnutrition, decreased protein synthesis capacity, systemic inflammation, comorbid illnesses associated with cachexia, or other factors such as inability to adhere to prescribed training volumes or intensities [188]. Given the prevalence of sarcopenia in this cohort [189], augmentation of lean mass adaptation to exercise using anabolic steroids, amino acid or protein

supplementation and other pharmacologic approaches to reduce inflammation or enhance protein synthesis may be required to extend the benefits of exercise treatment to robust improvements in body composition. At a minimum, diagnosing and treating existing nutritional deficiencies of protein, energy, and vitamin D may enhance the anabolic response to exercise.

31.7 Exercise, Medication, and Nutrient Interactions and Bone Health

Medication–exercise interactions need to be considered in the overall context of bone health. Some drugs, such as corticosteroids (see above) and thyroid hormone, increase the risk of osteopenia and fracture, and should always be accompanied by an appropriately intensive physical activity prescription. In the case of corticosteroids, this should be resistance training, due to the need to oppose both the myopathy and osteopenia of corticosteroid administration [142, 190]. By contrast, aerobic exercise has not been shown to significantly improve BMD in the setting of chronic prednisone therapy [191]. Both oral and inhaled corticosteroid treatment carry this risk of osteopenia and therefore the need for prophylaxis. There is evidence that excess alcohol consumption may adversely affect bone mineral density or increase risk of osteoporotic fracture [12, 141], and therefore moderation of alcohol intake is recommended. The adverse consequences of excess alcohol intake on testicular atrophy, myopathy, peripheral and central neurological function, gait, balance, coordination, and judgment, in addition to impairment of bone metabolism, may combine to produce a high-risk profile for injurious falls and fractures in the elderly. Finally, as is the case with muscle mass, weight loss, and inadequate intake of protein and energy will lead to losses of bone and increase hip fracture risk [36], and are associated with poor recovery and excess mortality after hip fracture. Thus, prevention and treatment of energy or protein malnutrition and early assessment of unintentional weight loss is part of the management plan for optimal bone mass and health.

Oral contraceptive (OC) use in young women has been associated with reduced bone turnover and lower BMD in this cohort. Exercise in the setting of oral contraceptives appears to have a complex and site-specific effect on bone. The negative effect at the femoral neck was only partially counteracted by a 24-month program of combined cycling, jumping rope, and high-intensity resistance training in 123 women aged 18–31 [192]. However, in a subsequent report of this same trial, the investigators noted that the exercise resulted in an increase of total body BMC, a decline in femoral neck BMD, while preventing increases in BMD and content seen in the spine of the OC–no exercise group [193]. The authors suggest that suppression of bone turnover and resorption due to OC use modifies the skeletal response to exercise, and that inadequate calcium intake may worsen this interaction. Effective countermeasures for this underappreciated pharmacological side effect are warranted, given the prevalence of OC use and declining calcium intake in young women during the precise years when peak spinal BMC is usually attained.

On the other hand, some investigators have sought to maximize bony adaptation via the combination of exercise, nutritional, and pharmacological treatments for osteoporosis. Most studies of exercise in fact have supplemented subjects with calcium, and in some cases vitamin D, to equalize baseline status of these nutrients. It makes sense to ensure adequate intake in these nutrients so as to optimize the skeletal milieu available for osteogenesis before exercise is begun. Specker has reported that exercise resulted in increased spinal BMD only in postmenopausal women consuming more than 1 g of calcium per day in an analysis of 16 trials [194]. It appears that adequate mineral must be present for bone remodeling to occur under the stimulus of mechanical loading, Given the prevalence of calcium and vitamin D deficiencies in the general population, the recommendation to ensure adequate dietary intake of calcium in all exercising men and women, and adequate vitamin D status

in older men and women, is supported by current knowledge of skeletal physiology and nutrient requirements [195].

The combination of exercise and postmenopausal estrogen replacement therapy for bone accretion has been studied by several groups of investigators. For example, Kohrt [196] reported a trial of 32 postmenopausal women assigned to groups by matching for body weight. The combination of exercise plus hormone replacement therapy (HRT) resulted in increased BMD at all sites except the wrist, with effects being additive for the lumbar spine and Ward's triangle and synergistic for the total body. Based on reductions in serum osteocalcin levels, these authors attributed increases in BMD in response to HRT and exercise plus HRT to decreased bone turnover (decreased bone resorption) and not increased formation. However, increases in osteoblast-mediated bone formation have been linked to exercise benefits by other investigators [16]. Additive or synergistic effects of estrogens and various forms of exercise, including resistance training, have been reported by others in both animal [197] and human studies [150, 198]. Thus, it appears that for women on HRT, additional skeletal benefits are likely when combined with either resistance training or aerobic exercise, along with the spectrum of nonskeletal health benefits of increased physical activity.

Bisphosphonates are now the mainstay of pharmacological osteoporosis treatment for most women at risk rather than HRT, and it is thus reasonable to consider combining this antiresorptive treatment with an anabolic effect of exercise on bone. In the earliest human report by Vico, young men were randomized to etidronate, exercise, both treatments, or placebo during 120 d of bedrest [199]. The two groups receiving bisphosphonates had decreased markers of bone resorption on iliac crest biopsy, while exercise alone resulted in increased bone resorption. In a subsequent study, Grigoriev [200] reported that exercise and etidronate improved calcium balance more than exercise alone in young men during 1 year of bed rest. Using a similar randomized factorial design to that of Vico, comparing etidronate, resistance training, both treatments, and placebo in 48 postmenopausal women for 1 year, Chilibeck [201] showed that etidronate significantly increased whole-body and lumbar spine BMC, whereas exercise had no direct or interactive effect on bone. However, only the resistance training improved muscle strength and lean body mass and decreased fat mass. Uusi-Rasi published a 12-months trial of jumping and alendronate in postmenopausal women [202]. Alendronate plus jumping preserved bone mass and exercise increased bone area in the distal tibia compared to controls, effects which were not sustained after discontinuation of both treatments [203]. A meta-analysis has now reported seven trials of combined anti-resorptive agents and exercise [107]. The increase in lumbar spine BMD of the combined-intervention group was significantly greater than that of the antiresorptive agent-alone group (fixed effect model: SMD = 0.55; $p < 0.0001$), and additional long-term trials appear warranted.

31.8 Practical Implementation of Exercise Programs for Bone Health

As reviewed above, habitual exercise has a modest but significant effect on BMD in many cross-sectional and prospective investigations, and both weight-bearing aerobic exercise, high-impact exercise, as well as resistive exercise have positive effects in experimental trials. The weight of the evidence suggests that while aerobic exercise may be effective as a preventive strategy in younger adults, it is likely that significant shifts in bone mineral compartment in older adults are more robust with weight-lifting exercise. In addition, it has been shown that high-impact forces to bone (jumping, using weighted vest with stepping, jumping, and resistive activities) are likely to have greater effects than low-impact or low-loading activities, particularly in children and pre-menopausal women. By contrast, simply wearing a weighted vest without the additional prescription of specific exercises has no impact on bone density or functional status [204]. The difficulty comes in the attempt to prescribe

high-impact activities (such as jumping while wearing a weighted vest) in older adults with both osteoarthritis of the hips and knees as well as risk of osteoporotic fracture and falling. It is doubtful that high-impact activities such as jumping would be feasible in such a patient profile, and could result in exacerbation of arthritis as well as fall-related injuries. In such cases, therefore, a *low-impact* but *high-loading* form of exercise (such as seated and standing weight lifting with machines or free weights) would be both effective and tolerable. In general, because the effects of muscle contraction on bone appear to be primarily regional (electromagnetic field stimulation of osteoblast function) rather than systemic, it is advised that muscle groups connected to bones of relevance to osteoporotic fracture be emphasized in such a program (e.g., spinal extensor muscles, hip abductors, hip extensors, knee flexors) as well as those related to gait and balance (ankle plantar flexors and dorsiflexors). Specific exercise recommendations and suggested modifications for common comorbid diseases of older men and women are presented in Table 31.4.

In addition to the above considerations, activity recommendations for the older age group should include avoidance of forward flexion of the spine, particularly while carrying an object (bowling, bending over to pick up something from the floor, sit ups with straight legs, etc.). Such actions increase the risk of anterior compression fractures of thoracic vertebrae in the presence of osteopenia. Similarly high-risk activities or hazardous environments that may lead to falls in those with poor balance are best avoided. Potential risks of exercise in individuals and suggested means to avoid such complications are presented in Table 31.5. It should be noted that the literature to date documents very few adverse events attributable to exercise, including resistance training and impact loading in older women, attesting to the relative safety of such prescriptions in supervised and unsupervised settings. However, few trials have included subjects with significant medical conditions that might increase the risk of exercise-related injury, and such trials are clearly needed.

The evidence presented in this review is consistent in the finding that the volume of exercise required for bony adaptation is small (only 12 min per week of jumping in one study [72]), whereas the need for high-impact or intensity of loading which is progressed over time is the critical factor. There is a great need to improve behavioral strategies to provide adequate instruction, supervision, and compliance with such an exercise prescription, as some trials have suffered from high dropout rates and low compliance, even when fully supervised. The skeletal adaptations are sustained only when the loading is continued, so that any impact on future fracture risk is dependent on long-term adherence to exercise prescriptions. Given the very short time that is needed for impact loading, finding ways to incorporate such episodes into daily activities may be more successful than planning structured exercise classes away from home. For example, inserting a few jumps during television commercials or hopping rather than walking up a flight of stairs may provide an effective stimulus if such habits can be effectively behaviorally reinforced. Such integrated lifestyle intervention programs should be based on the physiology of bone mechano-sensors, as currently known, since the requirements for bone remodeling are quite stringent compared to other health outcomes achievable through general physical activity recommendations. In addition, it is important to consider both skeletal and nonskeletal risk factors for osteoporotic fracture, and assess the responsiveness of each of these to targeted intervention programs (*see* Table 31.2). Given the multifactorial nature of this syndrome, it is likely that successful prevention programs will need to be multifaceted as well.

31.9 Conclusions

At all ages, an exercise prescription is important for the prevention and treatment of osteoporosis. A combination of lifestyle choices, organized sports, unstructured play, and household and occupational tasks can all contribute to a desirable exposure to physical activity that will be lifelong and robust

Table 31.4 Specific exercises for bone health and modifications for physical limitations

Exercise modality	Standard or optimal mode	Modification for arthritis	Modification for frailty/ neuromuscular impairment	Modification for cardiovascular/ pulmonary disease
Progressive resistance training	8–10 exercises for major muscle groups, including muscles attaching to greater trochanter and vertebral bodies, as well as gait and balance[a]	Provide exacting attention to form to prevent injuries	Usually little modification needed	Usually no modification needed
		May need to limit range to pain-free motion, provide good back support, adjust machines or free weights to accommodate joint deformities or restrictions	May need to alter certain exercises for neurological impairment	If angina or ischemia is provoked by exercise, keep intensity below the level at which this occurs
	Include novel planes of movement, free weights, standing postures if possible	Intensity may need to be individualized for some exercises	Supervision usually needs to be more intensive for safety and progression	May need to perform exercises in seated rather than standing positions due to fatigue or poor balance
	High intensity (approximately 80 % of peak capacity, progressed continuously)	May need to medicate for pain prior to exercise		Avoid breath holding, Valsalva maneuver, sustained isometric contractions, or tight handgrip during weightlifting
Aerobic training	Moderate to high intensity Weight-bearing	May need to reduce or eliminate weight-bearing or high-impact component: substitute brisk walking, stair climbing for jogging, step aerobics	May need to substitute seated exercises if weakness or poor balance prevents standing postures	Keep training intensity below the level that causes ischemia or severe dyspnea
	High ground-reaction forces (jogging, stepping, jump rope, etc.)		May need to begin with low–moderate intensity level and short sessions until improved	Walk or exercise beyond the onset of claudication if possible (1–2 min); then rest and repeat
				Avoid breath holding, Valsalva maneuver, sustained isometric contractions, or tight handgrip during
High-impact exercise	Jumping, stepping off boxes, jump rope	May need to reduce or eliminate high ground-reaction forces (heel drops instead of jumps)	Start with heel drops instead of jumps	Keep training intensity below the level that causes ischemia or severe dyspnea
			Perform exercises under supervision and while holding onto a support rail initially	
	Progressively increase height of jumps or boxes, hop on one leg	Substitute power training (rapid concentric muscle contraction against moderate to high load on weight-lifting machine) to produce rapid onset of high muscle contraction forces as in take off of jump, but with no impact	Gradually reduce hand support as tolerated	

Balance training	Combine progressively more difficult static and dynamic postures Reduce base of support Perturb center of mass Withdrawn vision Increase compliance of standing surface (decrease proprioception) by using pads, mattress, pillows to stand on Incorporate postures from yoga and T'ai Chi which emphasize the above principles	May not be able to place full body weight on osteoarthritic joints—use less painful leg to perform one-legged postures, assist weight bearing with use of cane Keep sessions short to avoid pain from prolonged weight bearing Reduce angle of flexion at knee during T'ai Chi movements	Perform exercises under supervision and while holding onto a support rail initially Gradually reduce hand support as tolerated	Usually none

[a]Most important exercises include leg press, squats, knee extension, hip abduction, hip flexion, dorsiflexion, military press, lat pull down, back extension, abdominal muscles
[b]One-legged standing, tandem walking, crossover walking, turning, stepping over objects, leaning to limits of sway, etc.

Table 31.5 Risks of exercise in osteoporosis

Potential risk	Preventive strategy
Injurious fall	Prescribe balance training prior to aerobic training if gait and balance are impaired
	Prescribe progressive resistance training for sarcopenia and muscle weakness
	Optimize lighting, visual aids, safety of exercise environment, climate conditions, footwear, judgment
	Review medications for agents which may cause falls, postural hypotension, or altered central nervous system function
Spinal compression fractures	Avoid forward flexion with loading of the spine
	Avoid twisting movements of the spine
	Emphasis good sitting and standing posture
	Avoid or modify sports/activities involving spinal flexion (bowling, biking, golf, gardening, vacuuming)
	Bend knees rather than spine to pick up or reach low objects
Dislocation of total hip prosthesis	Avoid internal rotation and flexion of the hip
Pain from osteoarthritis	Use low-impact, high-intensity exercises (such as weight lifting) rather than high-impact exercises (jumping, stepping, jogging)
	Emphasize brief, novel loading of bones with adequate rest periods rather than prolonged, repetitive loading bouts
Pain from hip fracture, spinal osteoporosis, or old compression fractures	Rule out new fractures or dislocation of surgical prostheses
	Brace or support spine during exercise if needed
	Use analgesia or local pain relieving techniques (heating, massage, etc.)

enough to counteract age and disease-related losses of bone. An initial emphasis on weight-bearing aerobic and high-impact activities in youth, shifting toward resistive loading and balance-enhancing exercises in old age, appears to address optimally the needs and capacities of the musculoskeletal system throughout the lifespan.

Evidence has been presented that a stabilization or increase in bone mass in pre- and postmenopausal women is achievable by either resistive weight-bearing aerobic or high-impact loading. Such effects on bone density (differences of 1–2 % per year associated with exercise) may be important for both prevention and treatment of osteoporosis and related fractures and disability, as reviewed in an increasing number of recent meta-analyses. Additional data are needed in men and frail elders, as is refinement of the exercise prescription for bone health in terms of the optimal modality, dose, frequency, and intensity of activity recommended. Even if exercise alone is an insufficient stimulus to maintain bone density at youthful levels, the combination of exercise effects on bone strength, muscle mass, muscle strength, and balance should lower the risk of injurious falls substantially in physically active individuals. Although the combination of resistance training and impact exercise has been shown to reduce fracture incidence in recent meta-analyses, large, long-term, randomized, controlled trials of any exercise modality with osteoporotic fracture itself as a primary outcome remain to be conducted, and are a priority for advances in this field.

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Chapter 32
Exercise, Nutrition, and Bone Health

Fiona L. Morris-Naumann and John D. Wark

Key Points

- Optimal bone metabolism is the result of hormonal, nutritional, and mechanical harmony, and a deficit in one area is usually impossible to overcome by improvements in others.
- Exercise during growth influences bone modeling locally at the regions being loaded, whereas calcium is thought to act systemically to influence bone remodeling.
- Exercise and calcium may not operate independently.
- Low dietary calcium intake or reduced bioavailability may minimize the adaptive response to exercise-induced bone loading.
- Adequate levels of calcium intake can maximize the positive effect of physical activity on bone health during the growth period of children and adolescents.
- Adequate levels of calcium intake can maximize bone density at the regions being loaded during exercise.
- Exercise, adequate nutrition, and optimal hormone levels are the components that influence bone outcomes.
- Making healthy nutritional choices, engaging in weight-bearing physical activity, and ensuring optimal hormone levels during growth provides a window of opportunity to build optimal bone mass, to reduce the risk of fracture later in life.

Keywords Exercise • Nutrition • Bone Health • Osteoporosis

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- Concurrent management of fracture risk with a physical activity prescription, adequate nutrition, and pharmacotherapy for osteoporosis when required offers the best approach to optimal bone health throughout adulthood.

32.1 Introduction

Osteoporosis is the most common metabolic bone disease and is a global health problem that will continue to take on increasing significance as the population ages [1]. Worldwide, osteoporosis causes more than 8.9 million fractures annually, with 1 in 3 women and 1 in 5 men aged over 50 sustaining an osteoporotic fracture [2, 3]. From a biomechanical perspective, fractures represent a structural failure of the bone whereby the loads applied to the bone exceed its strength [4]. Bone strength depends on a number of interrelated factors, including the amount of bone tissue (mass), the structure of bone (spatial distribution, shape, and microarchitecture), and the intrinsic properties of bone (porosity, matrix, mineralization, collagen, and microdamage) [4].

Osteoporosis is characterized by low bone mass (most often expressed as bone mineral density or BMD) and microarchitectural deterioration of bone tissue, leading to reduced bone strength, enhanced bone fragility, and increased fracture risk ([5]; Surgeon General Report 2012). The World Health Organization (WHO) defines osteoporosis as bone density that is 2.5 standard deviations (SDs) or more below the young adult mean value (T-score <-2.5). Patients with bone density between 1 and 2.5 SDs below average (T-score -1 to -2.5) are said to have osteopenia [6]. Fragility fractures are the hallmark of osteoporosis and are particularly common in the spine, hip, and distal forearm [7]. Unfortunately, the incidence of vertebral and hip fractures increases exponentially with advancing age [8].

The risk of developing osteoporosis is largely determined by the mass and size of bone acquired by adulthood, known as peak bone mass, and by the amount of bone subsequently lost over life [9, 10]. Peak bone mass can be defined as the greatest amount of bone mass achieved in a lifetime [9]. Bone mineral density almost doubles during puberty, through an increase in the size and volume of the bone [11]. This increase is associated with the increase in sex hormone levels and is almost completed with closure of the epiphyses. Low peak bone mass is an important determinant of later osteoporosis and risk of fracture [12].

By the end of the second decade of life, the majority of bone mineral density has been accumulated, with only minimal additional accumulation of bone mineral over the next 5–15 years during skeletal consolidation [9, 13]. This time course trend is illustrated in Fig. 32.1. Loss of bone with advancing age is a universal phenomenon, whereby bone resorption predominates over formation, resulting in bone loss. Bone mass loss accelerates in women during the perimenopausal years, with continued loss into old age [14]. Similar patterns are observed in men, without the accelerated loss linked to decline in ovarian function seen in women [15].

Bone Mass Versus Age With Optimal and Suboptimal Bone Acquisition [9].

Epidemiological evidence suggests that an interplay between heritable and environmental factors determines peak bone mass, with an estimated 60 % of the variance in peak bone mass variance determined by genetic factors [16, 17]. Studies in twins and mother-daughter pairs suggest that 40–80 % of the variability in the bone mass is determined by genetic factors. Heredity not only sets limits on how much bone a person acquires, but also on bone structure, the rate of bone loss, and the skeleton's response to environmental stimuli like nutrients and physical activity. The impact of genetics on development of peak bone mass is evident in longitudinal tracking of BMD, whereby those individuals who begin with lower BMD remained lower throughout the accrual of peak bone mass [18]. The known genes implicated in osteoporosis are numerous, including those for the estrogen receptor, transforming growth factor-β, and apolipoprotein E and collagen. Environmental factors then account for the remaining nongenetic influences, with nutrition, physical activity, and other lifestyle habits such as smoking being key contributors [19–21].

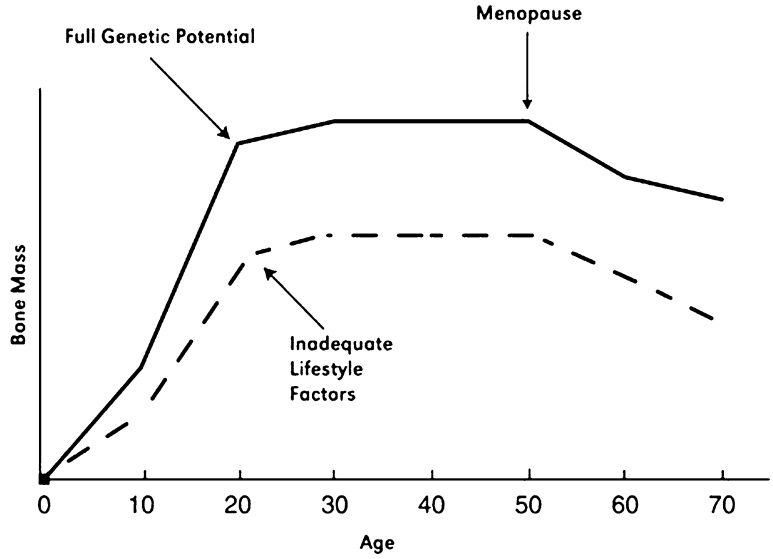

Fig. 32.1 Bone mass versus age with optimal and suboptimal bone acquisition across the life span [9]

There is a growing body of evidence linking nutritional intakes, particularly calcium and total energy, and physical activity, particularly the mechanical loading of the skeleton, to bone growth and to bone loss later in life. Both processes influence osteoporosis and fracture risk. Increasing evidence also suggests that the interaction between nutrition and physical activity is a stronger determinant than either entity alone [22]. While research has focused on the treatment of osteoporosis, equally important is a focus on promotion of optimal bone health during childhood and adolescence, and maintenance of bone mass in adulthood. This chapter reviews the effect of physical activity and the main nutritional determinants of bone health throughout the different stages of life and reviews the related strategies for the primary and secondary prevention of osteoporosis.

32.2 Physical Activity and Bone Health

Mechanical loading of the skeleton generally leads to favorable site-specific changes in bone density, morphology, and strength, whereas decreasing mechanical load, exemplified by spaceflight, bed rest, immobilization, and spinal cord injury, results in rapid resorption of bone, loss of BMD, and increased susceptibility to fracture [23–25]). In completely immobilized patients, bone mass loss may be up to 40 % in 1 year [23].

Bone experiences internal strain when mechanically loaded. As long bones are curved, they bend when axially loaded. This results in exposure of different tissue-level regions within the bone cross section to different levels of microstrain. Only those regions within the individual loaded bone that experience sufficient microstrain adapt. This has been demonstrated most evidently using the rodent ulna axial compression model, wherein tissue-level bone adaptation closely matches the tissue-level microstrain distribution [26].

Extensive research using animal models has shown that the skeleton's response to loading is regulated by several factors, including the magnitude and pattern of loading. Bone responds to mechanical stimuli via several principles: (1) bone preferentially responds to dynamic rather than static stimuli,

(2) only short durations of loading are necessary to initiate an adaptive response, and (3) bone cells accommodate to customary mechanical loading environments [27]. Therefore, when prescribing exercise to maximize bone mass it needs to incorporate high impact, dynamic loading which is novel. The adaptive response of bone to mechanical loading is also highly site-specific. This is clearly evident on the whole bone (organ) level, with only the bones that are actually loaded undergoing adaptation.

32.2.1 Exercise Prescription and Bone Health

Targeted exercise can have a positive impact on bone, increasing density, size, and mechanical strength and may be one of the keys to preventing fracture complications associated with osteoporosis [28]. If bone density and maximum tensile strength are increased before osteoporosis sets in, subsequent fractures could be minimized [29]. Cross-sectional studies report higher bone mass in athletic populations compared to non-athletes [30]. Comparative studies of athletic and nonathletic populations demonstrate significantly higher bone mineral density in the magnitude of 5–30 % higher, depending on the type and intensity of loading endured. The benefits are particularly pronounced in strength- or power-trained athletes, while endurance activities such as long distance running and swimming seem less effective with regard to peak bone density[31, 32]. However, it is not possible from these retrospective cross-sectional questionnaire-based studies to establish a direct cause-and-effect relationship between exercise and bone accrual.

Whilst research demonstrates that exercise has a positive impact on bone density, results are somewhat inconsistent and may reflect different exercise prescriptions. In order to have maximum impact on the skeleton, exercise prescription needs to be targeted to provide high-impact loading, at sites where the bone typically fractures and to be of a dynamic and unusual pattern [27]. Highly intensive, high-impact loading can be brought about by activities such as jumping and bounding, which elicit high ground reaction forces. In a high-intensity box jumping program, ground reaction forces were measured at 8.8 times body weight [33], and between 3.6 and 10.4 times body weight in elite gymnastics at training [34]. Based on Frost's [35] theory of mechanical usage set points, mechanical deformation (strain) must exceed a certain threshold to elicit a positive skeletal response. To demonstrate this point, Iuliano-Burns et al. [36] compared the bone response to a high-impact verses low-impact exercise and calcium intervention. No effects were found in the low-impact, stretching group, supporting the notion that low-impact, non-weight-bearing exercise does not promote an anabolic bone response.

To further demonstrate the importance of magnitude of loading for osteogenic responses, studies in trainee fighter pilots exposed to positive sustained accelerative forces of up to $6G_z$ over a period of 8–12 months have shown that positive G_z-induced loading has an osteogenic effect on bone in a site-specific manner. Regions that were maximally strained (head and spine due to helmet and mask assembly) had increased BMD and bone mineral content (BMC) [37, 38]. In contrast, spaceflight and bed rest result in decreased mechanical loading of muscles, which has a negative effect on bone [39, 40].

A second exercise prescription factor crucial for maximizing bone health is that the exercise needs to be site-specific. A clear example of this is in racquet-sport players, wherein the playing or racquet arm has significantly greater bone mass and size than the contralateral, nonplaying arm [41]. Starting age was also cited as having a positive impact on bone mass in the dominant arm of tennis and squash players, with early starting age providing greater benefits [42]. The site-specific nature of the bone response was also illustrated in the above study in the fighter pilots, whereby the sites exposed to the highest Gz loading sustained the greatest increase in bone mineral density, namely the cervical spine [38]. Rowing, because of the high compressive and shear forces placed on the spine (4.6 times body weight), has been shown to increase lumbar spine BMD but not BMD at other sites [43].

32.2.2 Childhood and Adolescence Bone Accrual and Exercise

As there is currently no cure for osteoporosis, there is a large emphasis on its prevention, including the optimization of peak bone mass. There is increasing evidence that regular weight-bearing exercise is an effective strategy for enhancing bone status during growth and possibly reducing fracture risk later in life [44]. Exercise interventions during early puberty have been linked to a window of opportunity to maximizing bone accrual, linked to the endocrine events of maturation [45]. Growth hormone (GH), insulin-like growth factor-1 (IGF-1), and sex hormones all have effects on bone accrual. BMC velocity begins to accelerate at age 10 or prepuberty, peaking around age 12.5 years or menarche. This BMC velocity coincides with rising levels of estrogen and testosterone, GH, and IGF-1 and may be an opportune time for bone to respond optimally to physical loading [45].

The premenarcheal years appear to be an opportune time to gain osteogenic benefits from exercise, corresponding to a time of greatest bone modeling, increasing concentrations of estrogen, and high growth hormone concentrations, and possibly an ideal time to establish positive attitudes toward exercise participation. A 10 % increase in peak BMC has been reported to be associated with a halving of fracture risk [46], or equivalent to delaying the onset of osteoporosis by 13 years [47]. This finding was supported by the work of Bass et al. [48], whereby cross-sectional research suggested that exercise conducted before puberty may confer residual benefits in bone density in adulthood, evidenced by comparing bone density in active prepubertal and retired female gymnasts to age-matched controls. In a retrospective study, Khan et al. [49], classical ballet classes undertaken between the ages of 10 and 12 years were independently and positively associated with a difference in adult hip BMD when compared to age-matched controls, further highlighting the importance of weight-bearing exercise during the pre- and pubertal years. Prior to and early in puberty, the adaptation in cortical bone geometry to loading appears to be mainly due to periosteal apposition [50].

Controlled exercise interventions provide the most stringent evidence of impact-loading exercise providing greater increments in bone mineral accrual than those observed in sedentary individuals. Morris et al. [51] investigated the prepubertal bone response to a 10-month, high-impact, strength-building exercise program in 71 premenarcheal girls, aged 9–10 years. The exercise group had significantly greater gains at the total body (TB; 3.5 %), lumbar spine (LS; 4.8 %), proximal femur (PF; 4.5 %), and femoral neck (FN; 12.0 %) sites compared with the controls TB (1.2 %), LS (1.2 %), PF (1.3 %), and FN (1.7 %). Although a large proportion of bone mineral accrual in the premenarcheal skeleton was related to growth, an osteogenic effect was associated with exercise. This was the first study to address exercise-induced gains in bone mineral density in the immature skeleton, highlighting this potentially important time for maximizing peak bone mass in girls. Bradney et al. [52] also reported substantial skeletal effects in response to an 8-month exercise intervention, in similarly aged boys. Meta-analysis of controlled trials on weight-bearing exercise and bone mineral accrual in children and adolescents further supports the benefits during growth for maximizing peak bone mass [53], benefits which have sustained lasting positive influences on the adult skeleton [54].

Although its bone health benefits are well documented and understood, exercise is not practiced by a majority of the population [55]. Of particular concern is that the general physical health and well-being of children is declining. Physical inactivity has been identified as the fourth leading risk factor for global mortality and this suggests that intervention strategies at an early age are needed to prevent long-term health concern. The challenge is then to get children to be physically active and benefit from the osteogenic effect on bone. Rowland and Freedson [56] urged that children must develop a lifestyle of regular physical activity to maximize long-term health benefits. To do this, they argued, means "turning children on" to physical activity by making it enjoyable and keeping them coming back because of an intrinsic desire to be physically active. Providing enjoyable experiences is a potent strategy for increasing activity levels in youth, their attitude about the value of exercise, and ultimately long-term health outcomes. Bone health is a topic particularly amenable to population-based

interventions, as there appears to be a widespread lack of knowledge about prevention approaches among both health providers and the general population [57].

32.2.3 Bone Health in Midlife: Maintaining Bone

Bone loss and fractures affect a large portion of the population, and they can be prevented during all stages of life. For middle-aged adults one of the primary health goals of an exercise program is to maintain bone strength. Without an exercise intervention, after the age of 40, bone mass decreases by an additional 0.5 % per year above the age and menopausal-related loss [58]. The prevalence of osteopenia and osteoporosis has been reported to be 15 % and 0.6 %, respectively, in premenopausal women and warrants attention within the 30–50 year age group [59]. While pharmacological therapy is usually not recommended in this population, reliance on lifestyle factors such as exercise is recommended. Whether appreciable increases in bone density can occur for this age group is equivocal and very much dependent on the mode of exercise, the impact loading of the exercise, the age of the participant, dietary factors, and history of physical activity [58]. In healthy adults, strength training can preserve or produce small increases in BMD, in the order of 1–3 %, at clinically relevant sites [60]. In a recent meta-analysis to determine the effects of exercise on BMD in premenopausal women, the findings suggested that exercise results in a small, but significant benefit in both FN and LS BMD [61]. However, the authors concluded that whilst it is plausible that the exercise-induced benefits might be beneficial, the clinical significance of such changes was unknown.

32.2.4 Bone Health in the Elderly: Maintaining Bone

Studies have shown that bone mineral density in postmenopausal women can be maintained or increased with exercise [62, 63]. However, we still lack definitive evidence on the effective dose of exercise that is required to bring about a slowing of age-related bone density loss [64]. It has been suggested that physical activity in the elderly may reduce the risk of osteoporosis-linked fractures in both men and women, and fall-related injuries [65–69]. However, there is no RCT evidence to date. In addition to strengthening bone, exercise also strengthens muscles, improves balance, and thus may reduce the overall risk of falls and fractures [68, 70, 71]. In the frail elderly, activity to improve gait, balance, and confidence has been shown to be valuable in falls prevention. Intensive exercise training can lead to improvements in strength and function in elderly patients who have had hip replacement surgery due to hip fracture [72]. Similarly, strengthening back muscles can reduce the risk of vertebral fractures and kyphosis [73–75].

One final benefit is in those individuals with osteoporosis and osteoporotic fractures [76]. In a recent consensus statement, exercise recommendations for individuals with osteoporosis and with osteoporotic vertebral fracture were developed from an evaluation of quality of evidence. Although there was limited evidence to quantify the risks of exercise in those with osteoporosis or vertebral fracture, the recommendations for exercise in individuals with osteoporosis or osteoporotic vertebral fracture were therefore conditional [76]. The panel strongly recommended a multicomponent exercise program including resistance and balance training for individuals with osteoporosis or osteoporotic vertebral fractures [76]. Although exercise probably confers a preventative benefit for osteoporosis and falls risk, the reality is that most adults do not meet the physical activity guidelines set at 150 min per week of moderate physical activity and may miss out on the exercise-induced benefits [55]. A framework for exercise prescription can be found in Fig. 32.2, followed by a summary of the key exercise recommendations in Table 32.1.

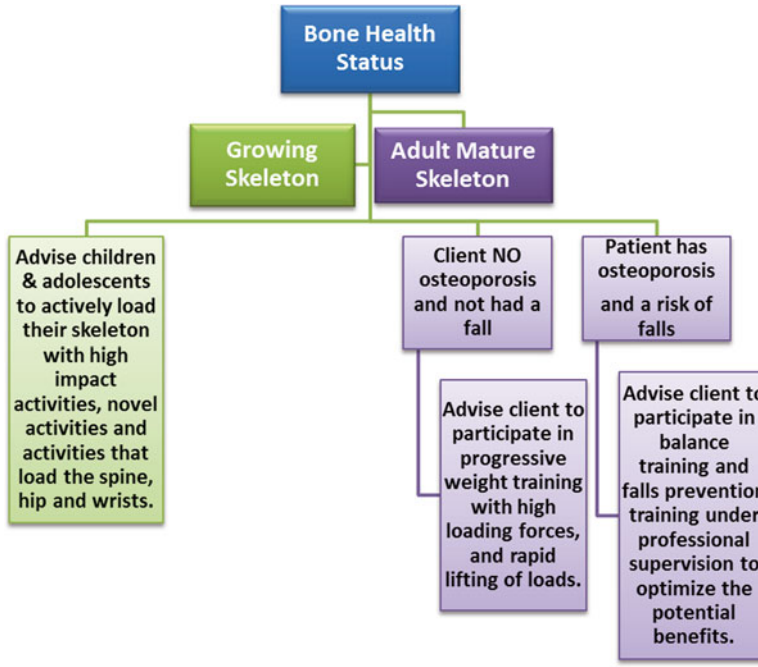

Fig. 32.2 An approach to exercise prescription for osteoporosis prevention and osteoporosis management

Table 32.1 Summary of the exercise recommendations for osteoporosis prevention and management

Exercise recommendations
• Weight-bearing physical activity has beneficial effects on bone health across the age spectrum
• Physical activities that generate relatively high-intensity loading forces, augment bone mineral accrual in children and adolescents
• Exercises can include jumping, skipping, bounding, rowing, or any activity that generate significant loads through the skeleton
• This exercise needs to target the sites most at risk for fracture (lumbar spine, hips, and wrists) and needs to provide a wide variety of activities that generate unaccustomed and novel mechanical forces through the skeleton
• Exercise-induced gains in bone mass in children are maintained into adulthood, suggesting that physical activity habits during childhood may have long-lasting benefits on bone health
• Adulthood is a period of bone maintenance and should incorporate progressive resistance training to ensure the bone remains stimulated
• Heavier weights, lifted rapidly, are far more beneficial for the skeleton than light weights in those individuals without bone fragility
• Targeted exercise in the elderly addresses many of the risk factors for osteoporotic fracture, including osteopenia, muscle wasting and weakness, poor gait and balance, falls, sedentariness, fear of falling, mobility impairment, and disability
• The focus of exercise during this phase should be strength and balance training, following the principles of falls prevention
• Once osteoporosis or osteoporotic vertebral fractures exist, a multicomponent exercise program needs to be engaged in, including resistance and balance training
• Certain exercises need to be avoided, such as activities that involve excessive forward flexion of the spine, twisting movements, dynamic abdominal exercises such as sit ups, exercises that require rapid change of direction or activities that could result in a fall and fracture

32.3 Nutrition and Bone Health

32.3.1 Nutrition

Nutritional intake is one of the most important modifiable factors in the development and maintenance of bone mass and the prevention and treatment of osteoporosis. Many of the nutrients consumed as part of a Westernized diet can potentially have either a positive or negative impact on bone health. Important nutritional factors include dietary calcium intake, vitamin D status, protein intake, total caloric intake, phosphorus, vitamins C and K, copper, zinc, and manganese. Calcium and phosphorus have obvious importance since they compose 80–90 % of the mineral content of bone. Protein is incorporated into the organic matrix of bone for collagen structure upon which mineralization occurs. Although each nutrient will be discussed separately, many nutrients interact with other nutrients, genetics, and environmental factors. The complexity of such interactions may explain why many studies have inconsistent findings regarding the effects of a single nutrient in bone health.

Optimal dietary intake of nutrients and energy for promoting bone health is important, with extensive research documenting the impact of dietary deficiencies for poor bone health. The detrimental impact on bone is thought to be through various mechanisms including alterations in the rate of bone metabolism, bone structure, endocrine function, calcium homeostasis, and possibly other bone-active mineral elements [77]. Whilst a focus has been on the importance of calcium for bone health, emphasis has moved to include total energy intake in particular. Evidence indicates that insufficient energy intake is detrimental to bone health via impaired secretion of GnRH, leading to a state of hypoestrogenism and a reduced synthesis of growth factors such as insulin-like growth factor-1 (IGF-1) [78].

32.3.2 Calcium

While many nutrients play a role in bone health, calcium has been singled out as a major public health concern today not only because it is a critical nutrient for bone but also because of national surveys that suggest that the average calcium intake of individuals is far below the levels recommended for optimal bone health [79]. The Institute of Medicine recommends a daily calcium intake of 1,000 mg throughout adult life, increasing to 1,200 mg in women over 50 years and men over 70 years [79]. Convincing evidence has emerged with respect to the effects of dietary calcium on bone health in all ages [79]. Calcium is an essential nutrient for bone health and the dietary requirement is determined mostly by skeletal needs. Skeletal accretion will only occur when adequate calcium is available at a threshold level. Although inadequate calcium intake is likely to be deleterious to bone, calcium intake significantly above the recommended level is unlikely to achieve additional benefit for bone health and may in fact increase the risk of cardiovascular events, especially myocardial infarction [80], though this issue remains contentious. Thus, strategies to increase calcium intake should be focused on people whose calcium intake is lowest. Calcium deficiency leads to a reduction in bone mass by increasing bone resorption to preserve the level of ionized calcium in the extracellular fluid. Lactose intolerance has been shown to be associated with low bone mass and increased risk of fracture due to low milk (calcium) intake [81].

Research including a large meta-analysis also suggests that the magnitude of osteogenic effects of calcium interventions on bone is generally greater in children than in adults, with the effect of the calcium supplementation on bone mass accrual most obvious in children with lower calcium intakes [82–84]. Studies in children and adolescents have shown that supplementation with calcium, dairy calcium-enriched foods or milk enhances the rate of bone mineral acquisition and peak bone mass [82, 85–88]. This observation is of interest since it is estimated that a 10 % increase in peak bone mass could reduce the risk of osteoporotic fractures during adult life by 50 %.

Because BMD is relatively stable throughout middle adulthood, less research exists regarding the effect of calcium on bone health in this age range. A meta-analysis of the effect of calcium on bone health in men and women aged 18–50 years indicated a positive relationship between dietary calcium and bone mass [89, 90]. In intervention studies, supplementation of calcium in premenopausal women maintained bone mass and prevented bone loss of about 1 % per year in comparison to controls or non-supplemented groups [89].

The loss of gonadal hormones and impact on bone health are most apparent during the postmenopausal years, with the decline of estrogen and testosterone contributing to accelerated bone loss. A meta-analysis in early postmenopausal women by Cumming [91] found a positive correlation between bone mass and calcium intake. The effect of calcium on bone loss is more pronounced during the late postmenopausal stage. Several researchers have demonstrated the benefits of calcium supplementation on bone mineral density in postmenopausal women, with the largest improvement observed when the baseline calcium intakes were the lowest [92–94]. Additionally, the combination of estrogen and dietary calcium is more effective in maintaining bone mass than either treatment alone [95, 96]. However, whether calcium supplementation can reduce osteoporotic fractures remains uncertain.

The important interaction between calcium and vitamin D also needs to be acknowledged. In a meta-analysis that included all the randomized trials in which calcium, or calcium in combination with vitamin D, was used to prevent fracture and reduce osteoporotic bone loss, the authors identified benefits of combining the two. In a meta-analysis, 29 randomized controlled trials were examined ($n = 63{,}897$) in studies that recruited people aged 50 years or older. The main outcomes were fractures of all types and percentage change of bone mineral density from baseline. In trials that reported fracture as an outcome (17 studies), treatment was associated with a 12 % risk reduction in fractures of all types. In trials that reported bone mineral density as an outcome (23 studies), the treatment was associated with a reduced rate of bone loss of 0.54 % at the hip and 1.19 % in the spine. The fracture risk reduction was significantly greater (24 %) in trials in which the compliance rate was high. The treatment effect was better with calcium doses of 1,200 mg or more than with doses less than 1,200 mg and with vitamin D doses of 800 IU or more than with doses less than 800 IU [97]. There is wide but not uniform consensus that both nutrients are important and beneficial.

32.3.3 Vitamin D

The integrity of calcium balance is also highly dependent on an individual's vitamin D status [98]. Vitamin D is essential for bone mineral metabolism through its role in calcium absorption and osteoclast activity. The active metabolite $1,25(OH)_2$ vitamin D_3 (calcitriol) facilitates active calcium absorption in the intestine by mechanisms including stimulation of the synthesis of calcium-binding protein. Ilich et al. [99] demonstrated that calcitriol is at its highest level during peak growth and suggested an important role in bone mass accrual in pubertal girls. Vitamin D is also involved in bone turnover, and a deficiency can cause secondary hyperparathyroidism as well as defective bone mineralization in both children and adults [100]. The main source of vitamin D is cutaneous synthesis, and most people throughout the world get their supply of vitamin D largely by the conversion of precursors in the skin to vitamin D, a process stimulated by exposure to UVB in sunlight.

For vitamin D, the recommended daily allowances are 600 IU/d for individuals pre-70 years and 800 IU/d for those aged 71 years and older [79]. Vitamin D levels and action decrease with increasing age, linked to lower exposure to sunlight, decreased ability to activate precursors in the skin, decreased ability of the kidneys and liver to hydroxylate vitamin D, lesser end organ responsiveness to calcitriol itself, reduced dietary intake, and diminished absorption from food. The most useful way to tell whether an individual or population group is getting enough vitamin D is by measuring levels of serum 25-hydroxyvitamin D (25 OHD; [79]). The Institute of Medicine Committee concluded that 25OHD levels of 20 ng/ml (50 nmol/L) cover the requirements of 97.5 % of the population and

should be used by clinicians as they consider the management of patients in their care [79]. These levels have been measured and indicate a high prevalence of vitamin D insufficiency in populations including nursing home residents, hospitalized patients, cultures that cover their skin for religious reasons, and adults with hip fractures [101, 102]. Supplementation of vitamin D in the elderly population who were deficient in vitamin D reduced falls risk and the rate of all fractures in several high-quality double-blind randomized control trials and was recommended for this population [103, 104].

32.3.4 Protein and Bone Health

Despite intensive investigation, the effect of dietary protein on calcium metabolism and bone balance remains controversial, including the potential differences between animal and vegetable sources of protein. Diets high in protein have generally been considered to be detrimental to bone, as an increase in dietary protein results in greater calcium excretion in the urine [105]. The source of the extra urinary calcium is not completely clear. A view held is that high intakes of protein (particularly animal) create a fixed metabolic acid load due to the high sulfur-containing amino acid content, which cannot be completely neutralized by the aging kidney and requires buffering. The large carbonate reservoir of the skeleton would be called on to buffer the acid, and skeletal calcium would, in turn, be lost in the urine. The result is increased bone resorption, decreased BMD, and increased fractures and is frequently used to justify a lower-protein diet [106].

On the other hand, increasing dietary protein increases insulin-like growth factor I, calcium absorption, and muscle strength and mass, all of which could potentially benefit the skeleton [105]. In a systematic review and meta-analysis of the relation between protein and bone health in healthy human adults, cross-sectional surveys reported a positive correlation between protein intake and bone mineral density (BMD) or bone mineral content at the main clinically relevant sites, with protein intake explaining 1–2 % of BMD variation [107]. A meta-analysis of randomized placebo-controlled trials indicated a significant positive influence of all protein supplementation on lumbar spine BMD but showed no association with relative risk of hip fractures [107]. Overall, the authors could find little support for a negative relation between dietary protein and bone.

32.3.5 Energy and Bone Health

There is a positive association between total body weight and BMD, with increased energy intakes favoring weight gain and higher BMD [108]. In contrast, weight loss of 10 %, typically results in a 1–2 % bone loss per year [109]. Conditions of malnutrition and more severe weight loss are considered risk factors for osteoporosis, linked to low macronutrient intake such as protein, low micronutrient intake such as calcium, and vitamin D. Like calcium, deleterious effects of protein malnutrition on bone mass during growth have been documented [110, 111]. The optimal time to intervene to reduce the burden of fractures appears to be during growth.

In a study in elderly men and women, higher dietary protein intake was associated with a lower rate of age-related bone loss [112]. Protein or caloric malnutrition also predisposes to falls and decreases soft tissue cover over bony prominences [113]. Protein intake is a major determinant of outcome after hip fracture, and serum albumin level is the single best predictor of survival in these patients [113].

Bone loss and increased bone fragility are well recognized in those with eating disorders, particularly in those with anorexia nervosa [114]. The etiology of bone loss and demineralization in anorexia is multifactorial, most likely attributable to low total body mass, low fat and lean mass, limited nutrient intake (particularly protein, calcium, and vitamin D), and prolonged hypoestrogenemia and hypocortisolemia [114]. The body weight history of girls and women with anorexia nervosa is the most

important predictor of the development of osteoporosis. In addition to girls and women, bone loss from eating disorders is now also recognized in men [1115]. The onset of anorexia nervosa frequently occurs during puberty, the time of life when maximal bone mass accrual occurs, thereby putting adolescent girls and boys with anorexia nervosa at high risk for reduced peak bone mass [116]. Pathological fractures typically occur within 7–15 years after the onset of the disorder [114].

32.3.6 Nutrition and Bone Health Across the Life Span

The evidence suggests that the composition of the diet can play an important role in building and maintaining bone mass throughout life, primarily by providing bone-building nutrients and by influencing absorption and retention of these nutrients. While good nutrition is important to bone health throughout life, the optimal type of nutrition and activity will vary across the life span, as will the impact that each will have on bone. The relationship between nutrition and bone health raises specific considerations at several stages across the life span: (a) the early life nutrition and maternal nutrition; (b) the growth phase that occurs during childhood and adolescence; (c) the maintenance phase that occurs during adulthood; (d) the mid-life bone loss phase that typically occurs in adults between age 50–70; and (e) the frailty phase that typically occurs in adults over age 70.

32.3.7 Early Life, Childhood, and Adolescence Bone Accrual and Nutrition

The role of calcium and other minerals in achieving peak bone mass begins before birth. Epidemiological studies have shown that maternal nutrition during pregnancy influences intrauterine skeletal mineralization [117]. term infants suggest that suboptimal maternal vitamin D status during pregnancy has adverse effects on offspring bone health in infancy and later childhood [118]. In a subpopulation, premature infants tend to have lower bone mineral content later in life, although this may in part be due to their tendency to be light and short for their age [119]. Low birth weight is also associated with low bone mass later in life [120].

Childhood and adolescence are particularly valuable times to maximize genetic bone mass potential through nutrition. Several studies in children and adolescents have shown that supplementation with calcium, dairy calcium-enriched foods, or milk enhances the rate of bone mineral acquisition [82, 85–88]. These studies were supported in a meta-analysis [121], which concluded that higher calcium intake increases bone mineral density in children and adolescents. Increases in BMD were more likely in populations with low baseline calcium intakes, and in most studies the increase did not persist beyond the calcium supplementation period [122]. Studies also examined the impact of higher calcium intake over time, concluding that the intake must be maintained for the positive effects on bone to persist [111, 122].

The formative years set the foundation for skeletal reserves and for lifelong eating habits. Observational studies have shown that increasing calcium and protein intakes are associated with a greater gain in bone mass and attainment of a higher peak bone mass [110, 111]. In contrast, low calcium and protein intake during childhood reduces peak bone mass growth and increases risk of fracture later in the life and is positively correlated with bone mineral mass at all ages [110, 123].

32.3.8 Adulthood and Bone Maintenance

Consuming adequate amounts of nutrients continues to be important during the young adult years when bone formation and bone resorption are balanced. The focus of this phase is therefore

maintenance of BMD. Unfortunately, there are very few studies in this age group. A meta-analysis of calcium and bone density in premenopausal women indicated that calcium had a positive effect on BMD in this group, as the supplemented groups lost less bone per year [90]. Calcium supplementation has been shown to have a positive effect on bone mineral density in postmenopausal women; however, results were linked to supplementation compliance [94]. In a study in elderly men and women, higher dietary protein intake was associated with a lower rate of age-related bone loss [112].

32.3.9 Fragility in the Elderly

Nutritional adequacy in the elderly is of particular importance as they are the group at greatest risk for falling, fracturing, and potential malnutrition. Earlier studies have shown a reduction in hip fracture risk associated with calcium and vitamin D [92]. Two subsequently published large trials have challenged these conclusions by being unable to detect significant anti-fracture effect in calcium and vitamin D treated individuals [124, 125]. Neither study targeted individuals at high fracture risk, and both studies had poor compliance to the supplementation. The clinical trial of the Women's Health Initiative was carried out in healthy postmenopausal women with an average calcium intake above 1,000 mg/day, 80 % of whom were under 70 years of age. When the analysis was carried out in only the compliant subjects, a significant (29 %) reduction in hip fracture risk compared with the placebo group was found [126]. A meta-analysis of 9 randomized clinical trials found that whereas supplementation with vitamin D alone was not sufficient to significantly reduce the risk of hip fracture in postmenopausal women, combined supplementation with vitamin D and calcium reduced the risk of hip fracture by 28 % and the risk of nonvertebral fracture by 23 % compared with supplementation with vitamin D alone [127]. Several factors can affect the body's ability to absorb dietary calcium, including vitamin D and estrogen. Deficiencies in either can reduce calcium absorption. The problem of reduced calcium absorption is more acute in older persons, who absorb less dietary calcium because their intestines are no longer as responsive to the action of 1,25-dihydroxyvitamin D [128].

Weight maintenance and good nutrition in the elderly is also an important goal, as it links directly to bone health. Low body mass index (BMI) is a well-established risk factor for fracture in postmenopausal women [129, 130]. In a retrospective cohort study, body weight and body mass index were associated with low bone mineral density and fractures in older women [131]. This relationship was also identified in a meta-analysis of BMI and fracture risk [130]. These studies emphasized the critical role that weight maintenance plays in osteoporosis prevention.

Good nutrition is also an important part of a successful rehabilitation program in patients who have had an osteoporotic fracture. In frail, elderly, hip fracture patients this is crucially important, as poor nutritional status can slow recovery, and increase susceptibility to further fractures [132]. Vitamin D should be prescribed whenever there is suspicion of inadequate status and particularly in elderly patients. About 800 IU/day is considered sufficient. A summary of the key nutritional recommendations for osteoporosis prevention and management can be found in Table 32.2.

32.4 Physical Activity, Nutrition, Hormones, and Bone Health

Optimal bone metabolism is the result of hormonal, nutritional, and mechanical harmony, and a deficit in one area is usually impossible to overcome by improvements in others. Exercise during growth influences bone modeling locally at the regions being loaded, whereas calcium is thought to act systemically to influence bone remodeling. Despite acting through different mechanisms, a growing

Table 32.2 Summary of the nutritional recommendations for osteoporosis prevention and management

Nutritional recommendations
• General preventive measures against osteoporosis should be emphasized whenever possible. Adequate dietary calcium intake is one of the mainstays for positive bone health
• Calcium needs may be met through a diet rich in calcium (e.g., milk, dairy products, calcium-fortified fruit juices) or by use of calcium supplements
• Sufficient calcium, vitamin D, protein, and total calories are essential for optimal bone growth
• Sufficient calcium, vitamin D, protein, and total calories are essential for optimal bone maintenance and prevention of age-related bone loss
• Vitamin D levels commonly deteriorate in older adults, and thus the requirement for vitamin D increases with age. Surveillance of vitamin D status and supplementation where indicated are recommended
• Alcohol consumption should be limited and good nutrition with adequate caloric and protein intake should also be promoted

body of research suggests that exercise and calcium may not operate independently [133, 134]. Low dietary calcium intake or reduced bioavailability may minimize the adaptive response to exercise-induced bone loading [134, 135]. Conversely, adequate levels of calcium intake can maximize the positive effect of physical activity on bone health during the growth period of children aged 3–5 years [136] and in adolescent girls [137]. Research also suggests that adequate levels of calcium intake can maximize bone density at the regions being loaded during the exercise [36]. In a group of normally active pre- and early pubertal boys, additional exercise and calcium supplementation resulted in a 2–3 % greater increase in BMC compared to controls at the sites loaded by physical activity than either the exercise or calcium alone. The authors concluded the dietary calcium may enhance the effect of exercise on BMC during growth [133].

In a cross-sectional study of male twins, exercise was a better predictor of bone mineral content (BMC) than are protein or calcium intakes [138]. It is thought that exercise produces region-specific effects, whereas higher calcium intake produces a more generalized effect in addition to the benefits of exercise.

In addition to exercise and nutrition, bone health is dependent on a third factor, that of normal levels of the hormones which effect bone metabolism. Any suppression of circulating estrogen and testosterone will contribute to bone loss [139]. Similarly, elevated glucocorticoid hormone levels associated with chronic health conditions such as inflammatory bowel disease or asthma, and perhaps mental health disorders, can lead to significant loss of bone over time, regardless of exercise or dietary calcium status [140].

32.5 Conclusions

Achieving optimal bone health and minimizing one's risk of osteoporotic fracture later in life depend on a lifelong approach. This approach relies on the establishment of an optimum level of bone during the growth years, with a subsequent goal to maintain and slow the rate of age-related bone loss thereafter. Exercise, adequate nutrition, and optimal hormone levels are the components that influence the bone outcome.

Making healthy nutritional choices, engaging in weight-bearing physical activity, and ensuring optimal hormone levels during growth provides a window of opportunity to build optimal bone mass, to reduce the risk of fracture later in life. Concurrent management of fracture risk with a physical activity prescription, adequate nutrition, and pharmacotherapy for osteoporosis when required offers the best approach to optimal bone health throughout adulthood.

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Chapter 33
Body Weight/Composition and Weight Change: Effects on Bone Health

Sue A. Shapses and Mariana Cifuentes

Key Points

- A low body weight in older individuals is a major risk factor for fracture, and the maintenance of weight can prevent bone loss.
- Newer information shows that obesity alters bone quality and is not always protective against osteoporosis and fracture, as previously thought.
- Weight reduction will have a different impact on bone, depending on the amount and whether it is involuntary or voluntary.
- Although mechanisms regulating bone loss are uncertain, it is clear that the method to achieve voluntary weight reduction (through different diets, medication, or increasing levels of activity) will determine the bone response.
- Extreme weight loss due to bariatric surgery leads to bone loss and the long-term implications are discussed.
- Alterations in bone quality and strength parameters due to weight reduction and regain suggest that bone lost is not recovered.

Keywords Body composition • Body weight • Bone • Fracture risk • Weight loss

33.1 Introduction

A high body weight and obesity are positively correlated with bone mass and density, whereas a low body weight increases fracture risk. In obesity, the greater bone mass may result from an altered dietary intake, the greater mechanical load on bone, or changes in hormones produced by the excess adipose tissue. The high bone mass in obesity is also found in children and is not gender specific. However, the impact of excess adiposity on trabecular and cortical compartments of bone presents a more complicated picture that leads to a higher than expected fracture risk. While the incidence of fracture is still highest in underweight individuals, fractures are numerous in the overweight/obese

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and are at least partially attributed to the majority of the population being in this weight range. In addition, obesity in children is associated with higher fracture rates than normal weight children so their risk is even greater than obese adults.

Weight reduction is recommended to reduce comorbidities, but also causes bone loss and fracture risk. The amount of bone loss due to weight reduction is influenced by the age, gender, and initial body weight. We present mechanisms that regulate bone metabolism during caloric restriction and factors that may attenuate bone loss such as interventions using different diets, types and levels of activity and medication. In addition, bariatric surgery presents a unique bone response with significant loss that has been shown to be proportional to weight reduction.

This chapter reviews the relationships between bone, body size, and composition. Those individuals who are at greatest risk of bone loss due to weight reduction as well as modifiable risk factors to attenuate bone loss are discussed. Areas of future research are suggested that include a focus on bone quality in the obese, and the long-term consequences of weight loss.

33.2 Body Weight, Bone Mass, and Fracture Risk

33.2.1 Obesity

Both osteoporosis and obesity are diseases of Western cultures. The prevalence of obesity and overweight is increasing worldwide and is nearly 70 % in the United States [1]. It is predicted that the current generation of children will grow into the highest number of obese adults in US history [2]. Obesity associated with visceral adiposity (upper body obesity or a high waist to hip ratio) increases the risk of comorbid conditions such as type 2 diabetes, hypertension, and dyslipidemia. A poor diet and sedentary behavior are also factors believed to contribute to the rising prevalence of osteoporosis. There is now evidence that these diseases are not mutually exclusive and that obesity does not protect against osteoporosis as had been thought for decades. Studies show a higher fracture incidence in obese children [3], and greater fracture risk at certain sites in obese than normal weight adults [4, 5]. The relationship between obesity and osteoporosis as well as the mechanisms involved are discussed below.

33.2.1.1 Body Mass Index (BMI)

Body mass index (kg/m^2) is a widely used tool to assess body weight adequacy in most subjects. In addition, it has been used as a means to predict comorbid conditions associated with excess body weight. Weight reduction is recommended for overweight and obese individuals (BMI >25 kg/m^2) which currently includes the majority of individuals in many developed countries. In older individuals or non-athletes, it can reasonably be assumed that excess weight corresponds to excess fat. However, the accuracy of BMI in measuring body fatness in older populations needs to be questioned because of the height loss experienced by some osteopenic and osteoporotic patients due to vertebral crush fractures. It may be more accurate to ask an older patient their height as a young adult and weight history to determine osteoporosis risk due to height loss. Recommendations for weight reduction may not be desirable for older, slightly overweight individuals who have become shorter due to atraumatic vertebral fracture.

33.2.1.2 Obesity and Bone

Studies show a higher bone mass with high body weight [6, 7] although the accuracy of bone mass measurements should be considered (as detailed in Sect. 33.4.3). The higher bone mass is attributed to either increased mechanical loading of body weight (including a greater lean body mass) and/or

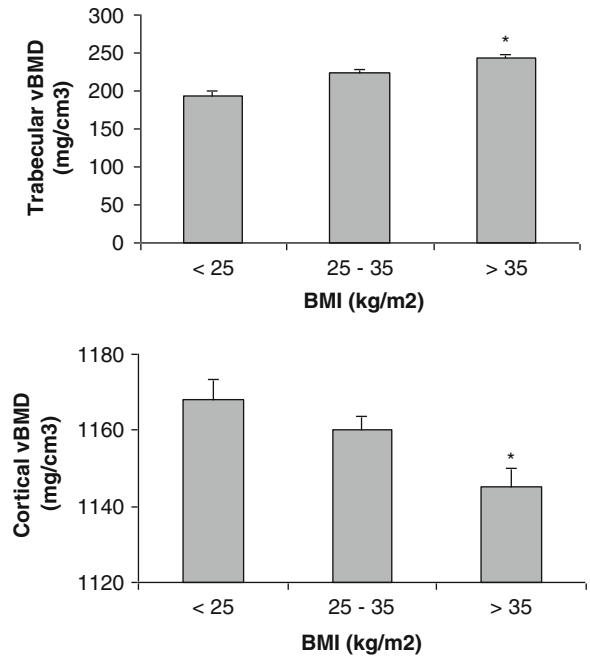

Fig. 33.1 Trabecular and cortical volumetric BMD in normal weight, overweight, and obese individuals. *Differs from BMI <25 kg/m^2 [9]

linked to a greater fat mass and greater conversion of adrenal androgens to estrogens in adipose tissue, as well as other potential mechanisms [8]. However, studies show that while the trabecular BMD is higher in obesity, cortical BMD is lower [9] (Fig. 33.1). This may contribute to newer evidence that bone quality is compromised in the obese and fracture risk is greater for a given density of bone [10, 11]. It is possible that sedentary behavior is the reason for the poor bone quality and the higher risk of falling in the obese (see Sect. 33.2.2.1).

Metabolic factors influence bone, and profiles vary by fat depot (visceral vs. subcutaneous) and gender [12, 13] and hence the effect of excess adipose tissue on bone may not be uniform in all heavier individuals. For example, multiple endocrine perturbations are found with visceral fat accumulation. These include elevated cortisol, androgens, and growth hormone in women, as well as low testosterone secretion in men [12], all of which are known to regulate bone mass. There are other hormonal differences in the obese that may increase bone mass, such as higher serum levels of calcitonin [14]. Some have found no differences in serum parathyroid hormone (PTH) between lean and obese subjects [14] whereas most agree there are increased PTH levels [15–17] and lower 25-hydroxyvitamin D3 (25OHD) [9, 15, 18, 19] in the obese. The lower 25OHD in obesity is well established and the response to vitamin D supplementation is attenuated [18]. The lower serum 25OHD is found in obese adolescents, with even lower concentrations in black than white obese children [20]. The decreased bioavailability of 25OHD in obese subjects may be due to its deposition into adipose stores [18, 19, 21]. It is possible that the decreased levels of 25OHD found in physically inactive compared active individuals [22] is due to their greater adipose stores. It is not clear whether these metabolic differences in the obese are detrimental or beneficial to bone.

In children, there is a higher bone mass in overweight and obese compared to lean individuals for a given chronological age [23, 24], but their growth spurt during puberty is less than in lean children [24]. In overweight and obese boys and girls there is a mismatch between body weight and bone development during growth, such that their bone mass and bone area are low for their body weight [25]. This may not be so surprising given that excess weight in obesity is due to higher fat mass. Excess adiposity and a high fat diet appear to be particularly detrimental to bone mineral density (BMD) and bone quality during growth, as demonstrated in rodent studies [26–28]. However, Ducher

et al. examined 93 overweight children and found that they had stronger bones than did their normal-weight peers; yet, this was not the case at the forearm that showed a high proportion of fat relative to muscle [29]. This finding is consistent with evidence that obese children are at an increased risk of distal forearm fracture [3]. One study found that adiposity in boys is associated with lower bone mass, whereas others have found lower cortical BMC and stress:strain index at the radius, which was worse in those adolescents with higher cardiometabolic risk factors [30, 31]. The risk factors associated with bone mass using multiple regression analysis included visceral adipose tissue ($\beta = -0.22$), waist circumference ($\beta = -0.23$), homeostasis model assessment of insulin resistance ($\beta = -0.23$), and high-density lipoprotein cholesterol level ($\beta = 0.22$) [31]. The cause for higher fracture risk in children may be related to a greater fat:muscle area at common fracture sites [29], an abnormal bone mineralization, lower bone formation rate [32, 33], and poor balance or force upon falling [34, 35]. These data are disturbing in light of the rising incidence of childhood obesity [30, 32, 36].

33.2.2 Low Body Weight

Evidence that the lean population is at greater risk of osteoporosis is strong. It is believed that genetics contribute to most of the variance in bone mass, particularly in premenopausal women, with 20–25 % being under environmental influences [37–39]. Therefore, it is especially important that individuals with low body weight make conscious efforts (i.e., prevention of further weight loss, high dairy intake, physical activity) to reduce their risk of osteoporosis.

33.2.2.1 Bone Mineral Density, Bone Turnover, and Fracture Risk in Lean Individuals

There is a large body of evidence showing that BMD is lower in lean than obese women [40, 41]. The extent that bone mass is proportionally greater at weight-bearing sites and not others may explain differences in fracture risk [5]. By measuring specific sites in the obese, one may be able to differentiate the effect of weight bearing compared to other variables such as endogenous hormones (i.e., estrogen) on bone. When evaluating the impact of low body weight on bone density, the data may be confounded by the possibility that there is a health condition causing low weight that may itself have an impact on bone. For instance, disease states such as some cancers and anorexia nervosa, as well as different populations characterized by low BMI, such as ballet dancers [42] and professional jockeys [43], have shown greater bone turnover, decreased bone density, and/or increased fracture risk. These states have been also associated with hormonal disorders or nutritional deficiencies, among others, which makes it difficult to ascertain the primary causal relationship. Interestingly, a study on the so-called constitutional thinness (BMI less than 16.5 kg/m^2) [44] revealed that despite greater body fat and no hormonal or other disorder as compared with their anorexic counterparts, these otherwise healthy women presented with several bone abnormalities such as low bone density and size (comparable to anorexic subjects), albeit a normal bone turnover. Given that the authors could not describe hormonal or body composition causality, they proposed that insufficient skeletal load and/or genetic factors may be associated with the observed bone impairment.

In lean older women, there is an increased annual rate of bone loss compared to obese women [37, 45], which is supported by their higher rate of bone turnover compared to heavier women [7, 46]. In contrast, among overweight and obese women, the relationship between high bone turnover and lower body weight is not observed, suggesting that bone mass is regulated differently in heavier women [7]. Consistent with this, a meta-analysis using worldwide data [47] established that the risk for any type of fracture increased with lower BMI; however the contribution to fracture risk was more important at low values of BMI than at values above the median. Low body weight increases the risk of developing

osteoporotic fractures compared to obese individuals (relative risk of 2–2.4) [41, 48]. A study in more than 900 asymptomatic postmenopausal women [49] confirmed that low BMI is a risk factor for vertebral fractures.

It is noteworthy that in lightweight individuals there is a higher risk of falling in obese, particularly those with a preferential distribution of body fat in the abdominal area [50], and in the obese that fall, the fracture severity could be greater [51]. On the other hand, the obese may be protected against fractures due to a cushioning effect of the fat surrounding crucial areas such as the hip, particularly in those with lower body obesity. For both lean and obese, the prevention of fractures and their severity should include prevention of falls.

It is generally agreed that weight bearing and greater amount of lean body mass (fat free soft tissue or BMC) in obesity is more protective against bone loss than excess fat tissue. Some studies, however, suggest a positive influence of fat mass on bone with aging [52–54]. Hormonal differences other than estrogen levels between lean and obese individuals may also contribute to the higher rate of bone remodeling in the lean, and should be addressed in future studies.

33.2.3 Body Size: Summary and Recommendations

A low BMI (<20) increases the risk of fracture, whereas a BMI of 25–35 (overweight or obese) may confer some protection. The recommendation that all individuals achieve a normal body weight may be inappropriate for those individuals at high risk for osteoporosis. To reduce fracture risk, it is possible that a higher body weight (within high normal or slightly overweight) is beneficial for certain postmenopausal women or older men, but not for younger individuals. On the other hand, obesity is associated with poor bone quality either due to endocrine alterations or poor lifestyle (diet and inactivity). The risk of fracture is influenced by both extremes in body weight (Fig. 33.2). These findings coincide with data showing that normal weight individuals have a greater life expectancy than thin or obese older individuals (BMI <20) [55]. It is suggested that weight recommendations should be addressed by considering both fracture risk and the comorbid conditions associated with excess body weight. For individuals who need to lose weight, intervention studies have shown that certain nutrients and exercise can ameliorate bone loss (see Sect. 33.4).

33.3 Body Composition

33.3.1 Cellular Level: Osteoblasts and Adipocytes

As humans age, there is a tendency to accumulate fat mass and decrease bone mass. This converse regulation can be observed at the cellular level, where the differentiation of the common mesenchymal progenitor goes towards either the osteoblast or adipocyte lineage, and many factors that induce one lineage, at the same time inhibit the other [56, 57]. One such example is the canonical Wnt-β catenin signaling pathway, whose activation in multipotent cells stimulates osteoblastic differentiation by increasing Runx2 expression, and inhibits the adipogenic fate by suppressing the key adipogenic transcription factors C/EBPα and PPARγ [58]. Thus, the signals to which mesenchymal cells are exposed will eventually determine the balance between bone and adipose tissue mass. Favoring adipocyte as opposed to osteoblast differentiation of bone marrow MSCs may affect bone turnover and favor the net loss of bone mass [56, 59]. There are several clinical observations that support the opposing regulation of adipocyte and osteoblast content in bone, showing greater bone marrow

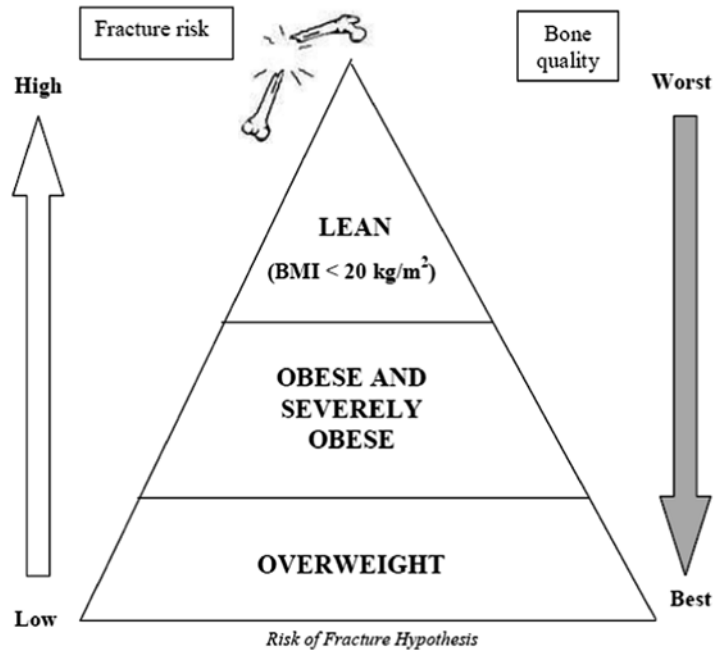

Fig. 33.2 Bone Mineral Density and Fracture Risk in different weight categories. Pyramid Fracture. Modified version from Shapses SA, Riedt CS, Schlussel Y, Gordon CL, Li WP, Brolin RE, Stahl T. Body weight and menopausal status influence trabecular and cortical BMD. In: Nutritional Aspects of Osteoporosis. Eds. Burckhardt P, Dawson Hughes B, Heaney RP. Elsevier, Amsterdam, The Netherlands, p 231–240, 2007

adiposity in osteoporotic women and other states associated with low bone mineral density such as diabetes, skeletal unloading, and glucocorticoid excess [60]. One of the therapeutic targets to treat type 2 diabetes mellitus is the activation of PPARγ by thiazolidinediones (TZDs). These compounds promote adipocyte differentiation, and at the expense of osteoblastogenesis [61]. Indeed, despite the alleged protective effect of obesity on bone mass, obese patients with diabetes mellitus who are treated with TZDs present an increased risk for fractures [60, 62]. Increasing evidence supports that aging-related bone loss and increased marrow adipose tissue is due to a switch in differentiation of stromal cells from the osteoblastic to the adipocytic lineage [63–65]. In addition, bone marrow fat has been correlated with visceral fat [66]. Its impact on bone is described in the next section.

Overall, potential drugs that inhibit marrow adipogenesis with the parallel enhancement of osteoblastogenesis could be a goal in the prevention of osteoporosis. However, such agents may impact organs or tissues other than bone or white adipose tissue. Consequently, therapeutic agents with potential stromal cell receptor targets must have tissue specificity. The plasticity between adipose and osteoblast cells is an interesting area showing the relationship between different components of body composition that could further our understanding of obesity and osteoporosis.

33.3.2 Relationship Between Bone, Lean Soft Tissue, and Fat Tissue Mass

The extent that lean or fat tissue mass influence bone mass is not uniformly reported in the literature. An important determinant of bone mass could be the amount of lean tissue mass, because it may reflect weight-bearing activity [67], or fat mass, because it is known to influence peripheral synthesis of estrogen and androgens [68]. Low muscle mass is a risk factor for low bone mineral density in young adult women, while higher fat is protective only when it is associated with substantial muscle mass [69]. Consistent with this, premenopausal and perimenopausal women show a

beneficial effect of increased body weight on bone mass, but only when it is comprised primarily of lean mass [70]. The strong association between lean mass (rather than fat mass) and BMD in younger women may be attributed to exercise, lifestyle factors, estrogen levels, or a combination of these factors. In postmenopausal women, Chen and coworkers [54] observed that body weight was a better estimate of bone mass than either lean or fat tissue alone. However the annual changes in bone mass were better predicted by changes in fat than lean mass in these postmenopausal women [54]. It has been suggested that even though most studies indicate that lean mass and strength are the main determinants of bone mass [71, 72], the influence of fat mass increases with aging [52–54] and is more important in women than men [8, 73, 74]. In postmenopausal years, this phenomenon may be explained through the influence of fat tissue in serum estrogen levels. In addition, the location of the fat tissue appears to help explain why the relationship exists only some of the time. The "lipid hypothesis of osteoporosis" suggests that obese individuals with upper body obesity and dyslipidemia may be at greater risk of bone loss than those with lower body obesity. A number of studies in different populations have linked obesity (particularly visceral adiposity) to low bone mass and fracture risk [75–79].

The study of the influence of lean versus fat tissue on bone mass is complicated by the fact that this effect varies depending on the bone site being evaluated [71, 80, 81]. Differences in trabecular content of bone as well as weight bearing of the specific site may confound the observation. Establishing the relative importance of site-specific fat to lean mass is important because it can lead to measures to prevent bone loss in certain physiological states and in high risk populations.

33.3.3 Calcium and Vitamin D Intake and Effects on Body Composition

Calcium supplementation decreases bone resorption, age-related increases in PTH, and bone loss, particularly when initial dietary Ca is low [82]. For more than a decade now, there has been discussion regarding the possible influence of Ca intake on other parameters of body composition, particularly on body weight and body fat [83–87] still without reaching a consensus. Animal studies support these observations that high dietary Ca accelerates weight and fat loss when compared to a low Ca, isoenergetic diet [88]. It has been proposed that a low Ca intake stimulates 25OHD and PTH, and that these calcitropic circulating substances in turn stimulate adipocyte Ca uptake. Elevated cytosolic Ca promotes fatty acid synthase transcription and activity and inhibits lipolysis [89]. Others have proposed alternative mechanisms such as increases in fat oxidation and fecal loss as well as a facilitation of appetite control [90]. Although promising, a retrospective examination of women in our weight reduction studies at two levels of Ca intake found only a weak insignificant association between Ca intake and body fat [87]. In addition, a large randomized controlled trial using 1,500 mg Ca/day versus placebo in weight-stable adults showed no effect of additional Ca on body weight [91]. The effect of dairy on promoting body weight and fat loss is not observed in long-term studies but may have some short-term benefits [92] and these effects may be due to the satiety associated with increased dietary protein from dairy, rather than its Ca content. An inverse relationship between vitamin D supplementation and body weight has also been suggested in one small trial [93], but most studies show no such relationship [94–97]. However, higher vitamin D intake may reduce risk of type 2 diabetes and cardiovascular disease [98]. The positive effect of vitamin D on type 2 diabetes is supported by randomized controlled trials in vitamin D deficient subjects. However, while vitamin D supplementation may be useful for those with impaired fasting glucose who are vitamin D deficient, studies are limited. There are large ongoing vitamin D supplementation trials worldwide (i.e., acronyms include VITAL, FIND, DOHealth, VIDAL, ViDA) that will address multiple health outcomes.

33.3.4 Genetic Markers of Bone and Body Composition

It is known that genetics contribute to the majority of variance in bone mass [37]. A few genetic markers of bone mass are also reported to be markers of body weight and composition, including several polymorphisms of the vitamin D receptor (VDR). It is known that an absence of the Bsm1 restriction site (BB) of the VDR genotype compared to its presence, bb, is associated with lower BMD and higher fracture risk [99, 100]. Interestingly, in younger premenopausal women, the BB polymorphism is associated with higher hamstring strength, body weight, and fat mass compared with women with the presence of the bb genotype [101]. Hence, it is possible that this polymorphism that indicates strength and higher body weight in young women does not have a protective effect in older women. Genetic markers of bone or fat mass could help determine the relationship between body composition and disease states such as osteoporosis and obesity.

33.4 Weight Reduction

It has been recommended that overweight and obese people lose approximately 5–10 % weight, in order achieve health benefits, in terms of blood pressure, lipids, and glucose control. Although regional differences in bone loss might be expected, with greater loss from more trabecular than cortical regions (or differences due to weight bearing), the data currently suggest that bone changes of about 1–2 % occur at all sites with a 10 % weight reduction. Studies in different populations show an association between the amount of weight reduction and total body bone loss [5, 102–113] and this can be seen in Fig. 33.3. The association between weight reduction and bone loss is variable in different studies due to different bone sites and imaging techniques of dual energy X-ray absorptiometry (DXA) [114, 115], the mix of different populations (men, pre- and postmenopausal women, different ages, different initial body weights), and types of intervention between studies. Additionally, age is known to influence bone loss and fracture risk, and indeed is an important

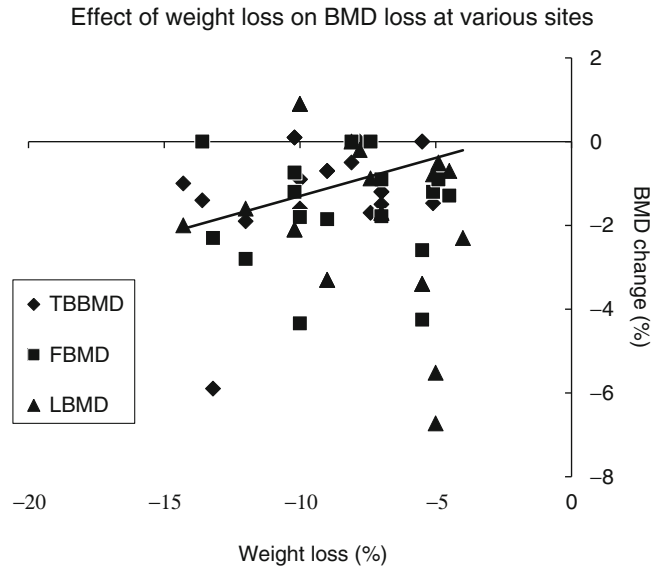

Fig. 33.3 Weight loss and bone mineral density change for total body (TBBMD, *filled diamond*), femoral neck (FBMD, *filled square*), and lumbar spine (LBMD, *filled triangle*) with voluntary weight reduction trials of 3–18 months duration [108–119]. Trend line given for TBBMD; $r = 0.40$

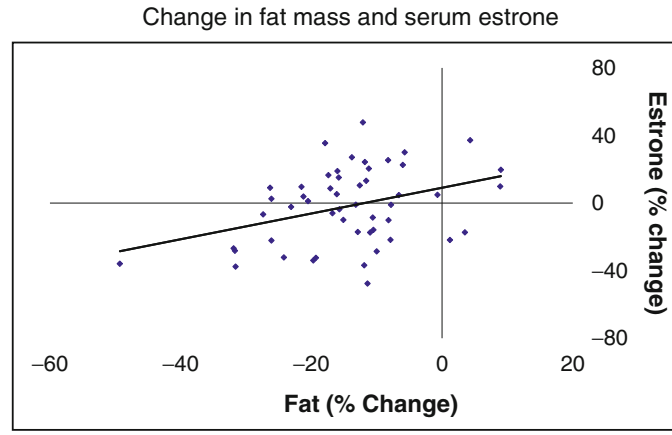

Fig. 33.4 Association between the loss of body fat tissue and changes in serum estrone after 6 months of weight reduction in overweight and obese postmenopausal women ($n = 112$; unpublished data)

component of the FRAX model to estimate fracture risk [116]. This is consistent with findings in rodent studies showing that energy restriction is more detrimental to BMD and strength parameters in old compared to younger rats [117]. These data are consistent with observations of bone density and strength in human cadavers [118] and with epidemiological studies showing that older women who lose weight have an increased risk of fracture [60, 119, 120]. Others have shown a greater effect on cortical than trabecular bone in young mice due to energy restriction. However, our findings in our older rodents show that trabecular bone is affected by energy restriction [121] and this is consistent with findings in older women who show a loss in trabecular, but not cortical bone after 1 year of 7 % weight loss [122]. Data suggest that hormonal changes (specifically estrogens) are influenced by body fat loss (Fig. 33.4). These endocrine changes negatively affect bone due to weight reduction, and this varies significantly between different study populations [7, 105, 106]. Others suggest that reduced weight bearing is the more important regulator of bone [52, 107, 110], but thus far studies have not been able to clearly discern differences in bone loss between sites with different weight-bearing stimulus (i.e., forearm compared to hip), although more data is becoming available.

33.4.1 Involuntary Weight Reduction

It is worth noting that besides the recommended 5–10 % weight reduction in overweight and obese individuals, the possibility of involuntary weight loss is a very important factor when considering the effect inducing bone loss. Involuntary weight loss may be associated to an underlying illness such as depression, cancer, or gastrointestinal disorders, all of which may cause weight loss-associated bone loss, but have been also shown to have an independent negative effect on bone [123–125]. Frailty is an additional factor playing a role in bone loss [126, 127] and risk of hip fracture in older women [120, 128] and men [129] due to involuntary weight loss, particularly when there is low initial body weight. Aging, sedentary behavior, and antigravity may also cause unintentional loss of body weight and muscle mass (sarcopenia) [130]. For the same degree of weight reduction, the negative impact on bone may be greater than expected in cases of involuntary weight loss when it is compared to the same weight lost intentionally in a healthy subject (Fig. 33.5). This would be particularly true if caloric restriction was accompanied by interventions known to attenuate bone loss (see Sect. 33.4.2).

Fig. 33.5 Voluntary (*left*) compared to involuntary (*right*) weight reduction may have a different effect on bone mass

33.4.2 Voluntary Weight Reduction

In overweight or obese individuals, weight reduction of approximately 10 % has been recommended because researchers have found it achievable and it reduces comorbid risk factors [131]. The amount of bone loss due to weight reduction does seem to vary between populations. For example, studies examining a population of postmenopausal women (without other populations) during weight reduction have found a 1–2.5 % loss of bone (total or lumbar spine) compared to a control, weight-stable group [105, 112]. Lean or overweight older premenopausal or perimenopausal women [111] respond to moderate weight loss (~5 %) in a similar manner as described for postmenopausal women, and show a loss of 0.8 % bone loss at the hip. Evidence of bone loss in younger obese premenopausal women (<45 years) is less consistently found [104, 106, 109, 112, 132], suggesting that weight reduction may not pose a risk factor for bone loss in estrogen replete women. In a study that exclusively examined overweight middle-aged men, moderate weight reduction (7 %) resulted in a 1 % bone loss (total body) [108], whereas preliminary data in our lab suggests that there is less BMD loss compared to older women. Many factors contribute to the extent of bone lost due to weight reduction, discussed below.

33.4.2.1 The Rate of Weight Reduction

The impact of weight loss on bone may also depend on the method for losing weight, and particularly how fast the weight is lost. As a plausible mechanism, we have unpublished data suggesting that a more rapid weight loss will be more detrimental to bone due to an activation of the Ca-PTH axis in women who lose moderate weight faster (0.7 kg/week) than others (0.3 kg/week). In addition, a faster compared to slower rate of weight loss results in a more negative nitrogen balance and loss of skeletal muscle mass [133] which would be expected to also result in greater bone loss. Studies have not specifically addressed whether a similar amount of faster or slower weight loss influences bone loss and

metabolism differently. This may be because while designing this type of study would be feasible, compliance would complicate the results and statistical analysis.

33.4.2.2 Calcium, Vitamin D, and Other Micronutrient Supplementation During Weight Reduction

Calcium intake is an important determinant of bone loss during weight-stable conditions and also during weight reduction. During moderately low calorie intake, women consume less Ca than during weight-stable conditions, yet both intakes are lower than recommended levels [109, 134]. In obese postmenopausal women, our double-blind placebo-controlled trial showed that 1 g of supplemental Ca/day prevented bone mobilization associated with a 10 % weight reduction [134]. In support of these findings, Jensen et al. [135] found that a 1 g Ca supplement/day in a group of pre- and postmenopausal women who lost 5.5 % of their body weight prevented bone loss at the femoral neck. Others have found that postmenopausal women losing weight sustain significant bone loss from the spine (but not forearm or total body) despite a total Ca intake of 1–1.2 g/day [112]. Importantly, energy restriction studies in the rat show both significant loss of bone and strength properties despite adequate dietary intakes of Ca and other nutrients [117]. This supports the hypothesis that bone loss is regulated by other factors during weight reduction. It is possible that other nutrients, or the amount or type of weight bearing, are contributing to BMD loss and the higher fracture risk after weight reduction. Importantly, there are no side effects of consuming Ca intake of 1.2 g Ca/day, even in individuals with a history of recurrent kidney stones due to hypercalciuria [136]. The Women's Health Initiative findings only found a higher risk of kidney stones in women consuming ~2,150 mg Ca/day [137]. Hence, the 1.2 g Ca/day should be consumed during caloric restriction to avoid a negative Ca balance.

The level of vitamin D intake and its role related to Ca intake during weight reduction is an important consideration. Although obesity is associated with lower serum 25OHD, its concentrations have been shown to rise after caloric restriction [97, 138, 139]. Mason et al. have shown that the serum 25OHD rise is due to the amount of weight loss and not the method when comparing exercise, diet, or exercise plus diet-induced methods [139]. In our study examining 400 IU/day compared to 2,500 IU/day vitamin D supplementation in women who were given 1.2 g Ca/day, the total (or net) absorption of Ca was calculated to estimate balance. Since it was a short-term study, BMD was not measured. In the vitamin D supplemented group, net Ca absorption was about 230 mg/day with 400 IU/day, which is higher than the 200 mg absorbed Ca/day estimated as necessary to offset obligatory Ca loss [140]. These data suggest that when Ca intake is at the recommended intake of 1,200 mg Ca/day, the use of higher doses of vitamin D than the recommended intake of 600 IU/day is not needed to avoid a negative Ca balance. However, the average Ca intake from dietary sources was only 650 mg/day in these women so without additional Ca supplements, only 140 mg of Ca would have been absorbed per day during caloric restriction. This 60-mg/day deficit would be expected to result in an additional 0.9 % bone loss/year. Hence, balancing intake of Ca and vitamin D at the recommended doses is the best approach to avoiding bone loss during caloric restriction and weight loss. Seasonal fluctuations in serum 25OHD result in lower levels in the winter months that are also associated with a rise in PTH, bone turnover, and lower bone mass [141, 142]. It is possible that weight reduction in the winter/early spring months (which is a popular time for dieting) is associated with a greater bone loss than a summer diet [106] and ensuring adequate vitamin D and Ca intake during the winter months will be necessary to prevent the changes to calcitropic hormones and bone loss [143]. Also, there is no evidence that the extent that serum 25OHD rises after moderate weight loss or after bariatric surgery (Roux-en-Y GB or gastric banding) attenuates bone loss [5, 144–146]. However, a study examining different levels of vitamin D intake on bone changes during weight loss could better inform about specific recommendations during caloric restriction, and a 1-year pilot trial is currently being examined in our lab. Finally, other nutrients (such as magnesium, zinc, vitamin K, etc.) may be limited during caloric restriction, yet their influence on bone mass has not been examined.

33.4.2.3 Macronutrients and Weight Reduction

In general, studies indicate there is little or no bone loss in obese younger individuals who lose a moderate amount of weight, and therefore weight reduction (in light of fracture risk) can be recommended with confidence for these individuals. Weight reduction in leaner women may result in more bone loss [127], and is the focus of our ongoing studies. It is suggested that all individuals interested in losing a moderate amount of weight will benefit from additional Ca [134, 135] or exercise [110, 111]. When discussing weight loss, it is important to focus on the type of diet used for this purpose, particularly because certain approaches may have a physiological impact that may affect bone metabolism, in addition to and independent from the weight loss itself. Besides the regular, well-balanced caloric restricted diet, which reduces energy intake with a proportional reduction in all macronutrients, there are other popular diets resulting in successful weight reduction. For example, with very low carbohydrate intakes during dieting or starvation that produce ketosis, bone loss has been observed [147–149]. The findings in the carefully designed study by Reddy et al. showed that 6 weeks of a ketogenic diet in healthy subjects results in a marked increase in acid load to the kidney [148]. Urinary Ca levels increased (~60 %) without a commensurate increase in intestinal fractional Ca absorption, resulting in a lower Ca balance. Markers of bone formation decreased, while resorption markers showed no change. Other investigators have shown that a low-carbohydrate, ketogenic diet in the treatment of morbidly obese adolescents results in a 15 kg weight loss over 8 weeks, but also increased Ca excretion and reduced total body bone mineral content [149]. A ketogenic diet is used to effectively treat epileptic children, but this leads to progressive bone loss, as shown over a 15-month period of time [147]. More recent evidence from weight loss studies suggests that high protein diets may not have the expected harmful effects on bone density or renal function [150] and we have shown a positive effect of higher protein intake [122]. Foster et al. [151] compared the effects of 2-year treatments with a low-carbohydrate or low-fat diet. With an approximate 11 % weight loss at 1 year and 7 % at 2 years, the authors did not find any differences in weight, body composition, or bone mineral density between the groups. In another 2-year study [152], healthy obese individuals on a low-carbohydrate, high-protein diet showed a 36 % increase in urinary Ca at 3 and 12 months, without changes in bone density or clinical presentations of new kidney stones. It is worth noting that there is evidence showing that an alkaline diet in weight-stable conditions is beneficial to bone [153–156]. Overall, an acidic diet in conjunction with caloric restriction may be expected to exacerbate the usual potential side effects of a weight loss diet, thereby increasing the risk of bone loss and kidney stone formation. Other diets that could potentially affect bone are those aided with medication. The pancreatic lipase inhibitor orlistat (OLS) induces an increase in bone resorption relative to formation [157] that is more dramatic than the change in the control group without OLS. Both groups showed a decrease in vitamin D status, but only the OLS group showed a significant increase in serum PTH. However, no changes in bone mass or density were seen after 1 year of OLS treatment with adequate Ca and vitamin D intake, apart from those explained by the weight loss itself. A vitamin D and Ca supplement should be taken during the treatment with orlistat for weight reduction.

33.4.2.4 Physical Activity and Weight Reduction

Studies have compared whether adding exercise to caloric restriction prevents the loss of bone mass due to weight reduction [158–162]. Under the assumption that a decrease in weight bearing is an important cause for weight loss-associated bone loss, maintenance of muscle mass particularly through an increase in resistance exercise should theoretically preserve bone mass. In postmenopausal women, aerobic exercise added to a caloric restriction program was able to prevent loss of BMD at specific sites such as the hip, which may be relevant for fracture risk [110]. In a lifestyle intervention study [111], it was found that those women losing weight (>8 %) who were more physically active

(primarily aerobic) lost less bone than the more sedentary women. Overall, in controlled trials of older individuals, if exercise is added to weight reduction regimens, it attenuates bone loss at the hip or femoral neck compared with diet alone. However, when diet plus exercise is compared with a weight-stable group [111, 160, 163], there is greater bone loss in those losing weight, suggesting that it can be attenuated but not prevented with added exercise. Importantly, the risk of falling in older obese individuals may be attenuated due to an increased level of physical function [164]. In summary, weight reduction with an increased level of physical activity will attenuate, but not prevent, changes in bone, whereas there is less data explaining how bone quality changes due to energy restriction with and without exercise.

33.4.2.5 Bariatric Surgery

With the widely known struggle is to lose weight, in particular for severely obese patients (BMI >35 kg/m^2) at high risk for developing metabolic, cardiovascular and other comorbid conditions, bariatric surgery is an effective alternative. The most common of these procedures include Roux-en-Y gastric bypass, laparoscopic adjustable gastric banding, and sleeve gastrectomy. Massive weight loss and drastic changes in food intake after surgery are not without important risks at several levels, particularly for bone metabolism. Several studies have reported bone loss after weight reduction in patients that have undergone these surgical procedures for obesity [165]. This issue has become more relevant in recent years, where the procedure has become more popular among less obese and even overweight individuals, as a "metabolic surgery" alternative for treating—or even curing—diabetes [166, 167].

A prospective study analyzed 59 women 1 and 3 years after gastric bypass surgery with a 35 % of weight loss (changes in BMI from 44 to 29 kg/m^2 in the first 12 months) [168]. Patients with bone disease at follow-up were older, which is in agreement with our observations of lower femoral neck bone mineral content >3 years post-surgery in postmenopausal but not premenopausal women [169]. Vilarrasa et al. concluded that the greatest risk for bone loss after surgery is for postmenopausal women and for those who lose more lean mass. However, even though more than 15 % and 30 % of patients developed femoral neck and lumbar spine osteopenia, respectively, the progression to osteoporosis or fracture was low [168]. This finding is supported by a cohort study where 2,079 patients followed for more than 2 years after bariatric surgery did not show a greater risk for fracture compared with controls [170]. Fracture risk may be related to the surgery (i.e., lap band vs. gastric bypass, limb length), initial body weight, or other factors [166]. Hence, the degree of malabsorption may induce bariatric surgery-associated bone loss, such as the lower availability of essential nutrients (particularly calcium and vitamin D), as well as alterations in calcium homeostasis alterations and vitamin D deficiencies that are common in obese patients [171, 172]. Moreover, bariatric surgery has been associated with changes in bone metabolism with secondary hyperparathyroidism and/or increased bone turnover [146, 169, 173–175]. Hyperparathyroidism probably contributes to the greater cortical bone loss in bariatric patients [176]. It has been observed that maintenance or elevations in vitamin D status after surgery is associated with less bone loss at the femoral neck [176]. The strong association found in many studies between the amount of weight loss and that of bone loss [168, 173, 177] suggests that skeletal unloading may be an important contributing factor. Moreover, the ability of weight loss to predict bone loss only at the hip, an important weight-bearing site, further supports this concept [144, 176, 178]. In addition, bariatric surgery induces dramatic changes in the production of several adipose-derived factors (e.g., adiponectin and leptin) and gut hormones (mainly peptide YY, Glucagon-like peptide-1, and ghrelin) that influence bone metabolism [179]. The possible impact of these changes is beginning to be addressed, as reviewed in [180]. It is relevant to note that despite the individual bone loss that accompanies weight loss, cross-sectional studies comparing post-surgical patients with BMI-matched controls do not show higher fracture risk associated with bariatric surgery [165]. There is no

specific recommendation for individuals who have undergone gastric bypass surgery, but a goal of consuming 1,500 mg/day Ca citrate when serum 25OHD levels are >50 nmol/L should be adequate to maintain Ca balance and avoid excessive bone loss. Close postoperative monitoring of bone metabolism parameters and bone mineral density should be performed in order to take proper measures for individual cases.

33.4.3 DXA Measurement Error

Dual energy X-ray absorptiometry (DXA) is the gold standard method for body composition and is most commonly used in bone research to estimate BMD and fracture risk. The sensitivity of DXA, however, may be reduced when there is excess soft tissue surrounding the bone in obesity, and can also be altered due to a very small amount of soft tissue surrounding bone in the lean or underweight individual. In addition, nonhomogeneous fat distribution or extremes in body size may reduce the sensitivity of DXA measurements [102]. In weight reduction studies, there is an additional concern that the soft tissue surrounding the bone is changing before and after treatment [107, 114, 115]. In some DXA studies, lard has been placed on top of the individual to determine the degree of error in bone measurements attributable to the change in the overlying fat tissue [107, 181]. In a carefully designed study by Yu et al., it was found that adding 6 kg or more (but not less) affected DXA, but not QCT measurements [182]. The findings show that the measurement of true volumetric BMD (g/cm^3) is more accurate when there are large changes in fat overlying bone than areal BMD (g/cm^2) that is measured by DXA. It should be noted that while many persons can achieve a 6 kg (or 13 lb) loss in total weight the amount of fat that would be lost in a single anatomical site would be less. Hence, since fat layering <6 kg did not affect measurement error, the precision concern is less likely to occur in most individuals undergoing moderate weight reduction. These authors also found that spinal BMD is more susceptible to measurement error due to overlying fat tissue than the hip [182]. This is of greater concern in the obese population who has a higher prevalence of osteophytes due to osteoarthritis [183] that can artificially elevate the BMD measurement [184]. Methods that are used to assess abnormal vertebrae (e.g., vertebral exclusion) causing measurement artifacts could be especially helpful in assessing spine BMD of the obese [5, 185]. In addition, there are other potential sources or error using DXA in the obese population such as positioning the person on the DXA bed, and we have seen errors made by untrained technicians. As necessary, we use straps around the arms and waist to reduce the spread of patients and to keep excess tissue within the scanning area. With the larger DXA beds of newer instruments, this problem occurs less often for individuals with BMI <40 kg/m^2. A three-dimensional image by QCT can provide more accurate data and allow for the distinction between cortical and trabecular bone, although this is not easily obtained at central sites without a significant increase in radiation exposure. Magnetic resonance imaging may also be able to overcome some of DXA shortcomings. Such distinctions are important to provide further insight into the precision of BMD and address bone quality and fracture risk in studies of obesity and weight reduction.

33.4.4 Weight Regain

The fact that weight regain after weight loss occurs in most cases raises the question of whether bone that is lost is also regained. In addition, it would be important to evaluate not only if net bone mass is regained, but also the ultrastructural properties and quality of the recovered bone. In postmenopausal women, it was observed that weight regain did not reverse the weight loss-induced reduction in

lumbar and spine BMD [102]. In addition, there are other trials without a control group that have addressed this, showing that weight regain leads to partial recovery of bone at some sites, but not others [104, 186–188]. In our preliminary data using a cohort-designed study, we have found that 2 years after weight reduction, there is no recovery of bone irrespective of weight regain. Hinton et al. found that adding exercise during the post-weight loss period did not affect the perturbations in bone turnover during partial weight regain over 1 year in premenopausal women [189]. The period just after weight loss may be important to better understand how bone metabolism differs and also may be a time when intervention to prevent bone loss should be considered. Similarly, a history of repeated weight loss and regain (i.e., weight cycling), which is common among overweight and obese individuals, may cause cumulative damage on bone and increase the risk for fracture [190]. Indeed, lower bone density has been observed in premenopausal women with a weight cycling history [191] and rodent studies have confirmed these observations, with reductions in bone quality and strength after regaining weight previously lost through energy restriction [192].

33.5 Conclusions and Future Directions

Low body weight in older individuals is a major risk factor for fracture, and may be due to low peak bone mass and/or increased rate of bone loss. The rising prevalence of both osteoporosis and obesity is attributed to a poor diet and sedentary behavior, and the previous belief that there is an inverse relationship between the incidence of osteoporosis and obesity is now challenged. Hormonal differences and/or increased weight bearing in the obese may explain the increased bone mass yet altered bone quality at certain anatomical sites. Fat mass may be a more important predictor of bone mass in older women, whereas lean tissue and physical strength are important determinants of bone in younger populations. Because increased marrow adipose tissue is associated with aging-related bone loss, further knowledge about the differentiation of stromal cells from the osteoblastic to the adipocytic lineage is indicated.

Involuntary weight loss and the method to achieve voluntary weight reduction (through different diets, medication, or increasing levels of activity) will affect bone mass differently. Caloric restriction alters bone-regulating hormones that have both direct and indirect effects on bone. A faster rate of weight loss is more catabolic to skeletal muscle mass, and may also be more detrimental to bone mass. During moderate weight reduction, there are at least a few methods to attenuate BMD loss, including assurance of adequate calcium and vitamin D in the diet, increased physical activity, and use of osteoporosis medications [122, 138, 160, 193], but these studies are limited since most do not address bone quality. This is an important direction for weight reduction research since we now know that obesity and diabetes are both associated with poor bone quality. In women who are already being treated for osteoporosis, these medications may be particularly important in the prevention of bone loss during times of restricted food intake and weight reduction.

Future studies of bone health in the obese should consider determining factors influencing bone quality. In addition, information explaining how race and ethnicity affect bone in the obese and during weight loss is a concern due to the rise in global obesity. Improvements in imaging techniques to measure bone mass and its quality is a promising direction of research that may improve the reliability of current methods when applied to obese populations and/or those undergoing weight loss or gain. Studies of the specific nutrient requirements in combination with exercise during weight loss are important for optimizing nutritional recommendations to reduce osteoporosis risk.

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Chapter 34
Nutraceuticals and Bone Health

Jeri W. Nieves

Key Points

- The use of dietary supplement or nutraceutical is common and exceeds 50 % in US adults with higher rates in individuals with chronic disease.
- Although calcium and vitamin D are important, various other nutrients may also provide benefit to the skeleton.
- The relationship between soy compounds and skeletal health is inconclusive.
- Dehydroepiandrosterone has not been shown to benefit the skeleton even in elderly with low serum levels.
- There is no clear skeletal benefit from various antioxidants, flavonoids, carotenoids, omega-3 fatty acids, and various vitamins with only limited observational data and small clinical trials.
- High homocysteine may relate to fracture risk, but whether this risk is reduced by any B vitamins is unclear.
- There is no clear relationship between bone health and nutritional intake of magnesium, boron, strontium, silicon, and phosphorus.
- Adults should consume adequate a intakes of protein, fruits and vegetables.

Keywords Nutraceuticals • Soy • B vitamins • Magnesium • Boron • Strontium • Silicon and phosphorus • Omega 3 • DHEA

34.1 Introduction

Adults should follow a varied diet with adequate protein and fruits and vegetables while ensuring an adequate intake of calcium and vitamin D in order to maximize their skeletal health. The importance of several nutrients in relation to bone health has been covered throughout this text. Information provided in this chapter is further explained in a recent review paper [1].

Data from NHANES, 2000, indicates that more than half of adults reported taking a dietary supplement in the past month, and even more individuals with a diagnosis of a chronic disease, reported use [2]. Many adults resort to the use of supplements and nutraceuticals to improve their bone health.

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Nutraceuticals are defined as any substance that is a part of a food that may provide health benefits, such as the prevention and treatment of disease. Isolated nutrients, dietary supplements, herbal products, and medical foods (available only by prescription) are all considered nutraceuticals. The dietary supplement manufacturer is responsible to ensure that the dietary supplement is safe and cannot make health claims that are false or misleading (Dietary Supplement Health and Education Act/DSHEA 1994). Supplements are products that contain a vitamin, mineral, amino acid, herb, or other botanical or dietary substance that will lead to an increase in the total dietary intake of that nutrient. The DSHEA does not have provisions for the FDA to "approve" dietary supplements for either safety or effectiveness [3].

34.2 Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) is a steroid that is a precursor to androgens in men and estrogens in women. Serum levels of dehydroepiandrosterone-sulfate (DHEA-S) fall with age. Commercials for DHEA include claims that it is an antiaging remedy, and will increase muscle, decrease fat, and improve energy, strength, and immunity. Although serum levels of DHEA have been related to bone loss and levels of bone resorption [4, 5], clinical trials provide inconsistent data regarding the benefit of DHEA and results vary by gender and skeletal site [6–11]. Any benefit of DHEA to bone may be limited to elderly individuals with low serum DHEAS [10]. A recent meta-analysis (1,353 elderly men, mean follow-up = 36 weeks) reported that there was no effect of DHEA supplementation in comparison with placebo on bone health [12]. The safety concerns with the use of DHEA include that it may adversely affect liver function, and lead to acne and masculinizing effects.

34.3 Phytoestrogens

Phytoestrogens are naturally occurring plant compounds that fall into three classes: (1) isoflavones (genistein, daidzein, glycitein) from soybeans and soy products; (2) chickpeas/lignans (enterolactone and enterodiol) that are found in flaxseed, cereal bran, and legumes; and (3) coumestans (coumestrol) from alfalfa and clover. Phytoestrogens can function either like an estrogen agonist or antagonist. American diets are very low in isoflavone intake (1–3 mg/d), whereas Asian diets contain an average of 30–60 mg/d of isoflavones [13–15]. There is limited data relating dietary phytoestrogens to fracture risk, but in one study there was a 36 % reduction in fracture risk in those with high compared to low intakes [16].

Reports of the benefit of soy to the skeleton indicate no benefit or only a modest benefit [8, 11, 17] and the variability may result from differences in study design and duration, sample size, initial bone mineral density (BMD), years since menopause, age, body weight, and the baseline intake of soy, calcium, and other nutrients. In conclusion, isoflavones that contain varying amounts of genistein, daidzein, and glycitein do not appear to benefit the skeletal of postmenopausal women [8, 11, 13–15, 17–27]. Genistein aglycone (54 mg a day) led to gains in BMD at the spine and the hip, similar to hormone therapy [28, 29]. Whether these reported benefits of genistein aglycone will be confirmed is not known. However, calcium supplements have added genistein and been marketed "to promote bone health."

Isoflavones may cause nausea or gastric irritability, stomach pain, vomiting, and constipation [30], although taking with food will minimize these effects. There have been no negative findings regarding breast or gynecologic endpoints in both epidemiologic studies and clinical trials with up to 3 years in duration in postmenopausal women [31–35].

A review of the effects of flax intervention on postmenopausal bone mineral density is inconclusive according to a review of the few randomized controlled trials that have been reported [36].

Red clover (*Trifolium pratense*) is part of the legume. One review of the red clover and bone health stated that there was limited evidence of efficacy [37]. In a recent 3-year trial of women at risk for breast cancer ($n=401$), 40 mg of red clover did not improve BMD as compared to placebo [38]. Red clover is probably safe but there is little evidence of a benefit.

Bone mass did improve with ipriflavone vs. placebo in two studies [26, 39], but not another [40]. The potential side effects of iproflavone include stomach pain, diarrhea, dizziness, and a potential lymphocytopenia [5] leading to concerns over its use.

Black cohosh or cimicifuga racemosa (CR) from buttercup is available in various forms and doses. Black cohosh may benefit the skeleton through its estrogenic activity [24]. Common side effects are headaches, gastric complaints, and weight problems and there are case reports of hepatitis and liver failure in women taking black cohosh [41].

34.3.1 Summary

Clinical trials of the skeletal effects of varying forms of soy isoflavones have mostly found no benefit, but there are also some conflicting reports. This may result, in part, from differences in the composition and dose of the soy product as well as differences in study populations and characteristics. Aglycone isoflavone equivalents were reported to be the most bioactive form of isoflavones in an NIH workshop [42].

34.4 Bicarbonates

An acidic environment has been associated with bone loss [43, 44]. In addition, bicarbonate (HCO_3) may improve bone health by decreasing 24-h urinary calcium, promoting calcium absorption [45], and perhaps lowering bone resorption [46, 47]. However, a 2-year study of potassium citrate supplements reported no change in bone turnover BMD as compared to placebo [48]. A recent review concluded that the use of supplements of potassium citrate or bicarbonate has not consistently shown a bone health benefit [49]. Further study may clarify any differences and tease out the effect of the bicarbonate versus potassium [48].

34.5 Minerals

Dietary sources of magnesium include green vegetables such as spinach, legumes (beans and peas), nuts and seeds, and whole, unrefined grains. However, over half of adults do not meet the RDA for magnesium [50]. A positive relationship between bone mass and dietary magnesium intake was found in young women and postmenopausal women and older men, although results are not consistent based on gender, race, or skeletal site [51–58]. Magnesium supplementation may be effective in individuals with low baseline serum magnesium levels [59, 60]. Overall, observational and clinical trial data concerning magnesium and BMD or fractures are inconclusive [57, 59–63], and in one study there was an increased risk of wrist fracture at levels exceeding the RDA [61, 64]. There is little evidence to support intakes of magnesium above the RDA for bone health and in general the RDA should be met with food [65]. However, a magnesium supplement may be required in those with low magnesium

levels including frail elderly with poor diets [62], persons with intestinal disease, alcoholics, or persons on treatment with diuretics or chemotherapy that depletes magnesium.

Boron is present in several foods, such as fruits, vegetables (potato and avocado), legumes, nuts, eggs, milk, wine, and dried foods [66]. Whether a low intake is of clinical concern is unknown although boron is not an essential nutrient. Boron (3 mg daily) may have a positive effect on bone [59, 67] or decrease urinary calcium loss [68] but there are no controlled trials. It is suggested that foods such as fruits, vegetables, and legumes be the source of boron, since these foods may provide additional benefit for the skeleton.

Cereals provide the greatest amount of silicon in the US diet, followed by fruit, water, beer, high-fiber grains, bananas, and vegetables. Silicon is a trace mineral that may be essential for bone health and collagen synthesis. Serum silicon concentrations decrease with age, especially in women [69]. Dietary silicon intake is positively related to BMD in some populations [70] but not others and results require confirmation [71]. It is also possible that silicon intake and estrogen act synergistically to benefit bone mineral density in postmenopausal women [72]. Women with low bone mass given low doses of silicon (<12 mg/day) did not have improvements in bone mass compared to controls [73].

Strontium has numerous salts including strontium ranelate, strontium citrate, and strontium carbonate. Strontium ranelate is a drug (not a supplement) that is approved in several European countries for the treatment of osteoporosis, but is not approved in the USA. In clinical trials, strontium ranelate reduced the risk of fractures [74, 75] and may be a modest antiresorptive agent. Strontium is incorporated into hydroxyapatite, replacing calcium. The most common side effects from strontium ranelate were nausea, diarrhea, headache, and skin irritation as well as small increased risks of venous thrombosis, seizures, and abnormal cognition [74, 75]. There are many strontium salts that are available through the Internet. However, their long-term safety and efficacy have not been evaluated in humans in large-scale clinical trials. Websites for many strontium compounds reference data from strontium ranelate clinical trials as proof of efficacy although they are marketing a different compound, not the formulation used in clinical trials. The dose, absorption, bioequivalence, and safety of the many forms of strontium available for purchase are not known.

Phosphorus is a component of dairy foods, meat, eggs, cereal, and processed foods and phosphoric acid is found in cola. The ratio of phosphorus as compared to calcium is often high in the typical diet [76]. Lower BMD was related to increased cola intakes in women but not men [77] and higher phosphorus intakes were related to higher fracture rates [78]. In a recent review article it was hypothesized that phosphorus added to the food supply may be contributing to the burden of osteoporosis in the US population and that consideration should be given to the calcium to-phosphorus ratio of meals [79]. These relationships require further study.

34.6 B Vitamins and Homocysteine

Food sources of pyridoxine (B6) are whole grains, fortified cereals, liver, soybeans, and beans. Folate (vitamin B9) dietary sources include dark green leafy vegetables, whole-grain breads, nuts, and fortified cereals and folic acid is added to fortified foods. Vitamin B12 (cyanocobalamin) is found in liver, shellfish, fish, beef, lamb, cheese, eggs, and some fortified foods.

Homocysteine has been linked to fractures in older men and women [80–83] and elevated serum homocysteine levels may be caused by deficiencies of folate (folic acid), vitamin B_{12}, or vitamin B_6. The role of B vitamins in bone health has been the subject of many recent research studies.

Low-serum folate was associated with higher homocysteine levels and higher risk of fractures [84] and poor bone health [85]. Poor vitamin B_{12} status has also been associated with low bone mass or osteoporosis [86–89], but this may relate to poor nutrition and increased frailty. Higher dietary intake of pyridoxine (B6) was associated with higher BMD and reduced fracture risk [90].

Folate has also been found to be more strongly related to BMD than any other B vitamin in most but not all studies [90–94]. In a Japanese study, pharmacologic doses of mecabolamin (a form of vitamin B12) for 2 years reduced hip fractures as compared to placebo in stroke patients [95]. Homocysteine levels and vitamin B12, but not folate, were related to bone density in postmenopausal women with osteoporosis in a recent meta-analysis [96].

Controlled clinical trials are needed to determine whether any of the B vitamins would reduce fracture rates [97, 98]. Without further evidence of a benefit for B vitamins it would be best to promote a healthy varied diet to assure adequate intake of B vitamins.

34.7 Vitamins and Antioxidants

Oxidative stress is a potential cause of many diseases and may be related to bone loss. An increase in reactive oxygen species (ROS) occurs with age and this may affect the generation and survival of osteoclasts, osteoblasts, and osteocytes [99]. Several antioxidants may relate to bone health including vitamin A, vitamin E, vitamin C, carotenoids, and quercetin (a flavonoid). The mechanism may be by creating a more alkaline environment, reducing urinary calcium excretion, or providing bioactive components (phenols and flavonoids). Alternatively, high nutrient intakes may be a marker for a healthy lifestyle.

Flavonoids occur in plant-based foods including fruits, vegetables, grains, herbs, tea, wine, and juices. Higher intakes of flavonoids have been positively associated with spine and hip BMD [100, 101]. Quercetin is a flavonoid and a strong antioxidant that is found in citrus fruits, apples, onions, parsley, sage, tea, and red wine, and in many food supplements. Quercetin may benefit bone health [102–106], although the data are weak and it would be preferable to get flavonoid compounds from fruits and vegetables.

Nutritionally essential omega-3 fatty acids are polyunsaturated fatty acids: α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). There are several sources of omega-3 fatty acids including fish, eggs, walnuts, and flax seed. Omega-3 fatty acids may up-regulate intestinal calcium absorption [107]. There have been several reports of a positive effect of omega-3 fatty acids on BMD [108–111], as well as a negative association between BMD and a higher ratio of omega-6 to omega-3 fatty acids [112, 113]. Dietary intakes of omega-3 fatty acids were slightly associated with femoral neck BMD; and when omega-3 supplement use was evaluated it was significantly associated with higher lumbar spine BMD in older adults in NHANES[114]. Calcium intake may interact with omega-3 fatty acids to improve bone health [115]. It would be better to obtain omega-3 from dietary sources and limit sources of omega-6 by reducing consumption of processed and fast foods and polyunsaturated vegetable oils (corn, sunflower, safflower, soy, and cottonseed).

Carotenoids exist in four forms: beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin that can each be converted to retinol (vitamin A). The other carotenoids lycopene, lutein, and zeaxanthin function as antioxidants, but are not converted to retinol (vitamin A). Carotenoids are found in various vegetables including carrots, sweet potatoes, spinach, kale, collard greens, papaya, bell peppers, and tomatoes. Lower serum lycopene, cryptoxanthin, and beta-carotene concentrations have been associated with lower BMD [116, 117] and foods containing carotenoids have been associated with higher BMD [118, 119]. Higher intakes of total carotenoids and lycopene were also associated with reduced fracture incidence in women and men [120]. Total vegetables and carotenoid intake were found to protect against hip fracture in men, particularly in lean men, while there was no association between dietary carotenoids or vegetables/fruits and hip fracture risk among women [121]. Given the available data, it would be best to get carotenoids from increasing the intake of the vegetables.

Retinol is the animal form of vitamin A and is found in liver, meats, eggs, milk products and fatty fish. Excess retinol A has been associated with decreased bone density and increased hip fracture rates

[122–125], although not always [126]. In a recent dose-response meta-analysis, there was a U-shaped relationship between serum retinol level and hip fracture risk [127]. The authors suggest that vegetable sources providing beta carotene would be preferred. Vitamins that contain no more than 2,000–3,000 IU retinol are also preferred, whereas beta-carotene doses are of no concern.

Dietary sources of ascorbic acid (vitamin C) include citrus fruits and juices, green vegetables (especially peppers, broccoli, cabbage), tomatoes, and potatoes. Vitamin C is important in collagen formation and may also provide skeletal benefit as an antioxidant. In a small clinical trial vitamin E (400 mg) and vitamin C (1,000 mg/day) taken for 6 months slightly reduce spinal bone loss [128] and reduced bone resorption in another study [119]. Low intakes of vitamin C are associated with bone loss and one study found that higher vitamin C was associated with fewer fractures [129–140]. Vitamin C intakes may interact with estrogen use, calcium, and vitamin E in women [141]. Subjects in the highest tertile of total vitamin C intake (mostly from supplements) had significantly fewer hip fractures (P trend = 0.04) and non-vertebral fractures (P trend = 0.05) compared to subjects in the lowest tertile of intake [142]. However, in the Women's Health Initiative Observational Study, increasing intakes of antioxidants (including vitamin C) were not associated with BMD [117]. In many studies, it is not possible to separate the effects of vitamin C supplements from vitamin C in fruits and vegetables [129, 141]. Therefore, the recommendation is to obtain the needed amounts of vitamin C from fruits and vegetables, rather than from supplements. Recommended intakes of five or more servings of fruits and vegetables per day should supply enough vitamin C for bone health.

34.7.1 Summary

Fruits and vegetables may reduce urinary calcium excretion, create an alkaline environment, provide specific nutrients, provide an antioxidant effect, and provide bioactive components (phenols and flavonoids) or they may simply be a marker for a healthy lifestyle. Therefore, foods, rather than supplements, may be the best way to get the benefit of antioxidant compounds and vitamins, because they may also provide skeletal benefit through other mechanisms.

34.8 Conclusion

Calcium and vitamin D play a clear role in bone health; in cases of low dietary intake of calcium, supplementation may be needed, but the first choice is to consume calcium from foods. In most cases vitamin D supplementation will be needed to ensure adequate intake. Many nutraceutical products may promote skeletal health. In addition to the usual differences in studies used in meta-analysis, studies of nutraceuticals have the added problem of the inconsistency of the formulations. Potential safety issues must be recognized when these nutraceutical products are taken in amounts that exceed dietary recommendations. In general, most nutritional products and specific nutrients, other than calcium and vitamin D, have inconsistent study results regarding their potential role in bone health. Furthermore, only short-term data are available for either efficacy or safety. It is important that medical providers are aware of the nutraceuticals commonly used by their patients and to have an understanding of the safety, efficacy and lack of regulation of these products. For many nutraceuticals, the marketed doses are equivalent to pharmacologic levels, not equivalent to what is found in food. Clearly for patients with osteoporosis or with a high risk for fracture nutraceuticals will not replace a medication proven to prevent fractures.

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Part VII
Nutrition Related Disorders and Secondary Osteoporosis

Chapter 35
Eating Disorders and Their Effects on Bone Health

Madhusmita Misra and Anne Klibanski

Key Points

- Low-weight eating disorders such as anorexia nervosa (AN) are associated with low bone density, impaired bone structure, and a higher risk for fracture in both adults and adolescents.
- Factors contributing to low bone density include changes in body composition, hypogonadism, a state of acquired growth hormone resistance with low insulin-like growth factor-1 (IGF-1) levels, relative hypercortisolemia, and changes in other hormones that may impact bone metabolism including leptin, peptide YY, oxytocin, adiponectin, insulin, and amylin.
- The best strategy to improve bone density and bone accrual rates is weight gain and restoration of menstrual function; however, residual deficits often persist. In addition, a significant number of patients remain low weight for many years.
- Calcium and vitamin D status should be optimized; however, supplementation of these micronutrients alone is not effective in improving bone density. Physiologic estrogen replacement is effective in increasing bone density at the spine and hip in girls with anorexia nervosa (AN) such that bone accrual rates approximate that in normal-weight controls; however, catch-up does not occur.
- Bisphosphonates (anti-resorptive) are effective in increasing bone density in adults, but not adolescents with AN. IGF-1 is a bone anabolic hormone, and IGF1 replacement increases bone formation in adolescents, and with estrogen increases bone density in adults with AN.

Keywords Anorexia nervosa • Bone density • Bone microarchitecture • Bone strength • Fracture • Adolescents • Estrogen • IGF-1 • Cortisol • Bisphosphonates

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35.1 Introduction

The prevalence of eating disorders has increased in the industrialized world in the last few decades [1–3], and based on DSM-IV criteria, 0.2–1 % of women in the USA were believed to suffer from anorexia nervosa (AN), an eating disorder associated with significant bone loss [4–8] and increased fracture risk [9–11]. The prevalence is likely to be even higher for AN based on DSM-V criteria [12], and may be as high as 4 % because amenorrhea is no longer required for the diagnosis [13]. In addition, AN has been increasingly reported in males, who, in one study, now comprise 5–15 % of this population [14], and also demonstrate significant bone loss [15, 16]. In a study from Canada, disordered eating attitudes and behaviors were reported in over 27 % of girls aged 12–18 years [17], with significant increases in Drive for Thinness, Body Dissatisfaction and Bulimia subscales (Eating Disorders Inventory) with age.

Of note, available data regarding bone density in patients with eating disorders are based on studies of eating disorders per DSM-IV and not the revised 2013 DSM-V criteria. DSM-IV categorized eating disorders as AN and bulimia nervosa (BN) and eating disorders not otherwise specified (EDNOS). DSM-V categorizes eating disorders under the broader category of feeding and eating disorders. These include pica, rumination disorder, avoidant/restrictive food intake disorder, AN (restricting type and binge-eating/purging type), BN, binge-eating disorder, and other specified feeding and eating disorder (atypical AN, BN of low frequency and/or limited duration, binge-eating disorder of low frequency and/or limited duration, and purging disorder). Table **35.1** summarizes the characteristic features of AN, BN, binge-eating disorder, and other specified feeding and eating disorders per DSM-V criteria vs. AN, BN, and EDNOS per DSM-IV criteria. Binge-eating disorder is typically not associated with low bone density and will not be discussed in this chapter. Pica, rumination disorder, and avoidant/restrictive food intake disorder are seen in younger children. However, as there are limited bone data in these conditions, they will also not be discussed.

Table 35.1 DSM-IV-TR vs. DSM-V criteria

DSM-IV-TR (eating disorders)	DSM-V (feeding and eating disorders)
Anorexia nervosa	*Anorexia nervosa* (*AN*)
• Refusal to maintain body weight at or above a minimally normal weight for age and height, e.g., weight loss leading to maintenance of body weight <85 % of that expected or failure to make expected weight gain during period of growth, leading to body weight <85 % of that expected • Intense fear of gaining weight or becoming fat, even though underweight • Disturbance in the way one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of current low body weight • In postmenarcheal females, amenorrhea, i.e., the absence of ≥3 consecutive menstrual cycles – Restricting subtype: During the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (self-induced vomiting or misuse of laxatives, diuretics, or enemas) – *Binge eating/purging subtype*: During the current episode of AN, the person has regularly engaged in binge-eating or purging behavior	• Restriction of energy intake relative to requirements leading to a significantly low body weight for age, sex, developmental trajectory, and physical health • Intense fear of gaining weight or becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight • Disturbance in the way one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight – *Restricting subtype*: Person does not regularly engage in binge eating – Binge-eating/purging subtype: Engages in binge-eating and purging behaviors (as in bulimia nervosa, but associated with low weight)

(continued)

Table 35.1 (continued)

DSM-IV-TR (eating disorders)	DSM-V (feeding and eating disorders)
Bulimia nervosa • Recurrent episodes of binge eating (on average ≥2 times/week for ≥3 months) • Recurrent inappropriate (compensatory) behaviors to prevent weight gain or compensate for binge eating (on average ≥2 times/week for ≥3 months) (such as self-induced vomiting, misuse of laxative, diuretics, enemas, or other medications, fasting, or excessive exercise) • Self-evaluation is unduly influenced by body shape and weight • Does not occur exclusively during episodes of AN – *Purging subtype* – *Non-purging subtype*	*Bulimia nervosa* (*BN*) • Recurrent episodes of binge eating (on average ≥1 times/week for ≥3 months) • Recurrent inappropriate (compensatory) behaviors to prevent weight gain or compensate for binge eating (on average ≥1 times/week for ≥3 months) • Self-evaluation is unduly influenced by body shape and weight • Does not occur exclusively during episodes of AN
	Binge eating disorder • Repeated episodes of binge eating (on average ≥1 times/week for ≥3 months) associated with marked distress • Not associated with recurrent inappropriate compensatory behaviors and does not occur exclusively in the presence of AN or BN
Eating disorders not otherwise specified (*EDNOS*) • Clinically important disordered eating, inappropriate weight control, or excessive concern about body weight or shape that does not meet all the criteria for anorexia nervosa or bulimia nervosa (includes binge-eating disorder) *Examples* • For female patients, all criteria for AN are met except that the patient has regular menses • All criteria for AN are met except that, despite significant weight loss, the patient's current weight is in the normal range • All of the criteria for BN are met except that the binge eating and inappropriate compensatory mechanisms occur < twice a week or for <3 months • The patient has normal body weight and regularly uses inappropriate compensatory behavior after eating small amounts of food (e.g., self-induced vomiting after consuming two cookies) • The patient engages in repeatedly chewing and spitting out, but not swallowing, large amounts of food • Binge-eating disorder: Recurrent episodes of binge eating in the absence of regular inappropriate compensatory behavior characteristic of BN	*Other specified feeding and eating disorder* • *Atypical AN*: Meets all criteria for AN except that despite significant weight loss, the individual's weight is within or above the normal range • *BN of low frequency and/or limited duration*: Meets all criteria for BM except that binge-eating and inappropriate compensatory behaviors occur on average <once per week and/or for <3 months • *Binge-eating disorder of low frequency and/or limited duration*: Meets all criteria for binge-eating disorder except that binge eating occurs on average < once per week and/or for <3 months • *Purging disorder*: Recurrent purging behaviors (e.g., self-induced vomiting, use of laxatives, diuretics or other medications) to influence body shape or weight in the absence of binge eating
	Pica *Rumination disorder* *Avoidant/restrictive food intake disorder*

Adapted from American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 4th ed, text rev., 2000, and Diagnostic and Statistical Manual of Mental Disorders, 5th ed, 2013 (Copyright 2000 and 2013: American Psychiatric Association. All rights reserved)

Adolescence is a time when peak bone mass accrues, with maximum accrual occurring between 11 and 14 years in girls and 13 and 16 years in boys [18]. Almost 25 % of peak bone mass is formed in the 2 years surrounding peak height velocity, and more than 90 % of peak bone mass is achieved by the time an individual is 18 years old [19]. Adolescence is thus a critical time of life for optimizing bone health, and insults suffered at this time may cause permanent deficits. Unfortunately, adolescence is also a common time for the onset of eating disorders [1].

35.2 Effect on Bone Density and Structure, and Bone Turnover

35.2.1 Anorexia Nervosa

AN consistently decreases bone mass in women [4, 7], in adolescent girls [5, 6, 8], and boys [16], and the degree of bone loss can be severe. Low BMD has been demonstrated at all sites and although bone loss occurs both in trabecular and cortical bone, trabecular bone appears to be preferentially affected. We have reported *T*-scores of <−2.5 and <−1.0 in 38 and 92 % of women with AN [7], and BMD *Z*-scores of <−1 in over 50 % of adolescent girls with AN at one or more sites [5]. Zipfel et al. [20] demonstrated lower bone density in women with the binge eating/purging subtype compared to the restrictive form of AN. In addition, in one study, 65 % and 50 % of adolescent boys with AN had BMD *Z*-scores of <−1 at the femoral neck and spine, respectively, compared to 18 and 24 % of normal-weight controls [16].

In adolescents with AN, profound and rapid bone loss occurs in a large proportion of girls suffering from this disorder. Bachrach et al. [8] reported that more than half of adolescent girls with AN with osteoporosis had been diagnosed with AN for less than a year. Bone loss is also more severe when AN begins in adolescence than when it begins in adult life, even with a comparable duration of illness [7]. The extent of bone loss depends on the duration of amenorrhea [5, 7, 8, 21, 22], body mass index [8, 21–23], and lean body mass [5, 24, 25].

With the availability of quantitative computed tomography (QCT) and high-resolution peripheral QCT (HRpQCT), data have emerged regarding bone size parameters, and bone structure and strength estimates in AN. Adolescent girls with AN have decreased cortical and increased trabecular area compared with controls, suggestive of increased endosteal bone resorption, likely from estrogen deficiency [26]. In addition, cortical porosity increases and trabecular thickness decreases, as does total and trabecular volumetric bone density [26]. Estimates of bone strength, namely stiffness and failure load, are also markedly decreased in AN [26]. Of concern, impaired bone structure in adolescents with AN occurs even before changes are seen in DXA measures of BMD [27]. Similarly, women with AN have impaired bone structure and strength, in addition to decreased cortical thickness and trabecular number [28, 29].

Although rarely useful clinically, surrogate markers of bone turnover in this population have provided insight into the underlying pathophysiologic processes leading to decreased BMD. Low levels of markers of bone formation have been reported in women with AN [23, 30], whereas levels of markers of bone resorption are elevated [20, 23, 30]. Similarly, in adolescents with AN, low bone formation has been reported [25, 31]. However, increased bone resorption is demonstrated less frequently in this younger population, suggesting a generalized reduction in bone turnover [25, 31]. In both adults and adolescents, the net result is decreased accumulation of bone mass and thus low bone density.

Consistent with data from DXA and QCT studies, fracture risk in adult women with AN is seven times that of healthy matched controls [32], and over 50 % of all women with AN have a BMD that is below the fracture threshold [30]. A population based cohort study in 1999 reported a 57 % cumulative incidence of fractures at the hip, spine and radius in women with AN 40 years after the diagnosis

of this eating disorder [10]. Vestergaard et al. reported increased fracture risk in patients with eating disorders, with the highest incidence rate ratio of fractures observed after diagnosis of AN, followed by eating disorders not otherwise specified (EDNOS) and bulimia (1.98, 1.7, and 1.44, respectively) [33]. We have reported a higher fracture incidence in adolescent girls with AN than in normal-weight, healthy adolescent girls (31 % vs. 19 %) [11].

35.2.2 Other Eating Disorders Associated with Low Bone Density

Although women with AN and concomitant bulimia have low bone mass, normal-weight bulimic patients may not [20, 34, 35]. Bone density is higher among normal-weight bulimic women (based on DSM-IV criteria) who exercise compared to sedentary women [35]. Davies et al. [36] examined bone mineral content (BMC) in women with AN alone, bulimia alone, and AN with bulimia. They reported lowest BMC in the AN-alone group, followed by women with AN with bulimia, and demonstrated that BMC in women with bulimia alone was not different from healthy controls. Per DSM-IV criteria, a large phenotypic section among patients with eating disorders was previously classified as having EDNOS. Of 76 women who qualified for this diagnosis, Marino and Zanarini [37] noted a history of restricting without low weight in 20 %, bingeing without purging in 37 %, purging without binging in 37 %, and low weight without loss of menses in 33 %. At least one study reported that in 33 patients with various eating disorder subtypes, bone density was lowest in the EDNOS group, followed by the group having AN and was highest in the group with bulimia [38].

35.3 Factors Contributing to Low Bone Density in Eating Disorders Associated with Weight Loss

35.3.1 Hypogonadism

Eating disorders in women may be associated with amenorrhea, especially when accompanied by weight loss. Amenorrhea of at least 3-month duration was, in fact, one of the diagnostic features of AN by DSM-IV criteria, although this is no longer required for the diagnosis of AN by 2013 DSM-V criteria. The hypogonadism in AN is attributed to acquired gonadotropin-releasing hormone (GnRH) deficiency and resulting "immature" patterns of LH pulsatility [39] with consequent low estrogen levels in adolescents and adult women with AN [31, 40]. Bone density correlates inversely with the duration of amenorrhea [5, 7, 8, 21, 22], indicating that hypoestrogenism and its duration contribute to bone loss. Audi et al. demonstrated an inverse relationship between estrogen levels and markers of bone resorption in pubertally mature adolescent girls with AN [41].

Estrogen inhibits bone resorption by an alteration in local concentrations of cytokines involved in osteoclast differentiation, activation, and apoptosis. Estrogen inhibits release of TNFα, IL-1, IL-6, and prostaglandin E2 (PGE2), and stimulates release of TGFβ and osteoprotegerin [42]. The net effect is inhibition of differentiation of osteoclast precursors to mature osteoclasts, inhibition of activation of mature osteoclasts and stimulation of osteoclast apoptosis. It is uncertain whether estrogen exerts any effect on osteoblasts, although a role for estrogen in inhibiting sclerostin secretion from osteocytes has been described. Sclerostin typically inhibits osteoblast differentiation by inhibiting Wnt signaling [43].

Estrogen deficiency also accompanies acquired GnRH deficiency in normal-weight women with hypothalamic amenorrhea due to stress or hyperprolactinemia. The extent of bone loss in AN, however, is far more severe at all sites than in comparable cases of hypothalamic amenorrhea that are not

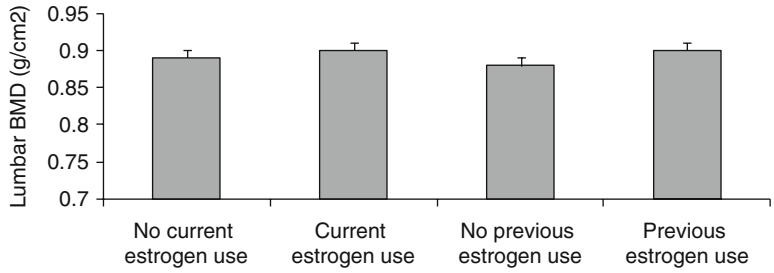

Fig. 35.1 Lumbar bone mineral density by estrogen use in women with AN. No differences were seen in lumbar bone mineral density (BMD) among women currently using estrogen, currently not using estrogen, with a history of previous estrogen use, and with no previous history of estrogen use (adapted from Grinspoon et al. Prevalence and predictive factors for regional osteopenia in women with anorexia nervosa. Ann Intern Med 2000;133:791. Copyright 2000 ACP-ASIM. All rights reserved)

associated with significant weight loss [24]. Administration of estrogen to women with hypothalamic amenorrhea without significant weight loss may improve bone density based on some studies [44], but not others [45]. Results have been much less promising in women with AN. In one non-randomized study, lumbar, but not hip bone density was reported to be higher in women who received oral contraceptives for a 30-month period compared to women who did not, although lumbar BMD was still lower than in normals [46]. In a cross-sectional study of 130 women, we reported no difference in BMD at multiple sites in women with or without estrogen exposure [4] (Fig. 35.1). Consistent with these data, we found no difference in BMD after 18 months of estrogen-progestin therapy in women with AN in a randomized trial [40] (Fig. 35.2), and over 9 months in another randomized study of oral contraceptive pills vs. placebo [47]. In women with very low weights, however, a post hoc analysis showed that estrogen therapy might have some protective effects [40]. Golden et al. [48] examined bone density in adolescent girls with AN before and after administration of an oral contraceptive pill (containing 20–35 mcg estrogen) for a mean duration of 23 months, and found no effects. Munoz et al. [49] reported no effect of an estrogen-progesterone combination in AN. Neither of these studies was randomized, blinded or placebo controlled. Subsequently, a randomized, placebo-controlled trial demonstrated that oral estrogen-progesterone combination pills were not effective in increasing bone density at the spine or hip in adolescent girls with AN [50].

The lack of improvement in bone density with estrogen-progesterone combination pills has, at least, partly been attributed to insulin-like-growth factor-1 (IGF-1) suppression by oral estrogen through its hepatic first pass effects [51, 52]. IGF-1 (as discussed below) is an important bone trophic hormone that is reduced in conditions of undernutrition such as AN. In contrast to oral estrogen, transdermal estrogen in physiologic doses does not suppress IGF-1 [51, 52], and has been shown to improve bone accrual rates at the spine and hip in adolescent girls with AN to approximate that in normal-weight controls [31] (Fig. 35.3). However, although bone density *Z*-scores were maintained over time with transdermal estradiol replacement (from normalization of bone accrual), *Z*-scores remained lower than in controls, and "catch-up" did not occur.

The lack of "catch-up" with estrogen therapy is likely because AN is associated with loss of other nutritionally dependent bone trophic factors that are not corrected with estrogen administration alone. Studies examining bone turnover have uniformly demonstrated decreased bone formation in AN [23, 25, 30, 31]. Markers of bone resorption are increased in women with AN [20, 23, 30], but are decreased in adolescent girls with this disease [31, 53]. Estrogen primarily acts by inhibiting bone resorption; its effects on bone formation are less clear. It is possible that without adequate bone formation, the anti-resorptive effects of estrogen are not sufficient to provide enough of a positive balance to result in increased bone mass.

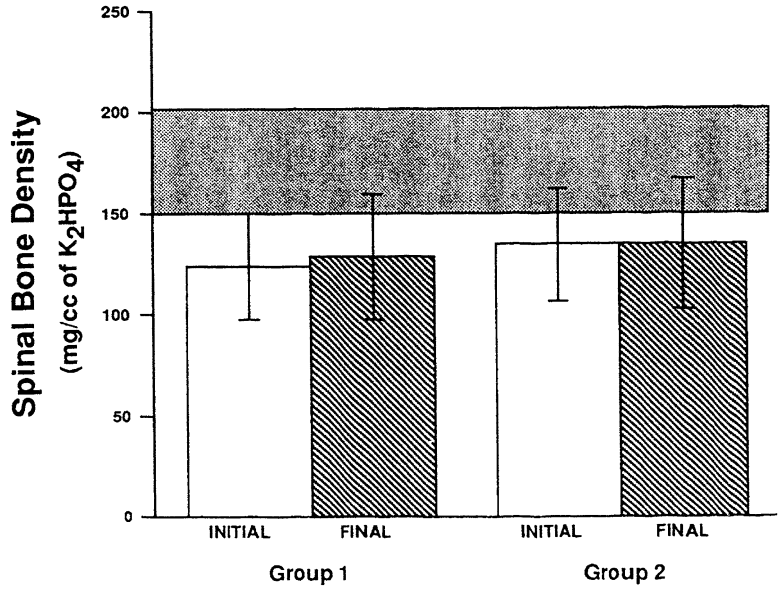

Fig. 35.2 Effects of estrogen administration (Group 1) vs. placebo (Group 2) on bone density in women with anorexia nervosa. No differences in bone mineral density were observed before and after 18 months of estrogen-progestin administration in a group of women with anorexia nervosa in a randomized, prospective placebo-controlled trial (reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 1995;80:900. Copyright © 1995 The Endocrine Society. All rights reserved)

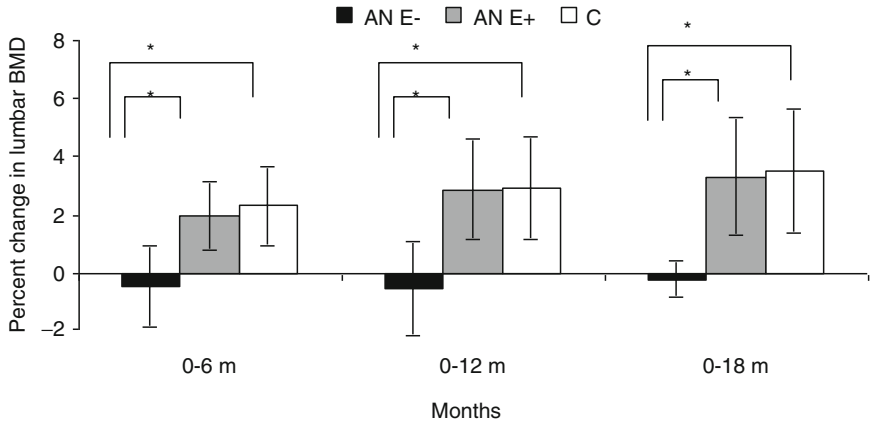

Fig. 35.3 Impact of physiologic estrogen replacement on bone density in adolescent girls with anorexia nervosa (AN). Girls with AN randomized to physiologic estrogen administration (AN-E+) had significant increases in bone density at the lumbar spine over 6, 12, and 18 months compared with those randomized to placebo (AN-E–), to approximate bone accrual rates observed in controls (C) (adjusted for baseline age and weight) (reprinted with permission from the J Bone Miner Metab. 26; 2430–2438; 2011. Copyright © by The American Society for Bone and Mineral Research, 2011)

A role for testosterone and dehydroepiandrosterone sulfate (DHEAS) in maintaining bone mass in hypogonadal women has been reported [54, 55] and testosterone values have been demonstrated to be lower in adolescent girls with AN as compared to healthy controls [25]. Like estrogen, the major effect of testosterone is a decrease in bone resorption; in fact, much of the action of testosterone occurs indirectly through its aromatization to estrogen. However, in addition to inhibiting osteoclast differentiation and increasing osteoclast apoptosis (effects similar to estrogen), testosterone also

affects osteoblast differentiation and proliferation. Consistent with these known effects of testosterone on bone, we noted a positive correlation between changes in levels of free testosterone and changes in markers of bone formation over a 1-year follow-up period in adolescent girls with AN in association with weight gain [25]. However, a recent randomized controlled trial of transdermal testosterone (in replacement doses) vs. placebo demonstrated that testosterone replacement was not effective in increasing bone density in adult women with AN after 12 months, despite a significant increase in lean mass in the group receiving testosterone [56]. Conversely, a recent study has reported maintenance of bone density *Z*-scores in adolescent girls and young adult women with AN following administration of oral DHEA with an oral estrogen-progesterone combination pill [57].

35.3.2 Undernutrition, Low Insulin-Like Growth Factor-I (IGF-I), and Growth Hormone (GH) Resistance

Undernutrition plays a key role in development of low bone mass in AN. Strong correlations have been noted between sensitive markers of nutritional status and bone density. Low body mass index [8, 21–23], decreased lean body mass [5, 24, 25], and poor caloric intake [58] are all associated with bone loss in AN, and lean body mass appears to be the major contributor to bone density, both in individuals with AN and in healthy controls [5, 24, 59]. We have reported correlations between changes in lean body mass over a 1-year period and changes in bone density [25]. The effect of lean body mass may relate to biomechanical forces exerted by muscle mass on developing bone. Bachrach et al. [60] demonstrated an improvement in bone density with weight recovery in girls with AN even before resumption of normal gonadal function, underscoring the critical role played by nutritional factors in bone loss in this disorder.

The acute profound effect of undernutrition on bone metabolism was demonstrated in a study by Grinspoon et al. [61] when acute fasting for 4 days led to a 50 % reduction in markers of bone formation in healthy young women. In adults, as well as adolescents with AN, markers of bone formation correlate strongly with markers of nutritional status such as BMI and percent body fat [30, 41], as well as with levels of IGF-I [25, 41]. Markers of bone resorption are elevated in AN in adults only [23, 30], and correlate negatively with BMI [23]. Nutritional rehabilitation in adolescent girls with AN is associated with an increase in bone formation markers [25, 62]. In contrast, bone resorption markers have been noted to decrease in one study [62] and increase in another [25].

A nutritional acquired hepatic growth hormone (GH) "resistance" has been described in adults and adolescents with AN [63–67], with low levels of IGF-I- [63–67] and GH-binding protein (GHBP) [63, 64] despite high levels of GH [63–67]. Weight recovery is associated with near-normalization of GH secretion [63] and an increase in IGF-I levels [30, 64, 68].

Both GH and IGF-I play an important role in bone formation [69]. IGF-I is also nutritionally regulated and thus serum levels are decreased in states of undernutrition including AN. GH directly stimulates proliferation of osteoblast precursors, and both directly and through IGF-I stimulates the differentiation of osteoblast precursors into active osteoblasts [69, 70]. The role of the GH-IGF-I axis on bone resorption is less clear. IGF-I also stimulates collagen synthesis by stimulation of the Type II IGF-I receptor on osteoblasts [71], and is necessary for longitudinal bone growth [72].

GH deficiency states are associated with low BMD, and BMD increases with physiological GH replacement [73]. Studies have demonstrated that IGF-I levels predict bone loss and bone turnover in adults and adolescents with AN [25, 30]. However, the positive association of GH levels with markers of bone turnover noted in normal-weight adolescents is not observed in adolescents with AN, suggestive of bone resistance to GH [65]. This is further corroborated by data from a randomized trial of supraphysiologic doses of recombinant human (rh) GH vs. placebo in adult women with AN, in which

5–6 times physiologic replacement doses of rhGH failed to increase IGF-1 or levels of bone formation markers [74].

Grinspoon et al. [30] and Misra et al. [75] reported that short-term rhIGF-I administration in replacement doses increases bone formation in adults and adolescents with AN without any effect on bone resorption. We have also shown that administration of rhIGF-I for 9 months increases bone density in adult women with AN only when co-administered with an oral contraceptive [47]. These data suggest that an effective therapy would optimally combine anabolic and anti-resorptive therapies.

35.3.3 Other Hormonal Factors

A number of other hormonal factors may contribute to low bone density in AN including high levels of cortisol, peptide YY (PYY), and adiponectin, and low levels of leptin, insulin, and amylin. Women and adolescents with AN have relatively higher cortisol values than normal-weight controls [67, 76–78], and hypercortisolemia is known to have multiple deleterious effects on bone. We have reported in both adolescents and adults with AN that higher cortisol levels are associated with lower levels of bone formation markers and with lower bone density [77, 78]. However, only 22 % of women with AN and severe bone loss had elevated cortisol values in one study [30], indicating that this is not the major contributor to bone loss in AN.

A reduction in levels of leptin may also play a role in bone metabolism in AN. Serum leptin is very low in individuals with the restrictive form of AN [79, 80], while levels are substantially higher in the subgroup of women with AN who also purge [80]. Peripheral leptin has a direct bone anabolic effect, particularly in appendicular bone [81, 82]. In contrast, central leptin negatively regulates bone density in the axial skeleton [82, 83]. Rh leptin administration in normal-weight women with hypothalamic amenorrhea increases levels of bone formation markers and lumbar bone mineral content, without increasing bone density [84, 85]. However, leptin administration is also associated with reductions in weight and fat mass [84, 85], a highly undesirable outcome in AN.

Other hormonal alterations that may contribute to low bone density in AN include increased levels of peptide YY (PYY) [86, 87] and adiponectin [88], and decreased levels of insulin [88], amylin [89], and oxytocin [90].

35.3.4 Calcium Metabolism

Most studies have not found an association between calcium intake and bone density in this disorder [4, 8, 59, 91]. Abrams et al. [92] performed calcium kinetic studies in adolescent girls with AN and demonstrated decreased calcium absorption and increased calcium excretion, suggesting an altered state of calcium metabolism. Castro et al. reported that a calcium intake of less than 600 mg per day was a significant predictor of bone loss in AN [22]. In a study of adolescent girls with AN and healthy adolescent girls [91], we showed that calcium intake was lower than the recommended daily allowance in 41 % girls with AN compared to 70 % of healthy controls, and vitamin D intake was lower than the recommended daily allowance in 23 % of girls with AN compared with 50 % controls. Thus, a higher proportion of healthy girls than girls with AN had less than recommended intake of calcium and vitamin D. Similarly, we found no significant difference in calcium or vitamin D intake between adults with AN and controls, although more patients with AN took calcium supplements [93]. In addition, supplementation of calcium and vitamin D did not improve bone density in adult women with AN [40].

Castro et al. [22] demonstrated that less than 3 h of physical activity per week was a risk factor for bone loss in AN, but other studies [8, 59] have not found an association of bone density and physical activity levels in this disorder.

35.4 Natural History of Bone Loss in Anorexia Nervosa and Effects of Weight Recovery

Studies have demonstrated that despite improvement in bone density with weight gain in AN, low bone density persists in a large proportion of women and adolescents [32, 60, 94–96]. We followed adult women with AN for a period of one and a half years, and demonstrated persistence of low bone density in half of all weight-recovered women at the end of the study [40]. In contrast, women with AN who did not recover weight had further decreases in bone density, suggesting that weight gain likely prevented further bone loss. In a group of 19 women with a past history of AN who had been weight recovered for 21 years or more, Hartman et al. [97] reported that femoral bone density was still significantly lower than in normals. Zipfel et al. [20] demonstrated an increase in BMD and *T* scores at the spine with weight recovery, with a decrease in prevalence of osteopenia from 35 to 13 % and of osteoporosis from 54 to 21 %. However, a large proportion of weight-recovered subjects continued to have low bone mass. Thus, the decrease in bone density is significant and often permanent in spite of weight recovery, and underscores the importance of early diagnosis and aggressive treatment of this disorder.

Studies in adolescents with AN likewise have failed to demonstrate a consistent improvement in bone mass with weight recovery. Bachrach et al. [60] found that half the girls with AN who were weight recovered still had BMD more than two standard deviations below the mean for age, though girls with weight recovery did demonstrate an improvement in lumbar and total BMD. Similarly, Jagielska et al. [6] prospectively studied 27 girls with AN, and demonstrated that while an initial decrease in BMD occurred in the first 7 months, sustained weight recovery in 11 subjects was associated with an improvement in lumbar and total BMD. We studied 19 adolescent girls with AN for a period of 1 year and found significantly lower bone densities in weight recovered AN compared to healthy controls at the end of the study period [25]. No increase in BMD occurred with weight recovery. However, weight recovered girls had significant increases in markers of bone formation and bone resorption over the study period as compared to controls, while non-recovered AN showed no such increase. In addition, an increase in a marker of bone formation (bone specific alkaline phosphatase) in the first 6 months of the study predicted an increase in lumbar bone mineral content over the next 6 months, suggesting that a longer period of weight recovery may be necessary to observe significant increases in bone density. In a subsequent 1-year prospective study of 34 adolescent girls with AN, we reported a significant decrease in bone density *Z*-scores over time in girls who did not gain weight or recover menses compared with 33 normal-weight controls, and a nonsignificant increase in BMD *Z*-scores in those who gained weight and recovered menses [96] (Fig. 35.4). Thus, a sustained period of weight recovery may be necessary before a definite increase in bone density can occur [20, 98].

35.5 Management of Bone Health in Low-Weight Eating Disorders

A decrease of one standard deviation in bone density results in a doubling of fracture risk. With the high prevalence of bone loss in girls and women suffering from eating disorders, monitoring bone density is of utmost importance in individuals suffering from such disorders, especially restrictive eating disorders. The increasing availability of dual-energy X-ray absorptiometry (DXA) has made this an accurate and readily available method of monitoring BMD without significant radiation exposure. Databases are available to obtain *Z*-scores by age and race in adolescent girls and after

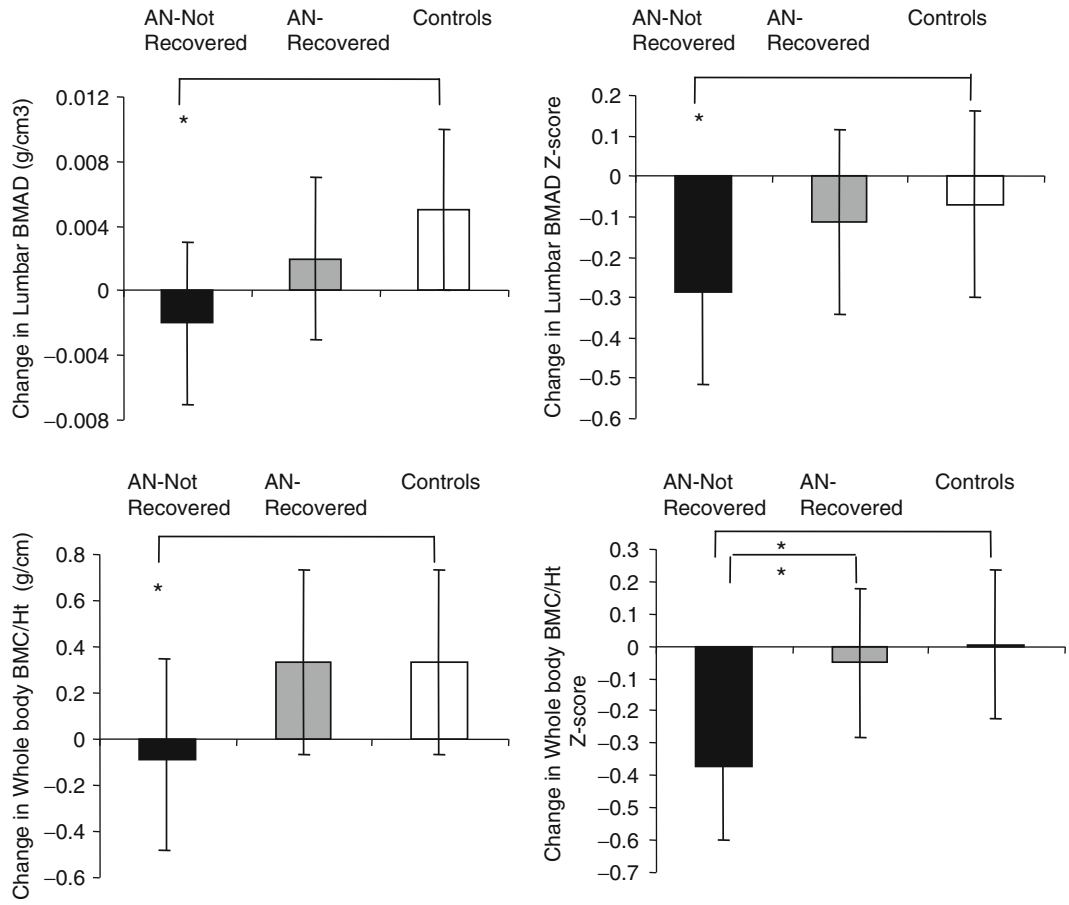

Fig. 35.4 Impact of weight gain and menses restoration on lumbar BMAD and whole-body BMC/height (and corresponding *Z*-scores) in adolescent girls with anorexia nervosa compared with controls. Girls who did not gain weight or regain menses (AN-not recovered) had significant decreases in bone density measures compared with controls, whereas girls with AN who gained weight and resumed menses had some improvement in bone density (reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2008;93(4):1231-7 0. Copyright © 2008 The Endocrine Society. All rights reserved)

controlling for height [19, 99]. A limitation of DXA readings is that it reports areal rather than volumetric BMD (bone mineral content/cross sectional area of bone in cm^2, rather than bone mineral content/volume of bone in cm^3). However, formulas are available to obtain an estimate of volumetric density or bone mineral apparent density based on the individual's height [100]. In children, adjustments based on maturity may be necessary when bone age is different from the chronological age.

While quantitative computed tomography (QCT) provides a direct estimate of volumetric bone density and size parameters (total and cortical cross-sectional area and cortical thickness), it is less reproducible and thus less useful than DXA in comparing bone densities over time. In addition, there is increased exposure to ionizing radiation by this method (particularly for QCT of the spine and hip), and it thus remains largely a research tool. However, QCT has been demonstrated to provide estimates of fracture risk in postmenopausal women above and beyond that provided by DXA measures of bone density [101, 102]. In addition, high-resolution peripheral QCT provides information regarding bone structure (including cortical porosity, and trabecular number, size, and separation) at peripheral sites such as the distal radius and tibia, while finite element analysis provides estimates of bone strength (such as stiffness and failure load). These methodologies remain research tools at this time.

Calcaneal ultrasound (CUS) is a noninvasive method for assessing bone density in adults. Its small size, low cost and the absence of ionizing radiation make it an attractive alternative to DEXA and QCT. The heel is made of cancellous bone similar to the spine, thus calcaneal bone density readings could act as a surrogate for lumbar spine bone mineral density. In adults, heel ultrasound measurements have been demonstrated to correlate with DEXA and predict fracture risk [103, 104]. Resch et al. [105] reported highly significant correlations between broadband ultrasound attenuation (BUA) and bone density at the spine and the hip in adult women with AN. Databases will need to be established for age before this technique can replace the more conventional methods of bone density assessment currently used for an adolescent population.

Markers of bone formation and bone resorption are important research tools in understanding the dynamics of bone loss in AN [106]. However, their clinical utility is limited by variations in these markers within groups and individuals and also by circadian and day-to-day variations. There might, however, exist a place for bone turnover markers in monitoring therapy for osteoporosis [107]. Variations in these markers with age, pubertal stage, growth velocity, and hormonal and nutritional status make them even less useful in a clinical setting in children and adolescents [108, 109].

35.5.1 Treatment

The optimal strategy to reverse bone loss in individuals with eating disorders is weight gain and resumption of menses. However, as described above, residual deficits may persist despite weight recovery. This is of particular concern in adolescent girls given the very narrow window of time to optimize bone accrual and subsequently peak bone mass. Because the underlying disease is difficult to treat and because residual bone deficits may be permanent, effective strategies to address this issue are critically needed. Calcium and vitamin D supplementation does not improve bone density in adults [40] or adolescents [25] with AN. Nevertheless, it is important to maintain an adequate intake of calcium and vitamin D in this population, and we recommend 1,300–1,500 mg of elemental calcium and 600–800 IU of vitamin D for all adolescents and adults with AN.

Birth control pills/oral estrogen-progesterone replacement when given alone has failed to improve bone density in AN in many studies [40, 47–50]. However, estrogen replacement as the transdermal estrogen patch (100 mcg 17-beta estradiol) with cyclic progesterone was successful in increasing bone accrual rates in adolescents with AN to approximate rates in normal-weight controls [31]. This is thus an effective strategy to maintain bone density *Z*-scores in girls with AN, although complete "catch-up" does not occur. Lack of "catch-up" is likely because of persistent abnormalities in other hormones such as IGF-1, cortisol and PYY. A randomized trial of transdermal estrogen has not been reported in adults with AN. Of note, although women with AN have lower levels of testosterone than controls, transdermal testosterone replacement was not effective in increasing bone density in adults with AN in a 12-month randomized placebo controlled trial [56].

We have shown that subcutaneous administration of rhIGF-I (30 mcd/kg b.i.d. for 7–9 days) increases levels of bone formation markers in adolescent girls with AN [75], and that subcutaneous administration of rhIGF-1 (30 mcd/kg b.i.d. for 9 months) improves bone density in adults with AN [47] when given in conjunction with a birth control pill (Fig. 35.5). Larger studies are ongoing to determine the therapeutic impact of combined use of rhIGF-1 with physiologic estrogen replacement.

Bisphosphonates inhibit bone loss by an inhibitory effect on osteoclasts and are highly effective in treating post-menopausal osteoporosis. Investigational studies of bisphosphonates include a study in adults with AN that showed a significant increase in bone density at the spine and hip following use of risedronate for a year [56] (Fig. 35.6), although a study in adolescents with AN failed to demonstrate a positive impact of alendronate on spine bone density [110]. This differential response to

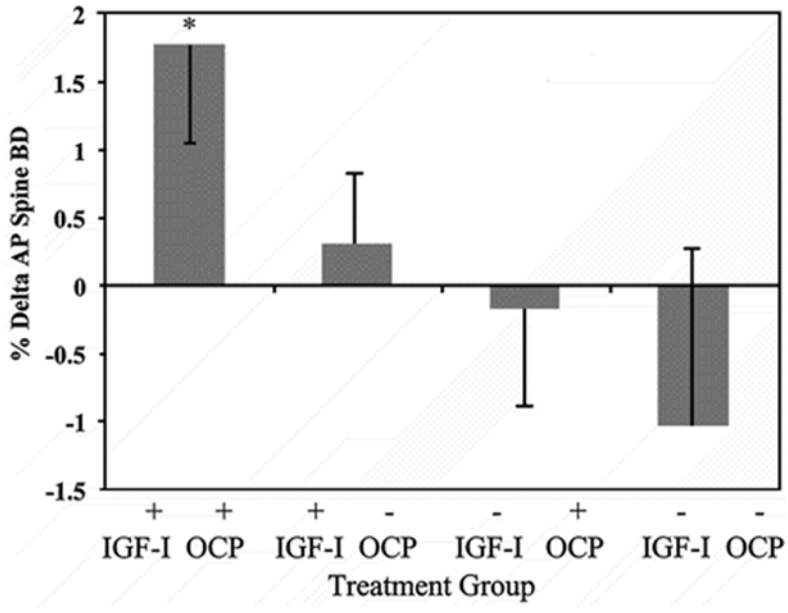

Fig. 35.5 Effect of rhIGF-I +/− OCP on BMD. Administration of recombinant human insulin-like growth factor-I (IGF-I) (30 mcg/kg b.i.d. subcutaneously) increased bone density in adult women with anorexia nervosa. The effect was most marked when IGF-I was given with a birth control pill (reprinted with permission from the Journal of Clinical Endocrinology and Metabolism, 2002;87:2888. Copyright © 1999 The Endocrine Society. All rights reserved)

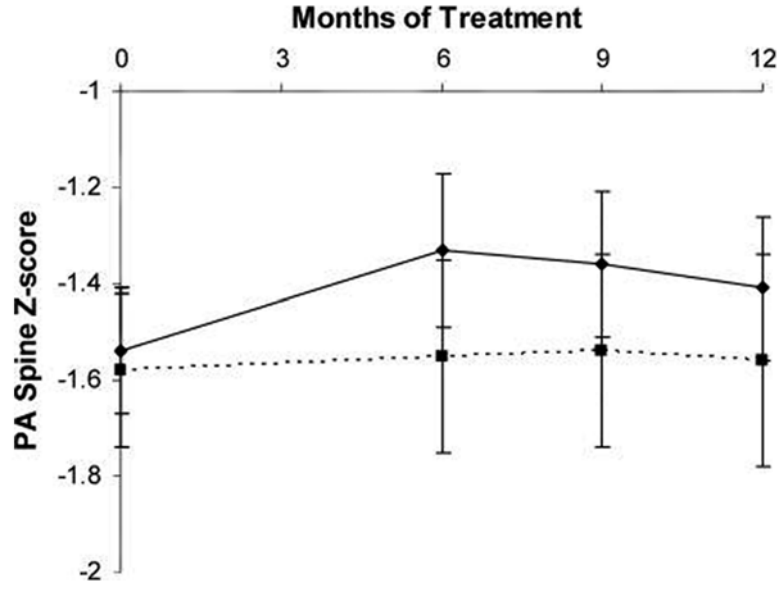

Fig. 35.6 PA spine BMD increased in women receiving risedronate (*solid line*) over a 12-month period compared with those receiving placebo (*dotted line*) ($P < 0.0001$). *Z*-scores are shown (reprinted with permission from the J Clin Endocrinol Metab. 2011 July; 96(7): 2081–2088. Copyright © 2011 The Endocrine Society. All rights reserved)

bisphosphonates in adults vs. adolescents may be consequent to increased bone resorption in adults with AN, but not in adolescents. Of importance, the long-term efficacy and safety of these agents in AN are currently unknown, and these agents should only be considered in severe osteoporosis after consultation with a specialist in metabolic bone disorders.

Teriparatide is now an approved intervention for treating postmenopausal osteoporosis, and a randomized placebo-controlled study in older women with AN has reported a significant improvement in bone density following 6 months of teriparatide use [111]. More studies are necessary to confirm these findings. Teriparatide currently has a black-box warning related to a possible increase in the risk of osteosarcoma in children with open epiphyses, individuals with unexplained elevations of ALP, those with Paget's disease, and individuals with a prior history of external beam radiation therapy or implant radiotherapy of the skeleton [112].

35.6 Conclusion

Bone loss is a common and serious complication of restrictive eating disorders, and it is important to monitor bone mineral density in individuals suffering from such disorders. The mechanism of bone loss is possibly multifactorial including hypogonadism, undernutrition, alterations of the GH-IGF-I axis, and, in some patients, hypercortisolism. DXA remains the most effective and reproducible method of monitoring bone density. Sustained weight recovery and adequate calcium and vitamin D supplementation are to be emphasized in all individuals suffering from low-weight eating disorders. The combination of bone anabolic and anti-resorptive therapy is the most promising therapy for bone loss in AN and is under investigation.

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Chapter 36
The Role of Nutrition for Bone Health in Cystic Fibrosis

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Key Points

- Cystic fibrosis (CF) is a common autosomal recessive genetic disorder that causes abnormal sodium and chloride transport due to mutations in the CF transmembrane conductance regulator (CFTR).
- Individuals with CF have altered osmolarity of body secretions, resulting in lung and gastrointestinal (GI) complications, lung infections, pancreatic insufficiency, maldigestion, and malabsorption.
- With improvements in medical care, life expectancy has increased and osteoporosis and osteopenia are increasingly recognized in those with CF.
- CF-related low bone mass is multifactorial and is influenced by nutritional status, disease severity, glucocorticoid use, hormonal status, inflammation, GI function, mechanical loading, and physical activity patterns.
- Nutritional intakes sufficient to acquire and maintain bone mass are essential to promote bone health, particularly among children with this disease as many long-term sequelae of CF accelerate bone loss in later life.
- Optimal calcium and vitamin D status can be impacted by alterations in gut integrity and fat malabsorption.
- The high-sodium intakes needed to maintain sodium balance may further limit calcium retention.
- Emphasis should be placed on maximizing bone acquisition early in life and on maintenance of appropriate body mass given the growth deficits that are often evident in those with CF.

Keywords Cystic fibrosis • Bone mass • Vitamin D • Calcium • Nutrition • Osteopenia • Osteoporosis • Nutrition • Fracture • CFTR

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36.1 Introduction

Cystic fibrosis (CF) is a common autosomal recessive genetic disorder in the USA and Europe although it also occurs globally [1–4]. It affects approximately 1 in 3,200, 1 in 9,200, and 1 in 15,000 live births among whites, Hispanics, and blacks in the USA, respectively [5, 6]. At present, approximately 30,000 individuals are affected by CF in the United States [7], and 80,000 are thought to be affected globally [8]. The disease results in abnormal sodium and chloride transport resulting from mutations in the CF transmembrane conductance regulator (CFTR) [9]. Although over 1,800 mutations of the CFTR gene have been identified, over 85 % of patients have at least one copy of the most common mutation, delta F508 (ΔF508) [7]. The large number of mutations in the CFTR gene contributes to the observed range of disease severity [10]. Mutations in the CFTR alter the osmolarity of body secretions, resulting in lung and gastrointestinal (GI) complications. Blocked bronchial airways, lung infections, and GI disturbances including pancreatic insufficiency, a common manifestation of CF, contribute to the maldigestion and malabsorption that are common among affected individuals.

Improvements in medical care have increased the median age of survival of individuals with CF to over 36 years of age, increasing from around 30 years of age in the last two decades. Neonatal screening for the disease has allowed for earlier access to treatment and has improved outcomes [7]. With an increasing life expectancy, secondary sequelae of the disease, including insufficient bone mass and increased risk of fracture [11], have become increasingly important to address to help maintain overall health and quality of life. Net loss of bone mass can result in osteopenia (generally defined in adults as a bone mineral density [BMD] 1–2.5 standard deviations, or "*T*-score", below the mean expected for a young adult) and osteoporosis (defined in adults as a BMD >2.5 standard deviations, or "*T*-score", below the mean expected for a young adult) [12]. In children, low bone mass is defined based on age-adjusted measures as a "*Z*-score" < −2 SD below the mean expected for a child of the same age [13]. The osteopenia observed in CF patients is multifactorial and is influenced by nutritional status, disease severity, glucocorticoid use, hormonal status, inflammation, GI function, mechanical loading, and physical activity patterns.

Nutritional intakes sufficient to acquire and maintain bone mass are essential to promote bone health in individuals with CF. This is particularly important in children with this disease, as many long-term sequelae of CF accelerate bone loss in later life. An average of 26 % of total body bone mineral is accrued within the 2-year period surrounding the period of peak bone mineral acquisition among healthy children [14], yet this is an age where clinical manifestations of CF may become increasingly apparent. Because of the magnitude of bone growth at this time, special emphasis should be placed on maximizing bone acquisition early in life, and preventative strategies for osteoporosis should be initiated in pediatric populations. In recognition of this problem, recommendations are now in place for caregivers to monitor bone health and to treat mitigating factors in children and adults with CF with compromised bone health [11, 15].

36.2 Metabolic Bone Disease in CF

Compromised bone health has been recognized as a consequence of CF for the last several decades, with increasing attention to its early identification and treatment. The most affected bone health occurs among patients with the worst disease severity [16], and may lead to kyphosis and fracture [11], thereby further affecting quality of life in these patients. Dual-energy X-ray absorptiometry (DXA) is the most common way of assessing bone mineral density or bone mineral content and forms the basis for diagnosing bone disease in this patient population. In adults (>20 years of age), BMD is compared to peak bone mass of a healthy reference group. Among children, defining compromised

bone mass is more challenging, generally requiring that their total body bone mineral content (BMC) be compared to a sex, age, and, ideally, height-matched reference group to generate a "*Z*-score," the number of standard deviation units beyond the mean for a healthy reference group. Serial DXA scans can be utilized to evaluate bone acquisition over time.

Bone turnover, or the balance between bone deposition and resorption, may be inferred using associated biomarkers [17], osteoclast precursor cells in circulation, which have been observed in excess during disease exacerbations [18], or stable calcium isotopic studies that estimate bone mineral deposition relative to resorption [19]. Available data suggest that bone formation may be lower in children with CF than their non-CF peers [19, 20], thereby limiting bone development during the formative pubertal years. During adulthood, bone resorption may be particularly excessive, especially during exacerbations of the disease [17, 18]. These findings are consistent with data from studies of bone density, which show less compromised bone density in childhood, increasingly compromised bone density during adolescence and into adulthood, and greatest bone deficits among those with the most severe disease.

36.2.1 Bone Mineral Density in Patients with CF

A variety of studies have shown compromised bone health in children and adults with CF, and these have been extensively reviewed [11, 15, 16, 20, 21]. In the USA, data from the national CF registry indicates that the prevalence of bone disease increases through late childhood and occurs in over 20 % of the adult population. Individual research studies have shown bone disease to occur in 40–70 % of the adult CF population [16], although such studies may be affected by sampling bias. Similarly, studies in children have also found low bone mass to occur. In pediatric groups interpretation of DXA data is challenging given the reduced amount of normative data on bone density and fracture in relation to age and pubertal status, and due to the variable definitions of low bone mass among children. Bone deficits are often reported during childhood [20, 22], with longitudinal data suggesting a loss of approximately 1 SD every 6–8 years from the age of 5 onwards [23]. A recent report summarized studies of bone health among children with CF and highlighted strategies for promotion of bone health in this group [20].

36.2.2 Causes for Osteoporosis and Osteopenia in CF Patients

There are a multitude of potential mechanisms for reduced BMD in patients with CF, some modifiable and some secondary to disease severity and treatment. These include a decrease in physical activity, exposure to glucocorticoids, hormonal imbalances, and malabsorption due to pancreatic insufficiency of nutrients that are critically important for bone health. However, ensuring proper nutrition can substantially benefit bone health. Subsequent sections will explore the role of appropriate growth in children and maintenance of body weight in adults (requiring sufficient energy from protein, fats, and carbohydrates) and specific micronutrients (vitamins and minerals) in optimizing bone health in CF.

36.3 Growth and Maintenance of Adequate Body Weight

To consolidate the skeleton and maintain a healthy body weight, optimal nutritional intakes are crucial. Body size is known to be a strong determinant of bone mass in adults, explaining approximately 50 % of the variance in bone mass among individuals at the population level (after accounting for

height) [24]. Maintaining optimal weight puts the appropriate loads on bone, maintains muscle mass, and allows participation in normal activity patterns, all of which benefit bone health.

Given the role of pancreatic insufficiency in reducing digestion and absorption of energy-rich fats, children and adults with CF are often shorter and thinner than their healthy peers. Landmark studies identified the link between nutritional status and survival in CF [25, 26], motivating the CF community to monitor and promote appropriate growth and weight maintenance. More recent longitudinal data support these findings, showing better growth, pulmonary health, and survival in children with better nutritional status at 4 years of age who were followed to adulthood [27]. The CF Foundation currently reports a median body mass index (BMI, kg/m^2) for children from 2 to 19 years at the 51.3rd percentile of the national reference data (http://www.cdc.gov/growthcharts/clinical_charts.htm), reaching the target of the 50th percentile and up from the 41st percentile a decade ago [7]. Median BMI in adults was 22.1 kg/m^2, approaching the goal of 23 kg/m^2 in males and 22 kg/m^2 in females. While BMI is a better measure of nutritional status than "ideal body weight", which was previously used, it does not capture relative deficits in height [28], which is an independent predictor of survival in CF [29]. Reduced height, as well as reduced lean body mass, is associated with lower total body bone mineral content in children with CF, linking compromised bone health with poor linear growth [30].

In children, being underweight can also delay the onset of puberty, further placing this group at risk for decreased BMD by delaying the puberty-associated increases in anabolic hormones, including sex steroids, like testosterone and estrogen, and insulin-like growth factor. Many studies have reported an approximate 2-year delay in puberty in adolescents with CF [31–34]. However, this has not been universally observed [27, 35], perhaps due to secular improvements in nutritional status.

Optimal nutritional intakes, supplementary feeds and parenteral intakes should all be utilized as needed to promote and maintain an adequate body weight in patients with CF, according to current recommendations [36]. Appropriate dietary intakes also provide the necessary nutrients required for the mineralization and maintenance of bone mass. Specific nutrients required for optimal bone mineralization include traditional nutrients linked to bone health such as calcium, phosphorus, magnesium, vitamin D, vitamin K, protein, and trace elements such as copper and zinc. The status of many nutrients integral to bone health is compromised in children and adults with CF which may further limit bone mineralization. At this time data on alterations in the physiology of these nutrients in individuals with cystic fibrosis are often lacking. Several of these nutrients are summarized here with respect to status and impact on bone health in CF patients.

36.4 Calcium

Calcium is the principal mineral in bone, comprising more than 30 % of bone mineral. The majority (>99 %) of the body's calcium content is located in bone. Because physiology is designed to maintain serum calcium and phosphorus concentrations at constant levels, this function will be maintained at the expense of bone. The ability to maintain and consolidate skeletal calcium stores is therefore dependent on optimal calcium intake, absorption of calcium from the GI tract, and sufficient retention of calcium to offset urinary, endogenous fecal, and dermal calcium losses. CF may adversely impact many of these pathways. Calcium status per se is difficult to evaluate, given the homeostatic control of this mineral in circulation. Tools for the direct assessment of calcium status include stable isotope studies—which allow for estimates of dietary calcium absorption, losses, and bone turnover—and serial DXA scans, where calcium content of bone is assumed to be ~30 % of total bone mineral content.

36.4.1 Calcium Absorption

Early studies assumed calcium absorption was reduced in patients with CF based on indirect evidence such as lower-than-expected urinary calcium excretion [37]. Aris et al. [38] evaluated calcium homeostasis in adults with CF by measuring the fractional absorption of ^{45}Ca and urinary excretion of calcium in CF patients and normal controls following a high-calcium breakfast. Seven young men and five young women were studied on two separate occasions with and without administration of pancreatic enzymes with 11 healthy young adults with normal BMD measurements as controls. Without pancreatic enzymes, subjects with CF showed significantly impaired calcium absorption (8.9 ± 0.2, compared to 11.8 ± 0.54 % in controls, $p = 0.02$). The addition of pancreatic enzymes did not fully compensate for this deficiency. A dual stable isotope study of calcium absorption was reported in a group of 23 clinically stable girls with CF (ages 7–18 years) [39]. In these children, fractional calcium absorption was similar to data reported in healthy girls matched for Tanner stage, averaging 27 %, 40 %, and 30 % in early, mid-, and late puberty, respectively [39]. More recent data from a dual stable isotope study in 7–13 year old children with CF did not show an impact of supplementation for 9 months with calcium (1 g/d), vitamin D (2,000 IU/d), or both, on fractional calcium absorption, which averaged over 35 % in each group [40]. Although relatively little data are available, studies suggest that calcium absorption is not impaired in children with CF, while in adulthood poor control of pancreatic insufficiency may compromise calcium absorption.

36.4.2 Calcium Losses

Data on the impact of CF on urinary calcium excretion have been conflicting, likely due to typical variability in calcium excretion across populations [37, 41–43]. Moreover, because children do not excrete calcium in proportion to intake as adults do, combining children and adults in studies of calcium excretion may obscure the ability to find correlates of calcium excretion. Several studies in patients with CF have suggested that there is an increased risk of Ca oxalate stones (cumulative incidence of 5.7 %) and oxalate crystalluria (4.2 %) [44–46]. This may be associated with enteric hyperoxaluria rather than excess urinary calcium, and low-oxalate, calcium-rich diets are advocated for this population [47].

Components of the diet influence urinary calcium excretion and may limit the amount of calcium available for bone deposition, the most important being dietary sodium. Individuals with CF are known to be susceptible to heat injury or illness owing to fluid imbalances [48, 49], and recommendations have been made to supplement CF patients generously with sodium chloride, particularly during the summer and in hot climates [50]. As in healthy adolescents [51, 52], dietary sodium is one of the strongest predictors of urinary calcium excretion in children with CF [39]. High dietary intakes of sodium may reduce the amount of calcium available for bone deposition [51], a finding that may have more of an adverse impact on bone health in groups already at risk for low bone mass.

Endogenous fecal losses of calcium occur when calcium of non-dietary sources is secreted into the GI tract from bile, pancreatic juices, or direct excretion into the GI lumen. In healthy adults and children, endogenous fecal calcium losses typically average 1.5 mg/kg/d, and these losses are only minimally affected by calcium intake [53–55]. Alterations in intestinal permeability, intestinal paracellular, or transcellular flux, and changes in GI calcium losses from pancreatic or biliary secretions may increase endogenous fecal calcium loses in patients with CF. One stable isotope study in children with CF has reported higher than expected endogenous fecal calcium [56]. Increased endogenous fecal calcium losses have also been reported in patients with chronic malabsorption syndromes

(severe Crohn's disease and protein-losing enteropathy resulting from intestinal lymphangiectasia) [57]. Intestinal permeability has been found to be increased in patients with CF [58], and duodenal outputs of calcium in individuals with chronic pancreatitis have been reported to be nearly doubled compared to healthy or diseased controls following saline, cholecystokinin, or secretin infusion [59–61]. Excessive fecal bile acid losses have also been demonstrated in patients with CF who have pancreatic insufficiency [62–64], which may also be a mechanism for calcium loss through the gastrointestinal tract.

Dermal losses of calcium also occur, accounting for a small proportion of obligate calcium losses daily. These have not been assessed in CF.

36.4.3 Bone Calcium Accretion and Turnover

In a small dual stable isotope study in prepubertal and pubertal girls with CF, bone calcium deposition was positively associated with net absorption of dietary calcium [19]. Net estimated gains in bone calcium were 103 ± 72 mg/d, 305 ± 69 mg/d, and 79 ± 162 mg/d for pre-, early, and late pubertal groups, respectively. Estimated by serial DXA scan, these girls retained an average 82 ± 65 mg/d of calcium in bone, compared to mean estimated calcium retention in healthy girls of 122 ± 60 mg/d [65].

Taken together, the relatively few studies closely examining calcium metabolism and bone deposition in CF support the notion that it is critical to optimize calcium intakes to allow for the greatest opportunity for calcium absorption and retention, and to overcome obligate losses that may be higher in those with CF than in healthy populations.

36.5 Vitamin D

Vitamin D plays essential roles in calcium homeostasis by increasing the efficiency of intestinal calcium absorption and influencing bone turnover by stimulating osteoblasts to induce the conversion of stem cell monocytes into mature osteoclasts [66]. Vitamin D can be produced endogenously from 7-dehydrocholesterol in the skin from UVB exposure, or may be derived from the diet, where it occurs primarily in fatty fish, egg yolks, and some mushrooms, or fortified foods like milk [67]. Regardless of the source, sunlight or ingestion, vitamin D in circulation is quickly hydroxylated in the liver to release 25-hydroxyvitamin D [25(OH)D], the major circulating form of the vitamin, which is measured to determine vitamin D status. To maintain optimal concentrations of circulating calcium and phosphorus, 25(OH)D is hydroxylated again in the kidney to form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D, or calcitriol], which then acts on cellular vitamin D receptors to promote a variety of calcemic functions. More recently, it has been recognized that tissues not involved in calcium metabolism may also locally produce 1,25$(OH)_2$D to promote autocrine and paracrine reactions unrelated to calcemic functions.

Because options for consuming vitamin D-rich foods are limited, and because the absorption of fat-soluble vitamins is inhibited by pancreatic insufficiency, use of supplements to maintain vitamin D status is essential in individuals with cystic fibrosis. Increased vitamin D status has been linked to bone health in those with cystic fibrosis [11], drawing increasing attention to the role of this vitamin among patients with CF.

36.5.1 Vitamin D Status in Patients with CF

There is firm evidence that vitamin D deficiency is common among patients who suffer from CF [11, 37, 68–73] and that multiple aspects of vitamin D metabolism may be adversely impacted by cystic fibrosis [74]. Vitamin D insufficiency may also be widespread in the general population, although definitions of deficiency and sufficiency for 25(OH)D are controversial [75, 76]. A recent case-control study showed poorer vitamin D status in children with CF who were maintaining their usual regimen of 800 IU per day vitamin D supplementation than in matched controls in the general population [77]. A recent Cystic Fibrosis Foundation consensus document developed evidence based recommendations for the diagnosis, treatment and management of vitamin D status in individuals with cystic fibrosis. To assess vitamin D status, serum 25-hydroxyvitamin D (25(OH)D) concentrations should be monitored annually, preferably at the end of winter when levels are at their nadir, with the goal of maintaining serum concentrations ≥30 ng/mL (75 nmol/L) [68]. Supplementation, preferably with vitamin D_3 (cholecalciferol) as opposed to vitamin D_2 (ergocalciferol), should be utilized to achieve and maintain these concentrations following current dosing and treatment recommendations [68]. Although treatment guidelines have been developed, there is still a considerable amount of trial and error in determining the optimal regimen for an individual, and frequent reassessment of status is required to assure the effectiveness of the prescribed regimen [68]. Exposure to sunlight or UV lamps has tended to be a less reliable way of improving vitamin D status [68], although expected seasonal variability has been observed in vitamin D status in the CF population, indicating that sunlight exposure can be an important means of enhancing vitamin D status in those with CF as in the general population [78]. Individuals with vitamin D deficiency that is refractory to supplementation should seek follow-up advice from a specialist with expertise in metabolic bone disease [68].

Adequate vitamin D status is also impacted by genetics. In particular, four genes involved in vitamin D metabolism (7-dehydrocholesterol reductase), transport (vitamin D binding protein) conversion (CYP2R1) and degradation (CYP24A1) of vitamin D contribute significantly to interindividual variability in vitamin D status [79, 80]. These genotypes have been identified in a Caucasian population but have not been explored in the context of CF specifically, although they may have the potential to affect the impact of supplementation strategies for individuals. Whether aspects of CFTR dysfunction unique to CF additionally affect vitamin D status or the metabolism of the vitamin by altering its absorption, production, utilization, losses, or functions at specific tissues is unknown, but is a ripe area for investigation.

36.5.2 Vitamin D Absorption

Suboptimal vitamin D status in the face of daily supplementation suggests that the vitamin may be malabsorbed, consistent with the frequent finding of fat malabsorption among pancreatic insufficient patients with CF. Patients with fat malabsorption syndromes such as celiac disease and biliary or pancreatic obstruction have been found to malabsorb oral vitamin D [81]. Studies in patients with CF specifically have also reported limited and highly variable absorption of oral doses of vitamin D_2 in particular [52], and even up to 400,000 IU of vitamin D_2 provided to patients over a 2 month period failed to substantially improve vitamin D status [82]. In children the impact of 50,000 IU vitamin D_2 given for a month had only a short-term impact on vitamin D status [83, 84]. A trial of calcitriol administration improved calcium absorption but did not affect vitamin D status itself [85]. Conversely, Hanly et al. [72] and, more recently, Stephenson et al. [86] have demonstrated the ability of vitamin D_3 to improve vitamin D status in CF patients, consistent with other reports [87, 88]. Thus, the current recommendation is for supplementation with vitamin D_3.

36.5.3 Vitamin D and Bone

While a variety of observational studies have linked vitamin D status to bone mineral content or density among those with CF [11, 20], less information is available on the impact of interventions with vitamin D_3, with or without calcium, on bone mineral density. Haworth et al. [89] studied the impact of supplementation with 1 g calcium and 800 IU daily in addition to the usual regimen of pancreatic enzymes and fat soluble vitamin supplements (900 IU/d vitamin D_3) for 12 months in a randomized, controlled trial in 30 adults with evidence of osteopenia ($n=15$ receiving supplemental calcium and vitamin D). They found non-significant improvements in BMD at the lumbar spine, hip, and distal forearm, and evidence of reduced bone turnover using bone specific alkaline phosphatase and cross-links as markers of bone acquisition and resorption, respectively. However, 25(OH)D status did not improve in the treatment group. Hillman et al. [40] provided 2,000 IU of vitamin D_3 with or without 1 g calcium to children aged 7–13 years for 6 months in a study with a crossover design, but no change was observed in 25(OH)D or bone parameters. It is likely that both studies were underpowered to find effects, and that the amount of vitamin D provided was insufficient to have an impact on status, given that from 800 to 2,000 IU of D_3 is now recommended for routine daily administration in those with CF beyond 10 years of age, and an upper daily limit of 10,000 IU is recommended as a maximum dose for those ages 18 years and above with the goal of maintaining 25(OH)D above 30 ng/mL [68]. At present, assuring appropriate status of vitamin D is emphasized but use of vitamin D as a treatment strategy for bone disease in CF was not advocated: in adult patients with severe bone disease, medical management with bisphosphonates may be necessary [90–93].

36.5.4 Vitamin D and Other Health Outcomes

Vitamin D is increasingly linked to other physiological processes; in particular, suboptimal vitamin D status may adversely impact lung function and inflammation and risk of infection [94, 95]. Indirect evidence of an infection-fighting role for vitamin D includes an inverse association of total IgG with serum 25(OH)D and total intake of vitamin D among nearly 900 CF patients, from 6 months to 66 years of age, after adjustment for other potentially confounding factors [96]. Additionally, serum 25(OH)D was also positively associated with lung function, as assessed by FEV1 (forced expiratory volume in 1 s). Pulmonary function and disease severity may impact motility and exercise, thereby affecting weight-bearing activities. A recent Cochrane review on the impact of vitamin D supplementation on respiratory outcomes and vitamin D toxicity was undertaken in those with cystic fibrosis, but existing data were insufficient to provide evidence for either vitamin D benefit or harm [97]. Vitamin D status has also been examined in relation to risk for CF-related diabetes, given a known role for vitamin D in pancreatic function [98]. Being vitamin D deficient nearly doubled the risk of having CF-related diabetes among Scandinavian children and adults with CF, and was associated with elevated glycosylated hemoglobin [99]. As data on the importance of vitamin D status in multiple aspects of the disease process in CF increases, this will allow for better understanding of the interrelated nature of bone health, muscle function, inflammation, and pulmonary infection risk.

36.6 Vitamin K

Vitamin K is essential for bone health, as this vitamin mediates the carboxylation of glutamyl residues on bone proteins, including osteocalcin, the most abundant noncollagenous protein in bone. Vitamin K is also needed for the carboxylation of the glutamyl residues on prothrombin. Because this is also a fat-soluble vitamin, its status may be compromised due to the fat malabsorption that occurs in

CF. An increased prevalence of vitamin K deficiency (as determined by prothrombin in vitamin K absence levels [PIVKA-II]) has been found in unsupplemented patients with CF [100]. Supplementation studies have found that vitamin K supplementation (with an average of 0.18 mg/d) improved measures of vitamin K status as evidenced by changes in PIVKA-II levels [101]. In addition, 4 weeks of vitamin K supplementation (5 mg/wk) increased the carboxylation state of osteocalcin in 18 patients with CF [102]. Higher levels of vitamin K than the 0.3–0.5 mg/d typically provided may be needed to fully replete children and young adults with CF based on data in pancreatic insufficient CF patients where only those taking high-dose vitamin K (>1 mg/d) achieved vitamin K status similar to that observed in the control group that did not have cystic fibrosis [103]. Few studies to date have related vitamin K status to measures of bone health in CF populations. A recent Cochrane review was undertaken to assess the efficacy of vitamin K supplementation in children and adults with cystic fibrosis [104]. Only two trials were included in the review; while both studies found an improvement in vitamin K status following supplementation, neither addressed indices of bone formation [104].

36.7 Zinc

Zinc is crucial for optimal skeletal maturation, growth, and development [105, 106]. Zinc also regulates gene expression [107], and is an essential cofactor for bone-related enzymes such as alkaline phosphatase [105]. Numerous case-report studies of zinc deficiency have been reported in infants and children with CF. These cases often present as acrodermatitis enteropathica-like rashes [108–112].

Zinc assessment is difficult due to the lack of sensitive biomarkers and the often non-specific signs of zinc deficiency; this provides challenges for assessment and dietary recommendations in those with cystic fibrosis. Studies to date have found that pancreatic-insufficient children with CF (ages 7–17 years) absorb significantly less dietary zinc in the absence of pancreatic enzymes, presumably because of undigested dietary fat or protein [113]. Moreover, pancreatic enzyme replacement therapy (>2 weeks) significantly increased plasma zinc levels compared to infants with CF who had not received enzyme replacement therapy [114]. Infants who did not receive enzyme replacement therapy had a 29 % prevalence of deficient plasma zinc concentrations (≤9.2 μmol/L) compared to a prevalence of 7 % in infants receiving pancreatic enzymes [115].

Zinc deficiency in patients with CF may be related in part to inadequate dietary intake, poor absorption of dietary zinc and in particular to increased endogenous fecal zinc secretion [47], a finding similar to that observed for increased endogenous fecal calcium losses. Stable isotope studies of endogenous fecal zinc secretion in breast- and formula-fed infants with CF found negative zinc balances in both groups of infants and substantially greater endogenous fecal zinc losses than those typically reported in breast-fed or formula-fed infants. Endogenous fecal zinc losses in CF infants were also correlated to fecal fat excretion [114, 115]. Because of the many bone-related enzymes and other physiological processes in the body that are dependent on zinc and the likelihood that that gastrointestinal effect of cystic fibrosis have an impact on balance of this mineral, more data are needed on the impact of zinc insufficiency in relation to bone acquisition and homeostasis in those with cystic fibrosis.

36.8 Copper

Copper deficiency can affect bone health, due in part to the essential role copper has in the enzyme involved in lysine and hydroxyproline crosslinking in collagen (lysy1 oxidase) [116, 117]. Copper also stimulates human mesenchymal stem cell differentiation toward the osteogenic lineage [118]. Case-control studies have found alterations in copper distribution and significantly lower copper–zinc superoxide dismutase

activity in mononuclear and polymorphonuclear cells in CF patients [119]. Decreased activity of copper-dependent enzymes, suggestive of abnormal copper homeostasis, has also been reported in pancreatic-insufficient adolescents with CF [120], and more recently in a group of 38 adults and children with cystic fibrosis [121]. In the latter study, 3 mg of Cu supplementation per day for 6 weeks failed to improve copper enzyme activity of erythrocyte superoxide dismutase and plasma diamine oxidase [121].

36.9 Magnesium

Magnesium is necessary for optimal bone health and most magnesium in the body is located in muscle and bone [122], but little data are available on magnesium homeostasis and bone health in individuals with cystic fibrosis. Oral magnesium supplementation (300 mg/d for 8 weeks) has been noted to improve respiratory musculature in children with cystic fibrosis [123]. Impaired magnesium status may impact bone mass via its association with hypocalcemia and impaired secretion of parathyroid hormone [124]. Data in children, however, have indicated that magnesium balance may be negative even among healthy children [125], and recent data in 63, healthy 4–8-year-old children found that the amount of this mineral that is ingested and absorbed is positively associated with pediatric bone mass [126]. Increased attention to magnesium metabolism in relation to bone mass is warranted in those with cystic fibrosis as status of magnesium has been found to be compromised as a result of CF itself [127] and by other CF-related treatments such as prolonged use of aminoglycoside antibiotics [128, 129].

36.10 Phosphorus

The majority of phosphorus in the body is located in bone as an essential component of hydroxyapatite [130]. Deficiency of this mineral can lead to rickets in children or osteomalacia in adults. For many years it was assumed that phosphorus homeostasis was largely controlled by the same hormones as those used to regulate calcium homeostasis. With the discovery of the phosphatonin, fibroblast growth factor 23 (FGF23) [131], it is now known that FGF23 synthesis by osteoblasts/osteocytes in bone regulates phosphate metabolism and has direct effects on vitamin D metabolism [132]. Elevations in phosphorus and calcitriol stimulate transcription, translation, and synthesis of FGF23 which in turn increases urinary phosphorus excretion and inhibits renal calcitriol synthesis by inhibiting the renal 1-α hydroxylase (CYP27B1) and stimulating the renal 24-hydroxylase enzyme (CYP24A1). Growth retardation is a common feature of hypophosphatemia [133], but little is known on how cystic fibrosis may impact overall phosphorus homeostasis and how the alterations in vitamin D homeostasis observed in those with cystic fibrosis may also impact regulation of phosphorus balance. Iron status and hypoxia are now also known to be key regulators of FGF23 [131], and both iron deficiency [134], and hypoxia [135], are common among those with CF. Multidisciplinary studies are needed to obtain data how anemia and lung disease and other CF related disease processes may impact FGF23, phosphorus homeostasis and bone metabolism.

36.11 Nutrition, Genetics, and Disease Severity

Additional information on the impact of CF on metabolism of nutrients required for optimal bone mineralization is needed in order to target interventions to maximize nutrient retention and bone health in this population. It is also important to consider that osteoporosis can occur even in the face

of adequate nutrition because of the strong genetic control of peak bone mass. Several genotypes significantly influence bone mass, including those for the vitamin D receptor, calcitonin receptor, parathyroid hormone receptor, and estrogen receptor [136], and genome-wide association studies are increasingly utilized to identify genetic determinants of bone mineral density and risk of osteoporosis or osteoporotic fractures [137]. In addition to genetic control of bone acquisition, genotype–phenotype relationships may also influence the severity and clinical implications of diseases such as cystic fibrosis. A North American CF Gene Modifier Consortium has been initiated to better identify modifiers of lung disease severity and other phenotypes in those with cystic fibrosis [138]. Better identification of genotypes that may be predictive of low bone mass (both related and unrelated to the CF disease process) will assist in targeting preventative therapies to those at greatest risk for low bone mass or osteoporosis.

36.12 Conclusion

With increased life expectancy among those with CF, more attention needs to be placed on optimal nutrition in support of bone health. Individuals with CF are at risk for osteopenia and osteoporosis which can lead to increased risk of non-traumatic fractures and adversely impact the quality of life. Although there are many potential causes for metabolic bone disease in patients with CF, early counseling should emphasize the importance of maintaining an optimal body weight and nutritional counseling should be provided if goals for appropriate BMI are not met. It is also essential to ensure that patients with CF consume appropriate amounts of calcium and vitamin D. Calcium intakes should, at a minimum, meet the recent adequate intake recommendations for this nutrient. Moreover, it is essential to monitor vitamin D status and correct vitamin D deficiency when present following recent guidelines [68]. Many other nutrients are known to be integral to bone health yet little is known about the impact of CF on the metabolism of these nutrients and how deficits in these nutrients further impact bone mass. Additional studies are needed to assess possible deficits in phosphorus, magnesium, zinc, copper, and vitamin K as these nutrients are required for optimal bone physiology.

Finally, it is essential that interventions designed to promote bone health in patients with CF be initiated as early as possible and particular attention is needed during the pubertal growth spurt when much of peak bone mass is obtained. Nutritional counseling and additional interventions as needed in this age group will assist in the attainment of maximal bone accrual during the pubertal growth spurt, thereby promoting bone health and improved quality of life for patients with CF. Treatment regimens and identification of those at greatest risk will continue to evolve as more information is obtained on the genetics of bone acquisition and on the function of CFTR in bone physiology.

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Chapter 37
Celiac Disease and Bone Health

Armin Alaedini

Key Points

- Celiac disease is an autoimmune disorder with genetic and environmental components. The prevalence of the disease is about 1 % in many parts of the world. It is characterized by innate and adaptive immune responses that are primarily triggered by the ingestion of dietary gluten, resulting in inflammation, villous atrophy, and crypt hyperplasia in the small intestine.
- Genes that code for human leukocyte antigens (HLA) DQ2 and DQ8 are strongly associated with and confer susceptibility for celiac disease.
- In addition to the characteristic intestinal symptoms, celiac disease is associated with extra-intestinal complications, including those affecting skeletal health. Reduction in bone mineral density and increased risk of bone fracture, caused by malabsorption-related alteration of calcium metabolism and immune-mediated mechanisms, are frequently seen in patients with celiac disease.
- The diagnosis of celiac disease relies on serologic testing and intestinal biopsy. Bone density evaluation is recommended within 1 year of diagnosis and treatment.
- Gluten-free diet is the recommended treatment for celiac disease, usually resulting in the gradual recovery of intestinal mucosa, which leads to elimination of calcium and vitamin deficiencies and progression towards normalization of bone density.

Keywords Celiac disease • Osteoporosis • Bone health • Malabsorption • Calcium • Vitamin D • Hyperparathyroidism • Gluten-free diet

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37.1 Introduction

Once considered to be a rare childhood enteropathy, celiac disease is now recognized as a common and complex autoimmune disorder that can arise at any age and may affect multiple organs [1]. In addition to intestinal symptoms, celiac disease is associated with various extra-intestinal complications, including bone and skin disease, anemia, endocrine disorders, and neurologic deficits [2]. Some of the associated complications of celiac disease can be linked to the characteristic mucosal lesion or the subsequent malabsorption that leads to nutrient deficiency. Others are thought to be due to common genetic background, most likely linked to the HLA region of chromosome 6, and other immune-related factors [3]. Recognition of the relationship between celiac disease and abnormalities in calcium metabolism and bone health dates back at least to the 1960s [4]. Reduced bone density and bone derangement are now recognized to be among the most common extra-intestinal complications found in newly diagnosed celiac disease patients. The latest information on the prevalence, pathogenic mechanism, clinical presentation, diagnosis, and recommendations for treatment of celiac disease in the context of bone health is reviewed here.

37.2 Prevalence and Genetics of Celiac Disease

Celiac disease is now estimated to affect approximately 1 % of the population in the United States and many other countries [5]. In addition to increased awareness about the condition, which has contributed to higher rates of diagnosis, the actual prevalence of celiac disease appears to be on the rise in the past few decades [6, 7]. Population-based studies indicate that the majority of celiac disease patients remain undiagnosed [8]. Hereditary susceptibility is closely associated with genes for specific class II human leukocyte antigens (HLA), DQ2 (DQA1 *05/DQB1 02) and DQ8 (DQA1 *0301/DQB1 *0302) [9]. The specific HLA molecules on antigen-presenting cells confer susceptibility for celiac disease by their ability to present specific immunogenic gluten peptides to gluten-specific T cells in the small intestine. An increasing number of non-HLA genes are also found to contribute to the genetic risk for celiac disease, although their significance and relevance remain to be characterized in more detail [10–12].

37.3 Clinical Presentation

Celiac disease can develop at any age and its phenotypic expression is highly variable. In adults, “symptomatic” or “classical” cases of the disease may present with chronic diarrhea, abdominal distention and pain, weakness, and malabsorption [13]. Diagnosis is more commonly made in women, although blood screening indicates that approximately equal numbers of males and females are affected [14, 15]. In very young children, the disease is often characterized by diarrhea, abdominal distension, and failure to thrive, while older children are more likely to present with pain, vomiting, constipation, and anemia [1]. In contrast to the typical form, in the now increasingly encountered “atypical” from of celiac disease, gastrointestinal symptoms may be lacking or less pronounced, while patients present more prominently with extra-intestinal features. These may include osteoporosis, short stature, anemia, infertility, and neurological problems, among others [16–46]. Because atypical presentations are now found to predominate, celiac disease is considered to resemble a multisystem disorder, rather than only a gastrointestinal one [47, 48].

37.4 Pathogenic Mechanism of Celiac Disease

Glutens are the major storage proteins of wheat and related cereals, comprising over 70 different molecules in any given wheat variety [49]. The main classes of gluten include α/β-gliadins, γ-gliadins, ω-gliadins, high molecular weight glutenins, and low molecular weight glutenins [50]. Because of their characteristic sequences of constituent amino acids, gluten proteins are not fully digested by the action of gastric, intestinal, and pancreatic enzymes. Instead they are broken into a number of relatively long peptides with strong immunogenic potential. It has been postulated that certain stress factors, including gastrointestinal infection, can lead to changes in intestinal permeability that allow the gluten peptides to enter the mucosa [2, 51]. The peptides are incidentally excellent substrates for the enzyme transglutaminase 2 (TG2). TG2 readily converts the neutral glutamine residues of gluten proteins, at specific sites, into negatively charged glutamic acid through deamidation [52]. The deamidated gluten peptides display increased affinity towards antigen presenting cells that express the HLA-DQ2 and -DQ8 molecules. Binding of the generated immunogenic peptides to these HLA molecules results in complexes that can subsequently activate gluten-specific CD4 T cells in the lamina propria [52]. In turn, these T cells can provide help to and activate gluten-specific B cells, the eventual result of which is the production of antibodies against gluten. In addition to anti-gluten antibodies, generation of antibodies against TG2 is a hallmark of celiac disease, which is believed to take place through the process of intermolecular help, as TG2-specific T cells have not been found yet [53]. Activation of T cells and generation of antibodies is accompanied by the release of immune complexes and various cytokines that result in the promotion of inflammation and villous damage in the small intestine [2, 50].

In addition to the central role of gluten-specific CD4 T cells in celiac disease, the innate immune response and intraepithelial lymphocytes are believed to be essential mediators of the characteristic mucosal damage. Gluten has been shown to induce the expression of IL-15 cytokine and nonclassical MHC class I ligands MIC and HLA-E by stressed epithelial cells in the small intestine [50, 54]. In turn, this can activate nonspecific intraepithelial CD8 T cells expressing the natural killer receptor NKG2D. The interaction of these lymphocytes with epithelial cells expressing the stress-induced MIC and HLA-E molecules results in the release of IFN-γ and cytotoxic molecules that contribute to epithelial cell damage [50]. A recent study indicates that, in addition to gluten proteins, the non-gluten α-amylase/protease inhibitors of wheat are strong activators of the innate immune response through the engagement of the TLR4-MD2-CD14 complex, which might have implications for the pathogenesis of celiac disease [55].

37.5 Effect of Celiac Disease on Bone Health

One of the most common complications of celiac disease is reduced bone mineral density (BMD), which affects as much as two-thirds of all patients at diagnosis [56, 57]. The prevalence of osteoporosis, based on pooled results from several studies using dual-energy X-ray absorptiometry (DXA), has been estimated as 28 % at the spine and 15 % at the hip in newly diagnosed patients [56]. Male and female patients appear to be at equal risk for osteoporosis, while postmenopausal women are at greater risk [56]. A number of studies have examined the prevalence of bone fractures among celiac disease patients. A single cross-sectional study of 165 patients with established celiac disease found 41 % to have a history of fractures, compared with 8 % of age- and gender-matched control subjects [58]. Eighty percent of fractures in the patient group were detected before the diagnosis of celiac disease or in patients who were not compliant with a gluten-free diet, whereas only 7 % of diagnosed and

diet-compliant patients experienced fractures [58]. An epidemiological study from Sweden, based on data from 13,000 patients, found that individuals with celiac disease, including children, are at increased risk of fracture, with incidence ratios for hip fracture being around two both prior to diagnosis of celiac disease and afterwards [59]. A meta-analysis found considerable heterogeneity among the various studies that have examined the topic, but confirmed a significant association between bone fractures and celiac disease [60].

The effect of celiac disease on bones may also have an adverse effect on growth in childhood and adolescence. Children with celiac disease appear to be shorter than matched controls [61]. A cross-sectional study found that men with celiac disease are shorter than men in the general population [62]. The malabsorption caused by intestinal damage and the ensuing malnutrition in celiac disease are thought to be the main drivers behind the observed shorter stature in patients. In addition, a dysfunction of the endocrine growth axis, possibly brought on by the celiac-associated malnutrition, has been proposed as a contributing element. The insulin-like growth factor (IGF) system is involved in promotion of cell proliferation and is crucial for growth. Expression of IGF-1, which is required for achieving maximal growth, and of IGF-2, required for early development, are both found to be decreased in celiac disease [63, 64].

It should be mentioned that Turner syndrome, a genetic disorder associated with celiac disease, can also be a cause of abnormal skeletal development in some patients. Approximately 6 % of individuals with Turner syndrome may have celiac disease [43]. Because of estrogen deficiency, low BMD, osteoporosis, and increased risk of fractures are closely associated with Turner syndrome [65]. In addition, short stature is a prominent feature of Turner syndrome [65].

37.6 Causes of Bone Loss in Celiac Disease

The etiology of bone loss in celiac disease is thought to be multifactorial. The major underlying cause is considered to be malabsorption, which is a well-recognized and established product of the characteristic villous damage in celiac disease. The loss of functional intestinal mucosa leads to an interruption in the uptake of calcium, as well as vitamins D and K, all of which are involved in the regulation of calcium homeostasis in the body. Calcium uptake takes place through both paracellular (passive) and transcellular (active) pathways [66]. Its absorption in the intestine is controlled by the action of bioactive vitamin D (1,25-dihydroxyvitamin D) on vitamin D receptors [67]. Therefore, in addition to the interruption of direct calcium absorption that is brought on by the villous damage in celiac disease, reduction in intestinal vitamin D absorption is another major factor that negatively affects calcium uptake. Some studies point to less than optimal vitamin D levels in many celiac patients. A recent retrospective cross-sectional study of 530 patients with celiac disease found almost 60 % to have vitamin D deficiency (<20 ng/mL) or insufficiency (20–29 ng/mL) [68]. The decreased levels of calcium and 1,25-dihydroxyvitamin D can trigger excessive secretion of parathyroid hormone (PTH), leading to secondary hyperparathyroidism. The ensuing elevation in PTH levels results in increased osteoclast activity, which ultimately enhances bone resorption to release skeletal calcium [69]. There is also evidence to indicate that vitamin K is involved in calcium metabolism and affects bone mineral density and bone accrual [70]. It is essential for the carboxylation of several proteins, including the bone matrix protein osteocalcin, which is involved in osteoblast differentiation [71]. A study of 54 children and adolescents with celiac disease found a quarter to have suboptimal vitamin K status at diagnosis [72].

In addition to malabsorption, secondary lactose intolerance, which appears to be present in many patients with celiac disease, may further exacerbate the problem by interfering with the consumption of calcium-rich and vitamin D-fortified dairy products by affected individuals [73].

The reduction in bone mass can also be a product of inflammatory changes in celiac disease. It has been proposed that cytokine expression changes that directly affect osteoclastogenesis and osteoblast activity are involved. Fornari et al. reported increased serum levels of IL-6 in untreated

celiac disease patients to correlate with decreased BMD [74]. Taranta et al. found the N-terminal telopeptide of procollagen type I and IL-6 to be elevated in affected patients, while IL-12 and IL-18 were reduced [75]. In addition, the ratio of receptor activator of nuclear factor-κB ligand (RANKL) to osteoprotegerin (OPG) (both proteins are implicated in the regulation of bone turnover) appears to be increased in affected patients [75]. Increased RANKL/OPG ratio is a marker of upregulation of osteoclastogenesis [76].

37.7 Testing for Celiac Disease

The definitive diagnosis of celiac disease requires serologic tests and duodenal biopsy, as well as clinical or histologic response to gluten-free diet. While highly sensitive and specific markers of celiac disease are now available and have become an essential component of diagnosis, intestinal biopsy continues to be widely recognized as the diagnostic gold standard. The summarized celiac disease diagnostic algorithm in Fig. 37.1 is based on the recommendations of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) [77] and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) [78] for the diagnosis of celiac disease.

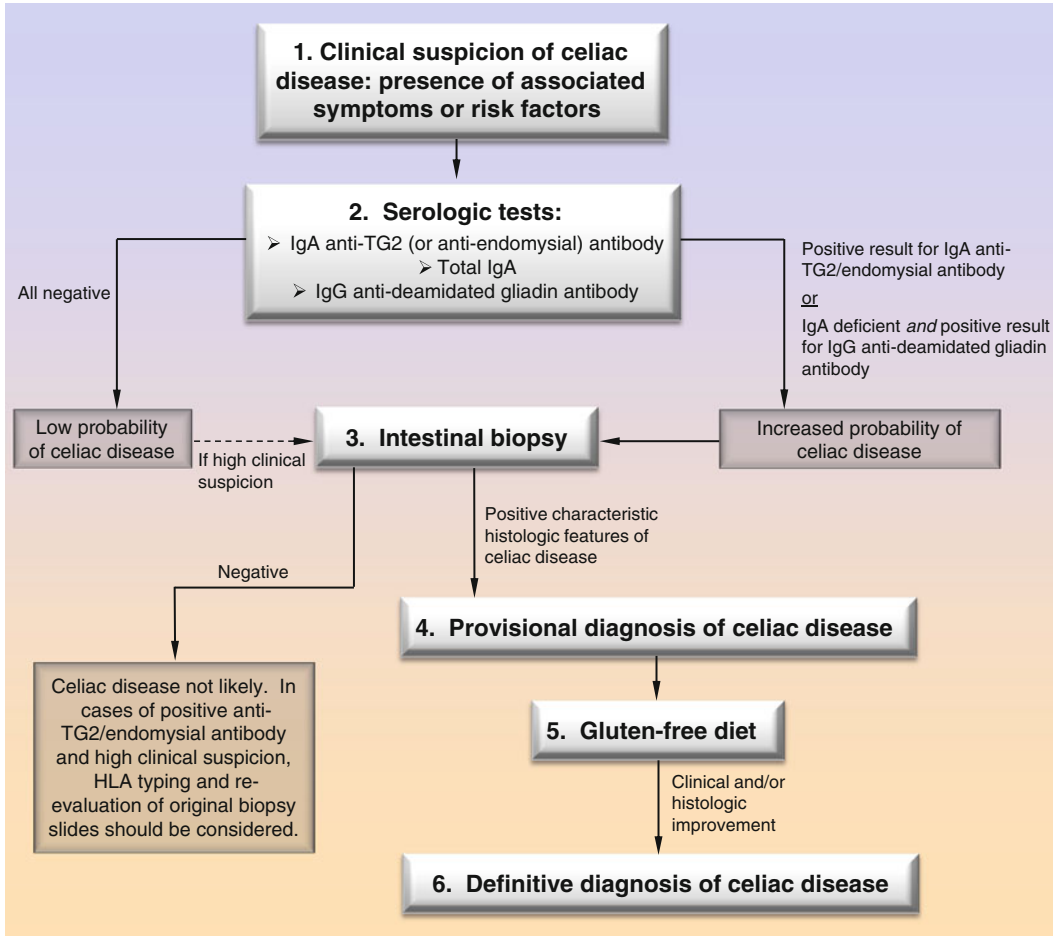

Fig. 37.1 Suggested algorithm for evaluation of patients suspected of having celiac disease

37.7.1 Serologic Tests

Celiac disease is usually suspected either due to the presence of the characteristic gastrointestinal symptoms, or because the patient belongs to an at-risk group. Individuals in the at-risk group include (1) those with celiac disease-associated conditions, including osteoporosis, and (2) first- and second-degree relatives of celiac patients. Once celiac disease is suspected, the patient should be tested for the associated serologic markers. Indications for celiac disease serologic testing in individuals with low BMD or history of fractures include low urinary calcium level, vitamin D insufficiency or deficiency, and elevated parathyroid hormone in the presence of normal calcium and vitamin D intake.

Currently, the most sensitive and specific serologic marker of celiac disease is the IgA anti-TG2 (or anti-endomysial) antibody [79]. Both the anti-TG2 antibody enzyme-linked immunosorbent assay (ELISA) and the anti-endomysial antibody immunofluorescence assay detect antibodies to the TG2 autoantigen [2]. The IgA anti-TG2/endomysial antibody tests have a sensitivity and specificity of over 90 % and are recommended for initial screening [80]. Because IgA deficiency is increased among patients with celiac disease [13], care should be taken in interpreting the results of IgA antibody tests. In the case of IgA deficiency, as determined by testing for total IgA, measurement of IgG antibodies to deamidated gliadin may be substituted [81]. While not as specific or sensitive as anti-TG2 antibodies, antibody reactivity to deamidated gliadin can increase the overall sensitivity of serologic testing for celiac disease and is especially useful in cases of IgA deficiency [82]. Antibody reactivity to native gliadin, while historically considered to be an important marker and a hallmark of celiac disease, has insufficient sensitivity and specificity in comparison to IgA anti-TG2 antibody and is generally not recommended for diagnostic purposes [80].

37.7.2 Intestinal Biopsy

A positive result for IgA anti-TG2/endomysial antibody, or IgG anti-deamidated gliadin antibody in case of IgA deficiency, is followed by intestinal biopsy. A biopsy can also be done in cases of negative serology but high clinical suspicion (Fig. 37.1) [2]. The characteristic histologic features of celiac disease range from near-normal villous architecture with increased intraepithelial lymphocytosis to total villous atrophy [83]. Positive identification of these abnormalities leads to a presumptive diagnosis of celiac disease, which should be followed by institution of a gluten-free diet. Because the associated histologic changes are not specific for celiac disease, definitive diagnosis can be made only after improvement in response to diet has been documented [3, 13]. A second biopsy to confirm histologic improvement is not necessary, except in cases where the clinical symptoms of celiac disease are not present [3].

If the results of serology and histology are not in agreement, it can be helpful to perform HLA typing [84]. Because nearly all celiac patients carry the HLA-DQ2 and/or HLA-DQ8 alleles, the absence of both markers has a very high negative predictive value, helping to rule out the disease in cases of equivocal biopsy results [80]. It should be noted, however, that because approximately 25–40 % of the general population also have these particular HLA genes, the presence of these markers by themselves does not predict celiac disease.

Most practice guidelines and position statements recommend routine testing of all celiac disease patients for nutritional status, including vitamin D, vitamin K, and calcium levels, as well as ferritin, folate, copper, thiamin, albumin, and vitamins B12, B6, A, and E [57, 78, 80]. Testing can be repeated every 6 months after the start of gluten-free diet to monitor normalization [57]. Bone density evaluation is recommended within 1 year of diagnosis and treatment of celiac disease [57, 78, 80].

37.8 Treatment

Lifelong strict exclusion of gluten-containing foods from diet is the only widely accepted treatment for celiac disease and celiac disease-associated osteoporosis [56, 85]. Patients are advised to avoid wheat and related cereals, including rye and barley, as well as any derivatives that contain gluten [85]. Intestinal symptoms generally improve within days to weeks after the initiation of gluten-free diet, although full mucosal recovery may take much longer [86]. Titers of antibodies to TG2 and gliadin usually decline rapidly following the elimination of gluten, but may require many months to become negative [87]. Most studies of BMD in celiac disease patients indicate an overall increase after initiation of a gluten-free diet [56]. The average increase in BMD is about 5 % in the first year, although the final BMD may remain below normal despite sustained dietary exclusion of gluten [56]. Levels of propeptide of type I procollagen have been found to correlate well with post-treatment bone mass gain in adult celiac disease patients [88]. IGF system abnormalities associated with celiac disease, including the lower IGF-1 and IGF-2 levels, which are believed to have a significant effect on growth, appear to normalize on gluten-free diet [63, 64]. Considering that much of the bone mass is gained during childhood and adolescence, early diagnosis of celiac disease and strict adherence to a gluten-free diet are particularly important. Upon initiation of gluten-free diet, children with celiac disease exhibit compensatory growth in height, during which the rate of growth exceeds the average rate [89]. A number of studies indicate that the risk of fracture in celiac disease patients is also reduced after the initiation of gluten-free diet, while others point to persisting increased risk of fracture, even years after diagnosis [56, 57, 66]. This increased risk may be due to persistence of mucosal damage in some patients. A recent cohort study of over 7,000 patients with celiac disease found villous atrophy on follow-up biopsy in 43 % and showed that persistent villous atrophy is predictive of hip fracture risk, but not fractures overall [90].

The efficacy of calcium and vitamin D supplementation in conjunction with gluten-free diet has not been demonstrated in controlled studies and no clear consensus about supplementation exists. A study of 24 women indicated that calcium absorption in celiac disease patients after 4 years of treatment remained lower than in 20 matched controls and suggested that increased calcium intake may compensate for the reduced calcium absorption in patients on gluten-free diet [91]. In the case of patients with vitamin D deficiency, it has been suggested that treatment should aim at restoring the levels of 25-hydroxy vitamin D to above 25 ng/mL, a level that would prevent PTH elevation and PTH-mediated bone resorption [56]. This may require vitamin D supplementation with large doses (50,000 U, 1–3 times per week) during the early stages of a gluten-free diet [56]. In cases of postmenopausal patients with celiac disease, other strategies in addition to instituting a gluten-free diet and vitamin supplementation, including pharmacological intervention, may be considered, although reliable data on the efficacy of such approaches are not available yet [57, 66].

Although the widespread use of wheat flour and related cereals in food products has been historically problematic for patients to adhere to a gluten-free diet, the substantially greater awareness of celiac disease and gluten sensitivity by the food industry and the general public in the past decade has made gluten-free foods increasingly more available. Many grains, such as rice, corn, quinoa, amaranth, sorghum, and buckwheat, are found to be safe for celiac disease patients and can be used as replacement for gluten-containing cereals [13]. Oat is generally considered to be well tolerated by most celiac patients, although some commercial preparations are reported to contain contamination from gluten-containing cereals [92]. In the USA, the Food and Drug Administration (FDA) has issued a ruling that defines the term "gluten-free" in the labeling of foods as meaning that "the food either is inherently gluten free; or does not contain an ingredient that is: (1) a gluten-containing grain (e.g., spelt wheat); (2) derived from a gluten-containing grain that has not been processed to remove gluten (e.g., wheat flour); or (3) derived from a gluten-containing grain that has been processed to remove

gluten (e.g., wheat starch), if the use of that ingredient results in the presence of 20 parts per million (ppm) or more gluten in the food. Also, any unavoidable presence of gluten in the food must be less than 20 ppm" [93].

A number of novel non-dietary therapies for celiac disease are in the developmental stage. These include oral proteases to further digest and detoxify gluten proteins, a zonulin antagonist that reduces intestinal permeability and thus the amount of gluten that is encountered by the immune system, a TG2 inhibitor, and gluten-sequestering polymers [94]. However, these are not likely to replace gluten-free diet as the main mode of treatment for celiac disease in the foreseeable future.

37.9 Conclusions

Reduced BMD and osteoporosis are among the most common extra-intestinal complications found in newly diagnosed celiac disease patients. A number of studies point to a significant association between celiac disease and bone fractures. Celiac disease can also have a negative effect on normal growth in childhood and adolescence. The cause of bone loss in celiac disease is believed to be multifactorial. In addition to abnormal calcium homeostasis, resulting from interruption in the uptake of calcium, vitamins D and K, and other nutrients due to the characteristic intestinal mucosal damage, immunologic mechanisms are considered to play a role in contributing to a reduction in bone mass in celiac disease. Practice guidelines recommend routine testing of celiac disease patients for nutritional status, including vitamin D, vitamin K, and calcium levels. Testing may be repeated every 6 months after the initiation of gluten-free diet for the purpose of monitoring the normalization process. Bone density evaluation is recommended within a year after the diagnosis and start of treatment. Currently, gluten-free diet is the only widely accepted mode of treatment for celiac disease and celiac disease-associated osteoporosis. Most studies of celiac disease patients indicate an overall increase in BMD following a gluten-free diet. The risk of fracture in celiac disease patients appears to decline after the initiation of a gluten-free diet, although it may remain higher than normal for an extended period of time after diagnosis. Strict adherence to a gluten-free diet is particularly important during childhood and adolescence, a time when much of the bone mass is gained. In conjunction with the exclusion of gluten from diet, calcium and vitamin supplementation may be considered in some cases. Some of the therapies currently undergoing development and clinical trial are likely to offer additional options for treatment of celiac disease and its various complications, including those affecting bone health, in the future.

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Chapter 38
HIV/AIDS and Bone Health: The Role of Nutrition

Stephanie Shiau, Stephen M. Arpadi, and Michael T. Yin

Key Points

- The life expectancy of the people living with HIV has increased; estimates indicate that most HIV-infected persons in the USA will be 50 years or older by 2015.
- HIV-infected individuals will remain at high risk for osteopenia and osteoporosis and fractures as their life expectancy increases.
- Those surviving early-life infection with HIV who initiate treatment at young ages may be especially at risk for adverse effects on bone health.
- Young men who acquired HIV early in life were reported to have lower peak bone mass by DXA.
- The etiology of osteoporosis in HIV-infected persons is complex and may involve both HIV disease itself and antiretroviral treatment.
- Traditional risk factors, such as smoking, hypogonadism, and low body weight, also play a role.
- Due to the higher risk of low BMD compared to HIV-uninfected populations and evidence suggesting higher fracture risk, DXA screening has been recommended by some but not all expert panels for HIV-infected postmenopausal women and men aged 50 years.
- Little is known on how best to optimize bone health.
- Nutritional considerations should be advised; provision of a diet that is rich in vitamin D, especially in areas with limited exposure to sunlight, may also help to ensure the best possible bone growth.
- HIV-infected individuals, especially children, should also participate in weight-bearing exercises and avoid detrimental behaviors such as smoking to improve their bone health.
- More research on specific nutritional interventions is needed.

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38.1 Introduction

Human immunodeficiency virus (HIV) is a retrovirus that infects immune cells such as CD4+ (helper) T lymphocytes, macrophages, and dendritic cells, leading to progressive failure of the immune system. Transmission of HIV can occur through sexual exposure, mother-to-child transmission, injection drug use, transfusion of contaminated blood products, and occupational exposure. Largely due to the widespread availability of effective antiretroviral therapy (ART) in the mid-1990s, HIV has been transformed from an invariably fatal illness to a manageable chronic illness in many parts of the world. The life expectancy of people living with HIV has increased; estimates indicate that most of the people living with HIV in the USA will be 50 years or older by 2015 [1]. Comorbid conditions in HIV-infected individuals have also changed markedly. Non-AIDS conditions, such as cardiovascular disease, liver disease, renal disease, and cancer, are now a greater source of morbidity and mortality among HIV-infected patients on ART than AIDS-associated complications [2]. Those living with HIV are also at increased risk for age-related complications such as osteoporosis [3]. The risk of certain aging-related conditions (diabetes mellitus, cardiovascular disease, fractures, renal failure, and frailty) has been noted to be higher in HIV-infected persons than uninfected peers and appear to develop at an earlier age [4, 5]. This had led to the hypothesis that aging is "accelerated" with chronic HIV infection [6]. The pathogenesis of these non-AIDS-related comorbidities is complex and the management of these conditions and comorbidities has become an essential component of HIV care.

This chapter focuses on nutrition and bone health in people living with HIV infection. Many studies have shown that HIV infection and ART are associated with lower bone mineral density (BMD) and increased prevalence of osteoporosis [7, 8] and increased rates of fracture [9–11]. Bone health is also a concern for children and adolescents, who acquire HIV infection either perinatally or early in life through sexual transmission, and have the greatest cumulative exposure to the negative effects of HIV infection and ART on bone metabolism.

38.2 Epidemiology of HIV and Bone Health

38.2.1 Adults

38.2.1.1 Cross-Sectional Studies

Low BMD has been well documented in many cross-sectional studies of HIV-infected individuals. The first reports of low BMD among HIV-infected individuals began to emerge in the early 2000s, just a few years after protease inhibitors became widely available [12–14]. Instead of separating out osteopenia (defined according to World Health Organization as a *T*-score between −1 and −2.5) and osteoporosis (*T*-score ≤ −2.5), most of the early studies included both osteopenia and osteoporosis (*T*-score ≤ −1.0) to define low BMD. When compared to the general population, early studies indicated the prevalence of low BMD was increased among HIV-infected individuals. A meta-analysis of cross-sectional studies conducted between 1996 and 2005 showed that 67 % of HIV-infected individuals had reduced BMD (*T*-score ≤ −1.0), and that this prevalence was more than six times higher than HIV-uninfected controls [8]. Subsequent studies have found a high prevalence of reduced BMD in people with HIV infection as well [15–17].

Though reduced BMD is well documented, fewer studies have estimated the prevalence of osteoporosis, (*T*-score ≤ −2.5). The aforementioned meta-analysis of cross-sectional studies reported a

prevalence of osteoporosis of 15 % in HIV-infected individuals; this was more than three times greater than in uninfected controls (OR 3.7, 95 % CI 2.3–5.9) [8]. A cross-sectional cohort study conducted in 2008 reported osteoporosis in 33.7 % of men (95 % CI 28.8–38.6) with a median age of 43 and 8.3 % of women (95 % CI 3.6–13.9) with a median age of 41 [18], suggesting a higher burden of osteoporosis for HIV-infected men compared to premenopausal women. A recent large study of younger patients aged 20–30 years also found more frequent osteoporosis in HIV-infected men than in control men (12.2 % vs. 5.5 %, $p = 0.033$) but no differences between HIV-infected women and control women [16].

38.2.1.2 Longitudinal Studies

The most dynamic phase of bone loss in HIV-infected individuals occurs with initiation of ART. In vitro studies suggest that various antiretrovirals have direct effects on osteoclast [19, 20] or osteoblast [21, 22] proliferation and function; however, the data are variable and sometimes conflicting, reflecting either true differences in the effect of specific antiretrovirals, or differences in experimental conditions (e.g., doses of antiretrovirals or experimental models used). Therefore, it is difficult to extrapolate these data to the clinical setting. Compelling data for the effect of specific antiretrovirals on bone metabolism come from randomized clinical trials of ART initiation comparing the effect of two or more regimens on BMD as measured by DXA over 1–4 years. Initiation of ART is consistently associated with a 1–6 % decrease in BMD at the spine or hip that occurs predominantly within the first 6 months after initiation of ART [23–27], followed by stabilization or improvement towards baseline BMD. There may be slight differences in magnitude of BMD loss between different antiretrovirals or ART regimens [23, 24]; however, interpretations of these differences are complicated by limited combinations used in clinical trials and differences study measures. Among antiretrovirals, tenofovir has been consistently demonstrated to be associated with a 1–2 % greater decrease in BMD compared to other nucleoside reverse transcriptase inhibitors (NRTIs) when used in combination in ART initiation [23, 24, 26]. The use of tenofovir alone or in combination with emtricitabine in HIV-uninfected individuals as preexposure prophylaxis is associated with a 1–2 % decrease in BMD over 48 weeks [28, 29]. Bone loss with initiation of ART may not be entirely attributable to direct effects of ART on bone cells. Immune reconstitution with ART is associated with an increase in inflammatory cytokines that may increase osteoclastogenesis and bone resorption. Supportive evidence comes from the observation that bone loss is greater in patients with lower CD4 counts at the time of ART initiation [30].

In younger HIV-infected individuals on established ART, however, BMD remains stable. A meta-analysis of six longitudinal cohorts reported similar rates of bone loss at the total hip or femoral neck between HIV-infected individuals and controls followed for 1.7–2.7 years [31]. There is less data on longitudinal changes in BMD in older HIV-infected individuals. In a study of postmenopausal women, the annualized rate of bone loss was 2.4-fold higher at the lumbar spine and 3.7-fold higher at the 1/3 radius in HIV-infected than uninfected postmenopausal controls [32]. In a study of older men, BMD changes were overall similar in HIV-infected men and uninfected controls, but the combination of heroin use and diagnosis of AIDS was associated with a decrease in BMD [33].

38.2.1.3 Fractures

An important clinical question is whether lower BMD associated with HIV infection or ART results in increased fracture risk. Two large database studies suggest that the prevalence of fractures is higher among HIV-infected individuals as compared to the general population [5, 34], especially among older individuals [34]. Several large cohort studies have compared the incidence of fracture among HIV-infected individuals to incidence in prospectively enrolled uninfected individuals [35, 36], individuals within the same clinic system [37], or the general population [10, 11]. Other cohorts have

reported incidence and predictors of fracture limited to HIV-infected individuals [38–40]. A recent meta-analysis of incident fractures in HIV-infected individuals had an incidence rate ratio of 1.58 (95 % CI 1.25–2.00) [9].

The main predictors for fracture among individuals with HIV include traditional risk factors such as smoking [11, 35, 37], use of glucocorticoids [39] or proton pump inhibitors [37], alcohol or substance abuse [39, 40], low weight or body mass index (BMI) [37], and comorbidities such as diabetes and liver disease [10, 11, 35, 37]. Also, infection with hepatitis C virus (HCV) was consistently identified as an independent risk factor for both fragility and non-fragility incident fractures [10, 11, 35, 38, 40–42]. The increased risk of fracture is approximately 1.5–2 times greater in HIV/HCV co-infected than HIV mono-infected individuals [11, 38, 41]. The overall burden of fracture among the HIV-infected population is likely to increase among older individuals as the HIV-infected population in many countries continues to age.

38.2.2 Children and Adolescents

38.2.2.1 Cross-Sectional Studies

Cross-sectional studies in the USA and Europe have found both lower bone mineral content (BMC) and BMD by DXA in perinatally HIV-infected children compared to controls [43–46]. Studies have also noted increased bone turnover among HIV-infected children and adolescents [47–49]. There are few studies from resource-constrained settings where undernutrition and childhood infections are highly prevalent. Of note, areal BMD (aBMD) by DXA may underestimate volumetric BMD (vBMD) in children with impaired growth and pubertal development due to the failure of aBMD to measure the anteroposterior diameter of bone. This is of particular interest for studies of HIV-infected children, as growth delays are frequently reported. A study of 58 HIV-infected Thai children and adolescents age matched to healthy children found a lower aBMD and aBMC by DXA and lower vertebral cross sectional area by quantitative computed tomography (QCT) in the HIV-infected group compared to the HIV-uninfected group, but no difference between groups in vBMD by QCT [50].

A large study of children at different pubertal stages compared total body and spine BMC and BMD by DXA in 236 perinatally HIV-infected children to 143 HIV-uninfected boys and girls frequency-matched by Tanner stage (1–5) and sociodemographic background [51]. HIV-infected boys had significantly lower total body BMC and total body and spinal BMD at Tanner 5, lower BMC at Tanner 3-4 and similar BMC and BMD at Tanner 1-2, compared to HIV-uninfected boys, after adjustment in their model. In contrast, HIV-infected and uninfected girls did not differ significantly on any bone outcome. These studies suggest that group differences in BMD may not become apparent until puberty, and the impact of HIV infection or ART may differ for boys and girls.

38.2.2.2 Longitudinal Studies

There are very few longitudinal studies of BMD in HIV-infected children. A study of 32 perinatally infected children aged 6.3–17.7 years observed a slight increase in BMD over 12 months at the spine and whole body, with the rate of change similar to that of controls [52]. A recent study published in 2013 found a similar result [53]. These studies suggest that group differences in BMD may not become apparent until puberty, and the relative impact of HIV infection or ART may differ for boys and girls. Evidence indicates recent HIV infection with sexual transmission during or after puberty in young men also results in decreased BMD and BMC. A comparison of BMD and BMC by DXA of 199 HIV-infected men aged 14–25 years with median time since HIV diagnosis of 1.3–2.2 years with HIV-uninfected controls found lower mean total body BMC *Z*-scores in the HIV-infected men on

ART compared to the HIV-uninfected men [28]. The study was not able to estimate the impact of HIV infection and ART initiation as there were too few men in the ART-naïve group.

Recent studies also suggest that impaired bone growth observed during childhood and adolescence results in reduced peak bone mass, the maximum amount of bony tissue at the end of skeletal maturation [54], in those who acquire HIV infection early in life. Young men who acquired HIV early in life either perinatally or through sexual transmission were reported to have lower peak bone mass by DXA [16, 55]. In addition, men infected with HIV early in life had similar cross sectional area of the radius and tibia as determined by high-resolution peripheral quantitative computed tomography (HRpQCT) than uninfected controls, but lower vBMD and markedly abnormal trabecular plate and cortical microarchitecture, and decreased whole-bone stiffness [55].

38.2.2.3 Fractures

Limited data on fracture risk is available in children. A study of Legg-Calve Perthes disease (LCPD), osteonecrosis of the hip, found that children with perinatal HIV infection have an increased risk for LCPD [56]. One study on fracture rates in a cohort of 1,326 perinatally HIV-infected and 649 uninfected children with a mean age of 5.8–7.1 found a similar rate of fracture in the HIV-infected and uninfected groups (1.2 vs. 1.1 per 1,000 person-years) [57]. Fracture risk may be increased in HIV-infected youth during adolescence, at a time when fracture rates are typically increased in boys. More importantly, risk of fracture in adulthood may be greater if impairments in bone acquisition during childhood prevented achievement of genetically determined peak bone size and mass. Even small differences in peak bone mass (10 %) have been postulated to delay onset of osteoporosis by as much as 13 years [58]; therefore, more research is needed to quantify fracture risk and optimize bone health in those who acquire HIV early in life.

38.2.3 Risk Factors for Low BMD

38.2.3.1 Adults

There are many factors that may contribute to the high prevalence of low BMD in HIV-infected adults, including the effects of chronic infection with HIV, both the direct effect of HIV-1-specific viral proteins on bone cells and indirect effects associated with chronic inflammation, immune activation (including activation due to gut microbial translocation), and high levels of pro-resorptive inflammatory cytokines; antiretrovirals, especially during the first 2 years after ART initiation; behaviors such as cigarette smoking and drug use; comorbidities such as diabetes and obesity; and co-infections such as HCV [7, 59–61].

38.2.3.2 Children and Adolescents

Studies of HIV-infected children and adolescents have also identified previously known factors associated with bone loss, such as vitamin D deficiency, delayed growth and puberty, low lean body mass, malabsorption, and physical inactivity. In addition to other hormonal influences, disturbances in somatotropic axis could also be important to bone accrual during childhood and adolescence, either directly or indirectly through effects on overall growth. Some studies have also identified an association with serum cytokine levels [48, 62], stage of HIV [43], class of antiretrovirals, or specific antiretrovirals. It is challenging to isolate the effects of antiretrovirals on bone metabolism from effects of HIV infection on growth in children, since the treatment paradigm involves lifelong therapy with multiple antiretrovirals [46, 47].

38.3 The Role of Vitamin D

38.3.1 Vitamin D Deficiency

Vitamin D plays essential roles in calcium homeostasis by increasing the efficiency of intestinal calcium absorption and influencing bone turnover by stimulating osteoblasts to induce the conversion of stem cell monocytes into mature osteoclasts [54, 63, 64]. Vitamin D deficiency (serum 25(OH)D <20 ng/ml) and insufficiency (<30–32 ng/ml) are common conditions in the general population [65–67]. Vitamin D deficiency is also reported in many cohort studies of HIV-infected individuals from the USA, Europe, and Africa [68–75], with prevalence in larger studies ranging from 24 to 74 %. However, studies that include HIV-uninfected controls with similar demographics and risk factors, do not report a higher prevalence of vitamin D insufficiency/deficiency among HIV-infected individuals [70, 71, 76].

In multivariate analyses from these and other studies, vitamin D deficiency is associated with older age [72], African American race or dark skin [70–74, 76–78], decreased dietary intake [76, 77, 79], decreased sun exposure [70, 79], evaluation during winter [72, 77, 78], and increased BMI [71]. Among HIV-infected individuals, several studies also found an association between low CD4 count and vitamin D deficiency [71, 78] or an association between higher vitamin D levels and a greater rise in CD4 counts after ART [80]. Findings from studies of HIV/HCV co-infected patients or HIV-uninfected patients with viral cirrhosis indicate that vitamin D levels, insulin-like growth factor 1 deficiency, vitamin D receptor polymorphisms, hypogonadism, hyperbilirubinemia, excessive alcohol use, tumor necrosis factor receptor p55, and HIV viral load may contribute to alterations of bone homeostasis [81–85].

38.3.2 Antiretrovirals and Vitamin D

Studies have examined the effect of various antiretrovirals on vitamin D metabolism. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) can activate CYP3A4 and CYP24 and reduce CYP2R1 function, leading to decreased 25(OH)D concentrations [86, 87]. There have been reports of osteomalacia in patients receiving efavirenz [88, 89]. In a longitudinal study, initiation of an efavirenz-containing regimen led to a mean decrease in 25(OH)D of 5 ng/ml after 6–12 months [90]. Switching from efavirenz-containing regimen to certain non-efavirenz containing regimens can lead to an increase in 25(OH)D concentrations [91]. The effect of efavirenz on vitamin D metabolism is hypothesized to occur through the induction of 24-hydroxylase, a cytochrome P450 enzyme that inactivates 25(OH)D and $1,25(OH)_2D$; this is similar to the effects of antiepileptics [90, 92, 93]; however, the exact mechanism is not known. Evidence indicates other NNRTI inhibitors such as nevirapine, etravirine, and rilpivirine do not decrease 25(OH)D concentrations as much as efavirenz [68, 78].

No strong association between the use of protease inhibitors and 25(OH)D concentrations has been established. It is possible that protease inhibitors may decrease the synthesis of or inhibit the metabolism of $1,25(OH)_2D$ since they are CYP450 inhibitors [94, 95]. Whether this results in clinically significant changes in calcium regulation or explains the association between protease inhibitor use and osteoporosis has never been established.

Among the NRTI antiretrovirals, zidovudine was found to be associated with lower 25(OH)D concentrations in one study [91]. However, tenofovir has not been found to be associated with lower 25(OH)D concentrations. Some studies suggest that tenofovir use may be associated with greater increases in PTH than other regimens [96, 97], while other studies have observed increases in PTH in association with initiation of various ART regimens, regardless of 25(OH)D concentrations [24]. A study of HIV-infected youth on antiretrovirals containing and not containing tenofovir found that

those with the highest plasma tenofovir concentrations had higher vitamin D binding protein, lower free $1,25(OH)_2D$, higher 25(OH)D, and higher serum calcium, which the authors suggest may indicate a drug-induced functional deficiency of vitamin D [98].

Despite the high prevalence of vitamin D deficiency in HIV-infected individuals, and the possible negative effects of antiretrovirals on vitamin D metabolism, low 25(OH)D has only been found to be associated with low BMD in a few studies [99, 100]. A more recent study of vitamin D status and bone mass in African HIV-infected women reported that HIV-infected women did not have lower BMD or 25(OH)D concentrations than HIV-negative controls [101]. Studies that utilized questionnaires on vitamin D supplementation as a proxy for 25(OH)D levels did not find an association between supplementation history and change in BMD or incident fractures [35, 102].

38.3.3 Vitamin D Supplementation

38.3.3.1 Adults

There are only a few published studies of vitamin D supplementation for musculoskeletal or cardiovascular outcomes in HIV-infected individuals. Results from the placebo arms of bisphosphonate studies in HIV-infected adults suggest BMD remains stable or increases 1–2 % over 1 year in those who take calcium and low doses (400–800 IU) vitamin D [103–105]. One study randomized 45 HIV-infected adults stable on ART with baseline serum 25(OH)D concentration <20 ng/ml to receive 4,000 IU of vitamin D3 or placebo and evaluated change in flow-mediated brachial artery dilation after 12 weeks [106]. A modest 5 ng/ml increase in 25(OH)D concentrations was detected in the treatment group compared to placebo, but no group difference was detected in change in endothelial function. Higher 25(OH)D concentrations may be needed to achieve an effect. In addition, the benefits of vitamin D supplementation may only be seen in those individuals at higher risk of skeletal and metabolic outcomes, such as the elderly.

38.3.3.2 Children

The potential effect of vitamin D supplementation on bone health has not been well studied in HIV-infected children. Havens found that 50,000 IU of vitamin D3 at 0, 4, and 8 weeks increased 25(OH)D concentrations above placebo but did not decrease bone turnover markers levels [107]. Arpadi et al. found 100,000 IU of vitamin D3 given every other month in combination with 1 gram of calcium given daily increased 25(OH)D concentrations in children and youth (age 6–16) but did not increase total body or spine BMC or BMD after 2 years compared to placebo, either before or after adjustment for stage of sexual maturation [79].

38.3.4 Screening and Treatment of Vitamin D Deficiency

Though efficacy data is limited, there are guidelines that support a structured screening approach for vitamin D deficiency in people with HIV infection. The Endocrine Society recommends 25(OH)D screening for all patients on ART, regardless of type of drugs [108], while The European AIDS Society recommends 25(OH)D screening for only for patients on efavirenz [109]. Although the guidelines do not suggest that target levels for 25(OH)D should be different for HIV-infected individuals, they acknowledge that higher supplementation may be needed for those receiving antiretrovirals that increase 25(OH)D metabolism such as efavirenz.

38.4 The Role of Calcium

Little is known about the specific relationships of dietary calcium intake and BMD in HIV-infected individuals. A study of 148 HIV-infected men aged 20–60 reported that 41 % had a calcium intake of less than 900 mg/day, with men on a protease inhibitor-based regimen reporting a slightly lower calcium intake [110]. Another study of 112 HIV-infected subjects and 76 HIV-uninfected subjects matched for age and sex evaluated bone turnover markers and BMD, lifestyle habits, and dietary intake found that among the calcium-rich food, yogurt intake was a protective predictor of BMD in HIV-infected subjects [58].

38.5 Other Nutritional Interventions

Apart from vitamin D and calcium, a number of other nutritional interventions may play a substantive role in bone homeostasis for people with HIV infection although data are limited. Renal losses of phosphate resulting in hypophosphatemia and hypomagnesemia due to ART or HIV-related acute and chronic renal disorders are reported [111]. The therapeutic potential of supplementation with phosphorus or magnesium, however, has not been studied.

Findings from a number of clinical studies of nutritional supplementation conducted in HIV-infected children may have implications for bone health. Studies conducted prior to the availability of potent ART reported improved weight gain among symptomatic HIV-infected children provided with increased calories by means of supplemental semi-elemental enteral feedings [112]. However, the extent to which there is any additional benefit from supplemental enteral feedings beyond the improved weight gain and statural growth associated with ART is unknown, nor is the impact on bone mass accrual or BMD [113–116].

Supplementation with zinc and vitamin A has each been reported to reduce diarrheal morbidity in children with HIV [117, 118]. Although improvements in short-term growth have only been reported in association with vitamin A [119], whether reductions in diarrheal illnesses can modulate bone metabolism by means of reduced microbial translocation and immune activation is a area for future investigations.

38.6 Conclusion

As life expectancy continues to increase, HIV-infected individuals will remain at high risk for osteopenia and osteoporosis, and fractures. This is likely to be an important source of morbidity. Those surviving early-life infection with HIV who initiate treatment at young ages may be especially at risk for adverse effects on bone health. The etiology of osteoporosis in HIV-infected persons is complex. Both HIV disease itself and antiretroviral treatment contribute to the multifactorial etiology. Traditional risk factors, such as smoking, hypogonadism, and low body weight, also play a role. Optimal screening and treatment recommendations are not well developed. Due to the higher risk of low BMD compared to HIV-uninfected populations and evidence suggesting higher fracture risk, DXA screening has been recommended by some but not all expert panels for HIV-infected postmenopausal women and men aged 50 years or older, particularly those with additional risk factors. There is a considerable knowledge gap on how best to optimize bone health. It is advisable to consider nutrition; provision of a diet that is rich in vitamin D, especially in areas with limited exposure to sunlight, may also help to

ensure the best possible bone growth. HIV-infected individuals, especially children, should also participate in weight-bearing exercises and avoid detrimental behaviors such as smoking to improve their bone health. More research on specific nutritional interventions is needed.

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Chapter 39
Dietary Factors and Chronic Low-Grade Systemic Inflammation in Relation to Bone Health

Robin M. Daly

Key Points

- Chronic low-grade systemic inflammation, which has been categorized by a two- to fourfold age-related increase in circulating inflammatory cytokines and acute phase reactants, has been implicated in the pathophysiology of a range of musculoskeletal-related disorders, including osteoporosis, sarcopenia, and fragility fractures.
- While the precise mechanism(s) underlying this age-related increase in inflammation (termed "*inflammaging*") are yet to be determined, genetic factors, pathological conditions and various hormonal, environmental, and lifestyle factors have all been shown to play a role.
- Over the past decade, there has been considerable interest into whether dietary interventions, including weight loss and caloric restriction alone or with exercise, and various dietary patterns, foods and nutrients, can modulate the inflammatory response within the body, particularly in people with diseases known to have a strong inflammatory pathogenesis (e.g., type 2 diabetes, cardiovascular disease, and cancer).
- Of the key dietary factors known to be important for bone and muscle health which have been investigated in terms of whether they exhibit anti-inflammatory properties, there have been mixed findings with regard to dietary and supplemental calcium and vitamin D, dairy products, dietary protein, vitamin K, magnesium and omega-3 fatty acids or their combination.
- Based on the available data, there is no consistent evidence that these dietary factors modulate inflammation in apparently healthy adults.
- However, a limited number of intervention trials have demonstrated that calcium and vitamin D supplementation, high dairy diets, increased dietary protein, vitamin K, and omega-3 fatty acids can produce modest reductions in circulating inflammatory biomarkers in people with osteoporosis, sarcopenia or the presence of another chronic disease(s).
- Given the emerging clinical evidence linking low-grade systemic inflammation to osteoporosis, sarcopenia and fractures in the elderly, further intervention trials are warranted to evaluate the long-term efficacy of different nutrients or their combination on markers of inflammation and their putative effect on modulating musculoskeletal health outcomes.

Keywords Inflammation • Cytokines • Osteoporosis • Fracture • Calcium • Vitamin D • Protein • Vitamin K • Omega-3 fatty acids • Magnesium • Aging

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39.1 Introduction

It is well established that there is a close interplay between the bone and the immune system, which has been termed "*osteoimmunology*" [130]. A range of chronic inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and chronic obstructive pulmonary disease, have been associated with systemic bone loss, osteoporosis, and increased fracture rates [46, 47, 53, 144]. Up-regulation of inflammatory cytokines has been established to be the common pathway mediating bone loss among these conditions. Indeed, various pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL-6), IL-1, and IL-17 can bind to bone marrow stromal cells and increase the expression of receptor-activator NF-κβ (RANKL) and macrophage-colony stimulating factor (M-CSF), which regulate osteoclastogenesis and osteoclast activity, and decrease the production of osteoprotegerin (OPG), which is a decoy receptor for RANKL that inhibits its effect, thereby promoting increased osteoclast activity and bone resorption leading to bone loss [83, 130].

It has also been established that the aging process per se is accompanied by marked changes, and a dysregulation of, the immune system (term "*immunosenscence*"), which can lead to chronic low-grade systemic inflammation categorized by a two- to fourfold increase in various inflammatory cytokines, their soluble receptors and acute phase proteins, relative to young and middle-aged adults [19, 124, 134]. Specifically, there are reports of an age-related increase in serum concentrations of IL-6, TNF-α, soluble IL-6 receptor, IL-1 receptor antagonist (IL-1ra) and IL-18 amongst others, as well as C-reactive protein (CRP) and fibrinogen and a reduction in anti-inflammatory cytokines such IL-10 and tumor growth factor-beta (TGF-β) [6, 38, 113, 140]. This age-related up-regulation of inflammatory cytokines and the resultant persistent, low-grade chronic systemic inflammatory state has been coined "*inflammaging*" [40]. From a clinical perspective, this systemic, chronic but low-grade inflammatory state has been identified as a major risk factor underlying many chronic diseases, including cardiovascular disease, diabetes, arthritis, cancer, dementia, Alzheimer's disease, metabolic syndrome as well as osteoporosis, fragility fractures, sarcopenia, frailty, disability, and even mortality [25, 124] (Fig. 39.1).

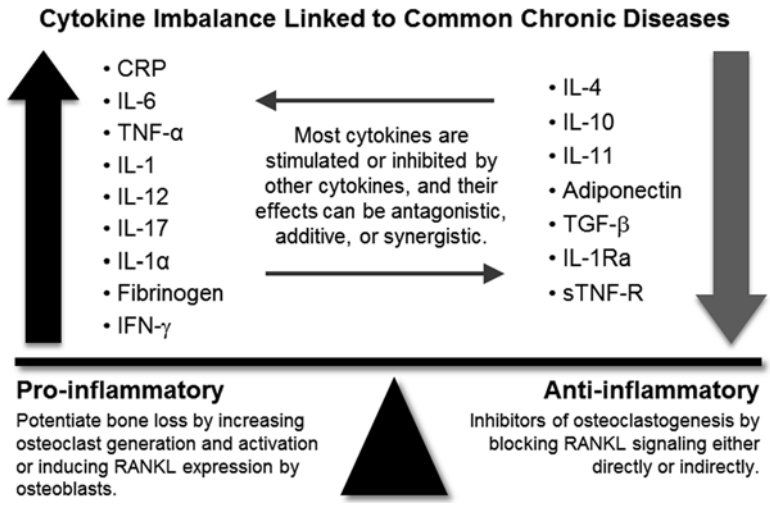

Fig. 39.1 Summary of common pro-inflammatory and anti-inflammatory cytokines and acute phase reactants. An imbalance between these pro- and anti-inflammatory cytokines can result in chronic systemic inflammation which can contribute to increased bone resorption and bone loss. *CRP* C-reactive protein, *IL* interleukin, *TNF-alpha (α)* tumor necrosis factor-α, *IFN-gamma (γ)* interferon-gamma-γ, *TGF-beta (β)* transforming growth factor, *IL-1Ra* IL-1 receptor antagonist, *sTNF-R* soluble TNF-receptor

In addition to advancing age and the presence of a chronic disease(s), a wide range of different factors have been reported to contribute to chronic low-grade inflammation in older adults, including genetic factors, decreased production of sex steroids, excess fat mass, smoking, poor nutrition, stress, and a sedentary lifestyle [18, 19, 38, 59, 76, 124]. Since some of these factors are modifiable, there has been considerable interest in identifying lifestyle strategies that might reduce this chronic low-grade systemic inflammation. Physical activity or exercise, particularly aerobic training, is recognized as one approach to reduce systemic inflammation in older people, with some reports of a dose–response relationship [12, 104]. There is also emerging evidence that various dietary interventions, dietary patterns, nutrient and micronutrients, including calcium, vitamin D, dietary protein, vitamin K, magnesium, and omega-3 fatty acids, all of which play an important role in regulating bone and/or muscle health, may also protect against systemic inflammation. This chapter will provide an overview of the effects of these dietary factors on inflammation, and their putative effects on slowing or preventing bone loss, osteoporosis, and related fractures.

39.2 Origins of Inflammaging

Currently the mechanism(s) underlying "*inflammaging*" remains uncertain, but it is thought to have multiple origins many of which are proposed to be related to the aging process itself. For instance, it has been hypothesized that it may be due to an impairment of the mechanisms that induce the inflammatory response [38] or a failure of anti-inflammatory mechanisms to neutralize inflammatory responses that are continuously triggered throughout life [19, 41]. While various genetic, lifestyle, environmental, and hormonal factors have also been implicated, it has also been shown that age-related cumulative oxidative damage or stress, which represents an imbalance in oxidant and antioxidant levels (excess free radical formation), can invoke an inflammatory response [77, 86]. While the role of oxidative stress on inflammation and disease is beyond the scope of this chapter, there is evidence that free radicals are involved in apoptosis of osteoblasts and osteocytes and activation of RANKL and osteoclastogenesis, and thus can contribute to bone loss [25, 44, 71]. For this chapter, the focus will be predominantly on the effects of dietary factors on common inflammatory mediators, including CRP, IL-6, TNF-α, IL-1β and IL-10, and their link to bone (and muscle) health.

While there are numerous reports that various inflammatory mediators increase progressively with age [6, 17, 38, 140], many age-related chronic diseases are also characterized by an increase in inflammatory cytokines. Thus, it has been suggested that this age-related increase in inflammation may be largely due to the presence of co-morbidities [19, 38]. In support of this notion, some previous studies in healthy well-nourished adults have failed to detect a difference in various circulating cytokines between young, middle-aged, and older adults [1, 87]. In an epidemiologic study involving 1,327 community-dwelling adults aged 20 to 85+ years, a battery of inflammatory markers [serum IL-6, soluble IL-6 receptor, IL-1 receptor antagonist (IL-1ra), IL-18, CRP and fibrinogen] were found to increase significantly with age in both men and women (Fig. 39.2) [38]. However, after adjusting for cardiovascular risk factors and morbidity, the association between age and many of these inflammatory markers was attenuated, which suggests that at least part of the pro-inflammatory state in older adults is likely to be related to the presence of risk factors for chronic disease. Genetic factors have also been shown to play a role. For instance, one twin study reported that 20–55 % of the variation in the inflammatory markers TNF-α, fibrinogen, and soluble intercellular adhesion molecule-1 (sICAM-1) were influenced by genetic factors [30].

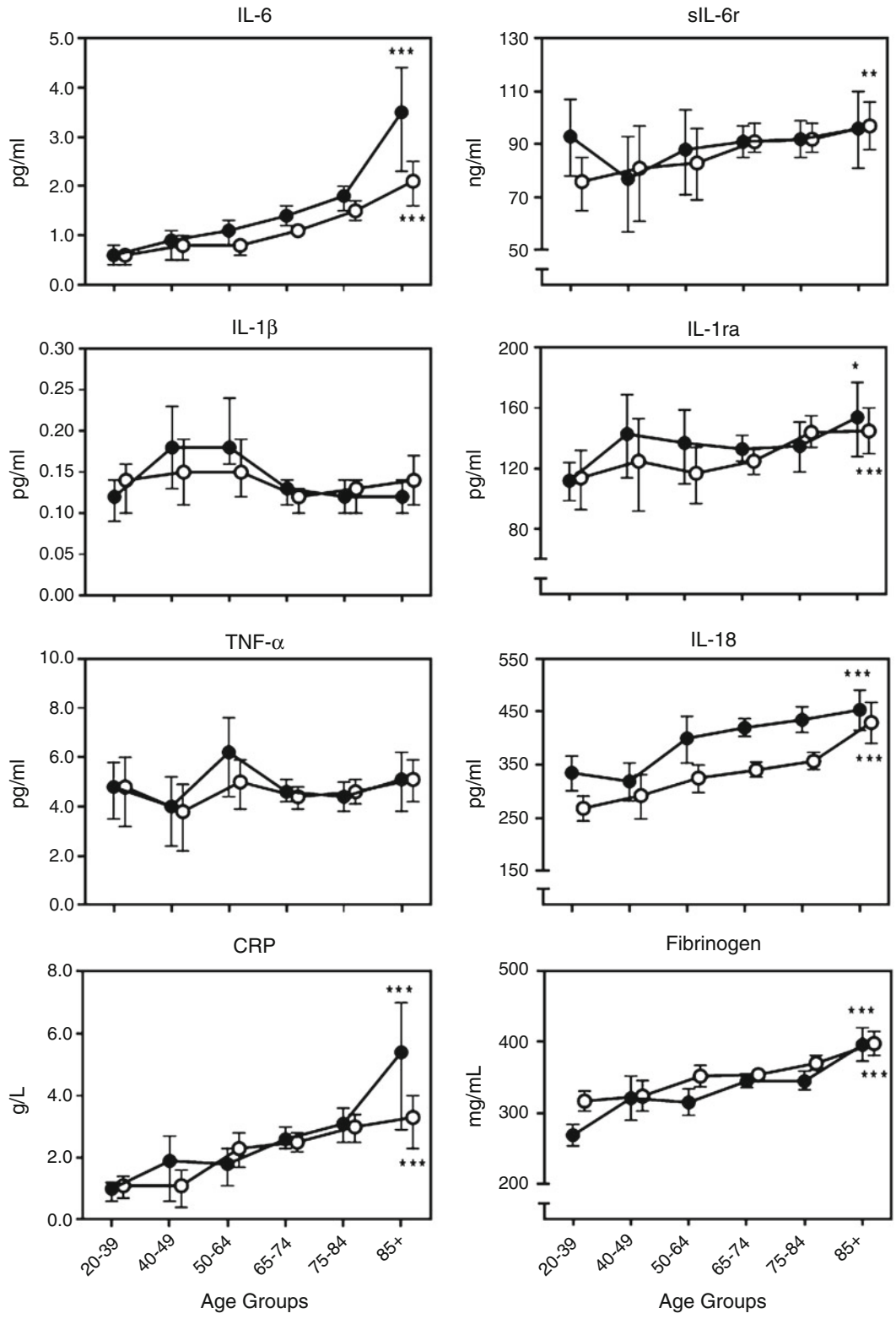

Fig. 39.2 Mean (with 95 % confidence intervals) serum levels of inflammatory markers according to gender (*black circles*, males; *open circles*, females) and age groups. IL (interleukin)-6; sIL-6r, soluble IL-6 receptor, IL-1 beta (β); IL-1 receptor antagonist (ra); TNF-alpha (α); IL-18, CPR, C-reactive protein. Values represent means and 95 % confidence intervals. Based on data from [38]. *$p<0.05$, **$p<0.01$, ***$p<0.001$ test for trend from general linear models

39.3 Effects of Inflammation on Bone and Muscle Loss and Fracture Risk

Over the past two decades there has been considerable evidence linking elevated circulating levels of pro-inflammatory cytokines with age- and menopause-related bone loss as well as reduced muscle mass, strength, and function. The following sections will provide a brief overview of the effects of low-grade systemic inflammation on bone and muscle health, osteoporosis, and fractures.

39.3.1 Inflammation, Bone Mineral Density, and Osteoporosis

There are several potential underlying mechanisms that may explain the association between elevated levels of pro-inflammatory markers with osteoporosis, accelerated bone loss, and fracture risk. For instance, in vitro and experimental studies in animals have shown that various pro-inflammatory cytokines such as IL-6, TNF-α, IL-1, and IL-11 can alter the bone remodeling balance by increasing the expression of RANKL and M-CSF and decreasing the production of OPG [26, 83, 131]. This can be confounded further by estrogen deficiency associated with menopause which has been associated with up-regulation of pro-inflammatory cytokines and an increase in osteoclast activation [26, 83, 131]. While more specific details about the role of various pro-inflammatory and inhibitory cytokines on regulating osteoblast and osteoclast differentiation and activity is provided in several excellent reviews [26, 83, 131], it is important to acknowledge that these findings largely reflect the influence of cytokines at the local bone microenvironment. In contrast, most human studies have measured serum cytokine concentrations and related these to BMD (or changes in BMD) and fracture risk.

To date there have been mixed findings from studies investigating the relationship between circulating inflammatory biomarkers (e.g., CRP, IL-6, and TNF-α) with bone mineral density (BMD) and bone strength. For instance, several studies have reported an inverse relationship between high sensitivity (hs)-CPR and/or IL-6 with BMD at various sites [74, 99] as well as DXA-derived estimates of femoral neck bone strength [62], whereas others have failed to find any significant association [42, 67, 85, 100] or differences in inflammatory cytokines between osteoporotic women and age-matched controls [70]. While these mixed findings may be related to differences in the populations studied and/or differences in potential confounders included in the analyses, the results from several prospective studies with follow-up periods ranging from 1.0 to 3.3 years have shown that increased levels of inflammation, particularly serum IL-6, are associated with bone loss [31, 45, 116]. There is also data showing that polymorphisms in cytokine genes such as IL-1 receptor antagonist (IL-1ra) and 174 GG polymorphism in IL-6, which alter the expression of a given cytokine, were related to lower BMD, increased bone turnover, and fracture risk [37, 72, 79]. Several studies in animals that do not express IL-6 and others using antibodies against specific cytokines and/or their receptors provide further evidence linking systemic inflammation with accelerated bone loss [64, 81, 107].

39.3.2 Inflammation and Fracture Risk

A number of prospective studies have also reported that elevated levels of inflammatory markers and/or their soluble receptors are associated with an increased risk of fracture [8, 22, 62, 90, 100]. In a study of 2,985 well-functioning white and black women and men aged 70–79 years followed over a mean of 5.8 years, Cauley et al. [22] reported that elevated inflammatory markers, particularly high receptor levels of the pro-inflammatory cytokines IL-2 sR, IL-6 sR, TNF sR1, and TNF sR2, were associated with a twofold higher incidence of non-traumatic clinical fractures, independent of known

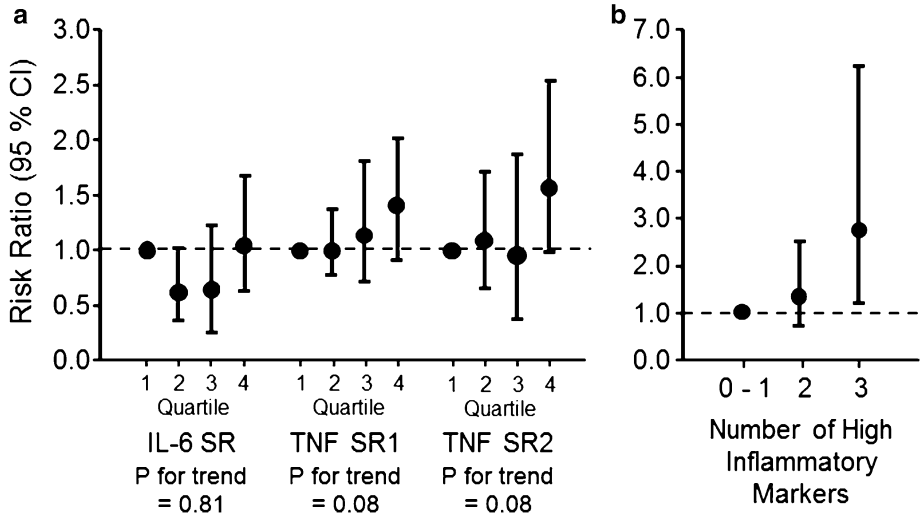

Fig. 39.3 Risk ratios (with 95 % confidence intervals) for hip fracture in women aged 50–79 years according to (**a**) quartiles of cytokine soluble receptor (SR) concentrations of interleukin (IL)-6 and tumor necrosis factor (TNF), and (**b**) the number of high inflammatory markers in the top quartile based on the distribution of cytokine soluble receptor concentrations. Based on data from [8]

risk factors including BMD. This is an important finding because cytokine soluble receptors may be more representative of a prolonged and severe underlying inflammatory state compared to the markers themselves which have a short half-life and often change transiently [22]. A second key finding from this prospective cohort study was that the risk of fracture was increased nearly threefold in participants with three or more inflammatory markers in the highest quartile compared to those with none or one marker classified as high. Although others have reported that an increase in a single inflammatory marker, particularly (hs)-CRP, is associated with an increase in fracture risk [62, 90, 100], the clinical relevance of this finding is that measuring several inflammatory markers may improve risk assessment. While a limitation of this study is that it focused on all non-traumatic fractures, the results from a nested case-control study in women aged 50–79 years who were followed over a median 7.1 years revealed that those with elevated levels (highest quartile) of inflammatory markers for three different cytokine soluble receptors (IL-6 sR, TNF sR1, and TNF sR2) had a 2.4–2.8-fold increased risk of incident hip fracture compared to women with zero or one elevated inflammatory marker [8] (Fig. 39.3). Although causality cannot be inferred from these prospective observational studies, these findings highlight that identifying strategies to reduce systemic inflammation may represent an important approach to reduce fracture risk in the elderly.

39.3.3 Inflammation, Sarcopenia, and Falls Risk

The finding that chronic systemic inflammation is related to bone loss and an increased fracture risk may be related in part to findings that elevated levels of various circulating inflammatory cytokines are associated with low muscle mass, size, strength and power [7, 23, 139], reduced physical performance [23], and increased disability in older adults [39]. This is also supported by the findings from a number of prospective studies with follow-up periods of 2–5 years which found that higher levels of inflammation (cytokines and their soluble receptors) were associated with accelerated losses in muscle strength

and lean mass [2, 101, 114, 115] and a greater decline in muscle function (walking speed) [135]. Although cause-and-effect cannot directly be inferred from these findings, several in vitro and in vivo studies have shown that an increase in inflammatory cytokines can directly inhibit muscle protein synthesis and induce cellular apoptosis in muscle (for review see Peake et al. [102]). Others have suggested that the influence of inflammation on muscle and functional performance may be mediated by changes in weight, particularly fat mass which secretes a large number of bioactive proteins termed adipokines (cytokines secreted by adipose tissue) [68]. For instance, it has been proposed that increased fat mass may result in a systemic "spill over" of inflammatory mediators to other organs/tissues and/or local inflammatory reactions within the muscle itself that could have a catabolic effect by impairing muscle protein synthesis [25, 102, 136]. In part support of this notion, Schaap et al. [114] found that higher levels of serum IL-6, CRP and TNF-α were associated with a greater 5-year decline in thigh muscle mass and strength, but these associations were attenuated after adjusting for changes in weight. Similarly, the findings from a 7-year study in 2,307 men and women aged 70–79 years showed that greater fat mass at baseline was associated with significantly greater loss in leg lean mass over the follow-up [75]. However, in this study the inclusion of various cytokines or insulin resistance in the model did not change the results, which suggests that the accelerated loss of lean mass with greater fat mass was not mediated by higher levels of adipokines or insulin resistance.

39.4 Nutritional Approaches Related to Osteoporosis and Fracture to Combat Inflammation

While there is evidence that poor nutrition is associated with chronic systemic inflammation, dietary interventions, including weight loss and caloric restriction, and maintaining a healthy diet are recognized to play an important role in reducing systemic inflammation. Indeed, a comprehensive review on the influence of dietary factors on low-grade systemic inflammation reported that there was sound evidence to support an anti-inflammatory role of healthy eating patterns, such as the Mediterranean diet and vegetarian diets, and specific dietary factors, including whole grains, fruit and vegetables, fish and omega-3 fatty acids as well as vitamins D, C and E [19]. Given that bone (and muscle) are responsive to various dietary factors and an individual's nutritional status, this chapter will provide an overview of the available evidence with regard to the influence of dietary and supplemental calcium, dairy foods, vitamin D, protein (including soy and milk proteins), vitamin K, omega-3 fatty acids, magnesium and a combination of these factors, on inflammatory markers and their association to BMD and fracture risk.

39.4.1 Dietary and Supplemental Calcium

While there are some reports that a high dietary calcium intake may have anti-inflammatory effects, it has been suggested that this may be mediated by a reduction in adiposity. For instance, in overweight and obese people it has been reported that an increased intake of dietary calcium or a high dairy diet may reduce inflammation (and oxidative stress) by promoting lipid metabolism and a loss of body fat [129, 145, 148]. This is supported by the findings from several laboratory studies in rodents which have shown that dietary calcium can inhibit lipogenesis and induce lipolysis by reducing 1,25-dihydroxyvitamin D-induced calcium signaling in adipocytes [122, 146]. Although others have been unable to replicate these findings, particularly with supplemental calcium, there is consistent evidence that increasing dietary calcium intake can promote modest weight loss through increased fecal-fat excretion [125]. Given that adipose tissue acts as an active secretory organ that releases many cytokines into the

circulation, it has been proposed that calcium may play a key role in modulating adipose tissue cytokine production [129, 147]. Indeed, studies in mice have shown that high-calcium diets can decrease the expression of pro-inflammatory factors, such as TNF-α and IL-6, in visceral fat, adipocytes and plasma, and stimulate the expression of the anti-inflammatory marker IL-15 as well as adiponectin [129, 147]. In the same rodent model, a high dairy diet incorporating nonfat dry milk was shown to be more effective for reducing inflammation and oxidative stress than supplemental calcium (calcium carbonate) [147]. While these findings suggest that increased dietary calcium, particularly a high dairy diet, may modulate adipocyte cytokine production in a mouse model of obesity, little is known about the influence of dietary and supplemental calcium on circulating inflammatory mediators in humans. However, if increased dietary calcium does play a role in modulating inflammation, it is possible that one of the mechanisms by which calcium reduces bone loss is via an anti-inflammatory effect.

It is well established that parathyroid hormone (PTH) regulates bone metabolism in response to fluctuations in serum calcium, but PTH can also regulate circulating concentrations of IL-6 and TNF-α, which in turn plays a key role in regulating the production of CRP [49, 50]. Thus, it has been hypothesized that suppression of PTH following calcium supplementation may play a role in lowering inflammation. However, the findings from a 12-month randomized controlled trial investigating the effects of 1,000 mg/day of calcium citrate on fracture incidence in 116 healthy postmenopausal women revealed that there was no significant difference in the change from baseline in CRP levels between the groups, and neither baseline nor changes in dietary calcium intake were related to CRP levels during the study [48]. To our knowledge, no other studies have specifically examined the effects of calcium supplementation on inflammatory biomarkers, but a number of intervention trial examining the effects of calcium plus vitamin D supplementation on inflammation have observed no marked improvement in healthy older adults [32, 43, 106].

In patients with chronic disease that often exhibit elevated circulating inflammatory markers, there is some evidence that calcium and/or vitamin D treatment may have anti-inflammatory effects. In a factorial 2×2 pilot, randomized, double-blinded, placebo-controlled trial involving 92 patients with colorectal adenoma, 6 months of supplementation with calcium (2,000 mg/day) led to a reduction in serum IL-6 (37 %), IL-8 (11 %), and IL-1β (27 %) relative to placebo [57]. Similarly, treatment with 800 IU/day of vitamin D_3 (relative to placebo) resulted in a decrease in CRP (32 %), TNF-α (13 %), IL-6 (32 %), IL-1β (50 %), and IL-8 (15 %), and combined calcium plus vitamin D decreased IL-6, IL-8, and IL-1β by 8 %, 13 % and 35 %, respectively. However, none of the above changes were significantly different from placebo, which may be explained by the small sample size. When the effects of calcium and/or vitamin D on a combined inflammation z-score incorporating all six cytokines (CRP, TNF-α, IL-6, IL-8, IL-1β, and IL-10) was analyzed, the overall z-score decreased significantly by 77 % in the vitamin D group ($P=0.003$). There was a 48 % ($P=0.18$) reduction in the calcium group and 33 % ($P=0.40$) decrease in the combined group (33 %, $P=0.40$), but neither were significant relative to placebo. While these findings provide some evidence that treatment with calcium and/or vitamin D may have anti-inflammatory effects (see Sect. 9.4.3 for further details), it is clear that further intervention trials with adequate sample sizes are needed to evaluate the anti-inflammatory properties of dietary and supplemental calcium (alone and with vitamin D) in people with diseases known to have an inflammatory pathogenesis.

39.4.2 Milk and Dairy Foods

There has been some concern that a high dairy diet may elevate inflammatory markers due to an increase in saturated fat and cholesterol. However, previous data from both cross-sectional studies and intervention trials have typically shown that increased consumption of dairy foods has either a neutral or beneficial effect of lower circulating inflammatory markers [33, 66, 78, 91, 98, 103]. Indeed, the

findings from a retrospective analysis of archival samples from two clinical trials conducted over 12–24 weeks revealed that high calcium (~1,100–1,200 mg/day) compared to low calcium (~400–500 mg/day) dairy diets reduced CRP levels by 11–29 % and increased adiponectin concentrations by 8–18 % during weight loss and maintenance in obese adults [147]. However, caution is warranted when interpreting these findings due to concomitant reductions in adiposity. In a 28-day acute blinded, randomized, cross-over trial comparing a high dairy (3 daily serves; calcium 1,200–1,400 mg/day) versus soy-based placebo diet (calcium 500–600 mg/day) in overweight and mildly obese adults, there was a significant reduction in both TNF-α and IL-6, as well as markers of oxidative stress, and an increase in adiponectin levels in the high dairy group; the opposite effect was observed in the soy-protein group [148]. In this acute study, there were no marked changes in body composition, which confirms that a high dairy diet may attenuate markers of inflammation in overweight/obese adults, independent of changes in fat mass.

Several longer-term intervention trials in overweight or obese people or those with a metabolic-related disease have reported a beneficial effect of a high dairy intake on various inflammatory cytokines. For example, the results from a 12-week randomized controlled trial involving an isoenergetic weight-maintenance diet with two contrasting serves of dairy in adults with metabolic syndrome revealed that the adequate dairy diet group (>3.5 daily serves) experienced a significant 21–47 % reduction in the inflammatory markers TNF-α, IL-6, CRP, and monocyte chemoattractant protein 1 (MCP-1) and a 53 % increase in adiponectin compared to the low dairy diet group (<0.5 daily servings) (Fig. 39.4) [128]. Interestingly, these beneficial effects were present after only 1-week of treatment and progressed over time. While several other intervention trials involving vitamin D or calcium plus vitamin D enriched milk or yogurt drinks have also observed a reduction in inflammatory markers in overweight/obese people or those with type 2 diabetes [66, 91], others have failed to detect any anti-inflammatory effects of a high dairy diet or an increased intake of low fat milk [103, 109, 133, 142]. A recent systematic review of eight randomized controlled nutrition intervention studies examining the effects of dairy products (milk, yogurt, and/or cheese) on biomarkers of inflammation in overweight and obese adults concluded that because of methodological factors and limitations associated with the available trials reviewed, it was not possible to make a definitive conclusion as to whether dairy products (or a specific type or amount of dairy) can attenuate inflammation [78]. However, this study did report that increased dairy consumption does not adversely affect markers of inflammation in overweight and obese adults. While the precise mechanism(s) by which dairy foods might attenuate inflammatory markers is not known, it has been suggested that it may be related to additional factors found in dairy products, such as angiotensin-converting enzyme inhibitors, protein and related bioactive peptides [19].

39.4.3 Vitamin D

The biologically active form of vitamin D, 1,25-dihydroxyvitamin D_3, is recognized to have immunomodulatory effects that can influence the differentiation and function of both innate and adaptive immune cell types and to modulate cytokine production (for a review on this topic refer to Hewison [55]). Indeed, data from human cross-sectional and prospective studies have shown that vitamin D status is linked to various autoimmune diseases, including multiple sclerosis, rheumatoid arthritis and type 1 diabetes [55]. While the underlying mechanism(s) by which vitamin D can alter cytokine production are yet to be fully determined, in vitro data suggests that it may be mediated by 1,25-dihydroxyvitamin D_3 coupling to the vitamin D receptor (VDR), which is located in many immune cells (monocytes, macrophages, T-cells, B-cells), to downregulate or transrepress inflammatory cytokines [89, 91]. It has also been shown that various immune cells have the capacity to regulate the activity of 1-α-hydroxlase, which converts 25(OH)D to 1,25-dihydroxyvitamin D_3 [89, 91].

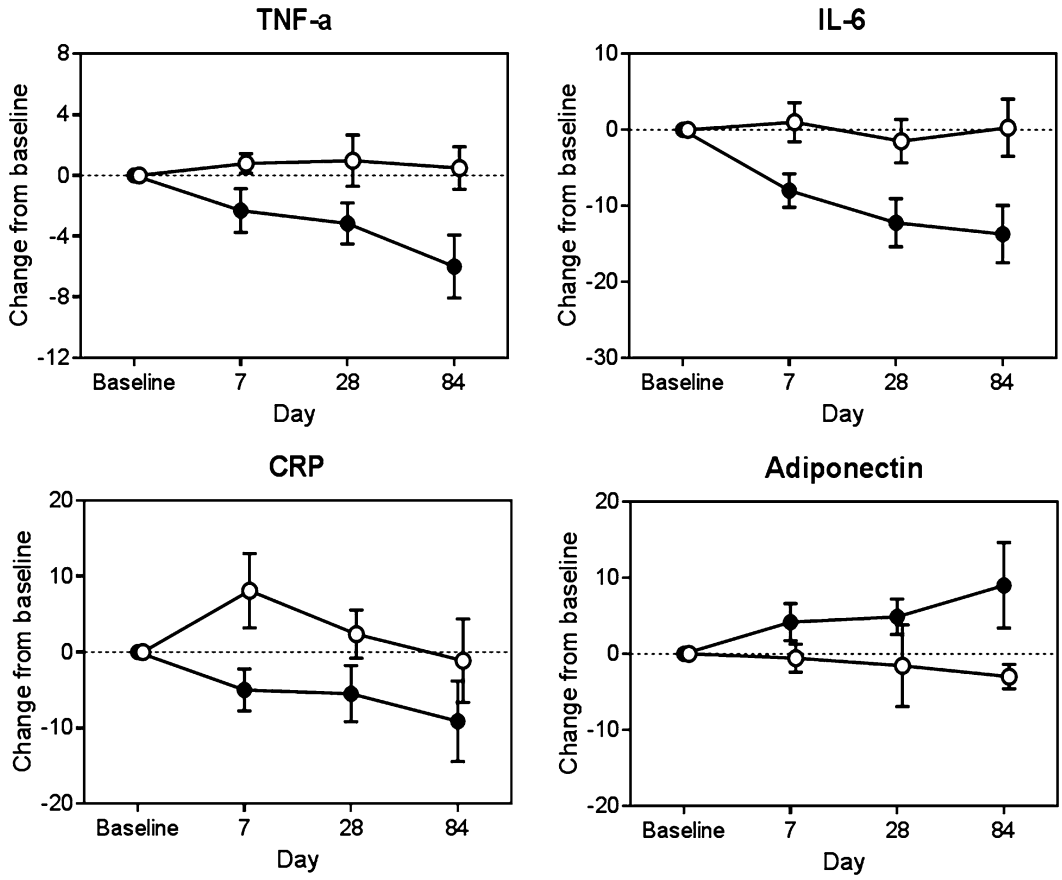

Fig. 39.4 Absolute changes in inflammatory biomarkers [tumor necrosis factor (TNF)-alpha (α), interleukin (IL)-6, C-reactive protein (CRP)] and adiponectin following a low dairy [*open circles* <0.5 daily serves] compared to adequate dairy [*black circles*, >3.5 daily serves] isoenergetic weight-maintenance diet in overweight and obese adults with metabolic syndrome. Values represent means with standard deviations. For all inflammatory markers, there was a significant diet effect [TNF-α, $P<0.01$; IL-6, $P<0.02$; CRP, $P<0.02$; adiponectin, $P<0.01$]. Based on data from [128]

As a result, there has been considerable interest in investigating whether vitamin D status or treatment with vitamin D exerts anti-inflammatory effects in humans.

To date there have been mixed findings from human observational and epidemiological studies examining the association between vitamin D status [serum 25(OH)D] and various inflammatory cytokines in asymptomatic adults and/or different subgroups with various pathological conditions [4, 60, 65, 105, 120]. In the Framingham Offspring Study involving 1,381 generally healthy community dwelling adults (mean age 59 years; 52 % women), plasma 25(OH)D concentrations were not consistently associated with various systemic inflammatory markers, including CRP, fibrinogen, IL-6, TNF-α and TNF-receptor-2 (sTNFR2) [121]. However, in a study of 6,538 British adults aged 45 years there was an inverse association between serum 25(OH)D and CRP and fibrinogen levels, but these findings were not significant after adjusting for adiposity, lifestyle, and social confounders [60]. Among 15,167 asymptomatic adults aged ≥18 years involved in the NHANES survey from 2001 to 2006, serum 25(OH)D at levels <52.5 nmol/L were inversely associated with CRP in both the univariate and multivariate analysis adjusting for traditional cardiovascular risk factors [4]. However, in the multivariate analysis there was a positive relationship between CRP and 25(OH)D at concentrations

above 52.5 nmol/L, suggesting that higher concentrations of 25(OH)D may be pro-inflammatory. While it is difficult to explain this latter finding, this study provides some evidence that the association between vitamin D status and inflammation may only exist at low serum 25(OH)D concentrations. However, these results must be interpreted with caution since only a single inflammatory marker was assessed and there was no adjustment for geographic location or time of year.

A number of short- and long-term intervention studies examining the effects of vitamin D supplementation on markers of inflammation in both healthy and "at risk" groups have also produced equivocal findings. Those which have consistently reported a beneficial effect of vitamin D treatment on pro- and/or anti-inflammatory markers have been conducted in people with various pathological conditions. For instance, there is evidence for an anti-inflammatory effect of vitamin D in people with chronic heart failure [117], chronic kidney disease [84], type 2 diabetes [119], multiple sclerosis [82], and prolonged critical illness [132]. Similarly, in a study conducted in postmenopausal women ($n=70$) with osteoporosis, 6 months of supplementation with 0.5 μg/day of calcitriol and 1,000 mg/day of calcium significantly reduced serum IL-1 and TNF-α levels and increased BMD at the lumbar spine, trochanteric and intertrochanteric regions; there were no changes in the calcium alone group [61]. In contrast, secondary analysis of a 3-year trial involving healthy, ambulatory community-based adults aged ≥65 years revealed that supplementation with 500 mg/day of calcium plus 700 IU/day of cholecalciferol had no effect on circulating CRP or IL-6 levels, despite improvements in BMD [106]. Similarly, a 6-month trial in postmenopausal women with osteoporosis comparing the effects of risedronate plus calcium (1,000 mg/day) and vitamin D (400 IU/day) versus calcium and vitamin D alone revealed no changes in serum IL-1β, TNF-α, RANKL or OPG after 3 or 6 months of treatment with calcium-vitamin D alone [32]. While treatment with risedronate was associated with a significant decrease in serum levels of RANKL and IL-1β and an increase in OPG levels, the changes in these inflammatory markers were not associated with changes in bone turnover at 3 or 6 months or BMD after 12 months. Collectively, these limited findings provide little evidence to support a cytokine mediated effect of vitamin D on bone health, which may be explained in part by the low doses of vitamin D (<700 IU/day) used in several of these trials.

To investigate whether higher doses of vitamin D might attenuate inflammatory cytokines, Zittermann and colleagues [150] examined the effects of supplementation with 83 μg/day (3,332 IU/day) of cholecalciferol for 6 months in overweight adults with low circulating 25(OH)D levels (mean 30 nmol/L) involved in a weight loss trial. Compared to placebo, vitamin D treatment resulted in a 10 % decrease in serum TNF-α, but no marked changes in IL-6 or CRP; both groups lost a similar amount of weight. In contrast, many previous intervention trials in relatively healthy or overweight/obese young and older adults have reported no marked changes in various inflammatory cytokines following supplementation with vitamin D at doses ranging from 200 to 4,000 IU/day [9, 10, 21, 43] or 20,000 to 40,000 IU per week [65] or even 100,000 IU twice over 10 weeks in patients with heart failure [143]. There are several factors that may explain these mixed findings, including: (1) differences in body composition and the health status of participants; (2) the baseline serum 25(OH)D concentrations of participants which may have already been sufficient, and (3) the baseline cytokines levels which may have been low prior to the intervention, providing little scope for improvement.

Currently there remains considerable debate with regard to the optimal serum 25(OH)D concentration for both skeletal and nonskeletal health benefits. However, it has been suggested that a 25(OH)D concentration as high as 80–100 nmol/L may be required for optimal immune function [56]. In a recent study designed to examine the specific mechanisms by which vitamin D might act on immune and inflammatory pathways, Zhang and colleagues [149] found that a minimum 25(OH)D concentration of 75 nmol/L in cell culture was needed to significantly inhibit IL-6 and TNF-α production induced by lipopolysaccharide (LPS), a molecule associated with bacterial cell walls that is known to promote an inflammatory response, in human monocytes. Despite this finding, a 12-month trial in healthy overweight adults aged 21–70 years with a mean serum 25(OH)D concentration of 56 nmol/L found that treatment with 20,000 or 40,000 IU of vitamin D_3 per week had no effect on a panel of 11

inflammatory cytokines, despite median 25(OH)D levels at follow-up of 141 and 98 nmol/L in the 40,000 and 20,000 IU dose groups, respectively [65]. While further long-term clinical trials are needed to investigate if there is a concentration of 25(OH)D and dose of vitamin D that might be effective for improving immune function, based on the current evidence it would appear that the immunomodulatory effects of vitamin D may only been seen when the immune system is stimulated, as is evident in people with a chronic disease(s), and/or when circulating 25(OH)D levels are deficient. Finally, given the findings from a recent 3-month trial which found that daily intake of fortified yogurt with 1,000 IU of vitamin D, with or without additional calcium, resulted in a significant reduction in serum hs-CRP, IL-6, IL-1β and fibrinogen and an increase in adiponectin in adults with type 2 diabetes [91], future studies should investigate the anti-inflammatory properties of foods fortified with calcium, vitamin D, and other nutrients identified to protect against inflammation.

39.4.4 Dietary Protein

Protein is one of the essential building blocks for bone that plays a central role in regulating circulating levels of insulin-like growth factor-1 (IGF-1), which is an important growth factor that regulates osteoblast function to optimize bone health. While it is beyond the scope of this chapter to review the effects of dietary protein on BMD and fracture risk, a recent systematic review and meta-analysis of population-based observational studies and intervention trials reported that a high total protein intake was positively associated with BMD and bone mineral content (BMC) at most skeletal sites, and that supplementation with protein had a small but positive effect on BMD at the lumbar spine [28]. While there was no significant effect of total protein intake on the risk of hip fracture [relative risk: 0.75 (95 % confidence interval), 0.47–1.21, $P=0.24$], this finding must be interpreted with caution as it was based on the results from only four studies. Nevertheless, given that protein is one of the key contributors to dietary acid load, which based on the acid-base hypothesis, may have a negative effect on bone health, particularly when calcium intake is inadequate, it is reassuring that there was little evidence for an adverse relationship between increased dietary protein and fracture risk.

Most of the beneficial effects of protein on bone have been attributed to its effects on increasing calcium absorption and circulating IGF-1 levels [54, 69, 118]. However, it is also possible that protein-induced increases in IGF-1 may indirectly have a positive effect on BMD and fracture risk via its anabolic effects on muscle and/or its role in reducing inflammation. For instance, there is evidence that higher levels of the pro-inflammatory marker IL-6 can decrease circulating levels of IGF-1, whereas low IGF-1 levels stimulate IL-6 [7, 29]. This suggests that IL-6 may oppose the effect of IGF-1 on bone (and muscle). There is also evidence that TNF-α can interfere with IGF-1 signaling and inhibit the signaling pathways downstream of the IGF-1 receptor, and thus decrease muscle protein synthesis which may lead to muscle loss [51]. While few human studies have investigated the interactive effects between IGF-1 and inflammation on bone health, several studies have reported a reciprocal relationship between IGF-1 and inflammation on muscle. In a population-based cohort of 526 adults aged 20–102 years, a reciprocal relationship was reported between IGF-1 and IL-6 on muscle strength and power [7]. When serum IL-6 levels were stratified according to tertiles, IGF-1 was positively related to muscle strength and power only in those in the lowest IL-6 tertile. Similarly, it has also been reported that low IGF-1 and high IL-6 concentrations were associated with an increased risk for incident walking limitations, mobility disability, disability in activities of daily living and death in older women [20]. In a 3-year prospective study in adults aged 65 years and over that investigated whether there was a synergistic relationship between dietary protein, inflammation and changes in muscle strength, a significant interaction was observed between dietary protein and serum CRP, IL-6 and TNF-α with the changes in muscle strength [11]. That is, in people with elevated inflammatory markers a lower protein intake was associated with a greater decline in muscle strength,

independent of the presence of chronic conditions. This suggests that chronic low-grade systemic inflammation can alter protein metabolism and may reduce the effects of protein on muscle, which may indirectly contribute to bone loss and increase fracture risk.

Whether a high protein diet or supplementation with different types and/or doses of protein has anti-inflammatory properties, either directly or indirectly via an increase in IGF-1, remains unknown. A systematic review and meta-analysis on the effects of higher versus lower protein diets on a range of health outcomes found there was no significant effect of higher protein diets on circulating CRP levels [112]. However, in overweight and obese premenopausal women, consumption of a diet higher in protein with an emphasis on dairy foods during a 16-week diet- and exercise-induced weight loss program improved markers of bone turnover as well as adipokine levels (adiponectin and leptin), serum OPG and RANKL, compared to those assigned to a low dairy diet [66]. Similarly, nutritional supplementation with amino acids (8 g of essential amino acids twice daily) for 18 months in 41 elderly outpatients with sarcopenia resulted in significant gains in lean mass with a parallel increase in IGF-1 and a reduction in TNF-α compared to those assigned to the placebo control group [126]. While further studies are still needed to evaluate the potential direct or indirect anti-inflammatory role of protein in humans, the above findings provide some evidence that diets higher in protein or dairy foods alone, or when combined with exercise, may help to reduce inflammation (most likely via an increase IGF-1 levels) and thereby enhance muscle and bone health.

39.4.5 Soy Protein and Milk Peptides

There are some reports that soy protein and its constituents, such as isoflavones, may also modulate inflammatory markers [19]. For instance, several clinical trials in postmenopausal women have reported that a soy-rich diet can reduce levels of TNF-α, IL-6 and CRP [5, 58]. In contrast, a review on the effects of soy foods and soy isoflavones on inflammation found that there was no consistent evidence for an effect on the cytokines IL-6 and TNF-α [13]. Similarly, the findings from several human trials have shown that specific milk or dairy proteins, such as whey or casein or milk peptides, have no marked effect on inflammatory biomarkers in overweight adults [97], mildly hypertensive people [80] or postmenopausal women [14]. Whether other specific branched chained amino acids, particularly leucine, which has a strong stimulatory effect on muscle protein synthesis, have anti-inflammatory properties and can modulate muscle health remain to be determined [92].

39.4.6 Vitamin K

There has been considerable interest in the potential role of vitamin K in preventing bone loss and fractures due to its function as a cofactor for the posttranslational γ-carboxylation of several vitamin K-dependent proteins, including osteocalcin, which is the primary noncollagenous protein in bone [52]. While more specific details about the role of vitamin K in regulating bone health has been reviewed elsewhere [52], the findings from a meta-analysis of randomized controlled trials ranging from 6 to 36 months revealed that supplementation with vitamin K had no marked effect on femoral neck BMD, but improved lumbar spine BMD [34]. However, the authors suggested that caution is warranted when interpreting these findings because there was considerable between study heterogeneity and publication bias. Nevertheless, several other reviews and meta-analyses have reported that supplementation with either vitamin K_1 (phylloquinone—which is found in vegetables) and vitamin K_2 (menaquinone—which is found in meat, eggs, dairy), particularly at higher doses (phylloquinone >1,000 μg/day or menaquinone >45 mg/day) and in combination with calcium and vitamin D, is associated with improvements in hip bone strength and a reduced incidence of clinical fractures [27, 63].

While the mechanism(s) by which vitamin K can slow bone loss and/or prevents fractures remains to be determined, it has been suggested that it may occur in part via suppression of inflammation as vitamin K has been shown to decrease the expression of genes for certain inflammatory cytokines [95]. Several human cross-sectional studies have also shown that high plasma phylloquinone concentrations, which represent a marker of vitamin K status, and/or a high dietary phylloquinone intake, were associated with lower circulating inflammatory markers, including CRP and IL-6, in middle aged and older adults [120, 121]. However, a follow-up intervention in healthy older men and women examining the effects of 3 years of supplementation with 500 μg of phylloquinone with additional calcium and vitamin D revealed there were no significant changes in circulating levels of IL-6 or CRP [121] or BMD [15]. Although a dose of 500 μg/day of phylloquinone is four- to fivefold greater than current recommended (adequate) intakes, the authors speculated that the lack of an effect in this study may relate to the dosage used and/or the relatively healthy status of the cohort studied [121]. Indeed, a previous phylloquinone supplementation study which reported a reduced rate of bone loss in postmenopausal women used a supplemental dose of 1,000 μg/day [16]. Despite these findings, given the limited data available it is currently not possible to make any firm conclusion about the anti-inflammatory properties of vitamin K and its influence on skeletal health.

39.4.7 Magnesium

Magnesium is an important mineral for skeletal health because of its role in regulating calcium homeostasis and the formation of bone mineral (hydroxyapatite). As reviewed elsewhere [94, 110], a number of human cross-sectional and epidemiological studies in pre- and postmenopausal women and elderly men have reported a positive relationship between dietary magnesium intake and BMD. However, the findings from longitudinal studies and a limited number of intervention trials with magnesium supplementation have produced mixed results; the greatest benefits appear to occur in people with low baseline serum magnesium levels. Although dietary sources of magnesium are widely available in food, particularly green leafy vegetables, whole grains and legumes, data from the 1999–2004 NHANES survey revealed that over half of all adults failed to meet the recommended dietary allowance (RDA) of 420 mg/day for males and 320 mg/day for females [93]. At present, the precise mechanism(s) by which magnesium deficiency may decrease bone mass is not clear, but there is evidence that magnesium deficiency is associated with an increase in both local and systemic inflammatory cytokines which may induce an increase in osteoclastic bone resorption. For instance, experimentally induced select dietary magnesium depletion in a rodent model has been shown to result in decreased bone mass which was coupled with changes in PTH and 1,25(OH)D, an increase in inflammatory cytokines and RANKL and a decrease in OPG [110]. It has also been shown that knockout of the TNF-α receptor in mice on a low magnesium diet is associated with reduced bone loss [111].

In humans, no study appears to have investigated whether there is a direct link between magnesium deficiency, inflammation and bone loss. However, there is consistent evidence showing that low serum magnesium concentrations and an inadequate dietary magnesium intake is associated with an increase in circulating inflammatory cytokines, particularly CRP [24, 73, 127]. For instance, among 3,713 postmenopausal women aged 50–79 years in the Women's Health Initiative Observational Study, dietary magnesium intake was inversely associated with hs-CRP, IL-6, and TNF-α-R2 in a dose dependent manner [24]. Similarly, in a cross-sectional analysis of 11,686 women aged ≥45 years participating in the Women's Health Study, CRP concentrations were found to be 12 % lower in women in the highest compared to lowest quintile of dietary magnesium intake [127]. Despite these results, it is difficult to determine whether these associations are due to magnesium alone or a combination of other nutrients since dietary magnesium intake is strongly related to other dietary nutrients, some of which may have anti-inflammatory properties [88]. Unfortunately, there are also mixed findings

from a limited number of intervention trials examining the effect of oral magnesium supplementation on inflammatory markers in apparently healthy overweight middle-aged women [88] and those with chronic disease, such as heart failure and prediabetes [3, 123]. Further intervention studies are still needed to extend our understanding of the influence of magnesium on inflammation and its link to diseases such as osteoporosis and fragility fractures.

39.4.8 Omega-3 Fatty Acids

Omega-3 fatty acids have been widely reported to have anti-inflammatory properties and up-regulate intestinal calcium absorption (for a review refer to Calder et al. [19]). Thus, there has been considerable interest in the role of omega-3 fatty acids in regulating bone health and metabolism. At present, the findings from human studies on the effects of a diet rich in omega-3 fatty acids or a lower omega-6 (n-6) to n-3 ratio (n-6 has been associated with increased inflammation) on BMD and fracture risk are mixed. For instance, there are some reports of a protective effect of omega-3 fatty acids against bone loss [36, 141] and fractures [35] while others have observed no significant effect or even an increased fracture risk [96, 137, 138]. Similarly, the results from a recent systematic review that included four randomized controlled trials with BMD as the primary outcome found that there was a beneficial effect of omega-3 fatty acids supplementation [primrose oil (high in linoleic acid) and fish oil] with calcium on BMD in only one trial [96]. Interestingly, this study was conducted in elderly women with osteopenia or osteoporosis, a group more likely to exhibit chronic systemic inflammation; all the others trials were conducted in healthy pre- and/or postmenopausal women or older men. Indeed, a systemic review on the effect of omega-3 fatty acids on inflammatory biomarkers found that they lowered circulating inflammatory markers in patients with acute and chronic diseases, but not apparently healthy subjects [108]. At present there is no consensus on the type or dose of omega-3 fatty acids required to lower systemic inflammation and/or improve bone health [108], but like most other nutrients it is likely that they will have their greatest benefits when inflammatory markers are elevated.

39.5 Conclusion

Aging is associated with chronic low-grade systemic inflammation (termed "*inflammaging*") that is now considered to be a key factor contributing to the development and progression of a range of musculoskeletal disorders, including osteoporosis, impaired muscle function, sarcopenia and fragility fractures. While the precise underlying factors contributing to systemic inflammation in older adults and the elderly are unclear, genetic factors, pathological conditions, and age-related changes in immune function together with a range of hormonal, environmental and lifestyle factors have all been implicated. Of the various lifestyle factors, there is a growing body of evidence demonstrating that dietary interventions, including weight loss and caloric restriction, and various dietary patterns, foods and nutrients may modulate the inflammatory response within the body, particularly in pathophysiological conditions when pro-inflammatory cytokines are elevated. Of the various dietary factors known to play a key role in regulating skeletal health which have been investigated in terms of their anti-inflammatory properties, there have been mixed findings for dietary and supplemental calcium and vitamin D, dairy products, dietary protein, vitamin K, magnesium and omega-3 fatty acids and their combination. In apparently healthy adults, there is no consistent evidence that these dietary factors modulate inflammation. However, a limited number of intervention trials have demonstrated that calcium and vitamin D supplementation, high dairy diets, increased dietary protein, vitamin K, and omega-3 fatty acids can produce modest reductions in circulating inflammatory biomarkers in people

with osteoporosis, sarcopenia or the presence of another chronic disease(s). Whether a reduction in these inflammatory markers translates into long-term beneficial effects on bone and muscle health or a reduction in fracture risk is not known. Given the established molecular and clinical evidence linking inflammation with bone metabolism, osteoporosis, sarcopenia and fractures, further intervention trials are warranted to evaluate the long-term efficacy of different nutrients or their combination on markers of inflammation and their relation to musculoskeletal health outcomes.

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Chapter 40
Impact of Nutrition on Medications for Osteoporosis

Jeri W. Nieves and Felicia Cosman

Key Points

- In many cases, depending on bone density and fracture risk, nutrition alone is not enough, and pharmacologic treatment may be required to prevent bone loss and osteoporosis-related fractures.
- Nutrition still plays an important role even in individuals being treated for osteoporosis.
- Many of the available osteoporosis treatments are capable of improving bone mass by 1–10 % at various skeletal sites over 3–5 years, but there must be an adequate nutritional supply of calcium and vitamin D in order to promote this bone gain.
- All pivotal phase III studies of the efficacy of treatments for osteoporosis have taken place in patients who were given calcium and vitamin D.
- There may be a minimal level of serum 25(OH)D required for optimal response to osteoporosis medication, likely between 25 and 33 ng/ml.
- The impact of calcium and vitamin D on pharmacologic therapy is inconsistent but in no cases was the efficacy reduced with adequate calcium and vitamin D.
- The recommendation to obtain adequate calcium and vitamin D through diet and/or supplements should maximize the beneficial effects of pharmacologic treatments.

Keywords Calcium • Vitamin D • Osteoporosis medication • 25(OH)D

40.1 Introduction

Nutrition is clearly important for skeletal health. Yet, in many cases, depending on bone density and fracture risk, nutrition alone is not enough, and pharmacologic treatment may be required to prevent bone loss and osteoporosis-related fractures. It is important to stress that nutrition still plays an important role even in individuals being treated for osteoporosis. In fact, Phase III pivotal trials for all of the

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currently approved treatments for osteoporosis were performed in conjunction with calcium and in most studies vitamin D. Therefore, bone mass increments and fracture reductions shown in these studies were achieved in the context of adequate calcium and vitamin D. Current guidelines for the treatment of osteoporosis recommend that patients being treated for osteoporosis should be supplemented with calcium and vitamin D, if needed, to bring intakes up to the current requirements.

In this chapter, we review epidemiologic studies and small clinical trials that have evaluated the efficacy of various osteoporosis treatments in individuals who have sufficient intakes of calcium and/or vitamin D as compared to those with insufficient intakes.

40.2 Interaction Between Nutrients and Treatments for Osteoporosis

Many of the available osteoporosis treatments are capable of improving bone mass by 1–10 % at various skeletal sites over 3–5 years. However, there must be an adequate nutritional supply of calcium and vitamin D in order to promote bone growth, similar to the amount that the skeleton needs during growth in childhood. Calcium is clearly a major substrate of bone and the skeleton contains 99 % of the body's calcium stores. Vitamin D is also needed for bone health, to aid in calcium absorption during periods of increased need, for example during BMD accrual due to the use of medication. Vitamin D also is likely to play a role in skeletal mineralization. In a recent meta-analysis, neither calcium nor vitamin D, when taken alone, were able to reduce the risk of fractures, but when given in combination calcium and vitamin D did reduce the risk of hip fractures [1]. Evidence is provided throughout this chapter to illustrate the relationship between pharmacologic osteoporosis treatments and adequate calcium or vitamin D intake, using available epidemiologic data.

The importance of adequate nutrition, as an adjuvant to osteoporosis medication following a fracture, was recently evaluated. A study of over 23,000 patients with recent hip fracture led to the finding of improved survival if calcium plus vitamin D *or* vitamin D supplements alone were purchased in combination with anti-osteoporosis drugs (hazard ratio and 95 % confidence interval 0.72 (95 % CI 0.50–1.03) in men and 0.62 (95 % CI 0.50–0.76) in women) [2].

40.3 Estrogen and Raloxifene

A review of several early clinical trials of estrogen (ET) and hormone therapy (HT) efficacy found that bone density response was greater in women who had an adequate intake of calcium compared to women who took estrogen with an insufficient amount of calcium [3]. Results from a subsequent clinical trial supported this and demonstrated that the combination of HT and vitamin D3 increased femoral neck BMD more than HT alone in women with osteoporosis [4]. In a small study of 169 women in Japan, supplementing raloxifene with alfacalcidol (1 alpha-hydroxy-D) showed greater improvements in parathyroid hormone suppression, but not bone density, compared to raloxifene given alone. The lack of a result for BMD may have been due to limited power for this outcome [5]. The effects of raloxifene on hip and spine BMD did not vary by vitamin D status at randomization ($P > 0.08$ and $P > 0.7$, respectively) in 7,522 postmenopausal participants of the Multiple Outcomes of Raloxifene Evaluation, a placebo-controlled trial of the effects of raloxifene on BMD and fracture [6]. In this trial, all women were provided daily supplements of 500 mg calcium and 400–600 IU cholecalciferol for 1 month before starting, and during treatment (raloxifene or placebo). Given that the major randomized controlled trials of raloxifene have been in women with adequate calcium and vitamin D intakes, these medications should be used in the context of adequate nutrition, particularly calcium and vitamin D.

40.4 Bisphosphonates and Denosumab

Many trials have looked at the combined effect of vitamin D serum levels or intake in response to bisphosphonates (BP). This is important given that about two-thirds of patients previously diagnosed with osteoporosis have inadequate vitamin D status [7].

40.4.1 Alendronate

In postmenopausal women ($n=701$) with a daily intake of 800 mg calcium and 400 IU vitamin D, the effect of alendronate (ALN) 10 mg daily, either with or without calcium (1,000 mg), was evaluated using the endpoints of bone density and bone turnover markers after 24 months [8]. In that trial, addition of calcium supplementation to alendronate did not significantly increase BMD compared to alendronate alone ($p=0.29–0.97$), but did result in a statistically significant, though small, additional reduction in a marker of bone resorption (urinary N-telopeptide). Vitamin D and BP use in combination may also impact calcium absorption. In a randomized, double-blind, clinical trial, 56 postmenopausal women with 25-hydroxyvitamin D [25(OH)D] concentrations of 25 ng/mL or less and low bone mineral density (BMD) received 5 weekly doses of placebo or alendronate 70 mg plus vitamin D3 2,800 IU (ALN + D). Prior to randomization, calcium intake was stabilized to approximately 1,200 mg/day and fractional calcium absorption was assessed at baseline and again after 1 month. The results were that the ALN + D group experienced an increase in calcium absorption at 5 weeks in excess of the increased absorption expected from vitamin D alone [9].

A post hoc analysis of 1,000 women from the Fracture Intervention Trial found that baseline vitamin D levels did not determine BMD or fracture response to alendronate, which was co-administered with daily intakes of 500 mg calcium and 250 IU cholecalciferol, although this is a relatively low dose of vitamin D [10]. In another study, only in patients with vitamin D deficiency was an advantage of alendronate plus calcifediol 0.266 mg weekly (~10,000 IU weekly) noted, based on a 25 % greater fall in the bone resorption marker CTX [11].

One study compared random assignment of ALN 70 mg once a week alone or plus calcitriol (1,25D3/0.5 mg daily) in 120 subjects. ALN plus calcitriol demonstrated a significantly higher increase in lumbar spine BMD and a greater decrease in parathyroid hormone levels than those receiving ALN alone [12]. An analysis from a Canadian cohort study found that in elderly patients with osteoporosis who were not responding to BP, the addition of 1,000 IU of vitamin D improved BMD at the lumbar spine [13].

In some studies the level of serum 25(OH)D was evaluated to determine if a certain level led to improved efficacy with bisphosphonate therapy. In one observational study of 210 patients, a positive response to BP therapy on BMD was seen in 47 % of patients. Furthermore, patients with a mean 25(OH)D ≥33 ng/ml had an ~4.5-fold significantly greater odds of a favorable BMD response [14]. In an evaluation of 52 postmenopausal women, treatment response with alendronate was positively related to basal serum 25(OH)D concentration. Furthermore, BMD changes were significantly greater in persons with serum 25(OH)D above 25 ng/ml, a finding that did not hold for a serum cutpoint of 30 ng/ml [15]. An inadequate response to BP treatment was also noted in a study of 120 women, particularly if 25(OH)D levels were not maintained over 30 ng/ml [16]. In 112 women on bisphosphonates, the skeletal response was maximized when serum 25 (OH) vitamin D concentrations exceeded 70 nmol/l (28 ng/ml) [17]. These data indicate that a minimal serum 25(OH)D level of approximately 25–33 ng/ml may be needed for optimal response to bisphosphonates.

Based on some of the above data, vitamin D was added to alendronate after a 15-week, randomized, double blind study of ALN + D ($n=360$) or ALN ($n=357$) once weekly showed similar

antiresorptive efficacy, improved serum 25(OH)D and no adverse findings, when compared to subjects given alendronate alone [18]. In terms of further benefits of combined therapy, a study of 2,579 subjects provided 70 mg alendronate and 7 capsules of 1 mcg alfacalcidol weekly achieved significant improvement in physical performance (chair stands and timed up and go) from their baseline [19]. This may have an impact on fall risk reduction as well.

40.4.2 Risedronate

In a study of 164 Korean adults, a combination of risedronate 35 mg and cholecalciferol 5,600 IU administered weekly for 16 weeks led to an increase in 25(OH)D level, but bone turnover did not decrease more than in the risedronate-alone group [20].

40.4.3 Zoledronic Acid

In a cohort of women from two clinical trials (zoledronate $n=154$, placebo $n=68$) with baseline serum 25(OH)D levels that exceeded 25 nmol/l, baseline dietary calcium intake and vitamin D status did not alter the effects of zoledronic acid [21]. In a randomized trial where 60 women were given either cholecalciferol (300,000 IU) or placebo, 5 days before an infusion of zoledronic acid, there was less intense musculoskeletal pain and lower levels of inflammatory markers in the cholecalciferol group [22].

40.4.4 Denosumab and Zoledronic Acid

In a retrospective analysis of 111 subjects, those subjects with serum 25(OH) vitamin D concentration <50 nmol/L had a mean PTH concentration level of 44 ng/L. Patients were then evaluated; if PTH levels were <44 ng/L these patients had significantly higher BMD response at the hip and lower bone turnover, compared to those patients with PTH ≥ 44 ng/L, whether treated with zoledronic acid or denosumab [23].

40.5 Teriparatide

Teriparatide is in a class of drugs considered to be anabolic, or bone forming, and therefore requires that adequate nutrition be present in order to meet the skeletal demand of increasing bone mass. There is little known about the interaction between teriparatide treatment and calcium or vitamin D status. In a small study there were 7 patients with a history of resolved secondary hyperparathyroidism attributable to vitamin D deficiency and they responded to teriparatide therapy no differently than the 88 patients without such a history [24]. Consistent with the mechanism of action of teriparatide, in the pivotal trial where all subjects received 1,000 mg calcium and 400–1,200 IU vitamin D, teriparatide treatment led to increases in $1,25(OH)_2D$ concentrations and decreases in 25(OH)D concentrations [25]. However, there were no consistent effects of these changes on bone turnover or BMD responses to teriparatide.

40.6 All Treatments Combined

Vitamin D repletion was reported to maximize the response to anti-resorptive therapy (alendronate, risedronate, raloxifene) in terms of both BMD changes and anti-fracture efficacy [26]. In this retrospective study, the adjusted odds ratio for incident fractures in vitamin D deficient as compared to vitamin D replete women all while on treatment was 1.77 (1.20–2.59, 95 % CI; $p = 0.004$).

40.7 Conclusion

In conclusion, there are a number of options for treatment of osteoporosis. The best approach is to reduce all possible risk factors, optimize nutrition, particularly calcium and vitamin D, and encourage an exercise program, which has an overall health benefit as well as impact on the skeleton and muscle. Pharmacologic therapies should be initiated in patients with osteoporosis and those at highest risk. Different pharmacologic therapies might be appropriate for women at different ages and according to their personal and family medical history. All pivotal Phase III studies of the efficacy of treatments for osteoporosis have taken place in patients who were given calcium and vitamin D. There may be a minimal level of 25(OH)D required for optimal response to osteoporosis medication, likely between 25 and 33 ng/ml. The impact of calcium and vitamin D on pharmacologic therapy is inconsistent but in no cases was the efficacy reduced with adequate calcium and vitamin D. Therefore the recommendation to obtain adequate calcium and vitamin D through diet and/or supplements should maximize the beneficial effects of pharmacologic treatments.

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Chapter 41
Nutrition and Bone Health in Space

Scott M. Smith, Martina Heer, and Sara R. Zwart

Key Points

- The effect of weightlessness on the human skeletal system is one of the greatest concerns in safely extending space missions.
- The ability to understand and counteract weightlessness-induced bone mineral loss will be vital to crew health and safety during and after extended-duration space station and exploration missions.
- Research on bone mineral loss during space flight has gone on for more than half a century, and recent studies have shown significant progress in developing countermeasures that have proved to be effective, including good nutrition and exercise.

Keywords Microgravity • Space flight • Weightlessness • Calcium • Vitamin D • Protein • Omega-3 fatty acids • Iron • Vitamin K

41.1 Introduction

The effect of weightlessness on the human skeletal system is one of the greatest concerns in safely extending space missions [1–11]. The ability to understand and counteract weightlessness-induced bone mineral loss will be vital to crew health and safety during and after extended-duration space station and exploration missions [1–7]. Research on bone mineral loss during space flight has gone on for more than half a century, and recent studies have shown significant progress in developing countermeasures that have proved to be effective, including good nutrition and exercise. We review the history of this research here and provide a summary of recent and ongoing studies, including efforts to counteract bone and calcium loss resulting from weightlessness.

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Unfortunately, the most obvious nutritional countermeasure—providing excess calcium—does not protect against bone loss [12]. This result is likely related to the decreased calcium absorption observed in space flight and in ground-based models [13–16]. Phosphate supplementation was also ineffective at reducing calcium excretion [17]. Combination therapy with calcium and phosphorus was also unsuccessful at mitigating bone loss and hypercalciuria [18]. Other nutrients, specifically sodium, protein, potassium, vitamin K, and omega-3 fatty acids, have also been proposed and/or tested as bone loss countermeasures [19], and are discussed in more detail below.

41.2 Space Flight and Bone and Calcium Loss

As a result of skeletal unloading during flight [20–24], bone mineral is lost, leading to increased urinary excretion of calcium [21, 23, 25]. It is often estimated that the rate of bone mineral loss in the total body during space flight is about 0.5–1 % per month [26–28]. After missions of 4–6 months, losses averaged across all skeletal sites are estimated to be 2–9 % [6], with significant site-to-site (Fig. 41.1) and individual variability. The bone loss and mineral shedding, including about 200–250 mg of calcium per day [14, 15, 25, 29–31], are accompanied by an increased risk of renal stone formation during and after flight [32–36]. The effects of space flight and return on bone and renal stone risk are not different between men and women [37].

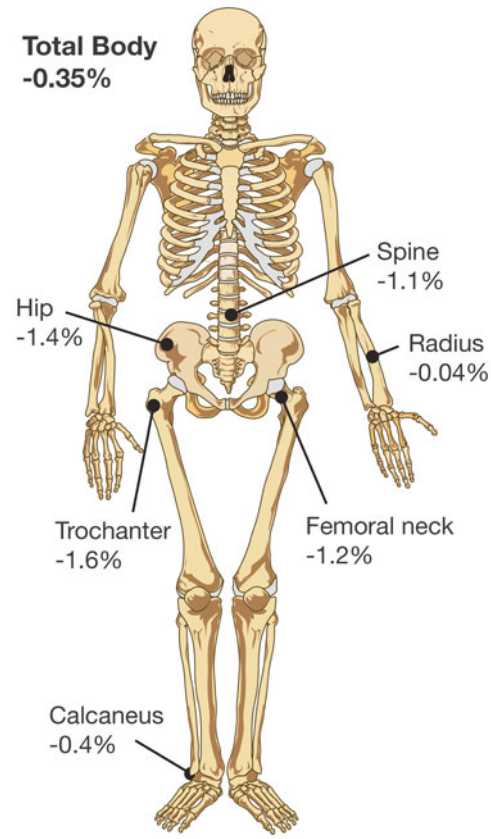

Fig. 41.1 Average bone mineral density losses in skeletal regions. Data are from Russian space station Mir and early ISS missions expressed as % loss per month [27, 38]

The Skylab studies showed that during space flight, bone mineral was not uniformly lost from all parts of the skeleton. Loss of bone tissue was greatest in mechanically loaded bones, but of the three crewmembers on the 59-day Skylab 3 mission, one lost a significant amount of os calcis bone mineral (−7.4 %) but the other two did not (+2.3 and +1.4 %). Calcium excretion in the urine was 200 % of the preflight value for the crewmember who lost os calcis mineral and 50 % of the preflight values for the other two crewmembers [21]. This subject-to-subject variability remains a hallmark of space flight-induced bone loss [39], and may provide insight into finding a means to mitigate this loss.

Negative calcium balance was observed during the Skylab [21, 23, 25, 30, 40–42] and Mir [14, 15] missions. During the 84-day Skylab 4 mission, calcium balance was −200 mg/day [25, 29, 30], but no significant calcium losses occurred during the 28-day Skylab 2 mission [21, 26]. Increased urinary and fecal calcium excretion accounts for most of the deficit in calcium [14, 15, 21, 23, 25, 33, 40, 42]. During the Skylab 4 mission, calcium losses correlated roughly with bone mineral losses [43] and increases in the excretion of hydroxyproline [29, 39]. Estimates of bone calcium loss from multiple studies and techniques converge on the estimate of about 250 mg of bone calcium lost per day during flight [14, 15, 25, 31]. When this rate of loss may slow down is not yet known, but it does not seem to be within the first 6 months of flight. Studies of bone metabolism have not been possible during space missions beyond 6 months, and the limited postflight bone assessment does not allow determination of the rate of loss during flight.

Bone loss and altered calcium metabolism occur in paralyzed individuals (as reviewed by Elias and Gwinup [44]), and a number of similarities can be found between these changes and those associated with space flight [45–48]. The loss of bone that occurs after spinal cord injury seems to stabilize after about 25 weeks, but remains above preinjury levels for years [49, 50].

Long-term follow-up data on bone recovery are far from complete [7, 51]. However, calcium balance data indicate that the rate of recovery is about +100 mg/day [14, 15]. By these estimates, on flights up to about 6 months duration, two to three times the mission duration is needed to recover the lost bone. Analysis of bone recovery data from dual-energy x-ray absorptiometry analyses suggests that although regional differences occur, the half-life of bone recovery after flight is on the order of 5–9 months [7, 52]. For longer exploration missions, however, the usefulness of these estimates comes into question, as space flight data are not available for these durations.

41.3 Space Flight and Bone Metabolism

Increased collagen cross-link excretion, and thus bone resorption, has been clearly shown to occur during space flight [14, 15, 53–56]. Hydroxyproline excretion was elevated during short-duration Shuttle flights [56] and Skylab flights of longer duration [25, 57, 40]. Calcium tracer kinetic studies also provided data indicating that bone resorption increases about 50 % during flight relative to before flight [14, 15]. Calcium balance studies showed calcium loss, and the initial data, along with data from animal models, suggested that a decrease in bone formation was the source of the calcium loss while bone resorption changed little. The role of bone resorption was later clarified by studying mature (as opposed to growing) animals and by examining resorption-specific markers [58].

Bone formation either remains unchanged or decreases during space flight [14, 15, 26]. Serum concentrations of bone-specific alkaline phosphatase and osteocalcin indicated that bone formation was unchanged during Mir flights, but increased after landing [14, 15]. Trends toward decreased levels of bone formation markers were noted in two Mir case studies [54, 55]. The results of studies using calcium tracer techniques showed bone formation in three Mir crewmembers [14, 15] to be equivocal (formation was unchanged or decreased). Together, increased resorption and decreased or unchanged formation yield an overall negative calcium balance [14, 15].

A potential confounding factor in early space flight studies is inadequate energy intake [59]. Inadequate energy intake can have negative effects on bone, and these are exacerbated by exercise [60, 61]. Although recent crews on the ISS have consumed close to 90 % of estimated energy requirements (discussed below), Shuttle, Mir, and earlier ISS crewmembers consumed about 70 % of caloric requirements [59].

The exact triggering mechanism for these changes in bone metabolism during space flight has yet to be identified, but the physiological and endocrine responses to them are as expected. The release of calcium from bone suppresses the secretion of parathyroid hormone (PTH), which results in lower levels of activated vitamin D (1,25-dihydroxyvitamin D), which leads to a reduction in calcium absorption from the gastrointestinal tract. Studies of calcium metabolism were conducted on Mir, and indeed, PTH, 1,25-dihydroxyvitamin D, and calcium absorption were all decreased [14–16]. Although it remains important to maintain calcium intake during flight, the lower calcium absorption during flight suggests that increasing calcium intake is not a viable countermeasure for weightlessness-induced bone loss, a fact proven in bed rest studies [62–64].

41.4 Bone Loss and Analogs of Space Flight

Space flight analog studies (such as bed rest) with humans have shown qualitative effects on bone and calcium homeostasis similar to those shown in flight studies [39, 65, 66], with quantitative effects generally being of smaller magnitude. Effects include loss of bone mass [67–70], decreased calcium absorption [13], increased urinary excretion of calcium and biochemical markers of resorption [13, 18, 23, 70–80], increased risk of renal stone formation [76, 79, 81], and decreased serum concentrations of PTH [71, 72, 74, 80, 82, 83] and 1,25 dihydroxyvitamin D [13, 71, 74, 83, 84].

That bone resorption increases during bed rest has been shown by histomorphometry [69, 85] and measurement of biochemical markers. Excretion of hydroxyproline [13, 18, 77] increases during bed rest, and excretion of collagen crosslinks [13, 53, 70–72, 80, 82, 83] is elevated about 20 % by the second day of bed rest [86], plateauing at about 50 % above control levels after a few weeks [71, 80, 82]. The increase in cross-link excretion during space flight is greater than 100 % [14, 15, 53, 54].

The concentrations of biochemical markers of bone formation are mostly unchanged during bed rest [13, 68, 71, 72, 80, 82, 83], but histomorphometry data from bone biopsies show that bone formation decreases [69, 74, 85]. This difference likely reflects the difference between site-specific (biopsy) and systemic (biochemical markers) indices of bone formation. After ambulation begins following bed rest, bone formation generally increases [13, 68]. When data from many studies (14–90 days of bed rest) were evaluated together, bone formation markers tended to increase over the course of bed rest [80, 70]. Whereas in the initial publications there was no statistically significant difference in BSAP over time, a retrospective analysis with data from multiple studies showed a statistically significant increase in serum BSAP (Morgan et al., in review). We speculate that this might reflect the beginning of a bone remodeling process, given that bone resorption does not similarly show analogous changes over time. Recent studies of sclerostin, an inhibitor of bone formation, have shown that it increases during bed rest [87, 88] and in unloaded animal models [89].

41.5 Exercise

All crewmembers on long-duration missions are required to exercise, a combination of resistance and aerobic exercises [90]. Exercise countermeasures have been implemented during flight as far back as the Skylab missions (Skylab was the first vehicle to allow enough room for exercise) [91, 92]. In addition to flight experiments, extensive ground-based testing has been done to evaluate the effectiveness

of exercise as a countermeasure for muscle, bone, and cardiovascular maladaptations that occur during space flight [4, 65, 93–96]. It was documented that treadmill and cycle exercise devices available on Mir did not prevent bone and calcium loss [14, 15, 27, 97, 98]. This was largely attributed to the fact that exercises with these devices are aerobic and not resistive types of exercise, and that treadmill running during flight does not provide the same ground-reaction force that is achieved on Earth.

In 2008, the advanced resistive exercise device (ARED) was launched to the ISS. This device accommodated additional exercise protocols, and more importantly, it had almost twice the loading capability of the previously used device, the interim resistive exercise device [99]. Comparing crewmembers exercising with each device has been somewhat difficult, given that more recent crews have maintained energy intake at levels >90 % of estimated requirements and have had better vitamin D status than earlier crews. Nonetheless, these better nourished crewmembers exercising with the ARED maintained body mass during flight (and came back leaner, with less body fat), and maintained bone mineral density in most regions and in whole-body scans, when assessed by DXA [99, 100]. When this result was published in 2012, the number of subjects was small, in part because many crewmembers participated in other countermeasure studies and were not included in this initial analysis. As additional crewmembers have completed flights they have been added into the analysis [37], and the findings hold—well-nourished crewmembers exercising with the ARED can indeed maintain bone mineral density after flight. Another result of the increased number of crewmembers flying long-duration missions is that data are now available to document that men and women have the same response to space flight with regard to bone loss, renal stone risk, and the ability of exercise to protect bone mineral density [37].

These data are very encouraging, but crewmembers exercising on the ARED do have alterations in bone biochemistry (and potentially bone architecture and strength). The exercise does not simply reverse the flight-induced alterations in bone metabolism. In fact, bone resorption is still increased above preflight levels, but bone formation is significantly increased.

The bone biochemical changes in crewmembers exercising with the ARED were very similar to what had been observed in bed rest studies testing resistance exercise. The exercise did not affect bone resorption, but did increase bone formation [99]. In the space flight study, as published, there was only a trend for increased bone formation ($p<0.06$) [99], whereas in bed rest, heavy resistance exercise 6 days a week led to a dramatic increase in bone formation markers [101], and in another study with resistance exercise every other day (combined with a treadmill protocol), roughly half the bone formation response [72] seen in the first study was obtained [101]. The exercise also did not have a significant effect on serum total calcium or urinary calcium. When data from additional crewmembers became available, they solidified the "trend" into statistical significance. Part of the explanation for the softer response during space flight than during bed rest is that the astronaut conditioning and strength trainers were initially reluctant to have crewmembers exercise too hard with the ARED, to minimize the risk of injury. Looking at the trend of changes in bone formation over the course of a 6-month mission, one sees a slow, steady increase over time [99], as opposed to results from the bed rest study, in which concentrations of bone formation markers plateaued the first time they were determined during bed rest (after 6 weeks of bed rest) [101].

Although this mode of bone remodeling, with increases in both resorption and formation, maintained bone mineral density during space flight, it may yield a bone with strength characteristics different from those that existed before flight. Studies to assess bone strength after flight are under way at NASA, to better understand what happens to bone under these conditions. Studies are also underway to evaluate optimized exercise protocols and nutritional countermeasures.

It should be noted that other physical and pharmacological countermeasures have been (and continue to be) tested in both ground-based and flight studies. Replacement of gravity by centrifugation ("artificial gravity") has been proposed as a multi-system countermeasure [102, 103], but particularly for bone. Although some initial studies have been completed, the optimal artificial gravity prescription for bone, including dose, duration, and frequency of centrifugation, remains to be identified [82, 104], along with its potential impact on nutrition and related systems [105, 106]. Vibration protocols, both

low and high frequency, have also been tested [107, 108], the latter serving as much as an exercise modality as a vibratory stimulus [109–111].

Pharmacological agents, the most common being the bisphosphonates, have also been tested for their ability to mitigate weightlessness-induced bone loss. Many ground analog studies of bisphosphonates (including bed rest studies and studies of patients immobilized because of spinal cord injury or other reasons) have been conducted, with generally positive findings [4, 10, 69, 112–121]. Ongoing discussion and debate surround the relative safety of these compounds for use in otherwise healthy individuals (astronauts), as opposed to the target population for whom the drugs were developed (most often elderly patients with disorders such as osteoporosis). In addition to resolving safety concerns, investigators have yet to determine the optimal drug, dose, and schedule of administration during space flight. Nonetheless, pharmacological agents may be required on exploration-class missions where a full suite of exercise equipment may not be available, or for individual cases in which a crewmember cannot exercise (for example, due to injury or illness).

41.6 Vitamin D

Vitamin D is critical for bone health and calcium metabolism, on Earth and in space. The absence of ultraviolet light exposure in the shielded spacecraft diminishes vitamin D stores in astronauts, as observed during the 84-d Skylab mission [40], Russian space station Mir missions [14, 15], and early ISS Expeditions [122]. Despite provision of vitamin D supplements on early ISS Expeditions (average supplement use was 3.0 ± 2.8 tablets per week of a 400-IU vitamin D supplement intended for daily use), the mean serum concentration of 25-hydroxyvitamin D for the ISS crewmembers was about 25 % less after landing than before launch [122].

In 2006, vitamin D recommendations to crews increased from 400 IU vitamin D/day to 800 IU vitamin D/day. In-flight 25-hydroxyvitamin D data provide evidence that 800 IU vitamin D/day is enough to maintain vitamin D status during long-duration space flight (Fig. 41.2) [99]. Despite this finding, similar to the situation for calcium, there is little evidence that maintaining vitamin D status, or providing excess vitamin D, will serve as a countermeasure to the bone loss of space flight.

An ideal ground-based model for individuals lacking ultraviolet light exposure is the Antarctic, where winter levels of ultraviolet B radiation are essentially zero. Two studies have been conducted at McMurdo Station, Antarctica, to determine the dose of vitamin D needed to sustain serum levels of 25-hydroxyvitamin D during a 5- to 6-month period when there is little to no ultraviolet B (UV-B) exposure, without increasing risks of hypercalcemia [123, 124].

Several ground-based studies (performed in Antarctica and at the Johnson Space Center) support the idea that a vitamin D dose in the range of 800–2,000 IU/day is tolerable and safe, and can maintain vitamin D status for 3–6 months even in environments with no UV light exposure [123–125]. This range is in line with the recent Institute of Medicine recommendations for vitamin D intake for North Americans [126]. One study conducted at McMurdo Station in Antarctica showed an interactive effect of serum cortisol and vitamin D status on immune function [124]. In that study, subjects with higher serum cortisol and lower vitamin D status presented with more latent virus reactivation in their saliva. It is clear that vitamin D may have an effect on other systems besides bone, but further research is required before evidence-based recommendations can be made for other systems.

Throughout the ISS program, supplemental vitamin D has been provided to astronauts to ensure that they have optimal vitamin D status. Efforts to provide vitamin D supplements are misinterpreted to infer that this might be a viable bone loss countermeasure, but this is not the case. Even when vitamin D stores during flight are adequate, the circulating concentration of the active form of vitamin D, 1,25-dihydroxyvitamin D, is decreased [14, 15]. As described in the section on calcium, this is likely the result of the increased release of calcium from resorbed bone, and results in decreased intestinal

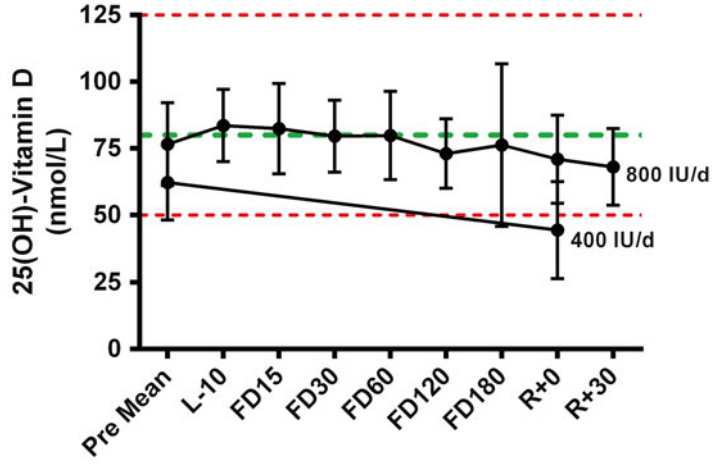

Fig. 41.2 Pre- and postflight data from medical operations required testing show that vitamin D status of crewmembers decreased after long-duration space flight, despite supplementation with 400 IU/day of vitamin D. In-flight data show that 800 IU/day is enough vitamin D3 to maintain status during long-duration space flight. *Red lines* depict Institute of Medicine-defined lower acceptable limits (with respect to bone health), and upper advised limit [126]. The *green line* at 80 nmol/L depicts what many perceive to be an optimal level with respect to parathyroid hormone suppression and non-bone health outcomes

absorption of calcium. Adequate stores of 25-hydroxyvitamin D will not affect this situation. Any attempt to directly provide the 1,25-dihydroxyvitamin D, or as in some cases on Earth, excess 25-hydroxyvitamin D levels, may lead to hypercalcemia, renal stones, soft tissue calcification, and at the extreme, even death. Controlled trials in bedridden subjects have also proven that several months of supplementation fail to affect bone metabolism. In one trial, bedridden elderly people took supplemental vitamin D (400 or 1,200 IU/day) or placebo for 6 months. Little effect was found on PTH, and no effect on bone markers [127]. In a similar 40-week trial of 1,000 IU of vitamin D2 or D3 (two groups), neither had an effect on bone markers [62]. The problem of weightlessness-induced bone loss must be solved, but vitamin D is not the answer. Nevertheless, even if bone loss is not stemmed, ensuring an adequate amount of vitamin D will remain important because of its effect on other physiological systems that are also affected by space flight [124].

Toxicity of vitamin D is typically less likely to occur than a deficiency [128–131], but use of supplements increases its likelihood. Excessive blood levels of vitamin D can lead to hypercalcemia, which can lead to nephrocalcinosis, arteriosclerosis, and soft tissue calcification. In one study conducted in Houston in healthy individuals, 50,000 IU/week for 4 weeks and then monthly increased the incidence of urinary calcium excretion that was higher than the normal range [125]. In that study, a 2,000-IU/day daily dose or a single 10,000-IU weekly dose did not increase the incidence of hypercalciuria.

41.7 Iron

Changes in iron metabolism and hematology occur soon after entering weightlessness, and a hallmark of this is a 10–15 % decrease in red blood cell mass during flight as a result of neocytolysis [132, 133]. One consequence of the decreased red blood cell mass is the subsequent transfer of the iron from those cells into storage proteins and processes. Evidence of this includes increased circulating concentrations of serum ferritin, an index of iron storage, after short- and long-duration space flights [122, 134, 135].

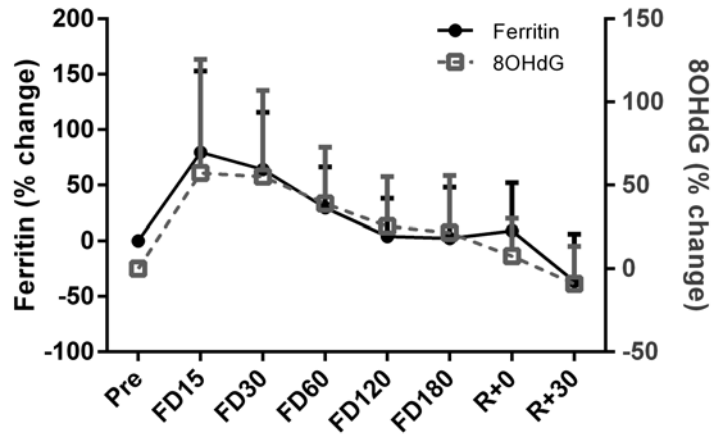

Fig. 41.3 The percentage change in ferritin and urinary 8OHdG before, during, and after long-duration space flight ($n=23$). "Pre" was determined from the mean of preflight data points (3 for ferritin, 4 for 8OHdG), and percentage change was calculated from that average [140]

Although the in-flight decrease in RBC mass is significant, the efficient postflight recovery suggests that it represents a physiological (as opposed to pathological) adaptation to weightlessness. After the first weeks of flight, RBC mass and body fluid volumes reach new plateaus (lower than on Earth), as shown by data from long-duration flights [136–139].

In-flight data show that iron stores increase early during a mission (within 15 days) and then return to preflight concentrations by the end of a 6-month mission [140]. In a recent study with 23 crewmembers on missions of 50–247-day duration, ferritin increased about 220 % in women and 70 % in men by flight day 15 [140]. At several time points, the transferrin index exceeded 1 μmol iron/μmol transferrin, which provides evidence for an iron overload [141]. Other acute-phase proteins (C-reactive protein and ceruloplasmin) were not changed during flight, indicating that the ferritin response was likely not just an inflammatory response. This study showed that the amount of increase in ferritin (area under the curve) was associated with the change in bone mineral density after flight, which was supported by the association between ferritin and other markers of iron status and markers of bone resorption. The change in ferritin over the course of a 6-month mission is presented below (Fig. 41.3), and this change was nearly identical to the change in urinary 8-hydroxy-2′-deoxyguanosine (8OHdG, a marker for oxidative damage) during space flight. Furthermore, the greater the increase in ferritin during flight (or the longer it was elevated—either case would result in a greater area under the curve), the greater the decrease in bone mineral density in the hip, trochanter, hip neck, and pelvis after long-duration space flight [140] (Fig. 41.4). These data are important to show that mean ferritin concentrations during flight that were not outside the normal clinical range were associated with evidence of oxidative damage and bone resorption. This association is supported by other studies in healthy ground-based populations [142–144].

41.8 Omega-3 Fatty Acids

The body of evidence demonstrating that dietary long-chain omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA), have beneficial effects on bone is now substantial [145–149]. The protective effect of EPA has been attributed to its anti-inflammatory actions and inhibition of signaling factors, such as NF-κB, that are associated with inflammation and downstream activation of the ubiquitin-proteasome system and osteoclasts [145, 150–154]. NF-κB deficiency in mice suppresses bone changes induced by unloading [155]. In vitro models show that EPA can inhibit

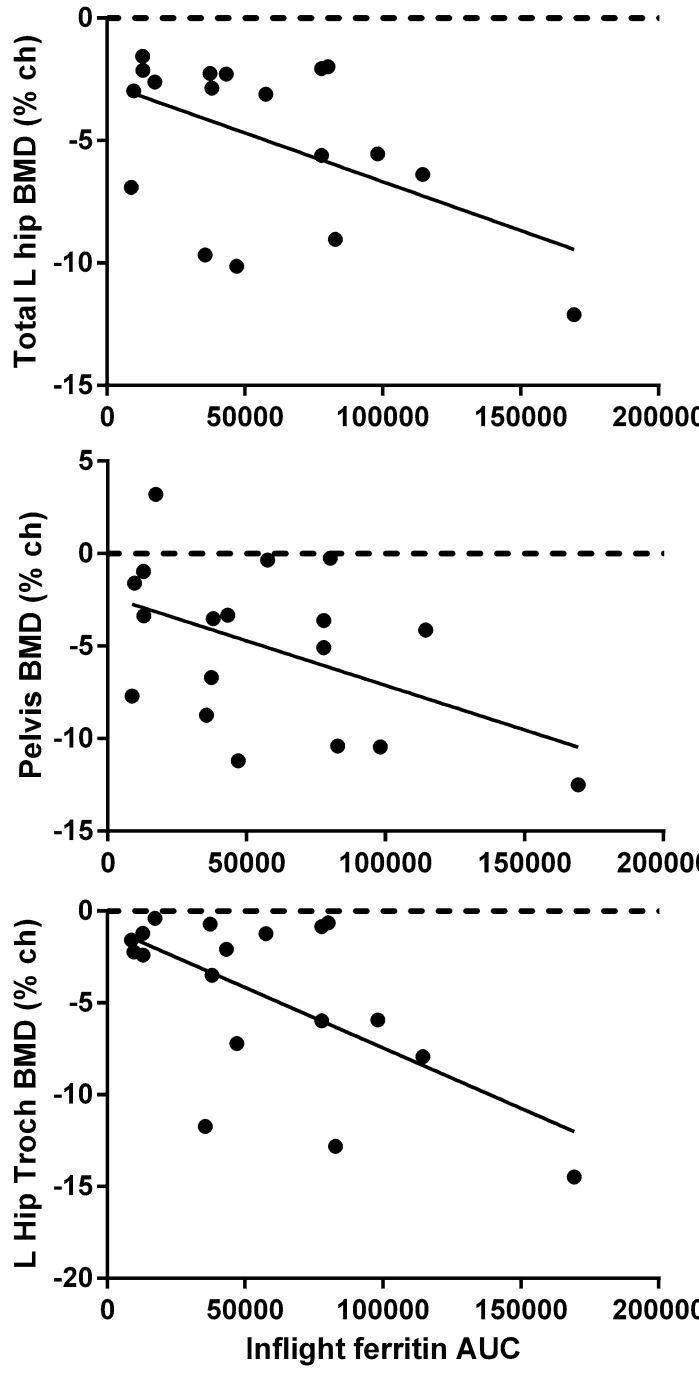

Fig. 41.4 The correlation of loss of bone mineral density at three sites (hip, pelvis, and trochanter) with increase in serum ferritin (expressed as area under the curve) during flight [140]

activation of NF-κB by several stimuli, including lipopolysaccharide, RANKL, TNFα, and arachidonic acid [151, 156–161].

Data from the ISS have documented that astronauts who ate more fish lost less bone (Fig. 41.5) [161]. These findings are supported by data from ground analog studies, including bed rest, in which the rate of bone breakdown was related to intake of omega-3 fatty acids [161]. In a series of cell

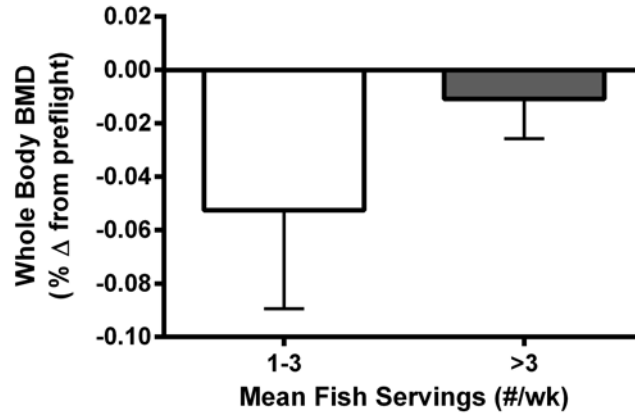

Fig. 41.5 Consumption of more servings of fish on the ISS was related to less bone mineral density (BMD) loss after landing. Data were adapted from [161]

culture studies, osteoclast activation was lower when omega-3 fatty acids were added [161]. More detailed studies are required during space flight, using controlled dietary sources of omega-3 fatty acids from the space food system or supplements, but the published data provide additional evidence of the potential importance of fish oils as a countermeasure, not only for bone loss but also for muscle, cardiovascular, and radiation risks of space flight.

41.9 Animal Protein:Potassium

The issue of dietary protein and bone health remains controversial despite decades of research [162–165]. Although the literature contains evidence that seems to conflict, we maintain that the ratio of dietary components (such as sulfur-containing amino acids) that lead to endogenous acid production to components (such as potassium-rich fruits and vegetables) that lead to endogenous base production affects acid-base balance. When the ratio is in favor of acid production, the body has a low-level acidic load that is detrimental to bone. This concept originated with the acid-ash hypothesis of dietary effects on acid-base balance [166] and has yielded an important area of nutrition research, which happens to have implications for space travelers.

Dietary intake of protein, specific types of protein, and patterns of acid and base precursors have recently been associated with the concentrations of urinary markers of bone resorption during bed rest [19, 167, 168]. In a study of identical twins, a strong positive correlation existed between markers of bone resorption and the ratio of animal protein to potassium intake during bed rest. The ratio of animal protein to potassium intake was even more strongly related to bone markers at the end of bed rest, when calcium excretion was highest. This finding supports the argument that calcium status could have an important role in determining the effect of protein on bone. If calcium is being resorbed from bone, then acid load can have a more detrimental effect on bone, similar to what has been observed in other studies of the effect of high-protein diets on bone [169, 170].

This effect of protein/acid load was further documented in a study evaluating the use of supplemental amino acids and carbohydrate (45 g/day essential amino acids and 90 g/day sucrose) to mitigate muscle loss [171]. The supplement contained 1.5 g methionine, which is about 1.13 times the recommended daily intake (supplementing the amount of methionine provided in the diet). The sulfur in methionine is converted in the body to sulfuric acid, and thus methionine is an acid precursor in the diet. It was evident that more methionine was broken down than was used by the body because urine

pH decreased in the amino acid-supplemented group. It was hypothesized that this low-grade metabolic acidosis [172] contributed to the higher urinary concentrations of bone resorption markers and calcium in the amino acid-supplemented group [171].

41.10 Sodium

Sodium intake of the general population in the Western world, including astronauts, is generally rather high compared to requirements, and is one of the prominent dietary risk factors for hypertension [59, 173]. High sodium intake leads to increased urinary calcium excretion and is therefore a well-recognized contributor to osteoporosis, particularly when calcium intake is low [174–178]. The mechanisms causing this are not yet fully understood. Recent data documents that high sodium intake will induce a low-grade metabolic acidosis [179–181]. Acidosis is a prerequisite to osteoclast activation [182–184].

When high sodium intake was combined with bed rest, another stimulus of increased bone resorption [86, 185], excretion of bone resorption markers had almost doubled (about 35 % increase because of inactivity plus about 50 % increase because of high salt intake) at the end of the 2 weeks of bed rest, relative to baseline [186].

The degree to which high sodium intake affects bone turnover during space flight is currently being examined in controlled diet studies on the ISS. Given the typical high intakes of sodium in astronauts to date, it is very likely that bone loss can be mitigated by lowering daily sodium intake. Because of the association of high sodium intake with bone loss and other undesirable effects in space flight, NASA has undertaken a major effort to decrease the salt content in the US space food products. The results of this effort should be apparent in coming years.

41.11 Vitamin K

Vitamin K has been implicated in bone health because it is involved in an enzyme-catalyzed reaction coupled to the gamma-carboxylation of the protein osteocalcin. However, its role is the subject of continuing debate, and at present a lack of clarity reigns [187–189]. Reports conflict on whether supplemental vitamin K has a protective effect on bone. In one study, daily vitamin K supplementation prevented changes in lumbar spine BMD over the course of 3 years in postmenopausal women [190]. Other studies have shown no effect of vitamin K supplementation on markers of bone turnover or BMD changes over 1- to 3-year periods [191, 192].

Initial findings from space flight studies indicated that astronauts had an overall vitamin K deficiency, and it was suggested that vitamin K might serve as a viable bone loss countermeasure. In one study, undercarboxylated osteocalcin was elevated (a sign of vitamin K insufficiency) throughout 3-week and 6-month Mir missions [185]. Data from the EuroMir 95 mission showed that markers of vitamin K status were decreased after 12.5 weeks of space flight, and that vitamin K supplementation (10 mg/day for 6 weeks) reversed these effects [193]. Vitamin K supplementation elevated γ-carboxyglutamic acid and decreased undercarboxylated osteocalcin, suggesting that vitamin K status was lower during space flight and was improved by supplementation [185, 193]. Early data from ISS astronauts revealed that on landing day their serum phylloquinone (vitamin K1) was 42 % lower than it was before flight, whereas urinary γ-carboxyglutamic acid did not change [122].

More recent data from a larger group of astronauts on the ISS and from several bed rest studies showed no major changes in phylloquinone, urinary GLA, or undercarboxylated osteocalcin [194]. These data indicate that although vitamin K is an important nutrient and intake needs to meet the body's requirements for it, supplementation during space flight is not needed, as excess vitamin K likely would not have an effect on bone.

41.12 Conclusion

The deleterious effects of space flight on the human body are significant. As a cross-cutting field, nutrition holds promise for helping to mitigate these effects. No system has more potential for nutritional mitigation than bone, and as highlighted briefly in this chapter, many modes of benefit are possible. Figure 41.6 shows a summary of effects of space flight (and countermeasures) on bone metabolism. Integrating these factors into the space food system and encouraging crewmembers to consume healthier diets has tremendous potential for future space missions in low Earth orbit and beyond. Likewise, a better understanding of the relationship between nutrition and bone health in space can only further enhance the understanding of this relationship on Earth, and may contribute to disease treatments for many affected individuals and to dietary recommendations for the general population.

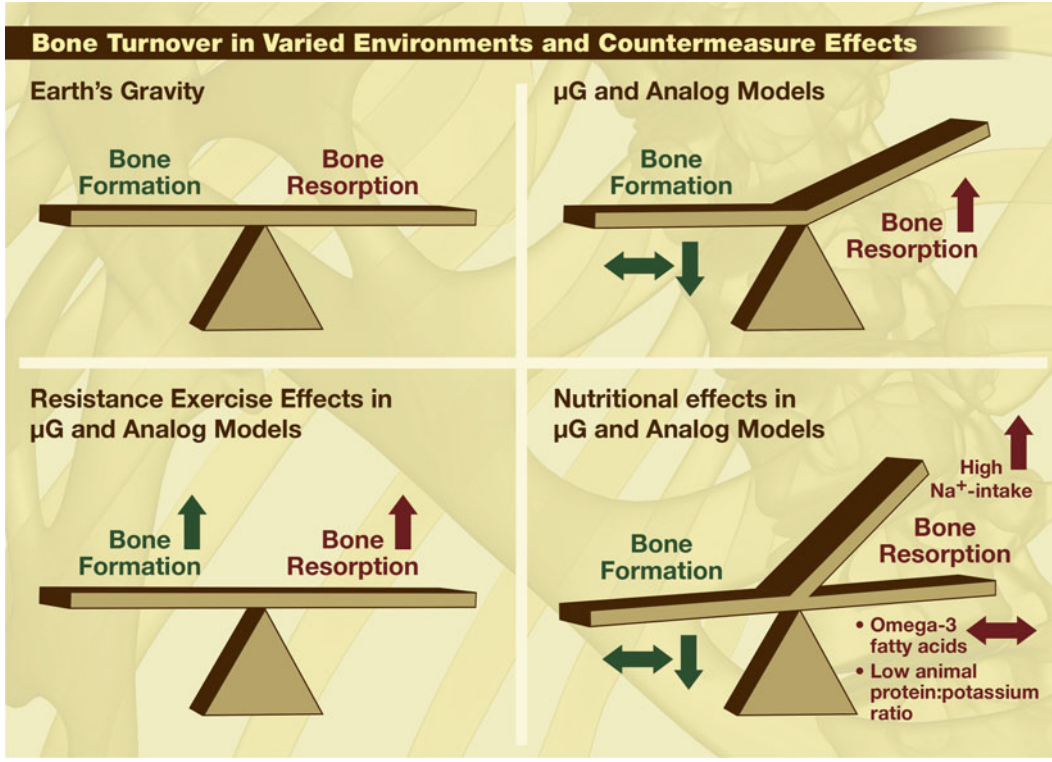

Fig. 41.6 Graphical depiction of changes in bone and bone metabolism during space flight. The *top left panel* shows the typical balance between bone formation and bone resorption, which results in unchanged bone mass. Other panels show the effects of space flight (and analogs) on these factors, along with the effects of exercise and nutritional countermeasures

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