Connie M. Weaver
Robin M. Daly
Heike A. Bischoff-Ferrari *Editors*

Nutritional Influences on Bone Health

9th International Symposium

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Editors
Connie M. Weaver
Nutrition and Science
Purdue University
West Lafayette
Indiana
USA

Heike A. Bischoff-Ferrari
Geriatrics and Aging Research
University of Zurich
Zurich
Switzerland

Robin M. Daly
Deakin University
Melbourne
Australia

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Foreword

The International Symposium on Nutritional Aspects of Osteoporosis: Origin, History and Scope

As an endocrinologist dealing with diabetes, obesity and bone diseases, I naturally developed an interest in the association between nutrition and bone health, which in the seventies and eighties was restricted to the role of calcium. I always felt that there was a gap with no regular international meeting dedicated to this topic, next to the much bigger research fields of bone cell biology, vitamin D or epidemiology, treatment of osteoporosis etc. When in 1990, I was asked by the Serono Foundation to organize an international endocrinology symposium, I seized the occasion and presented the idea of a bone nutrition meeting to Robert Heaney. He certainly was the figurehead needed for such a launch. He agreed to give it a try, and we organized the first meeting in 1991. Calcium was obviously the main topic, but not the only one. Three years later, the meeting was held again with a wider spectrum of topics. Vitamin D captured great attention, and other topics appropriate at that time, such as the role of other vitamins, proteins, various lifestyle factors and nutritional inadequacies in the elderly. From there on, we organized it every three years in Lausanne, Switzerland. In 1997, Bess Dawson-Hughes joined us as co-organizer. With her impressive knowledge and network she helped to enlarge again the spectrum to nutritional aspects of growth, genetic influences, the effect of isoflavones, etc.

From the beginning, we liked the broad range of participants from graduate students to senior scientists, coming from all over the world. We also tried to offer a frame that favors personal contacts and gathers highly devoted participants who always attend all sessions until closure, thus creating a community of intense exchange and discussion.

In 2009, Bob Heaney wanted to retire, and Connie Weaver, who had already been a speaker at all symposia since the very first one, completed the trio of organizers with enthusiasm and high competence. We thus kept organizing the symposium with the same frame, the same number of participants and posters, always under the auspices of IOF and NOF, and published each time a book with the proceedings. In the last two years, the online format of the book received more than 27,000 downloads, which reveals a substantial growth of interest in the field. For this 9th meeting in 2015, Bess Dawson-Hughes and my-self handed over the reigns to Connie Weaver. I am

particularly glad to see the continuity of this initiative under her leadership, together with Heike Bischoff-Ferrari and Robin Daly, who both know the symposium so well and bring a breadth of scientific knowledge.

This long experience taught us the many specific aspects of research in nutrition and bone health:

First, the three-year rhythm proved very well adapted to the speed of real progress in this field. Although research is produced all over the world, the number of research groups addressing this rather narrow topic is restricted. But it was easy to identify every three years new studies and surprisingly enough topics to compose a challenging program.

Second, clinical research in this field is complex and variable because it is performed with a whole food concept such as vegetarianism or Mediterranean diet, or with a class of nutrients, such as proteins, dairy products, fruits and vegetables, or with one specific food group, such as meat, milk, or finally with a single substance, such as calcium or a specific vitamin. It is also performed in all groups of age and for any length of time, varying between hours and days for acute metabolic effects and years for changes of bone mineral density.

Third, since the influence of nutrition on bone density or even fracture incidence is small, although significant, it only can be demonstrated through large cohorts. This requires epidemiologic studies or large cross-sectional studies. They capture the food habits over years, eventually lifetime, and sometimes can demonstrate nutritional influences which remain occult to follow-up studies. In addition, interventional studies are difficult, for reasons of questionable compliance and restricted numbers of participants, and can only be performed with a supplement, such as Calcium, or dairy products, or other specific food items. However, they do offer the possibility to examine the effects on bone metabolism over a short time. Nutrition interventions cannot compete with therapeutic trials as the effect size is small, baseline status is often not deficient, and funding for trials of sufficiently large sample size and duration is lacking. Consequently, nutritional trials set up using similar protocol as drug trials usually fail.

For all these reasons, scientists who study the nutritional influences on bone health are a very specialized group, which needs opportunities to gather and exchange their experiences. The “International Symposium on Nutritional Aspects of Osteoporosis” offers this opportunity. The first meeting in North America in the lovely city of Montreal proved that the legacy continues as reflected in these proceedings. It will hopefully continue to do so and further contribute to the awareness that nutrition is a significant contributor to bone health over the whole lifespan.

Lausanne, Switzerland Peter Burckhardt

Contents

Part I

Sarcopenia and Obesity

Sarcopenia: The Concept and Its Definitions

1

Marjolein Visser

Abstract

The concept *sarcopenia* was launched in 1989 by Irwin Rosenberg to indicate the process of age-related loss of muscle mass. In 1998, the first operationalization of sarcopenia was provided; the muscle index, which was calculated as appendicular muscle mass (assessed by dual x-ray absorptiometry) divided by body height squared, and the first cut points for low muscle mass were established. Since then, our understanding about the muscle-related changes with aging has increased and several algorithms have been proposed for the case-finding of sarcopenic older persons. Disappointingly, no consensus on the sarcopenia definition or muscle mass cut points has been achieved. This chapter provides a brief overview of sarcopenia research and describes the most recently proposed definitions of sarcopenia.

Keywords

Sarcopenia • Muscle mass • Muscle strength • Aging • Definition

The Concept Sarcopenia

In the 60s and 70s, body composition methodology became available to estimate body composition in vivo. Forbes repeatedly applied the total body potassium method (or ^{40}K method) to his own body and that of his colleagues to measure body composition changes with aging. While his body weight remained relatively stable with aging, he observed that his lean body mass was decreasing [1]. Several years later, Tzankoff & Norris showed that 24-h creatinine excretion was much lower in older versus

M. Visser, PhD
Department of Health Sciences,
Faculty of Earth and Life Sciences,
VU University Amsterdam, De Boelelaan 1085,
Amsterdam 1081 HV, The Netherlands

Department of Nutrition and Dietetics,
Internal Medicine, VU University Medical Center,
Amsterdam, The Netherlands
e-mail: m.visser@vu.nl

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younger individuals [2], suggesting a specific loss of muscle mass with aging.

In 1989, the process of age-related loss of muscle mass was named sarcopenia by Irwin Rosenberg. The word sarcopenia comes from the Greek words Sarx (flesh) and Penia (deficiency, poverty). Since 1989, research on sarcopenia has expanded and our knowledge on the age-related changes in muscle mass, muscle composition and muscle function has increased significantly.

Changes in Muscle Mass with Aging

Since the early work of Forbes and Tzankoff & Norris, several studies conducted in large prospective cohorts of older adults have provided us with accurate information on the loss of appendicular muscle mass with aging. Based on the whole-body dual-energy x-ray absorptiometry (DXA) results of the Health, Aging and Body Composition Study, a prospective cohort including 3,075 African-American and Caucasian men and women aged 70–79 years at baseline, the annual loss of appendicular muscle mass over a 5-year time period was estimated to be 0.7 % in women and 0.8 % in men [3]. In the same study, computed tomography (CT) was also used to assess mid-thigh muscle cross-sectional area at baseline and again after 5 years of follow-up, with an estimated annual loss of 0.6 % for women and 1.0 % for men [4]. Other studies have confirmed the more rapid loss of muscle mass in older men compared to older women.

An important determinant of the change in muscle mass is the change in body weight. Any kilogram of weight change generally consists of 75 % body fat and 25 % fat-free mass [5]. Indeed, in older persons, weight loss is generally accompanied by a loss of appendicular muscle mass, while weight gain is generally accompanied by muscle gain [6]. Thus, by interpreting any changes in muscle mass with aging, it is important to consider body weight changes or to adjust for body weight change.

Older persons have a lower muscle strength compared to younger persons. This was recently nicely illustrated by pooling handgrip strength data from 12 British studies covering an age range from 4 to 90 years [7]. Repeated isokinetic knee extensor strength measurements after a 5-year follow-up conducted in the Health, Aging and Body Composition Study showed an annual muscle strength loss of 2.7 % in women and 3.2 % in men [4]. This indicates that the prospectively measured annual loss of mass strength is about three times faster than the annual loss of muscle mass, highlighting that the strength per unit muscle mass is also declining with increasing age.

While cross-sectional studies shows that muscle mass and muscle strength are positively associated, prospective studies in older adults indicate that there is only a weak association between the loss of muscle strength and loss of muscle mass over time. Clarck & Manini categorized older men and women into three groups of muscle strength change based on six annual muscle strength measurements covering a 5-year time period. The groups were: stable strength (±1.5 % mean annual change), average decline (1.5–3 % annual loss), and severe decline (>3 % annual loss) [8]. Despite the clear differences in muscle strength change between the groups, no differences were observed in the change in muscle mass between these groups.

Accurate Assessment of Muscle Mass

The scientific literature shows that many body composition methodologies are being used to assess or estimate muscle mass. Table 1.1 provides an overview of the methods that are currently being used and provides some basic characteristics of each method. Some methods rely on the use of a prediction equation to predict (appendicular) skeletal muscle mass, such as the bio-electrical impedance method [9] and some anthropometric equations [10]. Individual prediction errors can be substantial (the standard error of estimate of these equations ranged between 2.5 and 3 kg) and these methods should therefore not be applied to interpret muscle mass or muscle mass changes of an individual. Unfortunately, because of their low costs and potential to be used at the bedside, these methods are still frequently being used to estimate muscle

Table 1.1 Characteristics of body composition methods used to assess muscle mass

Body composition method	Need prediction equation	Feasible in frail older persons	Radiation exposure	Regional (R), Total body (TB)	Precision[a]	Accuracy[b]
Anthropometry (e.g. mid-upper arm muscle circumference, or combination of measurements)	Yes (when measurements are combined)	+	No	R TB	– –	– –
Bio-electrical impedance	Yes	+ +	No	R TB	+	– –
24-h urinary metabolite excretion (e.g. creatinine)	No	–	No	TB	–	+
Creatine (methyl-d_3) dilution	No	–	No	TB	NA	+
Densitometry (e.g. underwater weighing, air displacement plethysmography)	No	– –	No	TB	– –	– –
Dual-energy x-ray absorptiometry	No	+ +	Yes	R TB	+ +	+
Computed tomography	No	+ +	Yes	R	+ +	+ +
Magnetic resonance imaging	No	+ +	No	R TB	+ +	+ +

[a]Precision: the degree to which repeated measurements under unchanged conditions show the same results
[b]Accuracy: the degree of closeness of the measurement to the true value
NA not available

mass in individual persons. Densitometric methods, such as underwater weighing and air displacement plethysmography can assess the amount of fat-free mass rather accurate and precise. However, fat-free mass should not be used as an indicator of muscle mass, as only about 50 % of fat-free mass consists of skeletal muscle mass [11]. The table indicates that only few methods are both precise and accurate, and thus are sensitive enough to detect small changes in muscle mass (for example to assess the impact of an intervention). The currently most recommended methods to measure muscle mass are dual-energy x-ray absorptiometry (DXA), computed tomography (CT) and magnetic resonance imaging (MRI).

Accurate Interpretation of Muscle Mass

When correctly interpreting the amount of muscle mass of an individual person, the persons' body height is very relevant. The taller a person is, the larger the amount of muscle mass will be. This has led to the development of the muscle index, appendicular muscle mass (assessed by DXA) divided by body height squared [12]. Similar to the body mass index, this index provides information on the amount of muscle mass independent from body height. A low muscle index ($\leq$5.45 kg/m^2 for women and $\leq$7.26 kg/m^2 for men) is frequently used to indicate sarcopenia [12–14], however, other cut-points for this muscle index are also being used [15]. Adjusting appendicular muscle mass (from DXA) or muscle cross-sectional area (from CT or MRI) for body height in statistical modelling to correctly interpret the amount of muscle mass is also a frequently applied method [6, 16].

A correction that is less often applied when interpreting muscle mass is the correction for body fat mass. Body fat mass and muscle mass are positively correlated, also in older adults. The extra muscle mass is simply needed to support the access body mass. Interestingly, older persons with more body fat mass also have a greater absolute muscle strength compared to their leaner

counterparts, however, the strength per unit muscle mass (relative strength) is lower [17]. When it is ignored that older persons with more body fat also have a greater muscle mass, the prevalence of low muscle mass (or low muscle mass index) indicating sarcopenia is generally zero in obese older persons. This was clearly shown by Newman and others [15]. However, it is well possible that in an overweight or obese older person the muscle mass can be too low for the current body weight or body fat mass. An approach to solve this problem is the use of residuals [15, 18, 19]. Using a regression model with appendicular muscle mass as the dependent variable, and body fat mass and body height as independent variables, the muscle mass residuals provide information on the amount of muscle mass in relation to a certain body fat mass and body height. A positive residual indicates that an older person is more muscular than can be expected based on this persons' body fat mass and body height, while negative residual indicates that an older persons is less muscular than would be expected based on this persons' body fat mass and body height. The latter person could be seen as sarcopenic. Sarcopenia based on the residual method was shown to be associated with functional limitations and well as incident mobility limitations and incident disability [15, 18, 19], and predicts disability better than non-fat-adjusted muscle mass [19].

Another approach that is being used to account for body size or body fat mass when interpreting the amount of muscle mass of an individual, is dividing muscle mass by total body weight, also called relative skeletal muscle mass [20], or dividing appendicular muscle mass by body mass index [21]. However, in older persons the between-person variation in body fat or body mass index is much larger than the between-person variation in (appendicular) muscle mass. Therefore, the value of this index is largely determined by its denominator (body weight or body mass index) and less so by its numerator (muscle mass). This may increase the risk of incorrect interpretation of study results when relating this index to clinical outcomes. For example, in a recent study among 1343 men and women aged 60 to 82 years, a low ratio of appendicular skeletal muscle mass divided by BMI was associated with an increased risk of several self-reported functional limitations, such as climbing several flights of stairs or walking more than one kilometer [22]. The authors concluded that the low ratio are 'suitable to detect patients at risk for negative outcomes such as frailty who might benefit from interventions targeted at improving lean mass' [22]. However, considering the above, it is questionable whether these patients would benefit most from such an intervention. The functioning level might improve more with an intervention targeting those with a high body mass index.

Recent Definitions of Sarcopenia

In the past decade, a shift can be observed in the concept sarcopenia. Originally, sarcopenia was defined as the age-related loss of muscle mass. Mostly for practical reasons, sarcopenia was operationalized as having a low muscle mass (divided by body height squared, divided by body weight, or using residuals) as this would only require a single assessment of muscle mass [12, 15, 20]. However, in the past years the sarcopenia definition has expanded to not only include low muscle mass but also low muscle strength and low physical function [13, 14, 23, 24]. Unfortunately, there is still no world-wide consensus on the definition of sarcopenia, which hampers clinical practice and scientific research. In the following paragraphs, the three most recently developed definitions will be described, including their differences and similarities.

In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) presented a practical clinical definition of sarcopenia based on a consensus [13]. The EWGSOP algorithm for sarcopenia case finding in older adults (or younger persons at risk) is shown in Fig. 1.1. The algorithm was endorsed by four participating professional medical societies.

The algorithm includes a measure of muscle mass, a measure of handgrip strength and a measure of gait speed (Table 1.2). For low gait speed the cut-point $\leq$0.8 m/s is used. For low muscle mass no specific body composition methodology

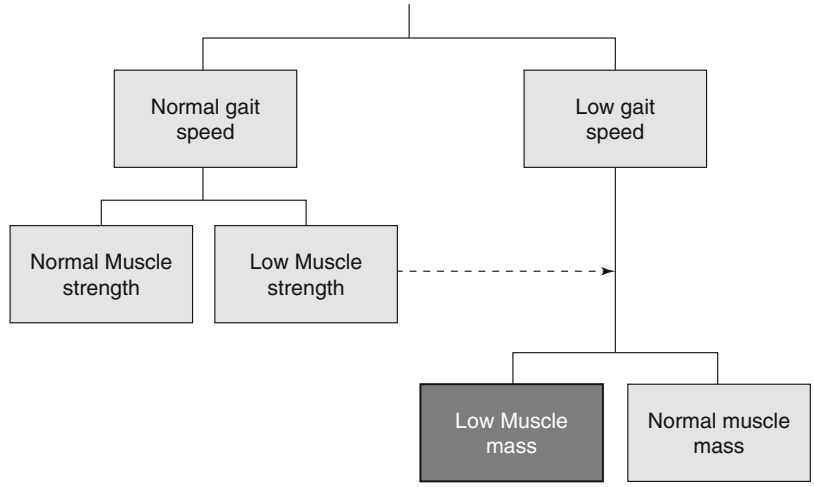

Fig. 1.1 Sarcopenia algorithm as proposed by the European Working Group on Sarcopenia in Older People (EWGSOP) in 2010 [13]

Table 1.2 Algorithm components and the suggested indictors and cut points of the three most recently developed sarcopenia definitions

Definition		Poor function	Poor muscle strength	Low muscle mass
EWGSOP [13]	Indicator	Gait speed (m/s)	Handgrip strength (kg)	DXA or BIA
	Cut point Men Women	≤0.80 ≤0.80	Not defined Not defined	Not defined Not defined
IWGS [14]	Indicator	4-m habitual gait speed (m/s)	NA	Muscle index (ALM/ht^2) (kg/m^2)
	Cut point Men Women	<1.0 <1.0	NA NA	≤7.23 ≤5.67
FNIH [24]	Indicator	Usual gait speed (m/s)	Sum of maximum strength value in either hand (kg)	ALM/BMI
	Cut point Men Women	≤0.80 ≤0.80	<26 <16	<0.789 <0.512

DXA dual-energy x-ray absorptiometry, *BIA* bioelectrical impedance, *ALM* appendicular lean mass, *NA* not applicable, *BMI* ody mass index

(either DXA or BIA) or cut-points are suggested. Also for low handgrip strength no specific protocol or cut-point are suggested. Persons are considered sarcopenic when they have a low gait speed in combination with a low muscle mass, or when they have a normal gait speed but low grip strength in combination with low muscle mass. Persons are considered severe sarcopenic when they meet all three criteria. A literature review conducted in 2013 showed that the prevalence of sarcopenia according to this algorithm was 1–29 % in community-dwelling older persons and 14–33 % in long-term care patients [25].

In 2011, the International Working Group on Sarcopenia (IWGS) published their definition of sarcopenia [14]. Their definition is the consensus of a group of geriatricians and scientists from academia and industry. Their algorithm includes a measure of muscle mass and a measure of gait speed (see Fig. 1.2, Table 1.2). In contrast to the EWGSOP algorithm, muscle strength is not included.

Low muscle mass is defined using the muscle index and the cutpoints developed by Baumgartner et al. [12]: ≤5.45 kg/m^2 for women and ≤7.26 kg/m^2 for men. Muscle mass should be assessed by DXA. For assessing low gait speed a walk test

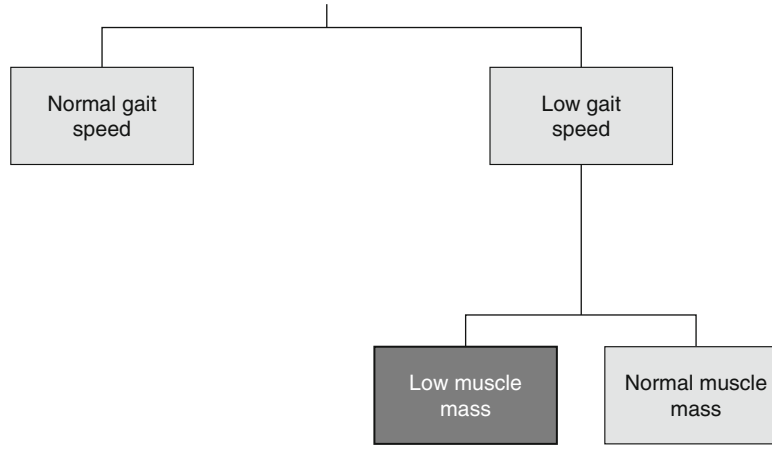

Fig. 1.2 Sarcopenia diagnosis as proposed by the International Working Group on Sarcopenia (IWGS) in 2011 [14]

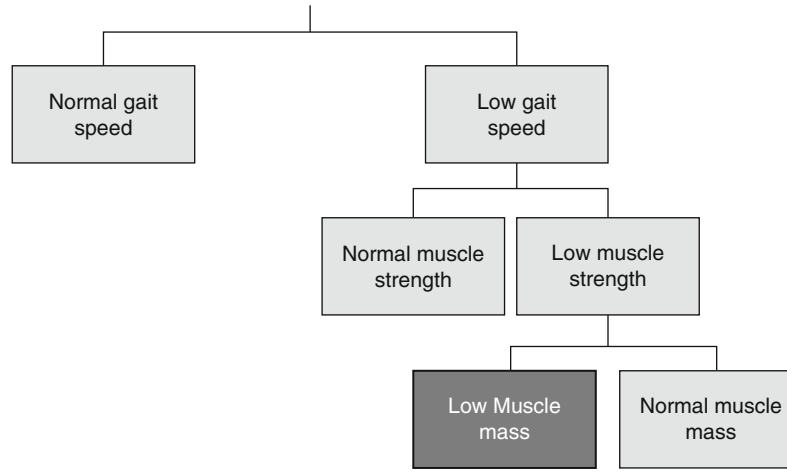

Fig. 1.3 Sarcopenia clinical paradigm as proposed by the Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project (FNIH) in 2014 [24]

using a 4-m course is suggested, with a habitual gait speed <1.0 m/s indicating poor gait speed. Older adults who have a poor gait speed as well as a low muscle index are considered sarcopenic. The prevalence of sarcopenia based on the IWGS definition will be lower as compared to the prevalence based on the EWGSOP definition, since IWGS does not include the 'poor muscle strength' pathway in their algorithm. For example, in a sample of 408 adults aged 65 years and older using the muscle index and Baumgartner cut-points to asses low muscle mass, the sarcopenia prevalence was 4.1 % and 7.8 % using the IWGS and EWGSOP definition, respectively [26].

Most recently, in 2014, the Foundation for the Institutes of Health Biomarkers Consortium Sarcopenia Project (FNIH) published their clinical paradigm (Fig. 1.3) [24]. The paradigm includes poor physical function, muscle weakness and low muscle mass. For poor physical function a usual gait speed ≤0.8 m/s was selected based on previous research (Table 1.2). To establish the cut points for muscle weakness (low handgrip strength) and low muscle mass (low appendicular muscle mass from DXA), statistical analyses were performed in a large dataset consisting of 26625 participants aged 65 years and older from nine collaborating studies [21, 27]. Recommended cut points for low handgrip strength are <16 kg for women and <26 kg for men. For low muscle mass, a ratio of appendicular muscle mass divided by body mass index <0.512 for women and <0.789 for men is suggested.

In a sample of 7113 men aged 65 years and older the prevalence of sarcopenia was 0.5 % according to the FNIH, 5.1 % according to IWGS and 5.3 %

according to the EWGSOP definition [28]. In 2950 women the corresponding prevalence rates were 2.3, 11.8 and 13.3 % [28]. The prevalence of sarcopenia based on the FNIH definition is lower compared to the prevalence based on the IWGS. This is not surprising since the FNIH definition is more strict; it has one criterion more than the IWGS definition (namely low handgrip strength). The prevalence of the FNIH definition is also lower than that of the EWGSOP definition, which is also to be expected since according to the FNIH definition a person has to meet all three criteria, while in the EWGSOP definition a person has to meet the low muscle mass criterion together with either the low gait speed or the low handgrip strength criterion to be considered sarcopenic.

Advantages and Disadvantages of the Recent Sarcopenia Definitions

In the scientific community there is some discussion about the use of these more elaborate definitions or algorithms of sarcopenia. Some are in favor of an expansion of the original sarcopenia definition and to also include measures of muscle strength and physical functioning. By applying the newly proposed algorithms, those older individuals with a poor muscle mass who also suffer from poor muscle strength or poor physical functioning can be easily identified. These persons seem to experience negative consequences of their poor muscle mass. The expanded sarcopenia definition can therefore be used in clinical practice to identify those older individuals who could potentially benefit from a treatment focusing on increasing muscle mass.

Others prefer to stick with the original definition of sarcopenia as proposed in 1989 – the age-related loss of muscle mass. The age-related loss of muscle strength should be distinguished from the loss of muscle mass and could be termed dynapenia [8]. Physical functioning, which includes measures of gait speed, is already a frequently used and valuable prognostic parameter in aging research [29]. Not combining measures of muscle mass, muscle strength and physical functioning into a single concept may facilitate research to increase our understanding about age-related muscle changes and how the change in each individual factor relates to important clinical outcomes. Other arguments against the use of a broader definition of sarcopenia focus on the inclusion of a general physical functioning parameter. While poor physical functioning may indeed be caused by muscle weakness [16, 27], many other non-muscle causes such as joint pain, dizziness, poor cognitive functioning or poor vision also negatively affect gait speed. It has therefore be questioned whether physical functioning should be included into a 'muscle concept' [19]. And finally, as loss of muscle mass and loss of muscle strength are hypothesized to influence the decline in physical functioning in old age, one should be cautious to include a physical functioning measure into the definition of sarcopenia [30]. By doing so, strong associations between sarcopenia according to the broadened definition and disability are to be expected as information on physical functioning is included in both the determinant and the outcome.

The Future of Sarcopenia Research

There is still uncertainty about the most optimal definition of sarcopenia to be used and which cut points should be applied to indicate low muscle mass or low muscle strength. This uncertainty currently hampers clinical research, clinical care and the prevention and treatment of sarcopenia. An important next step to be taken to in the sarcopenia research field is the pooling of large prospective datasets that include hard clinical end points such as mortality, (recurrent) falls, fractures, nursing home admission etc. and taking a data-driven approach to determine which muscle-related factors are most predictive of these outcomes and what cut points are optimal to indicate high risk individuals. This will allow the development of evidence-based criteria of sarcopenia, which may differ for different outcomes, on which treatment should be based.

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Defining Sarcopenia

2

Bess Dawson-Hughes
and Heike A. Bischoff-Ferrari

Abstract

Currently there is no standardized definition of sarcopenia. This hampers the clinical management of sarcopenia and limits the development and regulatory approval of interventions to reduce the progression of this common and debilitating condition in the elderly. Nine definitions of sarcopenia have been put forward by different individuals and working groups, but these definitions have not been examined or compared with respect to their ability to predict the rate of falling. Reduced lower extremity lean tissue mass, strength and function characterize sarcopenia, and they are known risk factors for falling. The purpose of this chapter is to describe the nine operational definitions of sarcopenia proposed in recent years, to describe the prevalence of sarcopenia by each definition, and to describe the degree to which each definition predicted incident falls over a 3-year period in a cohort of 455 community-dwelling men and women age 65 years and older. We also comment on the need for further research and the importance of reaching consensus on a globally accepted definition of sarcopenia.

Keywords

Sarcopenia • Lean appendicular mass • Gait speed • Grip strength • Falls

B. Dawson-Hughes, MD (✉)
Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington St., Boston, MA 02111, USA
e-mail: Bess.dawson-hughes@tufts.edu

H.A. Bischoff-Ferrari, MD, DrPH
Department of Geriatrics and Aging Research, University Hospital Zurich, Raemistrasse 100, Zurich 8091, Switzerland
e-mail: Heike.Bischoff@usz.ch

Introduction

The term sarcopenia, originally coined by Irwin Rosenberg [1], refers to 'paucity of flesh'. Sarcopenia refers to the state in which muscle mass has been lost; it is used most often in reference to the elderly.

With aging, there is gradual loss of muscle mass, muscle strength, and muscle power;

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however, these losses occur at different rates. In older adults, muscle mass declines at about 1 % per year and strength, the ability to generate force against a specific resistance, declines by 2.6–4.1 % per year [2]. Muscle power, the product of muscle force and velocity, declines more dramatically than muscle strength [3]. The composition of muscle fibers also changes with aging. There is selective loss in both the number and cross-sectional area of the fast-twitch type 2 fibers [4]. Type 2 fibers are important responders to loss of balance and their loss is thought to contribute to risk of falling.

Clinicians and health care workers recognize advanced sarcopenia when they see it, but there is currently no prevailing or generally accepted operational definition of sarcopenia. This creates problems in the management of patients with the condition, including making the diagnosis, coding the diagnosis, and developing the prompts in electronic medical records systems to trigger interventions to mitigate or reverse the condition. Additionally, the lack of a single definition hampers the development of pharmaceutical and other interventions to treat the condition, from the perspectives of both the developers and regulatory agencies. Currently there is no standardized classification of sarcopenia upon which to base the selection of study participants and there are no standardized clinical endpoints that are accepted by regulatory agencies for use in clinical trials testing potential interventions to reduce sarcopenia.

The purpose of this chapter is to (1) describe the muscle mass and functional assessment measures that have been used in sarcopenia definitions, (2) highlight falls as an important clinical consequence of sarcopenia, (3) describe the nine operational definitions of sarcopenia proposed in recent years, and (4) determine the degree to which each of these operational definitions predicts incident falls in a cohort of community-dwelling men and women age 65 years and older.

Muscle mass and functional assessment tools (Components of the operational definitions of sarcopenia)

Lean Tissue Mass

Current definitions of sarcopenia contain measures of either appendicular (arms and legs) lean mass (ALM) or total body lean tissue (TBLM). These are defined as the dual-energy X-ray densitometry (DXA) based non-fat, non-bone tissue weight divided by height (in meters) squared. The DXA measurements are highly reproducible (CV <1.0 %); however, DXA soft tissue composition measurements are subject to several sources of error. The method assumes that the hydration of lean body mass is uniform and fixed, but this is not true in older subjects or those who are sick [5, 6]. Although DXA can accurately predict total body weight, it may not accurately partition that weight into the 3 major compartments: fat, bone and other (includes lean). Error in the fat and other compartments was illustrated in an experiment in piglets who had DXA scans followed by chemical analysis of the carcasses [7]. Additionally, DXA cannot detect intramuscular fat which can account for 5–15 % of muscle mass in obese subjects [8]. Despite these limitations, DXA is an effective and widely used method of assessing soft tissue composition.

Short Physical Performance Battery (SPPB)

The SPPB test was developed by the National Institute on Aging for the Established Populations for Epidemiologic Studies of the Elderly. It captures domains of strength, endurance, and balance and is highly predictive of subsequent disability [9]. SPPB scores are also predictive of Nursing Home admission and mortality [9].

In this assessment, subjects are asked to perform a balance test (open, semi-tandem, and tandem stance), a timed 4-m walk (at the usual pace), and a chair rise test (timed 5 rises). Each of these tests has a maximum score of 4 points (total SPPB score is 12). To reduce inter-operator variability, a standard 'script' for administering the test is used. Strengths of this test are that (1) it has been validated in the older adults, (2) it provides continuous variables that capture strength and

balance during activities that are relevant to common daily living, (3) its components can be analyzed separately, and (4) the SPPB is widely used which facilitates cross-study comparisons.

Gait speed, a component of the SPPB, is used in several of the definitions of sarcopenia. Slow gait speed predicts poor outcomes in the elderly. The term dismobility has been applied to the state of a walking speed of <0.6 m/s [10]. In the National Health and Nutrition Examination Survey (NHANES), in the age range of 80–84 years, 15.9 % of men and 22.8 % of women age met the criterion for dismobility and among people ≥85 years of age, 31.0 % of men and 52.0 % of women had dismobility [10].

Grip Strength

Handgrip strength is a convenient and reliable measure of overall muscle strength. It is well correlated with other measures of strength including leg extension strength [11]. Grip strength declines with age and low grip strength values have been associated with falls [12] as well as with prolonged length of stay in the hospital [13] and increased mortality [14]. Handgrip strength of both dominant and non-dominant hands is determined with use of a hand held dynamometer. The higher of two or three consecutive readings with each hand is recorded as the maximum force produced. Grip strength testing is easily performed, takes little time, does not require expensive equipment, and is widely used.

Falls: An Important Clinical Consequence of Sarcopenia

Muscle weakness and frailty in older people lead to falls, disability and loss of independence. One in 3 community-dwelling people over age 65 and one in 2 over age 80 fall at least once each year [15]. In community-dwelling older women in Australia, the proportions falling each year were 28 % of those aged 65–74 years, 40 % of those aged 75–84 years and 48 % of those aged 85 years and older [16]. Serious injuries occur with 10–15 % of falls; for instance, 5 % of falls result in a fracture and 1–2 % of falls results in a hip fracture. Moreover, falls are independent determinants of functional decline and they lead to 40 % of all nursing home admissions [17]. Falls have important economic consequences. In the US, for example, falls are the leading contributor to the economic burden resulting from injuries in older adults [18] with most of this cost resulting from fractures, particularly hip fractures [19]. The leading causes of falling, based on an analysis of 16 trials are summarized in Table 2.1 [20]. Three if not four of the top four contributors (muscle weakness, gait deficit, and balance deficit and a history of falling) are related to functional deficits in the lower extremities, supporting the concept that falling is one of the consequences of sarcopenia [20]. Preserving muscle strength is an effective way to lower risk of falling and maintain physical function and independence in older persons [21].

Table 2.1 Common risk factors for falls identified in 16 studies

	Mean RR–OR	Range
Muscle weakness	4.4	1.5–10.3
History of falls	3.0	1.7–7.0
Gait deficit	2.9	1.3–5.6
Balance deficit	2.9	1.6–5.4
Use assistive device	2.6	1.2–4.6
Visual deficit	2.5	1.6–3.5
Arthritis	2.4	1.9–2.9
Impaired activity of daily living	2.3	1.5–3.1
Depression	2.2	1.7–2.5
Cognitive impairment risk factor	1.8	1.0–2.3
Age >80 years	1.7	1.1–2.5

From Guideline for the prevention of falls in older persons [20]; reproduced with permission
RR risk ratio, *OR* odds ratio

Operational Definitions of Sarcopenia

Nine operational or related definitions of sarcopenia have been proposed by different individuals and working groups. The first, by Baumgartner [22], was published in 1998 and a series of others

Table 2.2 Operational definitions of sarcopenia

	ALM(kg/ht^2)	TBLM	Fat Mass	Grip, kg	Gait speed, m/s	Reference Data
Baumgartner [22]	X					Rosetta study of 284 subjects
Men	≤7.26					
Women	≤5.45					
Delmonico 1 [23]	X					Health ABC
Men	≤7.25					
Women	≤5.67					
Delmonico 2 [24]	X		X			Health ABC
	<20 % tile of sex-specific residuals					
Cruz-Jentoft [25]	X			X[a]	X[a]	Not specified; we used Baumgartner ALM cut offs
Men	≤7.26			<30	<0.8	
Women	≤5.45			<20	<0.8	
Fielding [26]	X				X	Health ABC
Men	≤7.23				<1	
Women	≤5.67				<1	
Morley [27]	X				X	NHANES IV
Men	≤6.81				<1	
Women	≤5.18				<1	
Muscaritoli [28]	X	X[b]			X	Janssen
Men					<0.8	
Women					<0.8	
Studenski 1 [29]	X[c]			X		Pooled data from 9 studies
Men	<0.789			<26		
Women	<0.512			<16		
Studenski 2 [29]	X[c,d]			X		Pooled data from 9 studies
Men	<0.789			<26		
Women	<0.512			<16		

[a]This definition requires grip and/or gait speed
[b]Defined as a − 2 SD cutoff based on total body lean mass index ≤37 % for men and ≤28 % for women
[c]ALM adjusted for body mass index
[d]Low lean mass contributing to weakness

appeared between 2007 and 2014 [23–29]. These definitions utilize a measure of lean tissue mass with or without a functional test – either gait speed, grip strength, or both. The specific components of these definitions are summarized in Table 2.2. The definitions of low ALM have different cut offs and use different reference data bases. One includes DXA-derived fat tissue mass [24] and another uses total body lean mass (TBLM) rather than ALM [28]. Definitions that include functional measures use different function tests and different test cut-offs. Few studies have compared the performance characteristics of these definitions in relation to predicting hard clinical outcomes such as falls, quality of life, disability, or mortality. Hence there is currently no compelling rationale for selecting one of these definitions as the 'gold standard' definition of sarcopenia.

Which Operational Definitions Predict the Rate of Falls

We recently performed an analysis to determine the extent to which each of the 9 definitions of sarcopenia described above predicted the

incidence of falls over a 3-year period in a cohort of 445 community-dwelling men and women aged 65 years and older. The subjects had participated in the National Institute on Aging Boston STOP/IT study, a randomized placebo-controlled trial to determine the effect of supplementation with calcium (500 mg per day) plus vitamin D (700 IU of cholecalciferol per day) versus placebo on rates of bone loss from the hip [30]. The cohort was mainly white (430 were white, 11 were black, and 4 were Asian). In this trial, calcium and vitamin D reduced bone loss and fracture risk [30]. Calcium and vitamin D also reduced the risk of falling in the women but not in the men [31].

Study Measurements

At baseline, weight was measured with a digital scale and height with a wall-mounted stadiometer. ALM was determined by DXA with a DPX-L scanner (Lunar Radiation Corp, Madison, WI). Grip strength was measured twice with each hand and the higher reading was used in the analysis. Gait speed was assessed as the time required to walk 15 ft at the usual pace. The mean baseline clinical characteristics, ALM, grip strength, and gait speed values in the men and women are shown in Table 2.3. Falls were defined as "unintentionally coming to rest on the ground, floor, or other lower level" [32]. The falls assessment included instruction to participants to return a postcard after every fall. Upon receipt, staff then called the subject to assess the circumstances of the fall. Subjects were also questioned about falls on each 6-month visit throughout the study. The rate of falls rather than the number of first fallers was chosen as the primary outcome of this analysis because each fall adds risk of injury, fear of falling, and loss of independence. The prevalence of sarcopenia at baseline was calculated as the percent of sarcopenic individuals based on each of the 9 definitions, by gender. Comparative performance was assessed by multivariate Poisson regression analyses. There was no interaction of treatment during the trial (calcium + vitamin D or placebo) in the regression analyses, nonetheless, the analyses were adjusted for treatment as well as for gender.

Table 2.3 Mean (SD) baseline characteristics and measurements of the STOP/IT cohort, by gender

	Men (n = 199)	Women (n = 246)
Age, years	70.7 ± 4.6	71.1 ± 4.6
Weight, kg	81.9 ± 11.9	68.1 ± 12.5
Appendicular lean mass, kg	24.8 ± 3.3	15.9 ± 2.3
Appendicular lean mass/ht^2	8.2 ± 0.83	6.2 ± 0.76
Grip strength, kg	35.7 ± 6.5	19.5 ± 4.8
Gait speed, m/s	1.05 ± 0.22	1.00 ± 0.20

Prevalence of Sarcopenia by the 9 Definitions

The prevalence of sarcopenia in all participants differed for each definition. Among those definitions based on lean mass alone, the prevalence was: 11.0 % (Baumgartner), 11.7 % (Studenski 1), 16.9 % (Delmonico 1), and 21.4 % (Delmonico 2). Among the composite definitions (low lean tissue mass and decreased function), the prevalence was: 2.7 % (Morley), 3.1 % (Studenski 2), 5.0 % (Fielding), 7.1 % (Cruz-Jentoft), and 23.6 % (Muscaritoli). With one exception, the prevalence of sarcopenia was lower for the composite definitions, indicating that these definitions identify a more advanced stage of sarcopenia.

The prospective rate of falls in sarcopenic versus non-sarcopenic subjects for each definition of sarcopenia, after adjustment for treatment assignment and gender, is shown in Fig. 2.1. The findings were similar when 'fallers' rather than 'all falls' was considered [33]. For only two definitions did sarcopenia significantly predict the rate of falls in these community-dwelling older men and women, the Baumgartner [22] and Cruz-Jentoft [25] definitions. The relative risk of falling was somewhat higher by the Cruz-Jentoft composite definition than the Baumgartner definition involving ALM alone (1.82 [1.24–2.69] versus 1.54 [1.09–2.18], respectively). However, the prevalence of sarcopenia was lower by the composite Cruz-Jentoft definition than the ALM-based Baumgartner definition (7.1 % versus

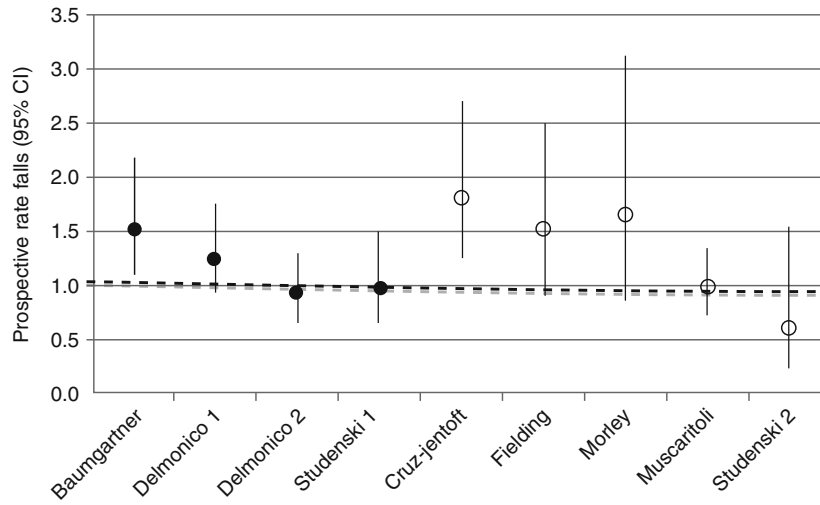

Fig. 2.1 Prospective rate of falls in sarcopenic versus non-sarcopenic seniors. (From Bischoff-Ferrari et al. [33], reproduced with permission)

11 %). It remains to be determined which of these definitions of sarcopenia is more predictive of rate of falls at any given sarcopenia prevalence.

Many other questions remain. Would a prior history of a fall in the last 6 or 12 months (not available in STOP/IT) have been as effective as the more elaborate single and composite definitions described above in predicting falls? Would gait speed and grip strength or other function tests alone be as effective as the combination of function test and lean mass?

Conclusion

The optimal definition of sarcopenia is one that identifies patients at high risk for significant adverse health events with enough lead time that effective preventive interventions could be instituted. Falls are a strong clinical endpoint because they are objective, discrete events that have enormous consequences for the patient, the health care system, and the economy. We have compared the ability of nine prevailing definitions of sarcopenia to predict rate of falling over a 3-year period in a cohort of community-dwelling older men and women. Two of the nine definitions significantly predicted the rate of falling, one involving measurement of ALM alone [22] and the other including ALM together with grip strength and/or gait speed [25]. In the STOP/IT cohort, the sarcopenia definition with the higher predictive capability [25] had the lower prevalence in this population, suggesting that it is identifying subjects with more advanced sarcopenia. Establishing a definition with the right balance between these parameters (prevalence and predictive capability) remains an important challenge. The ability of the prevailing definitions to predict falls in other cohorts and to predict other adverse outcomes such as injurious falls, declines in ability to carry out normal activities of daily living and other physical activities, and frank disability should be studied.

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Obesity, Insulin Resistance and Pediatric Bone

3

Richard D. Lewis, Joseph M. Kindler, and Emma M. Laing

Abstract

Numerous studies have demonstrated that obese children are at an increased risk for skeletal fracture; however, the basis for this observation has yet to be determined. The impact of excess adiposity on bone structure and microarchitecture are conflicting, though the majority of studies suggest that bone does not sufficiently adapt to excess adipose tissue, particularly in the upper vs. lower limbs. Emerging data also suggest that certain adipose depots, particularly within the visceral, bone marrow and skeletal muscle compartments, provide information beyond what is attained through measurement of total body fat alone. Examination of these specific fat depots may help clarify mechanisms related to fat-bone interactions. Specifically, cardiometabolic manifestations often accompany these "pathogenic" fat depots, and insulin resistance has been identified as a potential mediator in the relationship between fat and bone. Because prospective studies are limited, additional long-term investigations are warranted to better understand the manner in which excess adiposity influences pediatric bone development. This should be of particular public health concern since nearly one in five US children is considered obese and approximately one in four is at risk for developing type-2 diabetes.

Keywords

Obesity • Bone strength • Visceral adipose tissue • Insulin resistance • Pediatric

R.D. Lewis, PhD, RD, FACSM (✉)
J.M. Kindler, BS • E.M. Laing, PhD, RDN
Department of Foods and Nutrition,
The University of Georgia, 305 Sanford Drive,
Athens, GA 30602, USA
e-mail: rlewis@fcs.uga.edu; JMK5485@uga.edu; emonk@uga.edu

Abbreviations

BSI	Bone strength index
DXA	Dual energy X-ray absorptiometry
FFST	Fat-free soft tissue mass
FM	Fat mass

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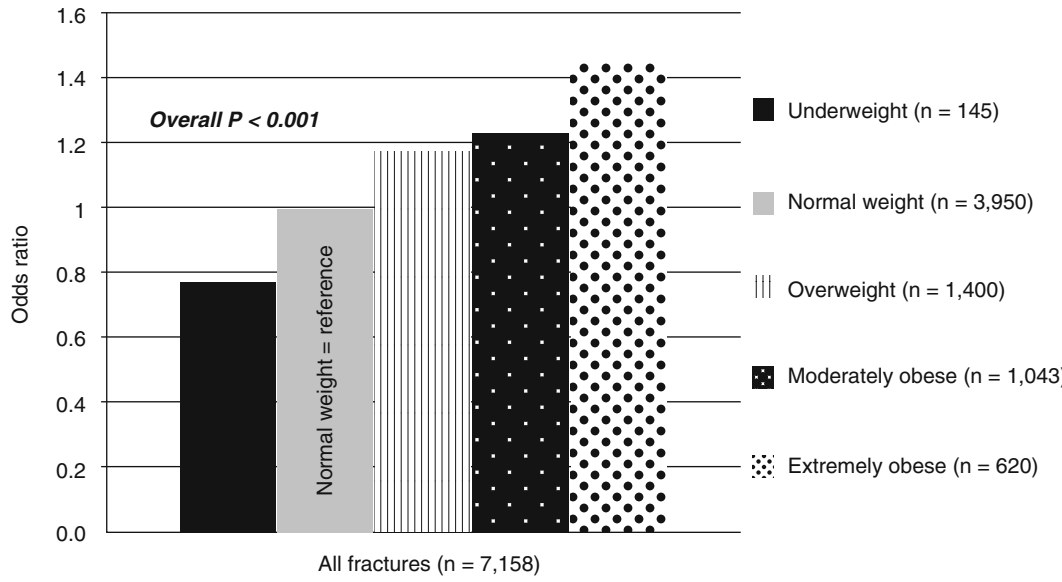

Fig. 3.1 Greater odds ratios in lower extremity fractures with severity of obesity (Data were obtained from Kessler et al. [9]). From electronic medical records of 913,178 patients ages 2–19 years Adjusted for sex, race, age, education, and Medi-Cal use (yes or no)

HRpQCT	High-resolution pQCT
PQCT	Peripheral quantitative computed tomography
SSI	Strength strain index

Introduction

Childhood obesity is a major public health priority as it is associated with an array of adverse secondary health outcomes. Approximately 25 % of US youth are considered obese, and as a result, the prevalence of prediabetes in children and adolescents has escalated to a similar rate, i.e., one in four children [1, 2]. Other chronic diseases, such as cardiovascular disease and hypertension are also linked to obesity, but musculoskeletal complications have not historically been associated with excess adiposity [1]. Several reports in the late 1990s and early 2000s suggest, however, that children with excessive adipose tissue are at a greater risk for sustaining a skeletal fracture [3–8]. Since the publication of these findings, others have shown a higher prevalence of both upper and lower extremity fractures in overweight or obese children versus their normal weight counterparts (Fig. 3.1) [9]. Why obese children are at greater risk of fractures is uncertain; however, both bone-dependent and bone-independent etiological factors have been implicated in the various proposed, yet interrelated, theories. The first hypothesis posits that obese children experience functional deficits that lead to an increased risk of falling and subsequent skeletal fracture [10]. A second suggests that obesity-associated lifestyle, cardiometabolic, endocrinological, and genetic factors interact in some way to compromise bone quality during youth, leading to an increased risk of fracture [11]. Adipocytes and osteoblasts originate from a common progenitor cell (bone marrow mesenchymal stem cells) and it is possible that adipogenesis occurs at the expense of osteoblasts differentiation. Adipocytes secrete inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1B, and IL-6, and other secretory factors that are known to promote adipogenesis and trigger RANKL stimulation of osteoclast proliferation and subsequent bone resorption (Fig. 3.2). The focus of this paper is to provide a review of the evidence summarizing: (1) the impact of excess

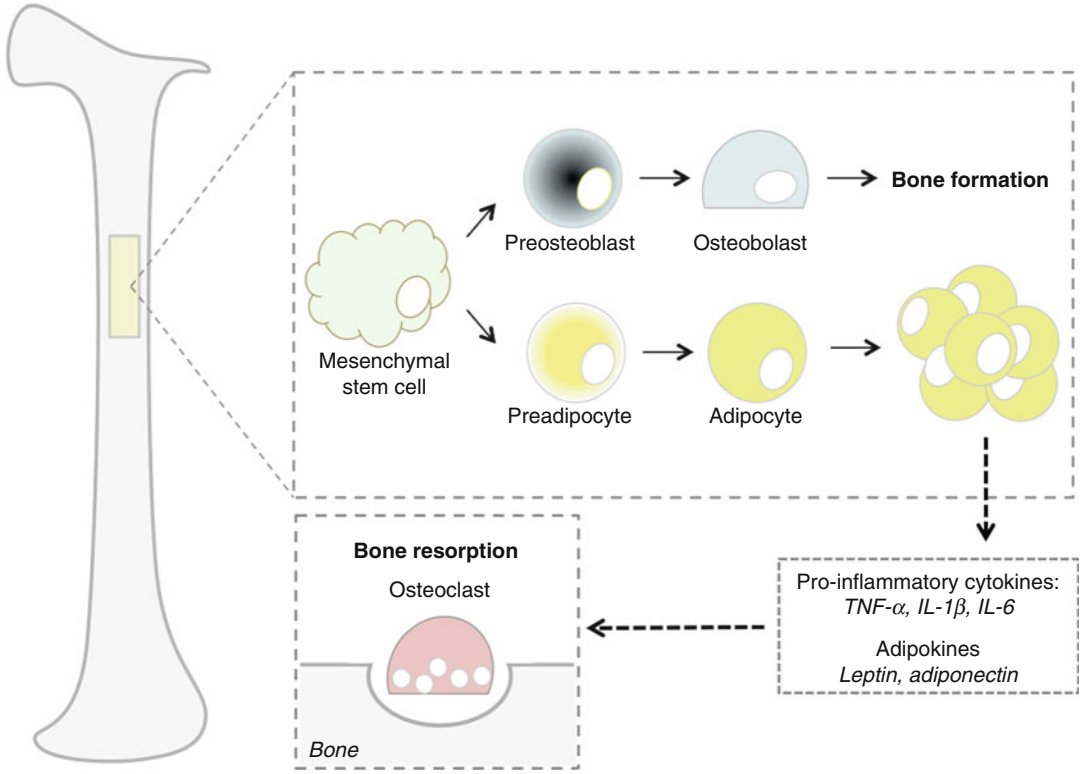

Fig. 3.2 Bone formation, adipose tissue accumulation, and bone resorption within the bone marrow microenvironment

adiposity on bone structure, microarchitecture and strength, (2) the importance of considering specific adipose depots in the context of the fat-bone relationship, and (3) if insulin resistance affects bone in youth.

Bone Mass, Structure and Strength

Early studies exploring the fat and bone relationship utilized whole body composition data derived from dual energy X-ray absorptiometry (DXA). While the relationships between fat-free soft tissue (FFST) mass and whole body areal bone mineral density (aBMD) or bone mineral content (BMC) are consistently positive [12–17], relationships between fat mass or % fat and whole body aBMD or BMC show conflicting results [18–23]. It is important to consider that variations in statistical procedures, study design, methods employed and control variables included in statistical models could be contributing to these inconsistent findings. For example, when correcting for body weight, obese vs. normal weight subjects have lower aBMD or BMC [20]; whereas, when correcting for height or FFST mass, obese vs. normal weight subjects have greater bone outcomes [18, 19]. Additional fat and bone studies have highlighted the importance of taking into account skeletal muscle, particularly since obese vs. non-obese children typically have greater lean body mass stores.

The ability of the skeleton to withstand exposure to unusual and high-impact forces depends largely on the geometrical and material properties of bone, most notably cortical bone size and volumetric density [24]. Findings from peripheral quantitative computed tomography (pQCT) studies in obese and non-obese children provide a musculoskeletal assessment of structural properties needed to better understand the influences of fat on bone. For example, Pollock et al. [25]

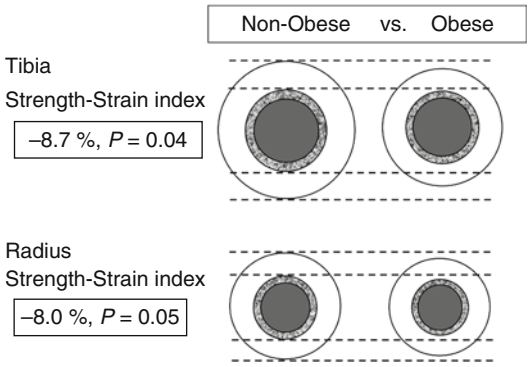

Fig. 3.3 Cortical bone strength in obese (n = 22) vs. non-obese (n = 93) late-adolescent females ages 18–19 years (Data were obtained from Pollock et al. [25]). SSI (mm^3) outcomes adjusted for muscle cross-sectional area

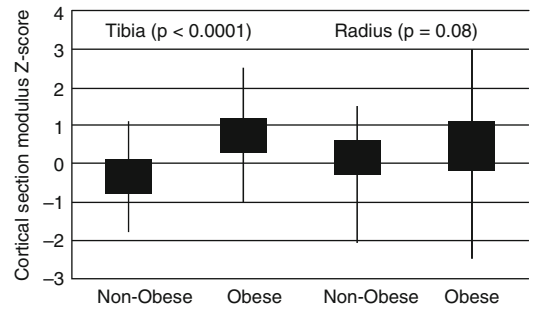

Fig. 3.4 Cortical section modulus at the tibia and radius in obese vs. non-obese adolescents (Data were obtained from Leonard et al. [28]). Obese (n = 91; BMI > 97th percentile; ages 10–15 years) Non-obese (n = 51; BMI > 5th and < 85th percentiles; ages 10–18 years) Sex- and race-specific (black and white) Z-scores relative to age, adjusted further by bone length

reported that obese vs. non-obese late-adolescent females had lower tibia strength-stain index (SSI) and radial bone-strength index (BSI) when correcting for muscle cross-sectional area (MCSA) (Fig. 3.3). These data imply that the extra body weight carried by obese females is not advantageous to bone strength and may contribute to fracture risk should a fall occur. By contrast, Sayers and Tobias [26] reported that when correcting for standing height, fat mass was a positive and negative predictor of mid-tibia periosteal and endosteal circumferences, respectively, in adolescents (mean age 15 years). It is important to note that FFST mass was not accounted for in this study [26]. Ducher et al. [27] showed that overweight children had greater cortical SSI at the tibia and trabecular BSI at the radius after accounting for differences in height. The authors concluded that the larger and stronger bones in the overweight participants were due to greater muscle mass, but not fat mass. To this point, adjusting for MCSA resulted in a significantly lower total bone cross-sectional area and cortical bone thickness at the radius in the overweight vs. the normal weight children.

The above findings support the importance of higher lean mass in obese vs. non-obese participants that drives bone strength differences in favor of the individuals with greater body fat; however, differences in sex, skeletal maturation, and physical activity levels are also important considerations. For example, Leonard et al. [28] compared bone structural and strength measures in obese and non-obese adolescents 10–15 years of age, and excluded children with high BMI z-scores with probable metabolic dysfunction. Tibia, but not radius, cortical section modulus z-scores were significantly higher in obese vs. non-obese adolescents when adjusted for sex, ancestry group, age, and tibia length (Fig. 3.4). Greater periosteal circumference was the primary bone outcome contributing to the greater section modulus in the obese participants; however, after statistical adjustment for calf MCSA, skeletal maturity, muscle strength, and moderate to vigorous physical activity, there were no differences in tibia bone strength between groups. These data are in agreement with earlier studies that show the more optimal skeletal properties in obese vs. leaner children is commensurate to lean more so than fat mass; but, also demonstrate that advanced skeletal maturation, greater muscle strength and lower moderate-vigorous physical activity levels in obese vs. non-obese subjects are important contributions to the obesity-bone relationship.

While the cross-sectional data described above provide important preliminary insight linking fat and bone, prospective studies provide more robust evidence that support these investigations. For example, Wey et al. [12] examined the radius over a period of 18–36 months in healthy 8–18 year-old children, and showed that in males and older females, longer–term exposure to higher body fat

was negatively associated with total bone area. These data suggest that obesity during childhood could lead to a smaller, weaker bone that becomes more apparent in late adolescence or early adulthood. Moreover, since fractures scale with body weight, and the beneficial effect of greater lean mass on bone structure in obese compared to non-obese children is more evident in the lower vs. the upper limbs, radial fractures in obese children pose a concern. However, at the tibia, Wetzenson and colleagues [29] reported greater trabecular and cortical bone strength in overweight compared to healthy weight children, 9–11 years of age. Over a period of approximately 16 months, the overweight vs. healthy weight children exhibited greater gains in cortical, but not trabecular bone area and strength, at the tibia. However, these greater increases in cortical bone area and strength were attributed more so to changes in lean mass vs. fat mass.

Bone Microarchitecture

To our knowledge, few studies have examined body composition and trabecular microarchitecture in children using high-resolution (HR) imaging techniques. Using HR peripheral quantitative computed tomography (HR-pQCT), Farr et al. [30] showed, in healthy children ages 8–15 years, an overall positive relationship between total body fat mass and trabecular bone microarchitectural measures after controlling for bone age, height, fracture history and appendicular lean body mass. At the radius and tibia in both boys and girls, fat mass was correlated with both trabecular separation (negative) and trabecular number (positive). Girls with greater fat mass also had a larger bone volume to total volume fraction at the ultradistal tibia, but not at the radius; however, this latter relationship was not observed in boys. The authors attributed this sex-related difference in terms of the BV/TV outcome to the negative associations between fat mass and trabecular thickness, which was apparent in boys but not girls. These differences, in the context of fat mass and trabecular bone thickness relationship, warrant consideration since the extra body weight accompanying obesity did not attenuate the negative relationship observed in this study, even at the lower body, ultradistal tibia, skeletal region of interest. In addition, the authors suggested that radius bone strength does not adapt to extra adiposity, and as such, the forearm vs. the lower leg is less suited to withstand the high forces generated in a larger individual, should a fall occur. This conclusion was later reasserted by Dimitri et al. [31], who showed significant differences in trabecular bone outcomes at the tibia, but not the radius, with obese vs. non-obese children showing greater trabecular number and lower trabecular separation. However, similar to the findings by Farr et al. [30], obese children in this study had significantly thinner trabeculae than their leaner counterparts. Moreover, there were no differences in BSI at the radius or tibia in obese versus lean children despite the extra 10 kg of lean mass in the obese children. Lastly, Evans et al. [32] showed higher tibia and radius trabecular number and lower trabecular separation in obese vs. normal weight young adult males and females, with greater differences occurring at the tibia vs. the radius. Despite skeletal muscle being an important determinant of appendicular trabecular bone microarchitecture [33], the authors in this study did not account for ~10 kg of additional lean mass in the obese subjects. Andersen et al. [34] reported similar findings with respect to greater trabecular number and less trabecular separation in obese vs. normal weight young adults. When accounting for body weight, the authors showed a lower estimated failure load at both the tibia and radius in obese vs. normal weight groups.

Whereas adult studies support the notion that obesity is advantageous to trabecular bone microarchitecture, particularly at the lower limbs, the limited data in children are conflicting. The clinical significance of the consistent inverse relationships between adiposity and trabecular thickness warrants additional consideration given that both studies in children showed this relationship along with positive associations between fat and other trabecular bone indices. Since cross-sectional findings may not be indicative of an effect over time, prospective studies are necessary to advance

our understanding of the fat-bone relationship in youth, particularly in terms of trabecular bone development.

Depot-Specific Adiposity

There is mounting evidence to suggest that certain adipose depots, and not necessarily total body fat, provide a better indicator of adverse health events associated with adiposity [35]. A scientific symposium held in 2011 at the Pennington Biomedical Institute addressed the issue of trunk fat and health risks based on data from the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) [35]. Findings from this symposium showed that children with greater trunk fat were more likely to have metabolic disturbances later in young adulthood. Gilsanz et al. [36] were the first to report this link in terms of cortical bone size and strength, i.e., that visceral adipose tissue (VAT) determined by computed tomography was negatively related to femoral structural and strength measures in 15–25 year-old females, which were in opposition to the positive relationships reported with subcutaneous adipose tissue (SAT) (Fig. 3.5). Two subsequent papers showed negative relationships between VAT and DXA-derived lumbar spine, hip and total body BMD [37] or total body BMC [38] in obese and overweight adolescents. A two-year prospective study in young girls demonstrated that higher android fat mass, a surrogate for VAT, was associated with greater increases in trabecular vBMD and BSI, but lower increases in cortical vBMD [39]. Consistent with the hypothesis that higher VAT is associated with impaired bone strength, Cohen et al. [40] demonstrated a negative impact of VAT on trabecular microarchitecture in young adult women. In this study, participants in the highest tertile of VAT had lower BV/TV and fewer and thinner trabeculae than those with lower VAT. The lower observed BV/TV might have been related to an overall decrease in bone remodeling, as both bone formation and resorption markers were lower in those with highest VAT. The results from this study oppose previous microarchitecture studies in healthy boys and girls [30] and obese young adult men and women [32, 34], and highlight the need to account for specific adipose depots.

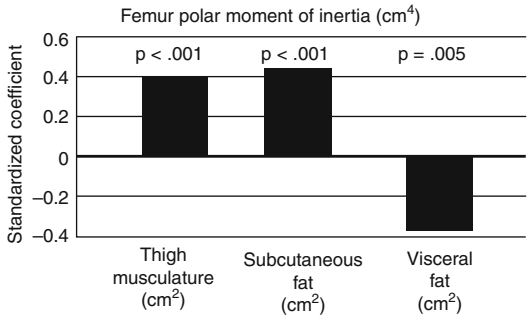

Fig. 3.5 Multiple linear regression model for femoral polar moment of inertia and thigh musculature, and subcutaneous and visceral adipose tissue (Data were obtained from Gilsanz et al. [36]). N = 100 females ages 15–25 years

Since adipocytes and osteoblasts are derived from common progenitor mesenchymal stem cells within the marrow cavity, osteoblast differentiation and subsequent bone formation may be altered by excess marrow adipose tissue (MAT). In adults, it was shown that greater marrow adiposity was inversely associated with both axial and appendicular skeletal outcomes [41]. The same research group reported positive associations for both baseline and change values in marrow density and femoral shaft cortical bone area over a period of up to two years in a younger group of healthy late-adolescent females 15–20 years of age [42]. The authors concluded that less marrow fat infiltration (i.e., greater marrow density) during growth is associated with larger bones. Young adult women with anorexia nervosa and significantly low total body fat mass, paradoxically have higher MAT at the lumbar spine and the femur [43]. In this study, MAT was inversely associated with spine and hip BMD ($r = -0.56$ to -0.71). Scheller and Rosen [44] noted in a recent review that the metabolism and functions of MAT are not fully understood and it is not yet clear if MAT accumulation leads to bone loss. The authors indicated that retention of some degree of MAT is necessary and beneficial with respect to skeletal and systemic metabolism.

Among the strongest determinants of pediatric bone strength is skeletal muscle mass, which is

typically measured via DXA. However, through various imaging modalities such as pQCT, appendicular MCSA can be acquired. One area of interest pertaining to skeletal muscle is that of skeletal muscle adipose tissue infiltration, which can be measured by pQCT at both the lower leg and forearm. Muscle density, a measure of muscle fat infiltration, considers the adipose tissue between and within muscle fibers. Prior work in adults suggests that skeletal muscle fat is greater in those with type-2 diabetes and is also associated with increased risk of fracture [45]. Of the few studies conducted to ascertain if excess muscle fat impacts bone strength in children, the findings to date have been inconsistent. For example, Farr et al. [46] reported in females 9–12 years of age that those with greater muscle fat (i.e., lower muscle density) had smaller, less dense and weaker bones at both trabecular and cortical bone regions of the femur and tibia. In contrast, in an older cohort of males and females approximately 18 years of age who took part in the Avon Longitudinal Study of Parents and Children (ALSPAC), muscle density was inversely associated with tibia cortical periosteal circumference, vBMD, BMC, thickness, and estimated bending strength [47]. The authors speculated that the differing results between the two cross-sectional studies were due to differences in participant ages and skeletal maturity. Two prospective studies published from the same cohort help clarify these inconsistent findings. In females 9–12 years of age, Farr et al. [48] showed that greater increases in muscle density over two years were associated with greater change in tibia and femur BSI. Additionally, girls in the highest fifth of 2-year muscle density change demonstrated the greatest positive change in tibia cortical vBMD. Alternatively, Laddu et al. [49] demonstrated that children with lower muscle density (i.e., greatest muscle fat) at baseline had the greatest positive change in various trabecular bone outcomes (Fig. 3.6). One caveat to these findings by Laddu et al. [49] is that over this two-year period, the girls with the lowest baseline muscle density exhibited the greatest increases in muscle density. These data show that increases in muscle density favor improvements in bone strength in young girls, primarily in trabecular regions of weight bearing limbs. To date, no prospective studies have been conducted that include males during pubertal growth or examine trabecular and cortical bone of the forearm. Including both sexes in future studies is important given the stronger relationships between muscle density and cortical bone outcomes in boys versus girls [47]. One of the key questions that arises in studies of ectopic fat and bone is whether or not metabolic

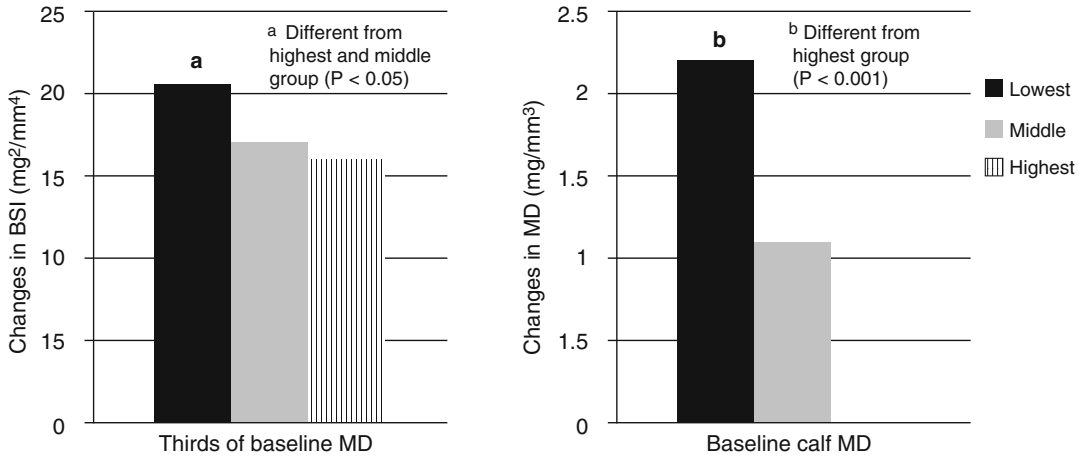

Fig. 3.6 Two-year changes in tibia BSI and calf muscle density (MD) across baseline MD in girls (Data were obtained from Laddu et al. [49]). N = 248 girls, ages 8–13 years at baseline. Adjusted for ethnicity, baseline measures of maturity offset, bone length, MSCA, 2-year change in maturity offset, and physical activity

disturbances secondary to excess adiposity are responsible for the effects of fat on bone.

Insulin Resistance and Bone

Of the few studies that have examined the relationships between metabolic health outcomes and bone in children, the vast majority has supported a negative relationship between insulin resistance and bone utilizing 2-dimensional bone imaging techniques (Table 3.1) [38, 50–57]. Among the first to examine these associations in a younger cohort of overweight American Latino children was Afghani and colleagues [50]. The authors showed that various measures of insulin resistance were inversely associated with total body BMC and aBMD. Lee et al. [54] likewise demonstrated that children with higher measures of insulin resistance had lower BMC at various skeletal regions of interest. Other studies in children with prediabetes [55] and who have multiple cardiometabolic risk factors associated with the metabolic syndrome [38] support these findings. Alternatively, Lawlor et al. [53] showed in the boys and girls who participated in the ALSPAC study that fasting insulin was a positive predictor of total body BA and BMC, but aBMD in females only. However, after accounting for the effect of fat mass, insulin was a significant negative predictor of aBMD, BA and BMC in all study participants. These latter findings are in agreement with those by Sayers et al. [57] who, in this same cohort of ALSPAC children, showed that fasting insulin was negatively associated with periosteal circumference, cortical BMC, cortical vBMD, and polar SSI. Based on the evidence acquired to date, it appears that insulin resistance in children is associated with suboptimal skeletal mass and structure, yet the mediating mechanisms explaining this relationship remain unknown.

Insulin resistance, hyperinsulinemia and hyperglycemia are conditions that typically occur secondary to obesity. Both obesity and type-2 diabetes are pro-inflammatory conditions, and provide one possible explanation for the fat-bone relationship, since exposure to pro-inflammatory factors may modulate bone metabolism. Another possible mechanism by which insulin resistance may impact bone involves the formation of advanced glycation end products. Circulatory glucose concentrations are tightly regulated throughout pubertal growth; however, hyperglycemia may lead to the glycation of bone collagenous proteins and result in compromised bone strength [58]. Pediatric studies relating bone quality with insulin resistance and hyperglycemia-related inflammation and bone glycation are limited, thus future studies should consider these mechanisms.

Summary and Conclusions

This review highlighted the general consensus that muscle is a key determinant of bone strength in children and adolescents undergoing rapid growth. Indeed, once skeletal muscle and advanced maturation are accounted for in studies of obese children, trabecular and cortical bone outcomes are similar to, or lower than, non-obese counterparts. Most studies in this review support the notion that obese children carry significantly more muscle and fat tissue; however, their skeletons do not adapt sufficiently to accommodate the potential extra forces generated by the extra body weight. The majority of fat-bone studies to date focused on total body fatness, though more recent studies considering specific adipose depots, such as VAT, MAT and skeletal muscle fat, are providing additional information on the fat-bone connection. While it seems as though excess VAT and skeletal muscle fat are associated with reduced bone strength, further work is warranted to clarify the role of these specific adipose depots in pediatric bone development. That is, it may depend on the severity of the fat accumulation and whether metabolic disturbances accompany excessive adiposity. Lastly, this review brought attention to the concept that insulin resistance is associated with lower bone mass and structural measures; however, the mechanisms responsible for these findings remain unknown. Future prospective studies that follow children throughout pubertal development and that are designed to address the issue of chronic exposure

Table 3.1 Insulin resistance and bone

Reference	Population	Bone imaging method and outcomes		Glucose regulation measures and outcomes		Summary of results
Afghani et al. [50]	Overweight Latino children with a familial history of type 2 diabetes N (boys/girls) = 106/78	DXA	Whole body BMC and aBMD	Oral glucose tolerance test Frequently sampled intravenous glucose tolerance test	Fasting glucose 2-h glucose Fasting insulin 2-h insulin Insulin sensitivity AIR Insulin AUC Incremental insulin AUC	In all participants, fasting insulin, 2-h insulin, AIR, AUC, and incremental AUC were negatively correlated with whole body BMC and aBMD. AIR and AUC were negative predictors of whole body BMC and aBMD, respectively.
Ashraf et al. [51]	African and European American female adolescents (n = 29; ages 14–18 years) and premenopausal adults (n = 49; ages 18–55 years)	DXA	Whole body BMC, aBMD, and BMAD	Mixed meal tolerance test	Insulin AUC Whole body insulin sensitivity index Fasting insulin	In adolescents and adults, insulin AUC was a positive predictor of whole body aBMD but fasting insulin was positively associated with aBMD only in adolescents. In the adolescent girls, insulin AUC and fasting insulin were positive correlates of whole body BMAD.
do Prado et al. [52]	Obese post-pubertal Brazilian boys (n = 41) and girls (n = 68); ages 13–18 years	DXA	Whole body BMC and aBMD	Fasting blood draw	Fasting insulin and glucose HOMA-IR	Fasting insulin and HOMA-IR were significant negative predictors of total body aBMD and BMC in boys but not girls.
Lawlor et al. [53]	English boys (n = 1100) and girls (n = 1205) approximately 15.5 years of age who took part in the ALSPAC	DXA	Whole body BMC, BA and aBMD	Fasting blood draw	Fasting insulin	Fasting insulin was positively associated with aBMD in girls, but not boys. However, fasting insulin correlated positively with BA and BMC in both sexes. After adjusting for fat mass, insulin was a significant negative predictor of aBMD, BA and BMC in both sexes.

(continued)

Table 3.1 (continued)

Reference	Population	Bone imaging method and outcomes		Glucose regulation measures and outcomes		Summary of results
Lee [54]	Korean adolescent boys (n=315) and girls (n=303) aged 10–19 years who participated in the 4th Korean National health and Nutrition Examination Survey	DXA	Whole body BMC Proximal femur BMC Lumbar spine BMC	Fasting blood draw	HOMA-IR	HOMA-IR was a negative predictor of total body, proximal femur and lumbar spine BMC in boys ages 13–16. HOMA-IR also correlated negatively with total body and proximal femur BMC in boys ages 17–19 years. HOMA-IR negatively correlated with BMC in normal and overweight boys and girls.
Pirgon et al. [56]	Obese adolescent boys and girls with (n=42) and without (n=40) NAFLD as well as healthy controls (n=30) approximately 12.3 years of age.	DXA	Lumbar spine aBMD	Fasting blood draw	HOMA-IR	HOMA-IR was negatively associated with lumbar spine aBMD in obese children with and without NAFLD, however, these relationships were substantially stronger in those with NAFLD.
Pollock et al. [55]	Prepubertal black and white boys and girls with (n=41) and without (n=99) prediabetes	DXA	Whole body BMC, BA and aBMD	Oral glucose tolerance test Fasting blood draw	Fasting insulin and glucose 2-h glucose Glucose AUC 2-h insulin Insulin AUC HOMA-IR	Children with prediabetes had significantly lower total body BMC and BA than those with normal fasting glucose concentrations. In the children with prediabetes, 2-h insulin and insulin AUC were negatively associated with total body BMC.
Pollock et al. [38]	Overweight adolescent black and white boys and girls with 0 (n=55), 1 (n=46) and >2 (n=42) cardiometabolic risk factors who were 14–18 years of age	DXA	Whole body BMC, BA and aBMD	Fasting blood draw	HOMA-IR *Cardiometabolic risk factors:* Waist circumference High density lipoproteins Triglyceride level Blood pressure Fasting glucose Fasting insulin	Children with 1 or ≥2 cardiometabolic risk factors had significantly lower total body BMC and aBMD than those with 0 risk factors. HOMA-IR was a significant and negative determinant of total body BMC in the total sample of overweight children.

Sayers et al. [57]	English boys (n = 1344) and girls (n = 1400) approximately 15.5 years of age who took part in the ALSPAC	pQCT (50 % tibia)	PC EC Ct.BMC Ct.vBMD pSSI	Fasting blood draw	Fasting insulin	Fasting insulin was a negative predictor of PC, cortical vBMD, cortical BMC and pSSI after adjusting for confounders. Through path analysis, insulin was associated with PC via muscle cross-sectional area (positive), muscle density (negative) and subcutaneous fat (positive).

DXA dual energy X-ray absorptiometry, *BMC* bone mineral content, *aBMD* areal bone mineral density, *AIR* acute insulin response, *AUC* area under the curve, *BMAD* bone mineral apparent density, *HOMA-IR* homeostasis model assessment of insulin resistance, *ALSPAC* Avon Longitudinal Study of Parents and Children, *BA* bone area, *NAFLD* -alcoholic fatty liver disease, *pQCT* peripheral quantitative computed tomography, *PC* periosteal circumference, *EC* endosteal circumference, *Ct.BMC* cortical bone mineral content, *Ct.vBMD* cortical volumetric bone mineral density, *pSSI* polar strength-strain index

to excess fat and metabolic disturbances will help improve our knowledge of the relationship between fat and bone and the mechanisms involved.

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Influence of Sarcopenic and Dynapenic Obesity on Musculoskeletal Health and Function in Older Adults

4

David Scott

Abstract

Age-related declines in skeletal muscle mass (sarcopenia) and strength (dynapenia) compromise musculoskeletal health. This may be exacerbated in the presence of obesity. Dynapenic obesity is more consistently associated with poor physical performance than sarcopenic obesity and so may be a better predictor of falls, but both sarcopenic and dynapenic obese individuals may have greater risk for osteoporosis and fractures than obese alone. Osteoarthritis may also be common in sarcopenic and dynapenic obesity. Weight loss and resistance training is the most effective therapy for improving body composition and muscle function, while reducing risk for falls, osteoporosis and osteoarthritis in older adults.

Keywords

Sarcopenic obesity • Sarcopenia • Dynapenia • Obesity • Falls • Fractures • Osteoarthritis

Introduction

Over one-fifth of years lived with disability worldwide are attributable to musculoskeletal conditions, which is second only to mental and behavioural problems in terms of disease burden [1]. Many musculoskeletal conditions, including osteoarthritis, disability and falls, and osteoporotic fractures, are more prevalent in older and obese individuals [2]. The confluence of ageing populations and obesity epidemics in developed countries indicate that the burden of musculoskeletal conditions will continue to grow.

D. Scott, PhD
Department of Medicine,
School of Clinical Sciences at Monash Health,
Faculty of Medicine, Nursing and Health Sciences,
Monash University, 246 Clayton Road,
Clayton, VIC 3146, Australia

Melbourne Medical School - Western Campus and
Australian Institute for Musculoskeletal Science,
The University of Melbourne,
St. Albans, VIC, Australia
e-mail: david.scott@monash.edu

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Components of the musculoskeletal system are highly inter-related and common genetic factors may influence age-related skeletal muscle and bone changes [3]. Sarcopenia and dynapenia are terms used to describe age-related declines in skeletal muscle mass and strength, respectively. Although sarcopenia and dynapenia are musculoskeletal conditions in their own right, they may contribute to the development and progression, and also be a consequence, of other musculoskeletal conditions [4]. Furthermore, when coupled with obesity (sarcopenic obesity and dynapenic obesity), deleterious effects on musculoskeletal health may be exacerbated. This review examines definitions of sarcopenic and dynapenic obesity, their associations with musculoskeletal conditions, and the role that lifestyle interventions can play in improving body composition and muscle function in older adults.

Sarcopenia and Dynapenia

The term "sarcopenia" initially described the process of age-related muscle wasting considered at the time to be the primary contributor to functional decline in older adults [5]. A consensus definition of sarcopenia does not exist, but several expert groups have proposed criteria in recent years reflecting general agreement that it is a now considered a multi-dimensional condition involving declines in both muscle mass and function [6–10].

The inclusion of poor muscle function as a component of sarcopenia has arisen from research demonstrating disparity in associations of muscle mass and strength with health outcomes in older adults. Low muscle function is a more consistent predictor of functional decline, falls and mortality than low muscle mass, and its associations with these outcomes are independent of muscle size [11]. For example, 5-year mortality risk increases by over 40 % per standard deviation decrease in quadriceps and grip strength in men and women aged 70–79 years, even after adjustment for muscle mass [12]. Furthermore, declines in muscle mass with age are not the primary cause of muscle function deficits; loss of strength exceeds the rate of loss of muscle mass by up to five-fold, and gains in muscle mass in older adults do not prevent strength declines [13, 14].

The disparities in associations and outcomes of age-related muscle mass and strength decline prompted Clark and Manini to propose that loss of muscle strength, or "dynapenia", should be considered a separate condition to sarcopenia [15], which is just one of many neuromuscular factors that contribute to dynapenia [16]. They proposed that measurements of hand grip and knee extension strength could be utilised to diagnose dynapenia in clinical settings, with subsequent investigations to determine the neurological and/or muscular causes of dynapenia [17].

The component of dynapenia not attributable to sarcopenia is often referred to as "muscle quality" and is assessed as muscle strength normalised to muscle mass, which declines with age [18]. The loss of muscle quality during ageing, like dynapenia, is thought to be attributable to age-related changes in components of the neuromuscular system including decreased activation of motor neurons and changes in skeletal muscle composition [19]. Infiltration of fat into skeletal muscle, known as inter- and intra-muscular adipose tissue (IMAT), is a component of muscle composition attracting increasing attention. IMAT may increase by as much as three-fold from young adulthood to old age [20] and is two-fold higher in obese compared to normal weight older adults [21]. High levels of lower-limb IMAT have been associated with mobility limitation even after adjustment for muscle size [22, 23], as well as poorer balance [24], falls [25] and hip fracture risk [26], and this may be related to deleterious effects of IMAT-associated inflammatory cytokines on skeletal muscle and bone [27]. IMAT in particular is a contributor to poor muscle quality likely to be significantly increased in sarcopenic and dynapenic obesity.

Sarcopenic and Dynapenic Obesity

The obesity epidemic in many developed countries has not resulted in reduced life expectancy [28]. In the US, the average number of years

spent living with diabetes has increased by 156 % in men and 70 % in women since the 1980s [29]. While mortality risk in older adults does not significantly increase with increasing body mass index (BMI), disability risk amongst obese individuals is increased by up to 2.5 times [30]. Obese older adults have two- to three-fold greater risk of functional decline over 3 or 4 years compared with non-obese older adults [31]. At least part of this increased rate of functional decline appears to be explained by poor muscle function. Obese older adults have significantly poorer lower-limb muscle quality (strength normalised to muscle mass) compared with healthy non-obese older adults, and even compared with frail non-obese older adults [32].

"Sarcopenic obesity" is the term used to describe low muscle mass relative to fat mass [33]. The combination of high fat mass with low skeletal muscle mass (the largest insulin sensitive tissue in the body) has led to interest in sarcopenic obesity as a potential risk factor for cardiometabolic disorders in older age [34]. It is also thought that sarcopenia and obesity may have an interaction effect on functional decline in older adults, given both conditions are independently associated with poor physical performance [35]. Certainly, it is logical that individuals whose muscle function is inadequate to support their body mass would experience functional limitation [36].

Stenholm and colleagues, in keeping with the evolving definition of sarcopenia, first proposed that sarcopenic obesity definitions should include muscle function rather than, or in addition to, muscle mass [37]. Some researchers have adopted the term "dynapenic obesity" to describe poor muscle function in the presence of obesity [38, 39], although others continue to refer to this state as sarcopenic obesity [40, 41], which is undoubtedly a source of confusion for clinicians. In support of the need for separate terms, we recently demonstrated discordance between sarcopenic and dynapenic obesity classifications in the Tasmanian Older Adult Cohort (TASOAC) study, a prospective population-based study of over 600 community-dwelling older adults [42]. Only half of participants who were classified as having dynapenic obesity also met criteria for sarcopenic obesity [42]. Furthemore, as reported in Table 4.1, in participants classified as having sarcopenic obesity or dynapenic obesity alone (i.e. not both conditions), despite similar body composition, dynapenic obese have significantly poorer lower-limb strength and muscle quality (strength normalised to muscle mass) than sarcopenic obese (unpublished data). Thus, dynapenic obese older adults may have similar muscle size but produce only around half as much absolute and relative force compared with sarcopenic obese, suggesting differences in neuromuscular function between the two populations. It is therefore likely that sarcopenic and dynapenic obesity are associated with different consequences for musculoskeletal health, and particularly physical performance and falls risk.

Table 4.1 Comparisons of body composition and muscle function variables for TASOAC participants defined as sarcopenic obese only or dynapenic obese only

	Sarcopenic obese only (mean ± SD) *N = 30*	Dynapenic obese only (mean ± SD) *N = 38*	P-value for difference
Total body fat (%)	39.4 ± 6.5	40.2 ± 5.1	0.725
Total appendicular lean mass (kg)	23.7 ± 4.1	25.0 ± 4.1	0.226
Lower-limb lean mass (kg)	16.4 ± 2.7	17.5 ± 2.9	0.103
Lower-limb strength (kg)	105.9 ± 43.6	50.4 ± 30.2	**<0.001**
Lower-limb muscle quality (kg/kg)	6.3 ± 2.0	2.8 ± 1.5	**<0.001**

Physical Performance

While some studies have provided evidence that low muscle mass in obesity is associated with worse physical functioning than sarcopenia or obesity alone [43–46], others have reported no differences for sarcopenic obese older adults compared to those with neither, or only one, of these conditions [47–49]. Conversely, the highest prevalence and incidence of mobility limitations

is consistently reported in sarcopenic obesity when defined by muscle strength [50–52]. Similarly, dynapenic obese older adults generally demonstrate worse physical performance than dynapenic or obese alone groups [38]. Dynapenic obese (defined as the lowest tertile of hand grip strength and BMI ≥ 25) Chinese older adults have greater likelihood of self-reported disability [53], slow gait speed and mobility disability [54], than those with dynapenia or obesity alone. In Japanese older adults, those with low hand grip strength and high BMI at baseline had almost four-fold increased odds for 2-year incident mobility limitations compared to those with normal strength and BMI, and these odds exceeded those for low muscle strength (two-fold increased odds) and high BMI (not significantly increased) alone [55]. A Korean study has indicated a significant interaction effect for dynapenia and obesity with activity of daily living scores, suggesting a synergistic effect of dynapenic obesity on poor physical performance [56].

We have directly compared physical performance across categories of sarcopenic and dynapenic obesity, and observed dynapenic obese older adults have significantly greater declines in performance on a physiological falls risk assessment compared to non-dynapenic and non-obese, but there were no differences for categories of sarcopenic obesity [42]. Recently, an analysis of more than 18,000 adults aged over 65 years in the COURAGE and SAGE studies has also revealed that low muscle mass and high body fat percentage is not associated with increasing disability on the World Health Organization Disability Assessment Schedule 2.0. Low muscle mass and high body fat combined with low hand grip strength and/or gait speed was associated with significantly increased disability however, suggesting that dynapenic obesity, but not sarcopenic obesity is associated with disability in older adults [41]. While further research is required to establish appropriate criteria for diagnosing sarcopenic and dynapenic obesity, it appears dynapenic obesity is a more consistent predictor of poor physical function than sarcopenic obesity, and this likely contributes to increased falls risk.

Falls, Fractures and Bone Health

Approximately 90 % of hip, forearm and pelvis fractures result from falls [57], and so identifying modifiable risk factors for falls is important. Evidence of increased falls and fracture risk in sarcopenic or dynapenic obesity is lacking because to date no prospective studies have examined this relationship, although data is available for individual components of these conditions. Higher skeletal muscle mass and strength are associated with maintaining balance [58, 59] and bone quality [59–61] during ageing. However, poor hand grip strength [62, 63] and lower-limb function [64, 65] may be more consistent predictors of falls and fractures than low muscle mass; falls and fracture risk appears to be generally increased for men with sarcopenia [59, 66–68], but the association for women is unclear.

Obese individuals demonstrate worse postural stability [69] than normal weight individuals. Accordingly they appear to fall more regularly, but do not report increased rates of injury from falls, particularly at high levels of adiposity [70, 71]. An 18-year follow-up of over 14,000 NHANES III participants reported overweight and obese adults have 58 and 50 % reduced risk of death due to unnatural causes, and that no deaths attributable to falls occurred in obese participants [72]. This protective effect of obesity may be related in part to reduced fracture risk, generally ascribed to increased soft-tissue padding and higher bone mineral density (BMD) [73, 74].

The role of obesity in improved bone quality and reduced fracture risk is controversial. Manitoba, Canada experience a decline in fractures between 1996 and 2006 but this was not found to be attributable to the increasing prevalence of obesity [75]. In the US, approximately half of all fractures occur in overweight and obese in those aged 65-74 years [76] and while obese women may be less likely to have a wrist fracture, they are more likely to experience ankle and upper leg fractures than non-obese women [73]. Similarly, another study reported that after adjustment for femoral neck BMD, the relative risk of any, vertebral, or upper-limb, fracture is

increased in older women by over 20 % per SD increase in BMI [74]. Fracture risk is also increased in type II diabetes despite higher BMD and even after adjusting for increased falls risk [77–79]

Advances in clinical imaging allow quantification of bone geometry and microarchitecture, as well as regional body fat depots, such as IMAT and visceral adipose tissue. Using peripheral quantitative computed tomography (pQCT), high BMI has been associated with lower cortical, but not trabecular, volumetric BMD in women [80], and high-resolution pQCT reveals that cortical porosity is similarly increased in type II diabetics compared to controls despite higher trabecular bone density [81–83]. A separate study suggested that the improved bone density and bone microarchitecture of obese post-menopausal women compared to normal weight women is not in proportion to the increase in fat mass [84]. Ectopic fat depots, which are more pro-inflammatory and closely associated with insulin resistance than subcutaneous fat [85], potentially have effects on bone quality. Overweight and obese men with high visceral adipose tissue have poorer bone microarchitecture and bone mechanical properties compared to those with low visceral adipose tissue [86] and IMAT is an independent predictor of 6-year incident hip fractures [26]. Thus, obesity is associated with improved BMD, but also with increased falls risk and poorer bone quality, which may explain why a substantial proportion of fractures occur in overweight and obese individuals. The poor muscle mass and quality of sarcopenic and dynapenic obese older adults may further increase falls and fracture risk in these populations.

In the New Mexico Elder Health Survey, both sarcopenia and sarcopenic obesity in men, but not women, was associated with increased likelihood of past-year falls compared to non-sarcopenic non-obese, with the highest (three-fold) increased odds for sarcopenic obese men [43]. In older adults participating in a falls prevention trial in New Zealand, sarcopenic obese individuals reported the highest number of falls, albeit non-significant compared to other groups in the previous 12 months.

A small study has suggested that obesity may offer some protection against low total body, hip and spine BMD in sarcopenic women [87]. Similarly, the highest prevalence of femoral neck osteoporosis (22 %) is observed in sarcopenic alone older adults, but it is notable that the prevalence in sarcopenic obese (17 %) is substantially higher compared to non-sarcopenic non-obese (12 %) and obese alone (7 %) [88]. Evidence to date suggests that sarcopenic and dynapenic obesity may be associated with the highest risk of falls, and also poorer bone health at least in comparison to obese alone individuals. Prospective studies of falls and fracture outcomes are required to elucidate the risk for sarcopenic and dynapenic obese older adults.

Osteoarthritis

Osteoarthritis (OA) is a major cause of disability in older adults [89] and associated with increased risk for falls [90, 91]. Obesity is a key risk factor for knee OA due to increased mechanical load on the joint and inflammatory and metabolic factors [92]. Obese individuals are seven times as likely as normal weight individuals to report a diagnosis of knee OA and, amongst those with OA, obese individuals report higher levels of pain, stiffness and functional deficit [93].

OA may contribute to sarcopenia and dynapenia progression in older adults; a mechanism may be avoidance of physical activity due to pain, resulting in muscle mass and strength declines [94]. Hip OA has been shown to be associated with significantly lower muscle mass and strength of the thigh and pelvic muscles [95]. In TASOAC, we observed that baseline knee pain, but not radiographic OA, predicted a greater decline in leg strength and muscle quality, although for women only [4]. Similarly, other studies report that significant associations between knee ROA, quadriceps weakness and physical performance are mediated by pain [96, 97].

The lower quadriceps strength of older adults with knee pain compared to those without appears to be explained in part by reduced muscle

Table 4.2 Prevalence of radiographic and symptomatic knee osteoarthritis in TASOAC participants according to sarcopenic and dynapenic obesity category

	Sarcopenic obesity categories			
	Non-sarcopenic, non-obese	***Non-sarcopenic, obese***	***Sarcopenic, non-obese***	***Sarcopenic obese***
	N=341	*N=187*	*N=171*	*N=83*
Severe pain (%)	18.5[d]	27.8	19.9	34.9[a]
Severe stiffness (%)	14.7[b,d]	28.3[a]	17.0[d]	33.7[a,c]
Severe dysfunction (%)	17.8[b,d]	31.9[a]	23.4	35.4[a]
Knee OA (%)	65.1	72.3	69.6	75.9
	Dynapenic obesity categories			
	Non-dynapenic, non-obese	***Non-dynapenic, obese***	***Dynapenic, non-obese***	***Dynapenic obese***
	N=323	*N=167*	*N=176*	*N=86*
Severe pain (%)	12.7[b,c,d]	26.3[a]	29.0[a]	29.1[a]
Severe stiffness (%)	10.5[b,c,d]	25.1[a]	24.6[a]	31.4[a]
Severe dysfunction (%)	11.2[b,c,d]	27.8[a]	33.1[a]	38.4[a]
Knee OA (%)	62.5[c,d]	71.3	74.4[a]	78.2[a]

[a]Denotes significant difference to non-sarcopenic/dynapenic, non-obese
[b]Denotes significant difference to non-sarcopenic/dynapenic, obese
[c]Denotes significant difference to sarcopenic/dynapenic, non-obese
[d]Denotes significant difference to sarcopenic/dynapenic obese

activation [98, 99], supporting the concept that function is compromised by pain associated with OA. It has been suggested that muscle inhibition occurs in OA as a result of changes in afferent input from the affected joint, leading to reduced efferent motor neuron stimulation of the attendant skeletal muscles [100]. Increased IMAT has also been implicated as a contributor to pain and radiographic changes in knee OA [101].

Thus, OA-related pain clearly contributes to dynapenia, although the association with sarcopenia is unclear. Sarcopenia and dynapenia may also be risk factors for the development of OA, and might exacerbate effects of obesity. It is thought that small or weak quadriceps muscles result in increased loading at the knee joint during weight-bearing activities, potentially leading to OA development [102]. In support, quadriceps weakness is present in patients demonstrating knee radiographic OA but not yet reporting pain [100], and greater quadriceps strength is associated with less cartilage loss and knee pain over 30 months in older adults [103].

An analysis of the Fifth Korean NHANES [104] has directly examined the association of sarcopenic obesity (defined as low appendicular lean mass relative to weight and a BMI ≥27.5) with radiographic knee OA in almost 3000 participants. The prevalence of sarcopenic obesity using this definition was only 3 % but compared to non-sarcopenic non-obese, sarcopenic obese and obese alone participants had odds of 3.5 and 2.5 for OA, respectively [104]. The substantial overlap of confidence intervals for sarcopenic obesity and obesity indicates a lack of a synergistic effect of sarcopenia and obesity on likelihood of OA. There was also no increased likelihood of knee OA in sarcopenic alone participants.

No study to date has examined whether similar relationships exist for dynapenic obesity and dynapenia alone, however preliminary analyses in TASOAC may be instructive. Knee OA was assessed at baseline by X-ray [105] and the validated Western Ontario McMasters Osteoarthritis (WOMAC) index [106]. Any joint space narrowing or osteophytes was classified as radiographic knee OA, and scores in the highest quartile for WOMAC questions were defined as severe knee pain, stiffness and dysfunction [4]. Comparing these outcomes by categories of sarcopenic obesity and dynapenic obesity (Table 4.2), the highest prevalence of symptomatic knee OA was

observed in sarcopenic obese and dynapenic obese participants, and this was generally significantly higher compared with non-sarcopenic/dynapenic, non-obese, but not sarcopenic/dynapenic alone or obese alone participants (Table 4.2). Radiographic knee OA prevalence did not differ across categories of sarcopenic obesity, but was higher for dynapenic obese and dynapenic alone individuals compared to non-dynapenic, non-obese. Our results are consistent with evidence that lower-limb muscle weakness, may be pathogenic in knee OA and precede pain and disability [100].

Given the known effects of obesity on OA, it is unsurprising that based on the few cross-sectional data available, knee OA prevalence is highest in those with sarcopenic and dynapenic obesity. However, the likelihood of knee OA in these individuals does not appear to significantly exceed that for sarcopenia/dynapenia or obesity alone. Nevertheless, dynapenia of the quadriceps appears to be a predictor of radiographic and symptomatic knee OA, and higher OA prevalence in sarcopenic and dynapenic obese individuals likely contributes to their increased risk for mobility limitation. Long-term studies are required to determine whether sarcopenic obesity and dynapenic obesity accelerate radiographic or symptomatic progression of this disease.

Lifestyle Interventions for Sarcopenic and Dynapenic Obesity

The key aims of interventions for sarcopenic and dynapenic obese older adults should be to reduce body fat and increase muscle mass, quality and strength. These goals present unique challenges because weight-loss induced by caloric restriction is effective for reducing body fat but may also result in declines in muscle and bone mass [107–109]. Thus, weight loss therapy alone is inappropriate to improve the musculoskeletal health of older adults with sarcopenic and dynapenic obesity.

However, combining caloric restriction with exercise appears to substantially ameliorate loss of non-fat tissue [110, 111]. Progressive resistance training in particular results in substantial gains in muscle mass, strength and physical performance even in very old individuals [112]. Multimodal exercise interventions including aerobic training, flexibility and balance tasks, in addition to progressive resistance training, results in smaller declines in lean body mass and BMD than diet alone, as well as significant improvements in strength, balance and mobility, in obese older adults [113]. Furthermore, high-velocity progressive resistance training, weight-bearing impact and balance exercises, may actually provide modest but significant gains in BMD, in addition to muscle strength and power, and balance, in older adults with risk factors for falls and/or osteoporosis [114].

Only two exercise interventions have been performed in sarcopenic and dynapenic obese older adults. The results from these small trials support the concept that caloric restriction may improve body composition and cardiometabolic health, but strength training needs to be included to improve physical performance [115], and that high-velocity training may elicit the greatest gains in muscle function [116], in these populations. Thus, exercise is likely the most effective strategy for both prevention and treatment of sarcopenic and dynapenic obesity, but needs to be carefully prescribed in older adults; for example large amounts of high-impact physical activity may not be appropriate in those with OA [117]. However, given exercise is recommended as a therapy for increasing bone health, reducing OA development and progression, and preventing falls and fracture [118, 119], participants with sarcopenic and dynapenic obesity are likely to obtain musculoskeletal benefits from exercise in addition to improving their body composition and physical function.

In addition to exercise, nutritional strategies, especially protein and vitamin D supplementation, may hold benefits for sarcopenic and dynapenic obese older adults. Current recommendations of 1.0–1.2 g/kg of body weight for healthy older people, and higher for those completing exercise programs, should be maintained even during caloric restriction [120, 121]. While a concern with high protein intake is that

fat intake may also increase and result in weight gain, a 4-month cluster randomised controlled trial demonstrates this is not necessarily true. Nursing home residents completing progressive resistance training combined with 160 g (approximately 1.3 g/kg of body weight) of lean red meat 6 days per week had greater gains in muscle mass and strength than those completing progressive resistance training only, and there was also a decrease in fat mass for the red meat group, with no net gain in energy intake due to a concurrent decrease in carbohydrate intake [122].

Vitamin D deficiency is common in both older and obese adults, and sarcopenic obese older adults have also been shown to have significantly lower vitamin D levels than those who are obese only [123]. Vitamin D may reduce both falls and fracture risk through actions on skeletal muscle vitamin D receptors and enhanced absorption of dietary calcium and phosphate resulting in improved bone quality [124]. Meta-analyses indicate that vitamin D supplementation has no effect on fracture risk [125] although a small number of studies suggest that proximal strength may improve in response to supplementation for those with low baseline vitamin D [126]. The lack of association may be related to the relatively low vitamin D doses administered in many previous studies, with doses of around 800 International Units (IU)/day the minimum recommended for falls and fracture prevention [127]. There is also a lack of evidence to support combined vitamin D supplementation and exercise therapy for improving body composition and muscle function [128, 129], although vitamin D supplementation in the order of ≥2000 IU/day may reduce inflammation in overweight and obese individuals [130, 131]. We and others have also reported enhanced responses to exercise for indicators of body composition and muscle function in older adults with normal compared to low vitamin D status [132–134]. While the need for vitamin D supplementation in the general population is unclear, sarcopenic and dynapenic obese individuals may benefit from correction of vitamin D deficiency.

Summary

The addition of the term dynapenic obesity to the already confusing area of sarcopenia research further justifies the reluctance of clinicians to assess these conditions in older patients. It is incumbent on researchers in this field to arrive at a consensus on whether dynapenia should be considered a separate condition to sarcopenia and, if so, to provide an evidence-based operational definition for dynapenia. Until these goals are achieved it is unlikely that significant advances will be made in our understanding of how sarcopenic and dynapenic obesity independently influence musculoskeletal health, and certainly clinical attention will remain poor.

Nevertheless, based on the available evidence it does appear that sarcopenic obese and dynapenic obese older adults display different characteristics, with sarcopenic obesity associated with better muscle quality relative to muscle size. This likely explains the more consistent association of dynapenic obesity with poor physical performance. There is a lack of prospective studies on OA, bone health and falls and fracture risk, but sarcopenic and dynapenic obesity appear to be associated with the highest prevalence of OA, and also poorer bone health relative to obesity alone. While further research is required, this supports the need for clinical assessment and management of sarcopenic and dynapenic obesity in the growing population of obese older adults in developed countries. The most effective method for preventing sarcopenic and dynapenic obesity is likely to be multi-modal exercise including progressive resistance training and caloric restriction, while maintaining intake of at least 1.0 g/kg of high-quality protein per day. Vitamin D supplementation may also be beneficial for those with low vitamin D levels.

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Part II

Protein

Evidence for a Link Between Dietary Protein and Bone & Muscle Health in Adults

5

Marian T. Hannan, Shivani Sahni, and Kelsey Mangano

Abstract

This chapter will provide an overview regarding bone, osteoporosis, musculoskeletal health and dietary protein intakes in adult men and women. A background on evolving thoughts and tested hypotheses on the Acid-base theory will be presented, followed by an overview of the major scientific findings of the effect of dietary protein intake upon bone and muscle health. This review will not cover dietary patterns, although this topic is of keen interest and population-based future studies are encouraged in this area of interest. It would appear that higher levels of dietary protein intake, within the range commonly consumed by U.S. adults, do not result in bone loss in older adults. These positive outcomes may be due to increased calcium absorption, circulating levels of IGF-1 and suppression of PTH. The link between dietary protein intakes and bone health is certainly more complex than simple association studies can tease apart and more in-depth work is still necessary to give insights that are helpful to populations interested in maximizing their bone health. Protein appears to make more of an impact on bone with adequate calcium intake.

The field of study for protein intakes and muscle outcomes in the adult population is an emerging area of research and one from which we anticipate a growing body of evidence in the future.

Keywords

Dietary Protein • Osteoporosis • Muscle health • Bone density • Dietary intakes

M.T. Hannan, DSc, MPH (✉) • S. Sahni, PhD
Institute for Aging Research, Hebrew SeniorLife, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
e-mail: Hannan@hsl.harvard.edu; ShivaniSahni@hsl.harvard.edu

K. Mangano, PhD
Department of Clinical Laboratory and Nutritional Sciences, University of Massachusetts, Lowell, MA, USA
e-mail: Kelsey_Mangano@uml.edu

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Introduction

Osteoporosis and low bone mass are currently estimated to be a major public health risk for almost 53.6 million US adults aged ≥50 years [1]. It is well appreciated that bone accrues across younger ages, appears to plateau for a number of years and then bone is slowly lost across the lifespan from middle-aged to older years. The impact of dietary influences across the lifespan is of great interest. The topic of protein and musculoskeletal health has been accumulating scientific interest that is beginning to match the general interest of the public. This chapter will address the peer-reviewed evidence for a link between dietary protein and bone as well as muscle health in adults.

Much of the research in the bone field has focused upon lifestyle and other individual characteristics that do not consider dietary influences. Yet, the effect of nutrition upon bone health has been a topic of scientific study for many years, mostly focusing upon the studies related to calcium and vitamin D. Professor Munger noted in a 1999 publication that "The preoccupation to date with calcium has resulted in less emphasis on the role of other nutrients in bone quality and osteoporosis" [2]. Yet important work has been conducted on not only calcium and vitamin D, but also many other important dietary factors, including work on energy, protein, carbohydrates, amino acids, fatty acids, vitamins A, folate, vitamin B6, vitamin B12, vitamin C, vitamin K phosphorus, copper, magnesium, zinc, boron, fluoride, iron, sodium, alcohol, homocysteine, caffeine, acid-alkaline ash, phytoestrogens, soy, herbals-botanicals, fiber, oxalates, just to name a select few. Yet, these nutrients are not equally important. In increasing numbers since the 1990s, scientists have also focused upon protein as an important macro-nutrient for bone and muscle health.

Policy-makers and governmental recommendations have also focused attention upon the important role of protein for overall health. In the United States, the earlier My Pyramid food guidance system, adopted in year 2005 ("My Pyramid Steps to a Healthier You" available at MyPyramid.gov), listed "meat & beans" and "milk" as separate entities to consider along with a stick figure climbing steps with hopes of incorporating physical activity. This U.S. recommendation was replaced in 2011 with the less complex message of a proportioned plate (see Choose My Plate available at ChooseMyPlate.gov) that specifically depicted daily portions of protein as well as dairy along with other food sources. This chapter will provide an overview regarding bone, osteoporosis, musculoskeletal health and dietary protein intakes in adult men and women. A background on evolving thoughts and tested hypotheses on the Acid-base theory will be presented, followed by an overview of the major scientific findings of the effect of dietary protein intake upon bone and muscle health, focusing largely upon epidemiologic studies. This review will not cover dietary patterns, although this topic is of keen interest and population-based future studies are encouraged in this area of interest.

Biological Mechanisms: Results From Calcium Absorption Studies

Dietary protein is known to have a significant role as related to bone. It is well known that nutritional deficiencies play a major role in the onset and progression of osteoporosis and bone loss [3–5]. Very low protein intakes were recognized to be detrimental to bone and muscle in older adults [6, 7]. However, prior to the mid-1990s higher protein intakes had been thought to be deleterious for older adults [8–10]. One of the first publications inferring the role of dietary protein on bone was work from Sherman [11] that reported that a high protein diet caused high urinary calcium excretion. In 1979, Bleich et al. published a paper in the New England Journal of Medicine showing increased urinary calcium excretion followed high protein intake [12]. Since these times, at least 26 clinical intervention studies in adult humans, mostly with controlled-diet, have been conducted where the investigators intentionally manipulated dietary protein and measured subsequent urinary calcium. Figure 5.1 depicts the findings and linear regression from these 26 studies [13]. Each point represents the mean of one of the groups reported in each of the

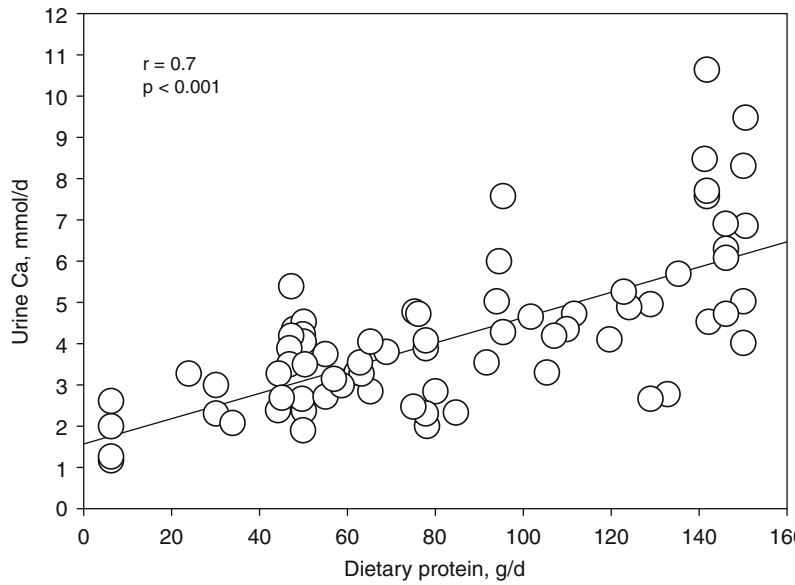

Fig. 5.1 Summarized data from 26 controlled clinical trials investigating the association between dietary protein intake with urinary calcium excretion with the regression line showing that a 25 g increase in dietary protein intake resulted in 0.8 mmol rise in urinary calcium [13] (Reprinted with permission)

studies. Overall, for each 40 g increase in dietary protein, urinary calcium increases approximately 50 mg. These data clearly establish that dietary protein is an important regulator of urinary calcium excretion in the short-term. However, it is worth noting that when meat is the source of the additional dietary protein, the attendant phosphorus load serves to blunt the rise in urinary calcium, so the slope of the regression line seen in Fig. 5.1 would not be as steep.

This work has contributed depth to the theme of Acid Base Metabolism. The Acid-base theory suggests that sulfur-containing amino acids in proteins contribute to acid load that may lead to bone loss [14, 15]. It is thought that dietary protein, because it is rich in the sulfur-containing amino acids, increases endogenous acid production and net renal acid excretion, leading to hypercalciuria. The high endogenous acid load would require buffering which might be obtained from bone. Thus, the skeleton could be potentially called upon to release alkali and as a consequence, bone resorption would be increased with a subsequent decline in bone mineral density (BMD), and an increased risk of fracture. Many biochemical and nutrition studies have shown that high protein intake is a powerful determinant of urinary calcium loss and a potential cause of bone loss. Yet it remains unclear how protein in mixed diets affects bone in adult humans. Is it that dietary protein affects calcium homeostasis and thus ultimately affects bone health? What is the source of the extra urinary calcium?

The traditional perspective is that the calcium was obtained from an increase in bone resorption, using the skeleton as an alkali reservoir to re-establish acid-base balance. Although there is adequate experimental evidence that an acidic environment increases osteoclast-mediated bone resorption [16, 17], the long-term consequence of this reliance on bone has not been established in humans. The human clinical trials in which acid-base status was pharmacologically manipulated with an alkaline source, such as potassium bicarbonate, found a fairly immediate decrease in bone resorption [18, 19].

If the Acid-base hypothesis were to hold true, we would expect over time, there to be a loss of bone density due to chronic elevated bone resorption.

However, data from population-based studies over a longer term do not produce these findings. At least ten epidemiological studies have shown that over 4 years or more, high dietary protein intakes were associated with higher BMD (not the reverse as predicted from the Acid-base theory) [20–29]. It is important to note that the laboratory-based studies were short-term, typically over several hours, to 1-day to a week, while the cohort studies examined annualized effects on

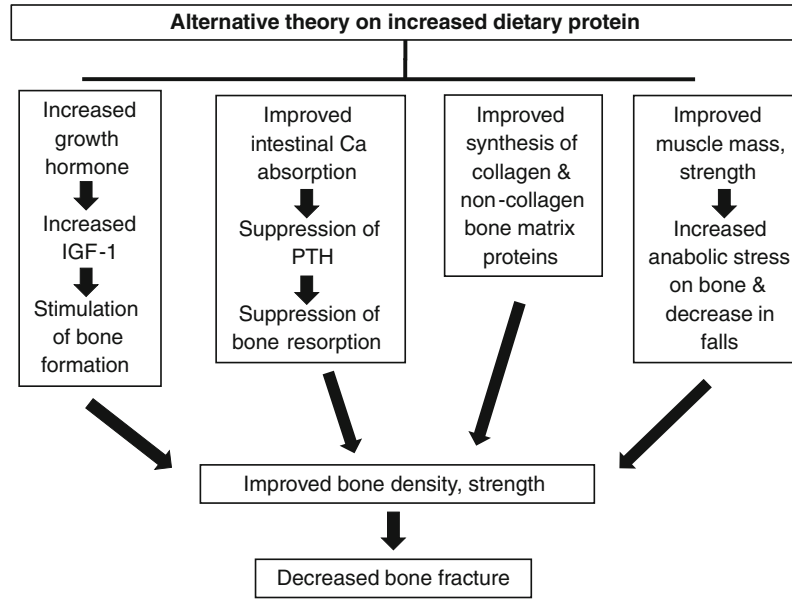

Fig. 5.2 Possible pathways for recent hypotheses on the effects of increased dietary protein upon bone and fracture risk in adults

bone loss or cross-sectional associations. To our knowledge, longer-term randomized trials addressing acid-base measures have either been short-term [30], or have had similar findings in agreement with the cohort studies; (for examples, please see Filip et al. [31] or Kerstetter et al. [32]).

A major question remained as to what the source (or sources) of urinary calcium may be, other than the skeleton, since it appeared unlikely to be derived from bone resorption over time. Kerstetter et al. [33] conducted an elegant study with the main objective to quantify the effect of a high protein diet on calcium kinetics in women using a novel calcium balance methodology, dual stable calcium isotopes. The cross-over study was designed to have 2 week lead-in with participants receiving a well-balanced standard diet, followed by 10 days of experimental diet which consisted of either moderate (1.0 g/kg) or high protein (2.1 g/kg) diet. The investigators collected fasting urine and blood samples at baseline and on Day-4. Participants received an IV dose of 42-Ca and three oral doses of 44-Ca. On Day-6, serum and urine samples were collected. The study found there was increased calcium absorption from gut during high dietary protein intake compared to the moderate protein intake, with no change detected in the bone turnover markers. The increase in calcium absorption seen in this study accounted for 93% of the increase in urinary calcium. This study showed that in the short-term, a high protein diet was not detrimental to bone turnover, and that high protein intakes augmented calcium absorption.

To better understand the biological underpinnings of the effects of a high protein diet on bone, the Kerstetter group conducted a 1-week diet controlled intervention trial with the goal to compare the short term effect of a low protein (0.7 g/kg), moderate protein (1.0 g/kg) and high protein (2.1 g/kg) diet on calcium homeostasis and kinetic measures of bone turnover in healthy women [34]. Their study participants were given controlled diets containing moderate amounts of calcium, sodium and protein for 2 weeks, followed by 4 experimental days of a protein diet. As expected, the rise in urinary calcium mirrored the rise in dietary protein intake. However, they also found that participants consuming the low protein diet had a dramatic rise in PTH and 1,25 dihydroxyvitamin D. In contrast, the high protein diet group showed suppressed PTH and increased urinary calcium. For those participants consuming the medium protein diet, there were no observed changes and no indications of adverse outcomes. This work and others led to recent hypotheses indicating that the calcium comes from increased calcium absorption and perhaps a combination of calcium absorption and bone resorption (see Fig. 5.2).

While calcium absorption studies are interesting and needed to help in our understanding of the link between dietary protein and bone health, our belief is that it is ultimately bone outcomes that matter. Therefore, a re-focus on the long-term effect of dietary protein upon bone would be helpful. Despite the shorter-term laboratory studies showing a clear effect between increased dietary protein in humans and increased calcium (both absorption and urinary output), as outlined above there is clearly clinical and experimental evidence from human studies that refute the acidogenic theory. Several studies concluded that high protein intake, especially in older adults, positively affected BMD and lowered risk of fracture over time [2, 27, 29, 35–39].

Dietary Protein and Bone Density

Several studies from the turn of the century changed the manner in which scientists and policy-makers thought about the effect of adult dietary protein consumption [2, 13, 27, 28]. As these studies were reporting results that went against the current scientific acid-base theory of that time, they underwent extensive peer review before achieving publication. One of the first publications was a cross-sectional study conducted by Kerstetter and colleagues that used the NHANES III sample that is representative of the U.S. population [28]. In this work, they examined the association between total femoral BMD and quartiles of dietary protein in adult women and found a distinct pattern of higher BMD with each quartile of dietary protein intake. Those women in the highest quartile had significantly higher femoral BMD compared to women in the lowest quartile.

In the same year, Hannan et al. reported findings that extended their cross-sectional work. Using the population-based Framingham Cohort [27], they investigated the relation between baseline dietary protein and 4-year change in BMD at several sites as well as examining total protein intake and protein from animal sources in older men and women. BMD loss over 4 years was regressed on percent protein intake, adjusting for age, weight, weight change, total energy intake, smoking, alcohol, caffeine, physical activity, calcium intake and in women, estrogen use. The mean age of their cohort was 74 years (standard deviation (SD) of 4.4), with typical values as seen in the population of older adults in the community for weight, total energy intake, protein intake (mean of 68.5 g/day ± SD 23.6) and calcium. The values of protein intake indicated that this population was not deficient but did not have very high levels of protein intake. They found a linear trend between quartiles of dietary protein intake and BMD loss at hip sites and lumbar spine sites. Similar patterns were observed for animal protein intake with BMD loss. This population-based study suggests that high levels of dietary protein intake, within the range commonly consumed, do not result in bone loss. On this basis, it was suggested that ensuring adequate dietary protein intake is an important component of bone health in older adults.

In the Rancho Bernardo cohort Study, Promislow et al. examined the associations of total, animal, and vegetable protein with BMD [29]. They further examined the variations in these associations with calcium intake in a community-dwelling cohort of 572 women and 388 men aged 55–92 years. This study reported a positive association of baseline animal protein intake with 4-year change in BMD that was statistically significant in women. In women, for every 15-g/day increase in animal protein intake, BMD increased by 0.016 g/cm^2 at the hip ($p=0.005$), 0.012 g/cm^2 at the femoral neck ($p=0.02$), 0.015 g/cm^2 at the spine ($p=0.08$), and 0.010 g/cm^2 for the total body ($p=0.04$). Conversely, a negative association between vegetable protein intake and BMD was observed in both sexes. Some suggestion of effect modification by calcium was seen in women but evidence for this interaction was not consistently strong.

The dietary protein and bone density studies indicate that higher levels of dietary protein intake, within the range commonly consumed by U.S. adults, do not result in bone loss in older adults. The link between dietary protein intakes and bone health is certainly more complex than simple association studies can tease apart and more in-depth work is still necessary to give insights that are helpful to populations interested in maximizing their bone health. Protein appears to make more of an impact on bone with adequate calcium intake.

Dietary Protein and Hip Fracture

Relatively few observational studies have examined the association of protein intake with the risk of fracture [2, 40–43]. Several of these population based studies have changed how scientists view the acid-base theory and long-term effects on fracture outcomes. One of the pioneers of the field, Dr. Ronald Munger reported on findings from the Iowa Women's Health Study, a population-based cohort study of 32,050 women following across 3 years (104,338 person-years) for fracture occurrence [2]. In examining both total protein intakes and animal protein intakes, they observed a clear trend for reduced risk of hip fracture as quartiles of protein intake increased (compared to quartile 1 of total protein intake, quartiles 3 and 4 both showed about half the risk for subsequent hip fracture, p-trend = 0.049). When evaluating the effect of animal protein, this study continued to show lower risk of hip fracture for the higher quartiles of dietary protein intakes (p-trend = 0.037) for the women in their study.

Wengreen et al. examined the role of dietary protein intake in patients with osteoporotic hip fracture in a case-control study in Utah [42]. Patients, 50–89 years of age, with hip fracture (831 women, 336 men) were included in this study along with age- and sex-matched controls (885 women, 449 men). The association between protein intake and risk of hip fracture was examined across quartiles of protein intake and stratified by age group (50–69 years and 70–89 years). This study reported that the odds ratios (OR) of hip fracture decreased across increasing quartiles of total protein intake for participants 50–69 years of age (OR: 1.0, (referent); 0.51 (95 % CI: 0.30–0.87); 0.53 (0.31–0.89); 0.35 (0.21–0.59); $p < 0.001$). No similar associations were observed among participants 70–89 years of age. Results from analyses stratified by low and high calcium and potassium intake did not differ appreciably. The authors concluded that adequate dietary protein was important for optimal bone health in older adults aged 50–69 years.

The population-based Framingham Study reported similar results. Misra and colleagues [36] reported that the quartile ranges for dietary protein intakes in the Framingham Study were similar to those reported in the Iowa Women's Health Study by Munger et al. Consistent with the findings from this study, as quartiles of dietary protein intake increased, the risk for hip fracture was decreased (compared to quartile 1, the hazard ratio for Quartile 2 was 0.70, for Quartile 3 was 0.56 and for Quartile 4 was 0.63, the p-trend was 0.07). When comparing the first quartile to the 3 higher quartiles of dietary protein (representing participants who met the RDA on average), the risk of hip fracture was higher in Quartile 1 compared to Quartiles 2–4 ($p = 0.02$).

Similar to the findings between dietary protein and bone density studies, work focused upon fracture outcomes also have found that higher levels of dietary protein intake, as commonly consumed, do not result in fracture in older adults. Rather, they indicate that adequate dietary protein intakes appear important in reducing the risk for hip fracture in older adults. The amount of data is limited and certainly fracture studies are also complex, such that more, carefully done studies are still needed to provide helpful insights.

Dietary Protein Intervention Studies – and a Plausible Role for Calcium

A well-designed study by Dawson-Hughes et al. in 2002 [44] compared BMD change in groups from a randomized clinical trial that compared the effect of protein intakes by an intervention of calcium supplement versus placebo. The results of this study are depicted in Fig. 5.3. The percent change in BMD at femoral neck, lumbar spine and total body was found to have a differential effect for the intervention of increased protein intake and calcium supplement. While it is difficult to separate the effects of dietary protein from dietary calcium as many dairy food sources obviously contribute to both, this was one of the first studies to consider supplemental calcium as part of the bone-protein story.

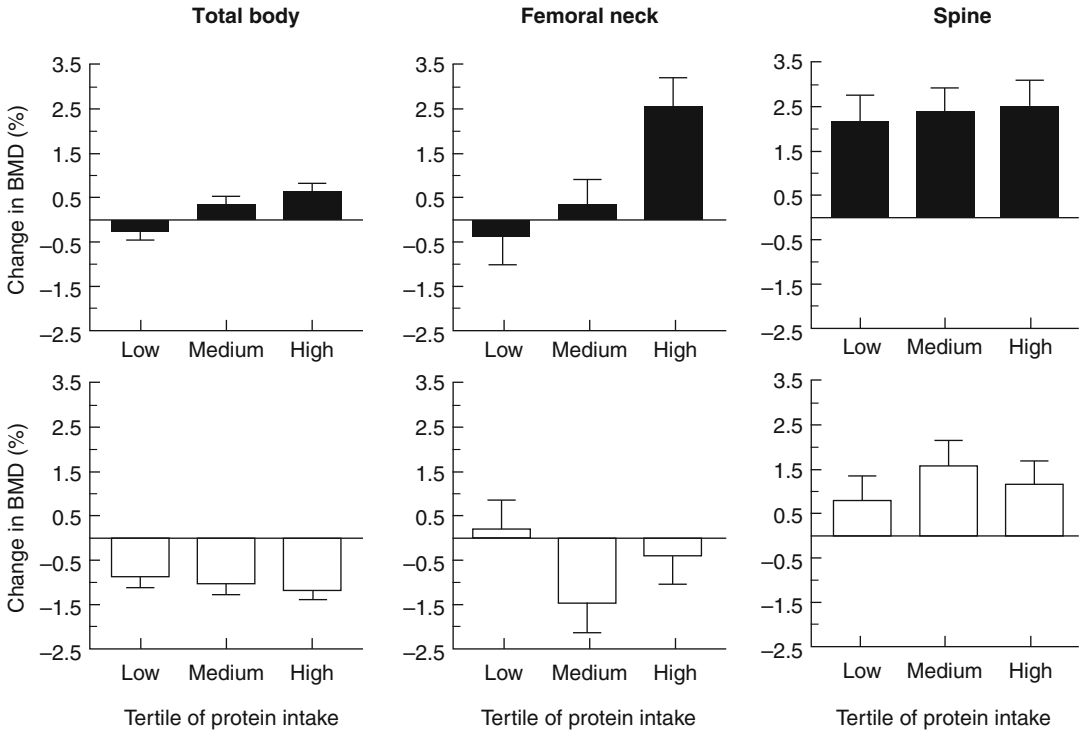

Fig. 5.3 Clinical trial in older adults showing percent change in BMD by protein intake tertiles by calcium supplement group (*top*) and placebo (*bottom*) (From Dawson-Hughes et al. [44])

Further work by Sahni et al. complemented this clinical trial [38]. These authors examined whether total calcium intake modified the associations between protein intake and incident hip fracture in the Framingham Offspring Study of over 3000 participants. They found that among those with the lowest calcium intakes (<800 mg/day), the participants consuming the highest tertile of animal dietary protein had 2.8 times the risk of hip fracture (HR of 2.8, 95% CI 1.20–6.74) compared to the lowest tertile in this group. However, in participants who consumed 800 mg/d of calcium or more, the findings are reversed with the highest protein tertile in this calcium group have a reduced hip fracture risk (HR of 0.15, 95% CI 0.02–0.92). These findings indicated that calcium modified the association of protein intake and risk of hip fracture in a community setting of older adults.

Similar interactions between protein intake and calcium intake have also been reported by other studies. In E3N (Etude Epide'miologique de femmes de la Mutuelle Ge'ne'rale de l'Education Nationale), which is a prospective study among members of the Mutuelle Ge'ne'rale de l'Education Nationale (MGEN) and includes French postmenopausal women (n = 36,217), high acid-ash diets were associated with an increased risk of fracture when calcium intake was low (<400 mg/1000 kcal), in which the relative risk (RR) was 1.5 for highest versus lowest quartile, 95% CI 1.17–1.94 [45]. Another cross-sectional study using the data from the NHANES [41] reported that in postmenopausal women (aged ≥50 years) who consumed less than 46 g/day of dietary protein, those with a total calcium intake of 1200 mg/day or more had a significantly higher risk of fracture than those with the lowest total calcium intake with an adjusted odds ratio (OR) of 5.98, (95% CI 1.15–31.13), whereas in women who consumed more than 70 g/day of dietary protein, those with a total calcium intake

of 1200 mg/day or more had a statistically insignificant lower risk of fracture with an adjusted OR of 0.69 (95 % CI 0.20–2.39). Overall this suggests that the relation between protein intake and bone health may vary differently in relation to calcium intake in older adults.

Summary of Dietary Protein and Bone

The connection between dietary protein and bone has become clearer over the past few decades with population-level studies [2, 27, 28, 36, 38, 44] showing good outcomes, and data from randomized clinical trials tending to show a small but positive effect. In U.S. groups of older adults, total protein intake and animal protein intake have been associated with higher BMD and minimal BMD loss over time, and lower subsequent hip fracture risk. It would appear that higher levels of dietary protein intake, within the range commonly consumed by U.S. adults, do not result in bone loss in older adults. These positive outcomes may be due to increased calcium absorption, circulating levels of IGF-1 and suppression of PTH. The link between dietary protein intakes and bone health is certainly more complex than simple association studies can tease apart and more in-depth work is still necessary to give insights that are helpful to populations interested in maximizing their bone health. Protein appears to make more of an impact on bone with adequate calcium intake.

Dietary Protein and Muscle

While the role of dietary protein upon muscle is well-known and appreciated from a theoretical point of view, the population studies are lacking. Many cohorts and clinical trials are underway with results to be reported soon. There is a wonderful review article by Rizzoli et al. in 2014 [37] that outlined our current state-of-the-art understanding of protein intake and muscle health. Figure 5.4 summarizes their review work, indicating that age-related causes of inadequate

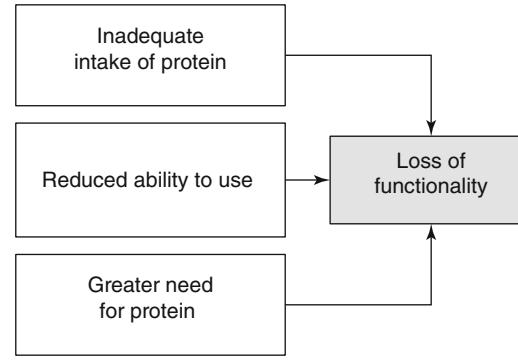

Fig. 5.4 Age-related causes of protein shortfall, leading to impairment of muscle, bone and immune function [37] (in process of obtaining permission to use)

intakes of dietary protein have important and fairly quick impact upon muscle, leading to impairment of muscle, bone and also immune function. It is known that protein and physical activity are the main anabolic stimuli for muscle in adults. Further, there is some evidence that exercise plus increased dietary protein may result in more muscle than either effect alone [46–48].

As individuals age, the anabolic sensitivity of muscle may become blunted but the effect typically continues to result with at least partial benefit to muscle. At current time, most examinations have focused on the extremes of dietary protein or on extremes of muscle or strength loss in elderly years. As discussed at length in the Chapter by Professor Robin M. Daly, there are a number of randomized trials and some meta-analyses showing mixed results that vary in effect by the type, dose and timing of protein intake as well as the baseline levels of protein intake (for example, please see Daly et al. [49]). More work on dietary protein interventions and muscle health is underway and we greatly anticipate the results.

Two recent epidemiologic studies from the Framingham study cohorts have further highlighted the importance of protein intake with muscle health. The first study determined the association between total protein, animal protein, and plant protein intake and lean mass of the legs and quadriceps muscle strength [50]. The study further considered whether the associations with

quadriceps strength could be explained by leg lean mass. This cross-sectional study included 1166 men and 1509 women from the Framingham Offspring Cohort (mean age 59 years; range: 29–86 years of age). In men and women, leg lean mass was higher in participants in the highest quartile of total protein intake and animal protein intake compared with those in the lowest quartiles of intake (adjusted least squares means in kg: Total protein—17.6 vs. 17.1 in men, P-trend: 0.005, and 11.7 vs. 11.4 in women, P-trend: 0.006; Animal protein—17.6 vs. 17.1 in men, P-trend: 0.002, and 11.7 vs. 11.4 in women, P-trend: 0.003). Plant protein intake was not associated with lean mass in either sex. In men and women, quadriceps strength was higher in participants in the highest quartile of plant protein intake compared with those in the lowest quartile (adjusted least squares means in kg: 22.9 vs. 21.7 in men, P trend: 0.01, and 19.0 vs. 18.2 in women, P-trend: 0.01); this association was no longer significant after adjustment for fruit and vegetable intake (P-trend: 0.06 in men and 0.10 in women). Although no significant association was observed for animal protein intake in either sex, non-significant protective trends were observed for total protein intake (P-trend: 0.08 in men and 0.10 in women). This suggests that dietary protein types may differentially affect muscle mass and strength. Whether plant protein is a marker of dietary quality or has direct effect on muscle strength (independent of lean mass) needs to be further clarified.

In the subsequent Framingham study, McLean et al. determined the association of dietary protein (total, animal, and plant) intake with change in grip strength over 6 years in 1746 men and women from the Framingham Offspring cohort [50]. In this study, greater protein intake, regardless of source, was associated with less decrease in grip strength (all p for trend $\leq .05$): participants in the lowest quartiles lost 0.17–0.27 % per year while those in the highest quartiles gained 0.52–0.60 % per year. In analyses stratified by age, participants aged 60 years or older (n of 646) had similar linear trends on loss of grip strength for total and animal (all p for trend $<.03$) but not plant protein, while the trends in participants younger than 60 years (n of 896) were not statistically significant. This suggests that higher dietary intakes of total and animal protein were protective against loss of grip strength in community-dwelling adults aged 60 years and older.

In conclusion, a variety of nutrients are important to bone health, muscle strength and reducing risk of hip fracture in adults. Contributions appear to vary by age but not sex. For better musculoskeletal health we should recommend a well balanced diet of adequate protein, perhaps with a particular focus on dairy, adequate fruits and vegetables, but more work is needed before giving definitive recommendations. It would be helpful to evaluate the effect of food patterns upon bone health, to understand relations of food intakes over time, especially in older adults. We need to understand what components of an adult diet are modifiable as well as what is not apt to change with interventions. We need to investigate the probable cause-and-outcome relations with bone as well as with muscle with an eye towards treatment and prevention. Larger, good-quality randomized controlled trials are needed to confirm these findings from smaller epidemiological studies so to ascertain optimal intakes for prevention of osteoporosis and maximization of musculoskeletal health.

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6 Dietary Protein, Exercise and Skeletal Muscle: Is There a Synergistic Effect in Older Adults and the Elderly?

Robin M. Daly

Abstract

Progressive resistance training (PRT) and dietary protein are both regarded as key factors for the maintenance of muscle health and function during ageing. Short-term acute studies have consistently shown that combining PRT with an adequate dose of high quality, rapidly digested, leucine rich protein sources can produce additive or synergistic benefits on skeletal muscle protein synthesis in older adults compared to PRT or dietary protein alone. However, there are mixed findings from the available randomized controlled trials (RCTs) conducted over ≥12 weeks with muscle mass, strength and/or function as the outcome. Of the 18 RCTs conducted in adults aged ≥60 years that we identified, only three reported a significant protein-exercise interactive effect on muscle mass and/or strength; none observed an additive benefit on muscle function. This chapter provides a brief overview on some of the reasons as to why there are inconsistent findings, with a focus on the type, dose, spread and/or change in protein intake, timing of consumption, pattern or distribution of intake and the potential influence of co-ingestion with other nutrients.

Keywords

Dietary protein • Amino acids • Protein supplementation • Resistance training • Older adults

Introduction

The age-related loss in muscle mass, strength and function, termed *sarcopenia*, has been associated with an increased risk of falls, fractures and frailty which can lead to a loss of independence, reduced quality of life, disability and increased mortality in the elderly [1–3]. Muscle loss has

R.M. Daly, PhD, FASMF
Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Highway, Burwood, Melbourne, VIC 3025, Australia
e-mail: rmdaly@deakin.edu.au

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also been implicated in various metabolic related diseases, including type 2 diabetes, metabolic syndrome and cardiovascular diseases [4]. In both men and women, muscle mass and strength begins to decline around the fourth decade of life, with a more accelerated loss occurring after the age of 50–60 years, which has been associated with a decline in functional capacity [1, 5, 6]. Although there is considerable inter-individual variability in the rate of loss in muscle, it has been reported that up to 40 % of muscle mass (or size) can be lost from young adulthood to old age [5, 6]. Thus, there has been considerable interest in identifying strategies to counteract muscle loss and the negative consequences of sarcopenia. At present, the precise mechanism(s) underlying age-related changes in muscle mass, strength and function have not been fully elucidated, but various neurological, hormonal and inflammatory factors, as well as physical inactivity and inadequate nutrition, particularly energy-protein malnutrition, have all been implicated [2, 7].

From a physiological perspective, muscle proteins are in a dynamic and continuous state of turnover, and advancing age has been associated with an imbalance between the rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). This can result in a net negative muscle protein balance that over time, leads to muscle loss [8]. As discussed in several excellent reviews [9, 10], progressive resistance training (PRT) and dietary protein are both recognized as potent stimuli for MPS; resistance exercise also increases MPB in the fasted state whereas protein and amino acids can inhibit (to a lesser extent) MPB, which has been mainly attributed to hyperinsulinemia. However, in older adults and the elderly it has been reported that the stimulatory effects of protein (amino acids) and resistance exercise on MPS are blunted, an effect that has been termed '*anabolic resistance*' [8, 9]. Recent data indicates that basal rates of MPS (and MPB) are not compromised in older adults, suggesting that age-related muscle loss is not due to impaired basal muscle protein metabolism but rather derangement in the muscle protein synthetic response to anabolic stimuli, such as exercise and food (or protein) [8, 11, 12]. To stimulate MPS and promote a positive net protein balance to optimize muscle mass, it has been suggested that older adults require a higher dietary protein intake, which when combined with resistance training, may represent an effective strategy to enhance muscle mass, strength and function in older people.

Does Protein Enhance the Effects of Resistance Training on Muscle in Older Adults?

Over the past two decades, there have been a number of well-designed randomized controlled trials (RCTs) which have examined whether additional dietary protein, protein (or amino acid) supplementation or multi-nutrient supplements enriched with protein can enhance the effects of PRT on muscle mass, strength and/or function in older adults and the elderly. The results from nine of these RCTs were included in a 2014 systematic review and meta-analysis which examined the effects of resistance training (for a period of 6 weeks or longer; mean 22 weeks) combined with protein or amino acid supplementation in adults aged ≥60 years [13]. The key finding from this meta-analysis, which included published trials up to January 2014, was that PRT combined with protein supplementation enhanced the effects on fat-free mass, but had no added benefits on muscle mass or strength. Table 6.1 provides a summary of the characteristics of the RCTs included in this meta-analysis and a number of other relevant and recent trials (up until August 2015) examining the protein-exercise interactive effects on muscle in older adults. This only includes RCTs involving adults with a mean age ≥60 years, which were at least 10 weeks in duration and involved a resistance training program, and included at least one group receiving protein (or amino acid) supplementation, dietary rich sources of protein (e.g., meat/beef) or a mixed product protein source (e.g., milk) that was compared to a group receiving no additional protein or prescribed a low protein or lacto-vegetarian diet.

Of the 18 RCTs which were included in Table 6.1, only three studies reported that additional protein combined with PRT led to

Table 6.1 Randomised controlled trials which have examined the interactive effects of increased dietary protein with resistance training on muscle mass, strength and/or function in older adults and the elderly

Reference	N	Age[a] (years)	Sex	Length (weeks)	Subjects	Training details	Baseline protein	Supplement details				Comparison group	Outcome[b]
						Frequency, dose and intensity		Type	Prescribed dose	Protein change	Timing		
Milk and dairy-based protein													
Campbell et al. [14]	12	64–66	M/F	12	Healthy sedentary	3 days/week, 3 sets, 8–12 reps, 80 % 1-RM	0.6 g/kg/day	Milk-based drink	1.6 g/kg	1.0 g/kg/day	NS	Low-protein (0.8 g/kg)	Negative
Rosendahl et al. [15]	191	85	M/F	12	Residential care	Multi-modal program, 5 times per fortnight	NS	Milk-based drink	7.4 g/day	6.1 g/day [c]	After exercise	Ex + Pl, milk or control + Pl	Negative
Iglay et al. [16]	36	61	M/F	12	Healthy untrained	3 days/week, 3 sets, 8 reps, 80 % 1-RM	1.1 g/kg/day	Egg, meat, dairy (via diet)	1.6 g/kg	0.1 g/kg/day	Consumed as part of meals	Low protein (0.9 k/kg)	Negative
Kukuljan et al.[17]	180	60–62	M	72	Healthy untrained	3 days/week, 2–3 sets, 8–20 reps, 60–85 % 1-RM	~1.3 g/kg/day	Fortified milk	13.2 g/day	11.9 g/day [c]	Morning and afternoon/ evening	Ex, fortified milk or usual care	Negative
Tieland et al. [18]	62	78	M/F	24	Frail elderly	2 days/week, 3–4 sets, 8–15 reps, 75 % 1-RM	1.0 g/kg/day	Milk protein concentrate	30 g/day	29 g/day [c] (0.3 g/kg)	After breakfast and lunch	Flavoured CHO drink	Positive
Leenders et al. [19]	60	70	M/F	24	Healthy untrained	3 days/week, 3–4 sets, 8–15 reps, 75–80 % 1-RM	~1.1 g/kg/day	Milk protein concentrate	15 g/day	0.18–0.24 g/ kg/day	After breakfast	Lactose and calcium drink	Negative
Buhl et al. [20]	29	72–73	M/F	12	Acutely ill	3 days/weeks, 3 sets, 8–12 reps (intensity NS)	0.74 g/kg/ day[d]	Milk-based protein	18.8 g/ day	14.1 g/day	After exercise	Standard care	Negative

(continued)

Table 6.1 (continued)

Reference	N	Age[a] (years)	Sex	Length (weeks)	Subjects	Training details		Supplement details				Comparison group	Outcome[b]
						Frequency, dose and intensity	Baseline protein	Type	Prescribed dose	Protein change	Timing		
Whey-based protein													
Candow et al. [21]	29	63–66	M	12	Healthy untrained	3 days/week, 3 sets, 10 reps, 70 % 1-RM	NS	Whey protein blend and egg albumin	0.3 g/kg	0.3 g/kg/day	Before or after exercise	Chocolate CHO drink	Negative
Chale et al. [22]	80	77–78	M/F	24	Mobility limited	3 days/week, 2–3 sets, 10–12 reps, 80 % 1-RM	~72 g/day	Whey protein concentrate	2×20 g/day	18 g/day	After breakfast and evening meal	Maltodextrin	Negative
Arnarson et al. [23]	161	73–74	M/F	12	Healthy untrained	3 days/week, 3 sets, 6–8 reps, 75–80 % 1-RM	1.1 g/kg/day	Whey protein concentrate + CHO	20 g/day (7 g EAA)	0.06 g/kg/day	After exercise	CHO drink	Negative
Verreijen et al. [24]	80	63	M/F	13	Obese untrained	3 days/week, 3 sets, 12–20 rep (intensity NS)	0.85 g/kg/day[d]	Whey protein, leucine + vitamin D	21 g/day	0.26 g/kg/day[e]	Before breakfast and after exercise	Isocaloric drink	Positive
Soy-based protein													
Fiatarone et al. [25]	100	87	M/F	10	Frail elderly	3 days/week, 3 sets, 8 reps, 80 % 1-RM	NS	Soy-based protein (liquid drink)	15 g	15 g	Evening	Ex, low energy drink or control	Negative
Meat-based protein													
Haub et al. [26]	21	65	M	12	Healthy untrained	3 days/week, 3 sets, 8 reps, 80 % 1-RM or fatigue	~1.0 g/kg/day	Beef-rich diet	0.6 g/kg	0.4 g/kg/day	NS	Lactoovo-vegetarian diet	Negative
Daly et al. [27]	100	72–74	F	16	Healthy untrained	3 days/week, 3 sets, 8–12 reps, 14–16 RPE	1.1 g/kg/day	Lean red meat	2×80 g/day cooked	0.2 g/kg/day	Lunch and dinner	CHO control	Positive

Casein													
Verdijk et al. [28]	26	72	M	12	Healthy untrained	3 days/week, 4 sets, 8–15 reps, 75–80 % 1-RM	1.1 g/kg/day	Casein hydrolysate	20 g/day	20 g/day (0.1 g/kg/day)	Before and after exercise	Flavoured water	Negative
Leucine													
Trabal et al. [29]	30	84–85	M/F	12	Nursing home	3 days/week, 2 sets, 15 reps, 65 % 1-RM	1.2 g/kg/day	Leucine	2×5 g/day	0.03 g/kg/day	After lunch and dinner	Maltodextrin	Negative
Essential amino acids													
Godard et al. [30]	17	71–72	M	12	Healthy untrained	3 days/week, 3 sets, 10 reps 80 % 1-RM or fatigue	1.1 g/kg/day[d]	Essential amino acids + CHO	12 g/day	% energy protein, 2 %	After exercise or at same time each day	Usual care	Negative
Kim et al. [31]	155	79	F	12	Sarcopenic women	2 days/week, 1 set, up to 8 reps, 14–16 RPE	NS	Essential amino acids	2×3 g/day	6 g/day	Twice a day (time not stated)	Ex, EAA or education	Negative

M male, *F* female, *Ex* exercise, *NS* not stated, *EAA* essential amino acids, *Pl* placebo, *CHO* carbohydrates, *RPE* rating of perceived exertion

[a]Mean age

[b]Outcome: positive effect on muscle mass or strength

[c]Change in protein intake based on mean compliance to supplement

[d]Protein intake in controls

[e]Difference in protein intake between intervention and control group

greater gains in muscle mass [18, 24, 27] or muscle strength [27] compared to PRT alone. No studies have shown that protein (or amino acid) supplementation augments the effects of PRT on muscle function in older adults or the elderly. Numerous factors are likely to contribute to these mixed findings, including heterogeneity in the cohorts studied (age, physical and nutritional status, current activity levels), differences in the study design (duration, setting and outcome measures), protein or supplement regimen (type, dose, timing and/or pattern of intake), co-nutrient ingestion (interaction of protein with other nutrients or a mixed meal) and/or the exercise dose (type, duration and frequency). This chapter will provide a brief overview on some of the potential reasons as to why some RCTs may have been successful and others failed to detect an additive or synergistic protein-exercise effect on muscle in older adults and the elderly. The focus of this chapter will be on the role of protein, particularly the type, amount, spread and/or change in protein intake, timing, pattern or distribution of intake and the potential influence of co-ingestion with other nutrients.

Type of Protein

One of the key factors that may explain the lack of a consistent interactive effects of dietary protein and PRT on muscle in older adults is the type of protein used. As shown in Table 6.1, a range of different proteins, such as milk-based drinks, whey protein alone or in combination with egg protein, casein hydrolysate, essential amino acids (EAA) including leucine, soy protein, fortified milk, dairy foods, milk protein concentrate and red meat/beef, have been used. As reported in a number of comprehensive reviews on the acute MPS responses to protein ingestion following resistance exercise (and at rest) in older adults [9, 32], protein sources which are rapidly digested and absorbed, such as whey protein, stimulate postprandial MPS to a greater extent than either soy protein or slowly digested proteins such as casein. In addition, skim milk, which typically contains about 80 % casein and 20 % whey, has also been shown to be more effective at increasing MPS than soy milk [33]. While it is important to recognize that acute measurements of MPS do not provide a direct quantitative estimation of chronic muscle adaptations (e.g., muscle hypertrophy) [34], collectively these results suggest that rapidly digested proteins such as whey protein or milk-based proteins, should augment the effects of PRT on muscle mass and strength in older adults and the elderly. However, as indicated in Table 6.1 there is currently no consistent evidence from the available RCTs that these high-quality protein sources used in combination with PRT produce an additive or synergistic muscle response in older adults or the elderly.

Other dietary protein sources that contain a relatively high proportion of essential amino acids (EAAs), such as meat/beef, have been shown to enhance MPS post-exercise [35]. While there is some evidence that ingestion of amino acids in a liquid rather than solid form results in a greater postprandial plasma amino acid concentration in older adults [36], a recent study reported that lean beef and milk (at an equivalent protein dose) produced similar increases in MPS [37]. Others have reported that minced beef is more rapidly absorbed and digested than intact beef resulting in enhanced amino acid availability and greater postprandial protein retention, but this did not translate into greater postprandial MPS rates [38]. Interestingly, in two separate RCTs that we conducted in elderly women and older men we found that PRT combined with the ingestion of intact lean red meat [27] (Fig. 6.1), but not fortified milk [17], was associated with a greater increase in muscle mass and strength than PRT alone. However, it is difficult to directly compare these findings due to differences in the participant characteristics, study duration and dose of protein provided. Nevertheless, the positive findings from the ingestion of lean red meat may relate to the fact that foods such as meat provide a high quality source of protein that contains complete and balanced proportions of all eight EAAs, including leucine.

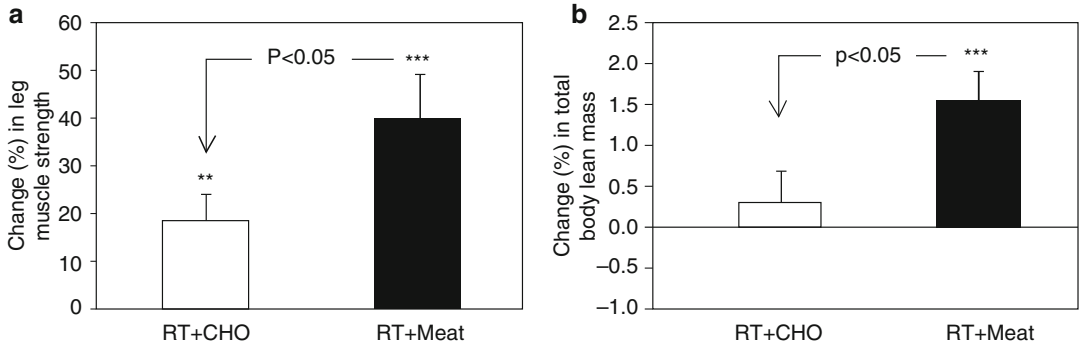

Fig. 6.1 Four-month changes (percentage) in leg muscle strength (**a**) and total body lean mass (**b**) following progressive resistance training combined with the consumption of lean red meat (RT + Meat) or PRT with the consumption of additional carbohydrates (RT + CHO) in elderly women [27].** P < 0.01, *** P < 0.001 within group changes from baseline

Leucine is a potent stimulator of MPS and a key amino acid that triggers the stimulation of important regulatory proteins that help to initiate MPS [39, 40]. While leucine supplementation alone appears to be ineffective for promoting an anabolic muscle response, leucine co-ingestion (above a certain threshold or leucine 'trigger') with dietary protein (bolus of amino acids) or a meal has been shown to further enhance postprandial MPS rates in the elderly compared to ingestion of dietary protein alone [41–43]. While long-term trials on the effects of leucine co-ingestion with PRT on muscle mass, size or strength in older adults are needed, a recent study of obese older adults involved in a 13 week PRT and weight loss program found that ingestion of a whey-protein, leucine and vitamin D enriched supplement each morning and after each of the three weekly training sessions preserved muscle mass compared to an isocaloric control group that lost muscle [24]. In this study, the leucine dose was 2.8 g, which is within the proposed leucine threshold of 2–3 g which has been reported to stimulate MPS in older adults [41, 42]. While these findings provide some evidence that a high whey and leucine rich protein supplement might be optimal, others have been suggested that if sufficient protein is provided following PRT, the type and composition of the protein consumed may be less relevant and the dose and timing may be more important since the sensitivity of muscle to the anabolic effects of protein (amino acids) is elevated for up to 24 h during recovery from exercise [44].

Amount of Protein

Several recent consensus and position statements have recommended that older adults consume at least 1.0–1.2 g/kg per day protein with at least 20–25 g of high quality protein at each meal, with higher intakes for those undertaking regular exercise [45, 46]. Indeed, acute studies have reported that the MPS response to protein or amino acid ingestion after resistance exercise increases in a dose–response manner in older adults. In a study of elderly men, postprandial MPS rates increased progressively following the ingestion of 10 g, 20 g and 40 g of whey protein [47]. In this study, at least 20 g of whey protein was required to stimulate an increase in MPS above exercise alone (10 g was ineffective), and increasing the dose to 40 g enhanced MPS rates by a further 32 %. In young adults a 20 g dose of high quality protein (whey) was shown to maximally stimulated MPS post-exercise [48], highlighting the importance of protein dose for older adults. On this basis, the lack of an additive or synergistic effect with PRT in a number of the published trials shown in Table 6.1 may relate to the insufficient dose of protein used. In most of the

supplementation trials conducted the protein dose delivered typically ranged from 15 to 30 g, which was often decreased further after accounting for compliance. A well-designed 6-month RCT in 80 mobility limited adults aged 70–85 years found that PRT combined with 40 g of whey protein daily divided into 20 g after breakfast and 20 g after the evening meal led to a 1.3 % gain in total lean mass compared to a 0.6 % gain in controls, but these differences were not statistically significant. There was also no evidence that the additional protein augmented the effects of PRT on muscle strength or function [22]. One reason why there may have been no added benefit of protein in this study is that the average increase in protein intake when supplement compliance (72 %) and dietary protein was considered was only 18 g per day. Total dietary energy intake was also reduced, which suggests that the supplement may be been a partial meal replacement. Overall, the total daily protein intake in the whey supplemented group in this study was <1.2 g/kg. This is important because an earlier meta-analysis of RCTs reported that protein ingestion at a dose of >1.2 g/kg per day enhanced the effects of PRT on muscle mass and strength in older adults [49]. This is supported by the findings from two more recent trials which found that increasing total protein intake from around 1.0 g/kg to ~1.3 g/kg per day enhanced the effects of PRT on lean mass and muscle strength in older women and the frail elderly [18, 27].

Protein Spread or Change Theory

Adequate dietary protein intake at baseline and the spread (e.g., difference in protein intake between groups) and change in protein intake (e.g., increase in protein intake from baseline after supplementation) are other important factors that may contribute to the lack of a consistent protein-exercise interactive effect on muscle outcomes in older adults [50]. As shown in Table 6.1, in many of the studies baseline protein intakes were around 1.1–1.2 g/kg per day, which is consistent with current recommendations, and the change in protein intake following supplementation (or difference from controls) was modest. Indeed, a review of 17 studies examining the effects of additional protein combined with PRT in young and older adults found that in those studies where there was no additional benefit of protein, the average percent increase in habitual protein (g/kg per day) was 6.5 % compared to a mean 59.5 % protein change in those studies which observed muscle benefits in response to a higher protein intake. Similarly, in studies where a higher protein intervention were deemed successful there was an average 66 % g/kg per day between group intake spread compared to a 10 % difference in those studies where additional protein provided no benefit over PRT alone [50]. These findings suggest that many previous trials may have been 'biologically flawed' in that they included participants who already had adequate protein intakes and/or failed to provide a sufficient dose to markedly increased protein intakes to a level different from controls (or baseline).

Timing of Protein Intake

The timing of protein intake (or supplementation) has also been identified as a factor that may influence the skeletal muscle responses to exercise. Several previous reviews have highlighted that ingestion of protein prior to, during and immediately or 4 h after PRT can enhance MPS rates and muscle hypertrophy in young adults [9, 44], but there are mixed findings from the limited studies that have been conducted in older adults. One 12-week study in elderly men found that ingestion of 10 g of protein immediately post exercise resulted in greater gains in muscle size and strength compared to those who delayed protein intake until 2 h after training [51]. However, several other trials have shown that ingestion of a protein supplement immediately before and after each exercise session failed to augment the effects of PRT on muscle hypertrophy in older adults [21, 28]. As shown in Table 6.1, many of the interventions did not consider the timing of protein intake in relation to the exercise session, but an interesting observation is that all the studies which did detect an interactive effect

on muscle distributed the protein intake across the day (e.g., breakfast and evening or after exercise). Further studies are needed to determine whether a pre- and post- training supplementation regimen might be more effective than morning and afternoon/evening for promoting exercise-induced muscle hypertrophy in older adults.

Pattern or Distribution of Protein Intake

Research has shown that resistance exercise can sensitize skeletal muscle to the anabolic properties of dietary protein (amino acids) for up to 24 h [52]. Thus it has been suggested that distributing protein intake throughout the day may provide added benefits compared to providing a single bolus dose (e.g., evening meal or post-exercise) [53]. In a 24 week study in 62 frail elderly people, Tieland et al. [18] found that twice weekly PRT combined with 15 g of protein consumed daily after both breakfast and lunch led to significantly greater gains in total body lean mass compared to PRT plus placebo; there were no additive effects on muscle strength or function. Total protein intake increased from 1.0 to 1.3 g/kg per day in the supplemented group and remained at 1.0 g/kg per day in the placebo group. Contrary to these findings, another 24-week intervention in healthy older adults (mean age 70 years) found that supplementation with 15 g of protein after breakfast each day did not promote greater exercise-induced gains in muscle mass, size or strength. The lack of an additive effect in this study may relate to the relatively healthier cohort studied, the adequate baseline protein intakes of the participants in this study (1.1–1.2 g/kg per day) and the modest increase in total protein intake throughout the study (0.18–0.24 g/kg per day) [19]. Interestingly, a recent acute study found that total protein intake (in the context of a mixed nutrient meal), rather than the distribution or pattern of intake, was more important in promoting acute protein synthesis in healthy older adults [54].

There is some evidence that the window of opportunity for protein feeding following exercise could extend to overnight (sleeping). The night period is generally associated with low MPS rates and a prolonged period when net protein balance remains negative [55]. However, ingestion of a bolus dose of protein prior to sleep has been shown to promote net muscle protein accretion during post-exercise overnight recovery in young adults [56] and augment muscle mass and strength gains during PRT in young men [57]. Whether such a feeding pattern could enhance exercise-induced muscle hypertrophy in older adults and the elderly is not known, but it is possible that the late evening snacking behaviours of older people could influence the protein-exercise anabolic muscle response.

Co-nutrient Ingestion

While various studies have investigated the effects of pre- and/or post-exercise feeding with protein (or amino acids) alone or a particular high protein food source (e.g., beef), dietary protein consumed as part of a product (e.g., milk) or meal often contains carbohydrates, fat and other nutrients which could influence the responses to exercise. Indeed, in young men it has been reported that consumption of whole milk (containing 8.2 g of fat) post-exercise led to a greater increase in amino acid uptake in the leg compared to fat-free skim milk (0.6 g of fat) [58]. While the reason(s) underlying the greater benefits of whole milk in this study are not clear, whether this would translate into greater gains in muscle mass or strength is not known. Others have suggested that carbohydrate co-ingestion can influence MPS and the muscle hypertrophic response to exercise [37, 44, 59–62]. Postprandial MPS rates in older adults have been reported to increase following carbohydrate and protein co-ingestion due to increasing circulating insulin levels [63]. Although an early study found that co-ingestion of carbohydrates and protein may reduce the anabolic response of muscle to protein (amino acids) in the elderly [62], others have failed to detect any effect (negative or positive) of carbohydrate plus protein ingestion on the acute synthesis of muscle proteins in young or older men following

exercise (or at rest) [59–61]. While a number of the trials listed in Table 6.1 included additional carbohydrates with protein supplementation or products/mixed meals which also contained carbohydrates and others vitamins and minerals, it remains unknown whether combining protein specifically with carbohydrates can enhance (or attenuate) the long-term effects of PRT on muscle mass, size or strength in older adults. However, a recent review of the effects of multi-nutrient supplementation with exercise on muscle mass, strength and function in older adults concluded that there was no evidence of an additive or interactive effect, with the exception of one study that was conducted in older adults with chronic obstructive pulmonary disease [64].

Summary and Future Directions

Given the clinical, functional and metabolic consequences associated with the age-related loss in skeletal muscle mass, strength and function, identifying strategies to optimize muscle health and function in older people is of great clinical relevance. Over the past decade, the findings from many acute studies have shown that combining PRT with the ingestion of high quality, rapidly digested, leucine rich protein sources can produce additive or synergistic benefits on post-exercise skeletal muscle protein synthesis in older adults beyond the effects of PRT or protein alone. In addition, it has been proposed that a total dietary protein intake of 1.2–1.5 g/kg per day, with 35–40 g of protein consumed in close proximity to exercise, may be the optimal dose for older adults to enhance the benefits of exercise on muscle outcomes. However, current evidence from the available RCTs which have examined whether additional dietary protein, protein (or amino acid) supplementation or multi-nutrient supplements enriched with protein can augment the effects of PRT on muscle mass, strength and/or function in older people have largely produced negative findings. Of the 18 intervention trials that we reviewed, only three reported a significant protein-exercise interaction on muscle mass or strength in older adults; none reported an additive benefit on physical function. As highlighted in this chapter, a multitude of factors related to protein, including differences in the type, dose, spread and/or change in intake, timing, pattern or distribution of intake, are all likely to have contributed to the heterogeneity in the findings. Furthermore, many of the studies were 'biologically flawed' in that they included healthy older adults who already had adequate protein intakes and/or did not provide a sufficient dose of protein to markedly increased protein intakes to a level different from controls (or baseline). To help inform the design of future nutrient efficacy trials, it is recommended that researchers carefully consider the key biological criteria outlined by Lappe and Heaney [65] which are unique to nutrients. These include: (1) use of a single form of the nutrient; (2) use of a low exposure control group; (3) ensure adequacy of dose for the treatment group; (4) demonstrate and document an altered intake to a sufficient 'therapeutic' level; (5) use of a uniform response (outcome) measure, and (6) optimization of co-nutrient status.

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Part III

Selected Nutrients

The Use of Calcium for Phosphate Control in Chronic Kidney Disease

7

Kathleen M. Hill Gallant

Abstract

Abnormal phosphorus metabolism in chronic kidney disease (CKD) is associated with adverse bone and cardiovascular outcomes, as well as higher mortality. Thus, phosphorus control in patients with CKD is an important component of disease management. Use of calcium to bind dietary phosphorus has been a common therapy for decades, but presents concerns over risk of vascular calcifications, cardiovascular events, and death. Calcium balance studies and clinical trials of calcium-based phosphate binders in patients with CKD, have added to the literature to guide the discussion of risks and benefits of using calcium in this patient population.

Keywords

Phosphorus • Calcium • Chronic kidney disease • Mineral bone disorder • Cardiovascular disease • Cardiovascular mortality • Renal failure • Phosphate binders

Introduction

Abnormal phosphorus metabolism in chronic kidney disease (CKD) is associated with adverse bone and cardiovascular outcomes, as well as higher mortality. Thus, phosphorus control in patients with CKD is an important component of disease management. Use of calcium to bind dietary phosphorus has been a common therapy for decades, but presents concerns over risk of vascular calcifications, cardiovascular events, and death. Calcium balance studies and clinical trials of calcium-based phosphate binders in

K.M. Hill Gallant, PhD, RD
Department of Nutrition Science, Purdue University,
700 West State Street, West Lafayette,
IN 47907, USA
e-mail: hillgallant@purdue.edu

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patients with CKD, have added to the literature to guide the discussion of risks and benefits of using calcium in this patient population.

Overview of Phosphorus in Normal Physiology

Phosphorus is an essential nutrient that plays critical roles in the body, including structure functions in bone mineral hydroxyapatite, DNA and RNA, and cell membranes; acid–base balance as an intracellular and titrable acid buffer; and in energy metabolism (e.g. ATP, GTP, cyclic AMP, creatinine phosphate). Phosphorus is obtained from the diet from a variety of foods, particularly from meats, dairy, nuts, and seeds. Additionally, a major source of dietary phosphorus comes from inorganic phosphate food additives [1–4]. Important in considering dietary phosphorus, is the level of bioavailability from different sources. Organic sources found naturally in plant and animal foods have lower bioavailability (~40–60%) than inorganic sources from food additives (~90+%) [5, 6]. The recommended daily allowance (RDA) for phosphorus for adults in the United States is only 700 mg/day [7], whereas usual intakes are estimated between 1000–1500 mg/day [8]. Due to the wide variety of foods naturally containing phosphorus, and the widespread use of phosphate-containing food additives, dietary phosphorus deficiency is rare. Conversely, the ubiquity of phosphorus in the food supply presents a challenge for people who have to limit their intake, namely patients with chronic kidney disease.

Phosphorus homeostasis involves several key tissues, including bone, gut, and kidney. When phosphorus is consumed in the diet, it is absorbed in the small intestine through active transport and passive diffusion processes, at a relatively high absorption rate of approximately 60% from a mixed diet. The three major phosphate regulating hormones, fibroblast growth factor-23 (FGF23), parathyroidhormone(PTH)1,25-dihydroxyvitamin D (1,25D), act to maintain serum phosphate levels with normal range. FGF23, a phosphatonin produced by cells of osteoblastic lineage, decreases renal reabsorption of phosphorus resulting in a phosphaturic effect. Similarly, PTH, secreted from the parathyroid glands in response to low serum calcium or elevated phosphate, also reduces renal phosphate reabsorption and increases urinary phosphate excretion. 1,25D acts mainly on the intestine to increase calcium absorption but also phosphorus absorption. Feedback loops amongst these three hormone work to orchestrate homeostasis: 1,25D stimulates FGF23 production, while FGF23 suppresses 1,25D; PTH stimulates FGF23 production, while FGF23 suppresses PTH; and PTH stimulates 1,25D production, while 1,25D suppresses PTH [9]. In normal physiology, the majority of phosphate homeostasis is achieved through renal regulation responding to high and low phosphate loads [10]. However, as kidney function declines, either through normal aging or by disease processes, the ability of the kidney to fulfill this role diminishes.

Abnormal Phosphorus Metabolism in Kidney Disease

Chronic kidney disease-mineral bone disorder (CKD-MBD) is a co-morbid condition in which patients with CKD exhibit abnormalities in laboratory values related to mineral metabolism (FGF23, PTH, 1,25D, phosphate, and calcium), bone disease along a varied spectrum, and vascular and soft-tissue calcification, leading to increased mortality [11]. Loss of phosphate control is considered to a be a key initiating factor in the development and progression of CKD-MBD, despite the fact that abnormal serum phosphate levels are not generally observed until late stages of the disease [12, 13]. Instead, early changes include increases in FGF23 and PTH and decreased 1,25D. These compensatory changes manage to maintain normal serum phosphate levels, but at the expense of the cardiovascular system and bone.

Risk of Excess Phosphorus

Serum phosphate has been associated with cardiovascular disease and mortality in numerous studies of various populations. Dhingra et al. [14]

studied 3368 participants of the Framingham Offspring Study prospectively for an average follow-up of over 16 years, who were free of cardiovascular disease and CKD at baseline, to determine the association between serum phosphate and cardiovascular disease risk. Higher serum phosphate was significantly associated with risk of cardiovascular disease. This was observed even over the normal reference range of serum phosphate. Notably, this association remained when subjects were restricted to only those with estimated glomerular filtration rate (eGFR) >90 mL/min and without proteinuria – indicating that the risk association observed in the general population is not simply driven by those with reduced renal function. A recent meta-analysis [15] of studies of the association between serum phosphate, cardiovascular risk, and mortality in people with normal kidney function, illustrates the consistent nature of these relationships across studies. Further, studies specifically in the kidney disease population have also shown higher serum phosphate is related to cardiovascular disease and mortality [16]. Kestenbaum et al. [17] demonstrated in a cohort of 6730 pre-dialysis CKD patients, that serum phosphate levels in excess of 3.5 mg/dL were independently associated with increased mortality. Increased risk of death has also been associated with higher serum phosphate in renal patients on dialysis [18].

While associations between serum phosphate and cardiovascular disease and mortality have been widely studied and show consistently that higher serum phosphate is adversely associated with these outcomes, the relationships between dietary phosphorus and CVD or mortality have been studied to a lesser extent. Recent studies have reported a positive relationship between dietary phosphorus intake and serum phosphorus [19], and an adverse relationship between dietary phosphorus intake and all-cause mortality [20] in the general population using NHANES data. Chang et al. [20] show that the risk of all-cause mortality begins to increase around 1400 mg/day, and that over 1/3 of the population consumed phosphorus in excess of this amount. However, few such investigations have been performed to date, and there is some inconsistency amongst those available [21].

Associational studies linking phosphate with CVD and mortality are supported by additional evidence of biological mechanisms to explain these associations. Phosphate has been shown to induce ectopic mineralization, and stimulate the transdifferentiation of vascular smooth muscle cells into osteoblasts capable of laying down collagen matrix for mineralization within the vascular tissue [22]. Further, CKD presents a condition of particular susceptibility to vascular calcification [23]. Moe et al. [24] have shown in rats with CKD that restricting dietary phosphorus can prevent the increase in vascular calcification as CKD progresses. In addition to effects on vascular calcification, abnormal phosphorus homeostasis can cause left ventricular hypertrophy (LVH) by way of FGF23. Studies in mice have shown that FGF23 directly induces LVH, and this is also supported by a positive association between FGF23 and left ventricular mass in patients with kidney disease [25]. Dietary phosphorus intake is associated with left ventricular mass in the general population [26], presumably mediated through phosphorus increasing FGF23.

Whether healthy people with normal kidney function should limit phosphorus intake below levels commonly consumed is unclear. However, available evidence suggests that this should be a continued area of inquiry. Additionally, it is important to note that many people in the general population have reduced renal function but are unaware. Plantinga et al. [27] reported that less than 8 % of people with moderate (stage 3) CKD and less than half of people in stage 4 CKD were aware they had the disease.

Phosphate Management in CKD-MBD

Phosphorus control in CKD is a major component in disease management. Strategies to control phosphorus in patients with CKD include dietary phosphorus restrictions, use of phosphate binder medications, and removal of phosphate through dialysis. A combination of these therapies are often used. Dietary phosphorus restriction can be effective in lowering serum phosphate. However,

it presents several major challenges. These include the wide prevalence of phosphorus in the food supply, patients' ability to adequately identify sources of dietary phosphorus, and inadequate protein intakes subsequent to phosphorus restriction. Phosphate binder medications are often used in conjunction with dietary restrictions. Patients take these medications with meals to bind dietary phosphorus in the gut and prevent its absorption. Finally, kidney dialysis used in late stages of the disease removes excess phosphate from the blood.

Calcium-Based Phosphate Binders

A variety of types of phosphate binders exist, and they are often classified as being either calcium-based or non-calcium based (which include other metals [aluminum, iron, or lanthanum] or the polymer resin, sevelamer). All types of phosphate binders currently used have been shown to be effective in lowering serum phosphate in patients with kidney disease. There are advantages and disadvantages to the different types of phosphate binders, such as costs, side-effects, and benefits in addition to lowering phosphate. Aluminum-based phosphate binders are not currently recommended for long-term use due to risk of adverse neurological effects. With this exception, the 2009 Kidney Disease: Improving Global Outcomes (KDIGO) Work Group concluded that there is "insufficient comparative efficacy data on clinical outcomes to make a recommendation for the use of a specific binder for all patients" [11]. With that, calcium-based phosphate binders are a popular choice for binder therapy, as they are relatively inexpensive compared to non-calcium based binder prescriptions. The National Kidney Foundation Kidney Disease Outcomes Quaility Initiative (KDOQI) guidelines state, based on expert opinion, that calcium-based binders may be considered for initial and primary binder therapy in moderate CKD (stage 3 and 4) and in kidney failure [28]. The most common are calcium carbonate (40 % elemental calcium), which has the advantage of low cost and availability over-the-counter, and calcium acetate (25 % elemental calcium), which has the advantage of potentially less calcium absorbed compared with calcium carbonate, but a slightly higher expensive. Calcium citrate, which is a popular form of calcium supplement due to its relatively high calcium absorbability, is not recommended in CKD because it has the potential to enhance aluminum absorption which carries risk for adverse neurological effects [11]. Calcium-based phosphate binders, in addition to reducing serum phosphate, reduce serum PTH, may prevent negative calcium balance and bone loss. They may also prevent low serum calcium, which is associated with mortality in patients with kidney disease [18]. Despite the comparative effectiveness, low-cost, and potential non-phosphate benefits of calcium-based phosphate binders, there is concern over their use with regard to risk for vascular calcification in patients with CKD. Block et al. [29] found in a randomized placebo-controlled trial of phosphate binders, that calcium reduced serum phosphate, but also resulted in increased coronary artery calcification (CAC) [29]. This was an important study as it represents one of the few placebo-controlled phosphate binder trials. Alternatively, Russo et al. [30] found that calcium did not exacerbate CAC in pre-dialysis CKD patients compared with controls, but CAC was higher in patients who received calcium compared with those who received a non-calcium based binder. Studies investigating effect of binder use on mortality have shown better survival in both non-dialysis and dialysis patients who receive binders (any) versus no binders [31, 32]. Since calcium-based binders represent a large proportion of binders used, this may be used to argue that these binders offer a survival advantage over using no binder at all. However, other studies have shown no benefit of calcium-based phosphate binder versus no binder [33], or worse survival in patients taking calcium versus a non-calcium-based binder [34, 35]. Another approach to determine risk of excess calcium in patients with CKD has been using calcium balance studies to measure calcium retention.

Calcium Balance Studies in CKD

Two recent calcium balance studies in moderate stage CKD show the effect of calcium intake on calcium balance in these patients. Spiegel & Brady [36] studied six patients with stage 3-4 CKD and six healthy controls in a randomized cross-over study of the effect of dietary calcium intake level on calcium balance. Participants were randomized to a controlled diet containing either 800 mg/day or 2000 mg/day elemental calcium and 1600 mg/day phosphorus for 1 week as outpatients, then studied for calcium balance over a 48-h inpatient period, during which stool and urine collections were made. Calcium balance was neutral in both healthy controls and patients with CKD when consuming a diet of 800 mg/day calcium, and was higher for both groups when given a diet of 2000 mg/d calcium. However, positive calcium balance was greater for patients with CKD. Additionally, there was no difference in 24-h urine phosphorus excretion between the two calcium diet levels in either healthy controls or patients with CKD. The higher calcium intake did result in lower serum 1,25D and intact PTH, but did not affect serum calcium or phosphate levels.

Hill et al. [37] studied eight patients with stage 3-4 CKD in a randomized cross-over study of both calcium and phosphorus balance. Patients were studied over 3 weeks on a controlled diet containing approximately 1000 mg/day calcium and 1500 mg/day phosphorus with either placebo or calcium carbonate (500 mg elemental calcium) 3×/day given with meals. The administration of calcium carbonate with meals is consistent with its use as a phosphate binder in CKD patients, rather than as a dietary supplement. The first week of each crossover period was an equilibration period to the controlled diet. During the last 2 weeks, patients participated in an inpatient setting, during which time all urine and feces were collected for calcium and phosphorus balance calculations. In addition, full calcium kinetics were performed on these patients using calcium-45 given orally and by IV, with serial blood, urine, and fecal measurements of the isotope over 3 weeks. Average calcium balance was neutral on the controlled diet (1000 mg/day calcium) with placebo, similar to the findings by Spiegel and Brady with a diet of 800 mg/day calcium. Increasing calcium intake by 1500 mg/day elemental calcium from calcium carbonate, however, produced positive calcium balance in these patients on the order of approximately 500 mg/day (Fig. 7.1). A small decrease in urine phosphorus excretion was observed, but overall phosphorus balance was unaffected by the calcium treatment (Fig. 7.1). Additionally, serum calcium and phosphate were not affected by treatment. The calcium kinetics performed in this study showed that bone balance was higher with calcium carbonate, but not to the magnitude of overall calcium balance, suggesting the potential for

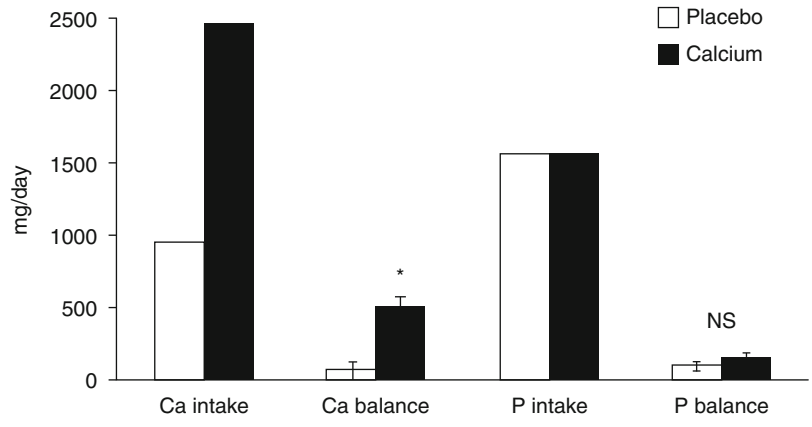

Fig. 7.1 Calcium and phosphorus balance in stage 3-4 CKD patients given placebo or calcium carbonate (1500 mg/day elemental Ca). Ca and P intake levels were controlled and shown here as a reference point. *p = 0.002; *NS* not significant (Data from Ref. [37])

risk of extraskeletal calcium deposition. Thus, these two balance studies taken together indicate that moderate stage CKD patients, on average, are in neutral calcium balance. Balance studies are by nature shorter term studies, so the long term consequences of these positive calcium balances cannot be directly determined. However, these findings are compatible with findings by others of increased CAC with calcium-based binders discussed above. Importantly, calcium carbonate used as a phosphate binder only modestly affected urine phosphorus and did not affect overall phosphorus balance. Based on urine phosphorus as a reflection of phosphorus absorption, it took an increase of 10 mg calcium to achieve only 1 mg of phosphorus binding, which is consistent with prior literature [30].

Conclusions

In recent years, concern over use of calcium to control phosphate in CKD has grown due to results of randomized controlled trials indicating risk of calcification and mortality, as well as calcium balance studies showing high positive calcium balance with increased calcium intake through diet or a calcium-based phosphate binder. However, it remains a popular binder due to its availability, low cost, and effectiveness in reducing serum phosphate. Further, there is controversy whether the risks outweigh the benefits [38, 39].

KDOQI guidelines state, based on expert opinion, that for patients with stage 5 CKD (late stage), total calcium intake (diet plus binders) should not exceed the Tolerable Upper Limit of 2000 mg/day [11] and calcium from binders alone not exceed 1500 mg/day. It should be noted that usual calcium intakes in patients with CKD cover a wide range and are similar to intakes observed in the general population [40]. Thus, it cannot be assumed that a patient with CKD has a low baseline calcium intake when prescribing calcium as a phosphate binder. Ideally, calcium intake would be assessed prior to determining appropriateness of use and dose of calcium-based phosphate binders. Use of food frequency questionnaires, such as the NIH Short Calcium Questionniare [41], could make implementation of calcium assessment into practice more feasible. Data from balance studies indicate that aiming for an adequate calcium intake in CKD patients, around 1000 mg/day (the current RDA for most adults [42]), may be considered a good approach for achieving neutral calcium balance, with the goal of protecting bone but limiting risk of calcium retention.

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Vitamin C and Bone Health

8

Shivani Sahni, Douglas P. Kiel,
and Marian T. Hannan

Abstract

Dietary and lifestyle factors have important contributions to skeletal health. Fruit- and vegetable-specific antioxidants, such as vitamin C, might help in preventing osteoporosis because vitamin C may decrease oxidative stress and subsequent bone-resorption. Vitamin C is an essential cofactor for collagen formation (an important component of bone matrix) and potentiates vitamin E activity in cells by regenerating α-tocopherol from its oxidized derivative.

In this chapter, we highlight findings from previous studies on vitamin C intake and bone measures to underscore our current understanding and emphasize the importance of vitamin C on skeletal health. Taken together, previous studies showed a positive association between dietary vitamin C and bone mineral density. Very few examined serum vitamin C status, vitamin C supplementation or bone loss. The reported associations were complex due to multiple interactions with smoking, calcium and vitamin E intakes and current estrogen use in women. One longitudinal study reported that higher vitamin C intake may be protective against bone loss in men with low calcium or vitamin E intake. There is an urgent need to replicate these findings in larger cohorts with data on bone loss over time. Studies have also suggested that vitamin C intake may be protective against hip fracture as well as other fractures, especially among current smokers and estrogen using women. Larger prospective cohort studies are required to further clarify these interactions. Lastly, good-quality randomized controlled trials are needed to confirm these epidemiological findings to ascertain optimal intakes for osteoporosis prevention.

S. Sahni, PhD (✉) • M.T. Hannan, DSci, MPH
D.P. Kiel, MD, MPH
Institute for Aging Research, Hebrew SeniorLife,
Beth Israel Deaconess Medical Center,
Harvard Medical School, Boston, MA 02131, USA
e-mail: ShivaniSahni@hsl.harvard.edu;
Hannan@hsl.harvard.edu; kiel@hsl.harvard.edu

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Vitamin C • Osteoporosis • Fractures • Older adults • Antioxidant • Dietary intake

Introduction

Worldwide, osteoporosis causes more than 8.9 million fractures annually, resulting in an osteoporotic fracture every 3 s [1]. Fifty one percent of these fractures occurred in Europe and the Americas, while most of the remainder occurred in the Western Pacific region and Southeast Asia [1]. By 2050, the worldwide incidence of hip fracture is projected to increase by 310 % in men and 240 % in women [2]. Therefore, osteoporosis and related fractures in older adults take a huge personal and economic toll with an annual cost of nearly $22 billion to the US healthcare system [2] and €37 billion to the European Union [3].

Recent findings from the population-based Cohort of Swedish Men (COSM) and Swedish Mammography Cohort (SMC) showed a dose-response association between fruit and vegetable intake and hip fracture such that an intake below the recommended five servings/day conferred higher rates of hip fracture [4]. Previous studies had also shown that higher fruit and vegetable intake has positive effects on bone mineral status [5–10]. Among other nutrients, fruits and vegetables are rich in vitamin C, a powerful water soluble antioxidant that is thought to prevent bone resorption due to its anti-oxidative properties as well as its role in collagen formation. While several studies have confirmed the association between vitamin C intake or status with bone mineral density (BMD) and fractures, the results have not been entirely consistent. This may be due to a complex association that involves interaction of vitamin C with smoking [11–13], postmenopausal estrogen use/hormonal therapy [11, 14, 15], calcium intake [12, 15, 16] and vitamin E intake [13, 17]. In this chapter, we highlight findings from previous studies on this topic to underscore our current understanding and emphasize the potential importance of vitamin C on skeletal health.

Vitamin C Effects on the Skeleton

Vitamin C may affect bone health via several mechanisms. *First*, collagen is the most abundant component of the extracellular bone matrix [18]. In adults, type I collagen represents about 90 % of the total protein matrix [18]. Vitamin C is an essential cofactor required for hydroxylation of proline and lysine into hydroxyproline and hydroxylysine respectively [19]. This post-translational modification of pro-collagen is crucial for the formation of mature collagen molecules and their assembly into fibrils [18]. Defects in this process, as seen in the disease scurvy, lead to the formation of unstable non-hydroxylated pro-collagen chains, which are degraded within the cell at normal body temperature. Without the structural support of collagen, blood vessels, tendons, and skin become fragile. This may also lead to weakening of bones and subsequent fractures. Previous animal studies have demonstrated that experimentally induced deficiency of vitamin C leads to impaired bone mass, cartilage and connective tissue [20, 21]. *Second*, Oxidative stress may increase bone resorption through activation of nuclear factor-kB protein, which is a crucial mediator of tumor necrosis factor-a and osteoclastogenetic activity [22–26]. Vitamin C, a strong antioxidant aids in decreasing this oxidative stress [27] and therefore may play a role in preventing bone resorption. *Third*, vitamin E is another strong antioxidant that has been linked with bone health [28, 29]. Vitamin C potentiates vitamin E activity in cells by regenerating α-tocopherol from its oxidized derivative [30].

Vitamin C and Bone Density

Evidence from Cross-Section and Case–Control Studies

Several cross-sectional and case-control studies have examined the association of vitamin C intake with dual-energy X-ray absorptiometry (DXA)-derived BMD at multiple bone sites. Most of these studies were focused on either total or dietary vitamin C intake. Most of the studies except one [14] showed positive associations between vitamin C and one or more BMD sites. Very few examined serum vitamin C status or vitamin C supplementation. However, the results of these studies are complicated by multiple interactions observed between vitamin C intake/status and other nutritional [13, 15, 16] and non-nutritional [11, 13–15] factors (Table 8.1).

Interaction with Smoking

In the Framingham Osteoporosis study, the association of vitamin C (total, supplemental, and dietary) intake with BMD at the hip [femoral neck, trochanter], spine, and radial shaft was examined in 334 men and 540 women (mean age = 75 years) [13]. Mean BMD was estimated, for men and women, by tertile/category of energy adjusted vitamin C intake. Negative associations were reported between total and supplemental vitamin C intake and trochanter-BMD among current male smokers (P-trend = 0.01). Among male nonsmokers, total vitamin C intake was positively associated with femoral neck BMD (P-trend = 0.04). No significant associations were reported among women. Smoking is an established risk factor of osteoporosis as outlined in our recent review [31].

Interaction with Smoking and Estrogen Use

Simon and Hudes analyzed data collected from 13,080 adults (aged 20–90 years) enrolled in the Third National Health and Nutrition Examination Survey (NHANES III) during 1988–1994. Because they identified three-way interactions among smoking, history of estrogen use, and dietary and serum ascorbic acid in post-menopausal women, they analyzed these relations stratified by smoking and estrogen use. Dietary ascorbic acid intake was independently associated with BMD among pre-menopausal women ($P = 0.002$). Among men, serum ascorbic acid was associated in a nonlinear fashion with BMD ($P < 0.05$). Among post-menopausal women without a history of smoking or estrogen use, serum ascorbic acid was unexpectedly associated with lower BMD ($P = 0.01$). However, among post-menopausal women with a history of smoking and estrogen use, a standard deviation increase in serum ascorbic acid was associated with a 49 % decrease in fracture prevalence ($P = 0.001$) [11]. Estrogen-use increases the turnover of ascorbic acid [32] and is associated with lowered ascorbic acid levels in animals and humans [33–35]. Studies have also demonstrated that smoking decreases the absorption and increases the turnover of ascorbic acid, thereby lowering blood ascorbic acid levels [36]. Therefore, interaction of vitamin C with smoking and estrogen use is expected. However, results of these interactions have been inconsistent.

Interaction with Calcium Intake

Using the data from Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial, Hall and Greendale examined the association of food frequency questionnaire (FFQ) based dietary vitamin C with BMD at the femoral neck, total hip and lumbar spine in women aged 45–64 years. They reported that with each 100 mg increment in dietary vitamin C intake, there was a 0.017 g/cm^2 increment in BMD at the femoral neck ($P = 0.002$) and total hip ($P = 0.005$). Association with lumbar spine BMD was not statistically significant ($P = 0.08$). This study further reported an effect modification by calcium intake. Upon stratification by calcium intake (>500 mg/day and ≤500 mg/

Table 8.1 Studies of BMD according to vitamin C intake or status in men and women

Study	Sample, age (years)	Vitamin C	Measurement/Site	Principal finding
New SA et al. (1997) [41]	994 healthy pre-menopausal women aged 45–49 years	FFQ based total vitamin C	BMD lumbar spine, femoral neck (FN), femoral trochanter, and femoral Wards	Non-linear association observed. Participants in 3rd quartile of total vitamin C intake had higher lumbar spine BMD ($P<0.002$) and femoral neck BMD ($P<0.01$) compared to those in the lowest quartile
Hall and Greendale, (1998) [16]	775 women aged 45–64 years from the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial	FFQ based dietary vitamin C	BMD femoral neck, total hip and lumbar spine	Each 100 mg increment in dietary vitamin C intake, was associated with a 0.017 g/cm^2 increment in BMD ($P=0.002$ femoral neck; $P=0.005$ total hip). Association with lumbar spine BMD was not statistically significant ($P=0.08$). Effect modification by calcium intake, significant associations were seen with higher calcium intake (>500 mg/day) but not with lower calcium intake
Simon and Hudes (2001) [11]	3204 pre-menopausal women, 2906 post-menopausal women and 5739 men from the Third National Health and Nutrition Examination Survey (NHANES III)	Dietary vitamin C from 24-h recall and serum ascorbic acid	BMD hip	Three-way interactions among smoking, history of estrogen use, and dietary and serum ascorbic acid were identified. Dietary ascorbic acid intake was associated with BMD among premenopausal women ($P=0.002$). Among men, serum ascorbic acid was associated in a nonlinear fashion with BMD ($P<0.05$). Among post-menopausal women without a history of smoking or estrogen use, serum ascorbic acid was unexpectedly associated with lower BMD ($P=0.01$)

Morton et al. (2001) [15]	994 post-menopausal women	Vitamin C supplement use	BMD ultradistal and midshaft radius, hip, and lumbar spine	Vitamin C users had higher BMD at the femoral neck ($P<0.02$) and total hip ($P<0.06$). Women taking both estrogen and vitamin C had higher BMD levels at all sites. Among current estrogen users, those also taking vitamin C had higher BMD levels with marginal significance achieved at the ultradistal radius ($P<0.07$), femoral neck ($P<0.07$), and total hip ($P<0.09$). Women who took vitamin C plus calcium and estrogen had the highest BMD at the femoral neck ($P<0.001$), total hip ($P=0.05$), ultradistal radius ($P=0.02$)
Wolf et al. (2005) [14]	11,068 women aged 50–79 years from Women's Health Initiative	Dietary and total vitamin C intake from FFQ	BMD whole body, L2–L4 spine, total hip, femoral neck, trochanter, total hip BMD t score	No significant associations with diet or total vitamin C intake (P range = 0.08–0.33). Effect modification by use of hormone therapy. Current hormone therapy associated with higher BMD at multiple bone sites in women with high total vitamin C intake (P range = 0.03–0.05)
Sahni et al. (2008) [13]	334 men and 540 women (mean age 75 years) from the Framingham Osteoporosis Study	Total, dietary and supplemental vitamin C intake from FFQ	BMD femoral neck, trochanter, spine, and radial shaft	Interaction by sex and smoking. Total and supplemental vitamin C intakes were negatively associated with trochanter-BMD among current male smokers (P-trend = 0.01). Among male nonsmokers, total vitamin C intake was positively associated with femoral neck BMD (P-trend = 0.04)

day), women with higher calcium intake had an increment of 0.0190 g/cm^2 in femoral neck BMD ($P=0.002$), 0.0172 g/cm^2 in total hip BMD (P = 0.01) and 0.0199 g/cm^2 in lumbar spine BMD (P = 0.02) per 100 mg vitamin C. No relation between BMD and vitamin C was evident in the lower calcium stratum for any of the bone sites examined. Vitamin C aids in the absorption of calcium, therefore, interactive effects of these two nutrients upon BMD have been reported with cross-sectional studies reporting a beneficial role of higher vitamin C intake with higher calcium intakes.

Interaction with Calcium Intake and Estrogen Use

In the Rancho Bernardo study, Morton et al. evaluated the independent relation of daily vitamin C supplement use with BMD in a population-based sample of 994 post-menopausal women. Daily vitamin C supplement intake ranged from 100 to 5000 mg; the mean daily dose was 745 mg and average duration of use was 12.4 years. Vitamin C users had BMD levels ~3 % higher at the mid-shaft radius, femoral neck, and total hip ($P<0.05$) after adjustment for confounders and covariates. In the fully adjusted model, significant differences remained at the femoral neck ($P<0.02$) and marginal significance was observed at the total hip ($P<0.06$). Women taking both estrogen and vitamin C had significantly higher BMD levels at all sites. Among current estrogen users, those also taking vitamin C had higher BMD levels at all sites, with marginal significance achieved at the ultradistal radius ($P<0.07$), femoral neck ($P<0.07$), and total hip ($P<0.09$). Women who took vitamin C plus calcium and estrogen had the highest BMD at the femoral neck ($P=0.001$), total hip ($P=0.05$), ultradistal radius ($P=0.02$), and lumbar spine. Vitamin C supplement use appears to have a beneficial effect on levels of BMD, especially among postmenopausal women using concurrent estrogen therapy and calcium supplements [15].

Evidence from Longitudinal Studies of Vitamin C and Bone Loss

To our knowledge only two studies have examined the effect of vitamin C intake on bone loss (Table 8.2). Kaptoge et al. examined the association of dietary vitamin C intake (from 7-day food dietary) with total hip BMD loss over 3 years in 470 men and 474 women (aged 67–79 years) [37]. No associations were reported in men but in women, low intake of vitamin C was associated with faster rate of total hip BMD loss. Women in the lowest tertile (7–57 mg/day) of vitamin C intake lost BMD at an average rate of −0.65 % p.a., which was significantly faster compared to loss rates in the middle (58–98 mg/day) and upper (99–363 mg/day) tertiles of intake, which were −0.31 % p.a. and −0.30 % p.a., respectively (P = 0.016).

In the Framingham Osteoporosis study, Sahni et al. examined the association of vitamin C (total, supplemental and dietary) intake with BMD loss over 4 years in 213 men and 393 women (mean age was 75 years). In contrast to the study by Kaptoge et al. [37], results from the Framingham Osteoporosis Study showed vitamin C to be protective against bone loss but only in men not women. In this study, higher total vitamin C intake was associated with less femoral neck and trochanter-BMD loss in men with low calcium (all P-trend ≤ 0.03) or vitamin E intakes (all P-trend = 0.03, Fig. 8.1). Higher dietary vitamin C intake tended to be associated with lower femoral neck-BMD loss (P-trend = 0.09). These associations were attenuated but retained borderline significance (P-trend <0.1) after adjusting for potassium intake (a marker of fruit and vegetable intake), suggesting that vitamin C effects may not be separated from other protective factors in fruit and vegetables. No significant associations were observed among women. In light of the studies highlighted in this review that examined vitamin C with BMD and BMD loss, there is a strong evidence that higher vitamin C intake is associated with higher BMD. However, the associations are complex due to several reported

Table 8.2 Studies of change in BMD according to vitamin C intake in men and women

Study	Sample, age (years)	Vitamin C	Measurement/Site	Principal finding
Kapotge et al. (2001) [37]	470 men and 474 women aged 67–79 years from the EPIC-Norfolk study	Dietary vitamin C from 7-day food diaries	3-year BMD change in total hip	In men, no effect of dietary vitamin C on total hip bone loss. In women, low intake of vitamin C was associated with greater bone loss. Women in the lowest tertile (7–57 mg/day) of vitamin C intake lost BMD at an average rate of −0.65 % per annum (p.a.), which was significantly greater compared to loss rates in the middle (58–98 mg/day) and upper (99–363 mg/day) tertiles of intake, which were −0.31 % p.a. and −0.30 % p.a., respectively (P = 0.016)
Sahni et al. (2008) [13]	213 men and 393 women (mean age 75 years) from the Framingham Osteoporosis Study	Total, dietary and supplemental vitamin C intake from FFQ	4-year BMD change in femoral neck, trochanter, spine, and radial shaft	Interaction by sex, calcium and vitamin E intake. Higher total vitamin C intake was associated with less femoral neck and trochanter-BMD loss in men with low calcium (median vitamin C intake across tertiles = 74,139, and 200 mg/day, all P-trend ≤ 0.03) or vitamin E intakes (all P-trend = 0.03). Higher dietary vitamin C intake tended to be associated with lower femoral neck-BMD loss (P-trend = 0.09). These associations were attenuated but retained borderline significance (P-trend < 0.1) after adjusting for potassium intake (a marker of fruit and vegetable intake)

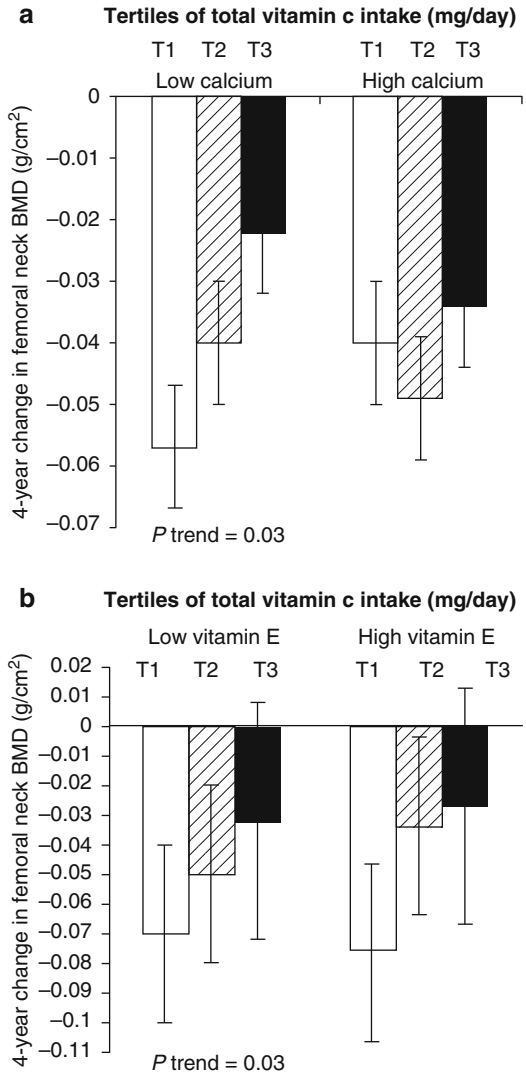

Fig. 8.1 Adjusted mean 4-year changes in femoral neck by tertiles of total vitamin C intake among men stratified by (**a**) total calcium intake and (**b**) total vitamin E intake. Low calcium group: total calcium intake ≤ median intake (661 mg/day); high calcium group: total calcium intake > median intake. Low vitamin E group: total vitamin E intake ≤ median intake (7.7 mg TE/day); high vitamin E group: total vitamin E intake > median intake. Models were adjusted for age at the baseline (years), BMI (kg/m^2), height at the time of enrollment (m), total energy intake (MJ/day), baseline physical activity index, alcohol intake (none/moderate: <26.4 g/day of alcohol; high: ≥26.4 g/day of alcohol), smoking (never/former/current smokers), and intake of total vitamin D (mcg/day), caffeine (mg/day), and multivitamin use (yes/no). Models for panel **b** were further adjusted for total calcium intake (mg/day). Values are means ± SE, n = 201. Analysis was based on a general linear model with Dunnett's adjustment for multiple comparisons. *Different from T1, $P < 0.05$

interactions with smoking, calcium and vitamin E intakes and current estrogen use in women. Some of these interactions were also replicated in longitudinal studies of bone loss. While two prospective cohort studies provided strong evidence for vitamin C intake reducing hip bone loss, there is still a need to replicate these data in other larger cohorts. These data may be sufficient to support a randomized controlled trial of vitamin C supplementation. In the study by Kaptoge et al. [37] and by Sahni et al. [13], the bone protective effects of vitamin C intakes were seen at intake levels that were greater than the dietary recommended intakes of 75 mg/day for women and 90 mg/day for men. This information can be useful in designing future randomized controlled trials to ascertain optimal intakes of vitamin C for osteoporosis prevention.

Evidence from Intervention Studies

To date there has been one double-blind randomized control trial of vitamin C (along with vitamin E) and bone density in 30 men and women [38]. The treatment groups received 400 IU of vitamin E daily and either 500 or 1000 mg/day of vitamin C for 12 months. The group with the highest vitamin C intake had significantly less hip bone loss compared with the placebo group but no significant association was seen for lumbar spine BMD. Another study by Chuin et al. was a 6-month randomized controlled of 34 women that examined the effect of vitamin C in combination with other treatments and bone density [39]. Vitamin group received ascorbic acid (1000 mg/day) and a-tocopherol (600 mg/day). Exercise and placebo group received 60 min of resistance training three times/week and placebo (lactose) and exercise and vitamin group received 60 min of resistance training three times/week and ascorbic acid (1000 mg/day) and a-tocopherol (600 mg/day). Lumbar spine (LS) BMD decreased significantly by 1 % in the placebo group but remained stable in the other three intervention groups. Taken together, evidence from these two trials investigating vitamin C's effect

on bone density loss is unclear. While these two studies show promising results for vitamin C intake (at dosages much higher than usual dietary intake), they did not examine the effect of vitamin C alone and the study by Chuin et al. was not double blinded. The studies had small sample size and limited duration of treatment. Future trials are needed to examine interventions of vitamin C supplementation in larger studies of longer duration. Such good-quality double blinded randomized controlled trials will aid in confirming the epidemiological findings reported thus far and will help in ascertaining optimal intakes for osteoporosis prevention.

Vitamin C and Fracture Risk

As noted above, several studies have examined dietary vitamin C's effect on bone density but relatively fewer studies have examined effects on fracture. Most of the studies with fracture outcomes had either cross-sectional or case-control designs. Some fracture studies also reported significant interactions with smoking or estrogen use (Table 8.3). In a case cohort study from the Swedish Mammography Cohort [17], Melhus et al. examined 247 cases (44 current and 42 former smokers) and 873 controls (93 current and 127 former smokers) aged 40–76 years. Significant interaction between current smoking status and both dietary vitamin E ($P=0.02$) and vitamin C ($P=0.03$) intake was reported. Among current smokers, hip fracture risk increased with low intake of vitamin E [3.0 (95 % CI: 1.6–5.4)] or vitamin C [3.0 (1.6–5.6)]. In contrast, the OR decreased to 1.1 (0.5–2.4) and 1.4 (0.7–3.0) with high intakes of vitamin E and C, respectively. Current smokers with a low intake of both vitamins E and C, the risk of hip fracture was much higher; OR = 4.9 (2.2–11.0).

The only prospective cohort study on this topic was from the Framingham Osteoporosis study. This study evaluated associations of vitamin C intake (total, dietary, and supplemental) with incident hip fracture and non-vertebral osteoporotic fracture, over a 15- to 17-year follow-up [40]. 366 men and 592 women (mean age 75 ± 5 years) completed a food frequency questionnaire (FFQ) in 1988–1989 and were followed for non-vertebral fracture until 2003 and hip fracture until 2005. Tertiles of vitamin C intake were created after adjusting for total energy (residual method). Over follow-up 100 hip fractures occurred and 180 non-vertebral osteoporotic fractures occurred. Among men and women in this study, subjects in the highest tertile of total vitamin C intake (median = 313 mg/day) had a significantly lower risk of hip fracture as compared to subjects in the lowest tertile of intake (median = 94 mg/day) (HR T3 = 0.56, 95 % CI = 0.31–0.98, P = 0.04, HR T2 = 0.73, 95 % CI = 0.44–1.20, P = 0.21, P for trend = 0.04, Global test P value = 0.0001). Similarly, subjects in the highest tertile of total vitamin C intake (median = 308 mg/day) had lower risk of non-vertebral osteoporotic fracture as compared to subjects in the lowest tertile of intake (median = 95 mg/day) (HR T3 = 0.66, 95 % CI = 0.43–1.01, P = 0.06, HR T2 = 0.93, 95 % CI = 0.65–1.36, P = 0.73, P for trend = 0.05, Global test P value < 0.0001). Subjects in the highest category of *supplemental vitamin C* intake (median = 260 mg/day) had significantly lower risk of hip fracture as compared to non-supplement users (HR T3 = 0.31, 95 % CI = 0.13–0.73, P = 0.007, HR T2 = 0.50, 95 % CI = 0.20–1.24, P = 0.13, P for trend = 0.02, Global test P value < 0.0001). Similarly, subjects in the highest category of supplemental vitamin C intake (median = 260 mg/day) tended to have lower risk of non-vertebral osteoporotic fracture as compared to non-supplement users, but this only approached significance (HR T3 = 0.58, 95 % CI = 0.30–1.11, P = 0.10, HR T2 = 0.80, 95 % CI = 0.38–1.71, P = 0.57, P for trend = 0.07, Global test P value < 0.0001). Dietary vitamin C intake was not associated with fracture risk (all $P>0.22$).

Taken together, these studies suggest that vitamin C intake may be protective against hip fracture as well as other fractures. These protective effects were more evident among current smokers and estrogen using women. Larger prospective cohort studies are required to further clarify

Table 8.3 Studies of vitamin C and relative risk of fractures of the hip and other sites

Type of Fracture/Study	Study design	Sample	Results
Hip fracture			
Michaelsson et al. (1995) [42]	Case-control study, the Swedish Mammography Cohort	247 cases and 893 matched controls aged 40–75 years	Higher vitamin C intake was associated with increased hip fracture risk compared to low intake (adjusted OR = 1.9, 95 % CI: 1.2–3.1)
Melhus et al. (1999) [17]	Case cohort study, the Swedish Mammography Cohort	247 cases (44 current and 42 former smokers) and 873 controls (93 current and 127 former smokers) aged 40–76 years	Effect modification by smoking. Among current smokers, low dietary vitamin C was associated with a higher hip fracture risk; OR = 3.0 (1.6–5.6). No association was seen with high dietary vitamin C; OR = 1.4 (0.7–3.0). Effect modification by vitamin E. Current smokers with a low intake of both vitamins E and C, had increased risk of hip fracture; OR = 4.9 (2.2–11.0)
Zhang et al. (2005) [12]	Case-control	1215 male and female cases aged ≥50 years and 1349 age- and sex-matched controls without hip fracture	Effect modification by smoking. Ever smokers: Vitamin C intake did not have a significant linear association with hip fracture. Never smokers: Protective trends seen with higher intake (5th quintile, OR = 0.65 (95 % CI, 0.38–1.13), P trend = 0.02
Sahni et al. (2009) [40]	Prospective cohort, the Framingham Osteoporosis Study	366 men and 592 women (mean age 75 years)	Over follow-up 100 hip fractures occurred. Subjects in the highest tertile of total vitamin C intake had significantly fewer hip fractures (P trend = 0.04) compared to those in the lowest tertile of intake. Subjects in the highest category of supplemental vitamin C intake had significantly fewer hip fractures (P trend = 0.02) compared to non-supplement users. Dietary vitamin C intake was not associated with fracture risk (all P > 0.22)
Any fracture			
Simon and Hudes, (2001) [11]	Cross-sectional, third National Health and Nutrition Examination Survey (NHANES III)	3778 pre-menopausal women, 3165 post-menopausal women and 6137 men aged 20–90 years	Effect modification by smoking and history of estrogen use in post-menopausal women. Among ever smokers with a history of estrogen use, a standard deviation increase in serum ascorbic acid was associated with a 49 % decrease in fracture prevalence
Sahni et al. (2009) [40]	Prospective cohort, the Framingham Osteoporosis Study	366 men and 592 women (mean age 75 years)	Over follow-up, 180 non-vertebral osteoporotic fractures occurred. Subjects in the highest tertile of total vitamin C intake had significantly fewer non-vertebral fractures (P trend = 0.05) compared to subjects in the lowest tertile of intake. Subjects in the highest category of supplemental vitamin C intake had significantly fewer non-vertebral fractures (P trend = 0.07) compared to non-supplement users. Dietary vitamin C intake was not associated with fracture risk (all P > 0.22)

these interactions with hip fracture as well as other fractures.

Conclusion

Dietary and lifestyle factors have important contributions to skeletal health. Higher vitamin C intake appears to be associated with higher BMD and lower bone loss. These associations seem to be modified by other nutritional and non-nutritional factors. Limited work suggests that usefulness of vitamin C intake in bone loss prevention, particularly in men with concomitant low intakes of calcium or vitamin E. However, more studies are needed to confirm these findings. Vitamin C intake also appears to be protective against hip fractures and other fractures, though there is a dearth of prospective cohort studies in this area. Some studies have suggested that association of vitamin C with bone measures is non-linear. Overall the protective effects of vitamin C have been observed at levels much higher that the dietary recommended intakes. Well designed randomized controlled trials are needed to confirm these epidemiological findings and to ascertain optimal intakes for osteoporosis prevention.

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Acid-Base Balance of the Diet: Implications for Bone

9

Bess Dawson-Hughes

Abstract

With aging, men and women develop a mild and progressive metabolic acidosis. This occurs as a result of the combination of declining renal function and ingestion of acid-producing diets. Acid-producing diets are generally low in fruits and vegetables in relation to their content of cereal grains and protein. There is extensive evidence that severe metabolic acidosis causes bone loss, but the impact of the chronic, low-grade acidosis on bone mass and fracture risk in older individuals has not been established and remains controversial. In a recent dose-finding trial in healthy older men and women, the dose of 81 mmol/day of potassium bicarbonate ($KHCO_3$) produced the greatest reduction in urinary excretion of N-telopeptide (NTX), a biochemical marker of bone resorption over a 3-month period. Findings from the two available placebo-controlled, 2-year alkali intervention trials are conflicting, with one null and the other indicating favorable effects of potassium citrate on rates of bone loss and on trabecular bone density. Reductions in bone turnover markers and calcium excretion in the absence of changes in calcium absorption have been observed in short-term studies. Further work is needed to assess the long-term skeletal effects of lowering dietary acid loads.

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B. Dawson-Hughes, MD
Jean Mayer USDA Human Nutrition Research
Center on Aging, Tufts University, 711 Washington St.,
Boston, MA 02111, USA
e-mail: Bess.dawson-hughes@tufts.edu

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Acid-base balance • Bone turnover • Calcium excretion • Potassium bicarbonate • Bone mineral density

Introduction

With aging, men and women develop a chronic low grade metabolic acidosis. In a review of published studies, Frassetto et al. found that, between the ages of 20 and 80 years, the circulating [H+] increased 6–8 % and [HCO3-] decreased 12–16 % [1]. The mild metabolic acidosis in older adults is thought to result from the combination of declining renal function and the daily ingestion of an acid-producing diet. Between the ages of 20 and 80 years, the GFR decreased by an average of 50 % [2], and in the process, the kidney was less able to excrete an acid load [3]. In the National Health and Nutrition Examination Survey (NHANES), acid-producing diets, estimated from protein and potassium intake assessed by a 24-h recall, were associated with lower serum bicarbonate levels in middle-aged and older adults but not in younger men and women [4]. In contrast to the findings of Frassetto et al. [1], serum bicarbonate levels in NHANES generally increased with aging [4].

Acid-producing diets can generally be characterized as having a relatively high content of protein and cereal grains in relation to their content of fruits and vegetables. Sulfur, found in cereal grains and protein, is metabolized to sulfuric acid whereas fruits and vegetables are metabolized to bicarbonate and other alkaline salts [5–7]. It is worth noting that although dietary protein is acid-producing, protein has a net positive effect on bone, mediated by its stimulation of the anabolic insulin-like growth factor 1 [8]. Remer and Manz assessed the potential renal acid load (PRAL) of many common foods [5]. The most acid-producing foods (i.e., those with the highest PRAL values) they assessed were hard cheeses. In contrast, raisins, other dried fruits, and spinach had the lowest PRAL values [5]. Milk and yogurt were found to be almost neutral.

The most direct measure of the acid-base balance is, of course, arterial blood pH. Oral administration of sodium bicarbonate supplements increased blood pH in a dose-related manner in healthy adults both at rest and during vigorous exercise [9]. Acquiring arterial blood is painful for study subjects and is therefore not feasible for most clinical investigation because subjects would be difficult to recruit and would tend not to return after the first measurement. The measurement of net acid excretion (NAE) in 24-h urine collections is currently the gold standard measure of acid-base status for clinical research. It reflects the acid-base balance of food actually consumed, as opposed to PRAL estimates which are based on what people said they ate. We measured NAE levels of 171 men and women age 50 and older at the time of their enrollment into one of our trials [10] and found that 96 % had positive NAE values, consistent with their consuming acid-producing diets. This supports the findings of others that the diets of many older adults are net acid-producing.

By a long-standing hypothesis, the daily influx of a dietary acid load triggers bone resorption in older adults and this is an adaptive response to preserve neutral pH. Bone is recognized to be an alkali reservoir. In the process of neutralizing the mild metabolic acidosis, bone mineral is lost and calcium from bone is excreted in the urine. The mechanisms by which a mild metabolic acidosis affects bone have been delineated. An acidic environment impairs osteoblast function [11–13] and it increases bone resorption [14] through activation of a recently identified proton receptor on osteoclasts [15]. Acid also has a direct physico-chemical effect that promotes bone resorption [16].

In this chapter, I will review the impact of supplementation with the alkaline salts, potassium bicarbonate ($KHCO_3$) and potassium citrate

(Kcitrate), on NAE and on bone-related outcomes, with particular focus on the optimal dose of $KHCO_3$ and the influence of the starting NAE level on the response to supplementation with $KHCO_3$.

Effect of Acid Base Balance on Bone Related Outcomes

Calcium Excretion

Bone is an alkali reservoir that, as indicated above, responds homeostatically to an acid environment by increasing resorption. In the process, calcium is released into the circulation, filtered by the kidney, and excreted in the urine. This has been demonstrated in the acute setting by Sebastian et al. who studied postmenopausal women on high protein metabolic diets [17]. When treated with $KHCO_3$, calcium excretion dropped rapidly and dramatically; the pattern reversed when the alkali supplements were discontinued. Frassetto et al. later demonstrated that the effect of alkali on calcium excretion was dose related, with linear inverse association over the supplement dose range of 0 through 90 mmol per day [18]. Moreover, the pattern was persistent over 12–36 months of supplementation. Similarly, Moseley et al. reported sequential declines in calcium excretion across Kcitrate doses of 0 (placebo), 60, and 90 mmol per day over a 6-month period [19]. The two available meta-analyses are concordant in noting a significant positive association of NAE with calcium excretion [20] and that supplementation with alkali lowered calcium excretion [21].

Calcium Balance

Calcium balance was assessed in 52 men and women aged 55 years and older who were selected to have high-normal calcium excretion based on the median values of a similar population [19]. They were randomly assigned to treatment with placebo, or 60 mmol or 90 mmol per day of Kcitrate for 6 months. Calcium balance was assessed at the beginning and end of the study. At 6 months, calcium excretion was significantly reduced in both Kcitrate groups whereas calcium absorption was unchanged. Calcium balance was significantly more positive in the 90 mmol dose group, and there was a similar trend toward improved calcium balance in the 60 mmol dose group [19]. The more positive calcium balance after 6 months indicates that the effect of alkali on bone is not transient, and suggests that longer term supplementation (or diet modification) might reasonably be expected to improve bone mass.

Bone Turnover Markers

Bone Formation

Several small studies have reported no significant impact of supplementation with alkaline salts of potassium on the bone formation marker, serum osteocalcin [10, 17, 22]. With the newer biochemical marker of bone formation, serum amino-terminal propeptide of type 1 procollagen (P1NP), findings have been varied. Jehle et al. reported that supplementation with 60 mmol per day of Kcitrate increased serum P1NP levels steadily over time and after 2 years of supplementation, serum P1NP was approximately 14 % higher than placebo [23]. This is consistent with the concept that decreasing acidosis would promote osteoblast activity [24]. Macdonald et al. found no impact of up to 55 mmol per day of Kcitrate on serum P1NP levels over a 2-year period [25]. We recently reported that supplementation with 81 mmol per day of $KHCO_3$ for 3 months significantly reduced serum P1NP levels by 11 % [26], which is consistent with the concept that formation is coupled to resorption (as discussed below). The fact that on the 3-month visit in our study, urinary NTX and serum P1NP were significantly correlated ($r=0.321$, $P<0.001$) supports the 'coupling' interpretation as does the fact that 3-month change in urinary NTX was significantly correlated with change in serum P1NP ($r=0.165$, $P=0.012$). Further work is needed to better understand the observed divergent effects of alkali on biochemical markers of bone formation.

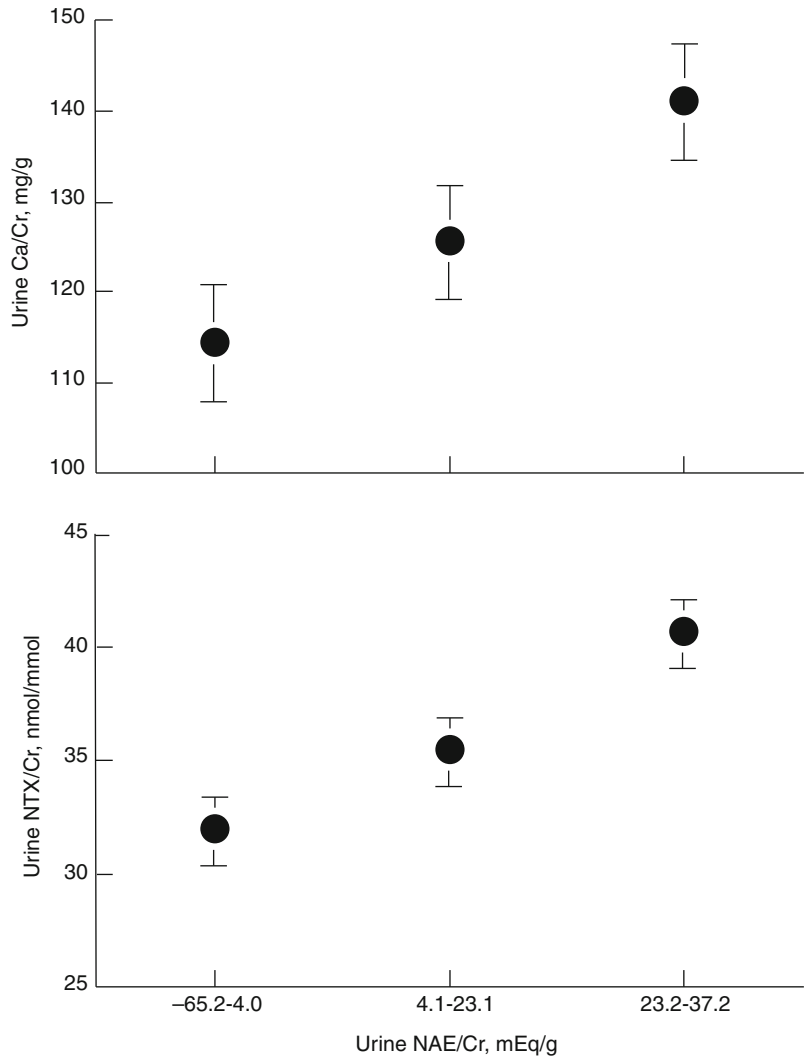

Fig. 9.1 Mean 24-h urinary N-telopeptide to creatinine (NTX/Cr) and calcium/Cr ratios by net acid excretion/Cr (NAE/Cr) tertile after 3 months of treatment with 67.5 mmol per day of bicarbonate or no bicarbonate in 171 subjects. NAE/Cr was significantly associated with Ca/Cr and NTX/Cr (after adjustment for sex, baseline value, sodium/Cr and potassium/Cr, $P<0.001$) (From Dawson-Hughes et al. [10]; reproduced with permission)

Bone Resorption

There is substantial and reasonably consistent evidence that supplementation with alkaline salts of potassium reduces urinary NTX, serum carboxy-terminal collagen crosslinks (CTX), and other biochemical markers of bone resorption. This has been observed in short-term studies [10, 17, 22] and in studies of 6-month [19] and 2-year duration [23]. Another 2-year study however identified no change in urinary NTX after supplementation [25].

Role of Dose Although we were not able to identify the optimal dose directly, we suspected that the dose tested in our earlier trial, 67.5 mmol per day, was inadequate [10]. That impression was based on finding a positive association between urinary NTX and NAE in all subjects after 84 days of treatment with bicarbonate or no bicarbonate (Fig. 9.1) shows urinary NTX/Cr and Ca/Cr levels by tertile of NAE/Cr. Since higher doses of bicarbonate sequentially lower NAE, this figure suggests that a bicarbonate dose higher than the 67.5 mmol per day administered might further lower urinary NTX, or bone resorption.

Subsequently, Moseley et al. assessed serum CTX in the 52 subjects in their calcium balance study [19]. Serum CTX decreased significantly in both Kcitrate groups when compared with placebo; however, the magnitude of the reduction

was numerically greater in the 60 mmol/day group (32 %) than in the 90 mmol per day group (14 %) [19].

We recently completed a dose-finding trial in which the resorption marker, 24-h urinary NTX, was the primary bone outcome [26]. This was a double-blind randomized placebo-controlled study in 244 healthy men and women age 50 years and older with eGFR ≥ 50 ml/min. The subjects were randomized to placebo or 1 mmol/kg or 1.5 mmol/kg of $KHCO_3$ daily for 3 months; 233 subjects completed the study. The mean age of the participants was 67 years and 48 % were females. The median administered doses in the low and high dose $KHCO_3$ groups were 81 mmol and 122 mmol per day, respectively. At baseline, urinary NTX levels in the 3 groups were similar. Changes in NTX in the three groups after 3 months of treatment, adjusted for baseline NTX, sex, and change in urine creatinine were: placebo −14 ± 11 nmol, low dose $KHCO_3$ −53 ± 11 nmol, and high dose KHCO3 −43 ± 11 nmol per day. Only the low dose differed significantly from placebo (P = 0.012). P1NP also declined significantly only in the low dose group (P = 0.004, adjusted for baseline P1NP and sex). Urinary calcium declined significantly in both $KHCO_3$ groups (P < 0.001, adjusted for baseline urinary calcium, sex, and changes in urine creatinine and calcium intake). To further explore the effect of $KHCO_3$ dose, we show mean urinary NTX reductions after 3 months of treatment from our two trials in subjects taking 67.5 mmol [10], and median doses of 81 and 122 mmol per day [26] in Fig. 9.2 [10, 26]. Collectively, our data support the use of 81 mmol per day for optimal reduction in bone resorption in older subjects who are consuming typical acid-producing diets that are common in the US [4]. There is no indication in our studies that increasing alkali intake to more than 81 mmol/day results in additional suppression of bone turnover.

Identifying the NAE Level Associated with the Greatest Suppression of Bone Resorption When we examine the NTX levels by category of NAE after 3 months of treatment in the 233 subjects in our dose-finding trial (Fig. 9.3), NTX levels are highest in subjects with NAE levels ≥ 15 mmol per day and are significantly lower in the NAE range of 10 to 15 mmol per day [26]. There is a further modest lowering of NTX with declining NAE levels to the neutral range of −5 to 5 mmol per day. At alkaline NAE levels below −5 mmol/day, there was no further reduction in NTX and even a suggestion that NTX may be rising, although the rise was not statistically significant. These findings support our hypothesis that there is a J-shaped relationship between NAE and the biochemical marker of bone turnover, NTX, and that the nadir of the J is at or very near neutral NAE, around −5 to 5 mmol/day.

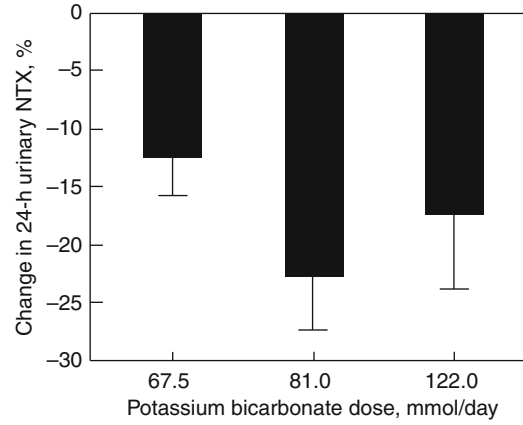

Fig. 9.2 Three-month changes (±SEM) in 24-h urinary N-telopeptide (NTX) by dose of $KHCO_3$ [67.5 mmol per day dose in 37 subjects, median 81.0 mmol per day dose in 79 subjects, and median122 mmol per day dose in 75 subjects; a cross-study comparison] [10, 26]

The Diet Characteristics of Subjects with Neutral and Higher NAE Levels In our dose-finding study, 36 % of the subjects had NAE levels at baseline that were −5 to 5 mmol per day, the range associated with the lowest urinary NTX [26]. Based on our record-assisted 24-h diet recall assessments, these subjects consumed an average of 8.1 servings per day of fruits and vegetables (F + V) and 5.5 servings per day of grains. Similar intakes of fruits and vegetables and grains are recommended in the Dietary Guidelines (9 and 6 servings, respectively). The F + V/grain servings ratio in our subjects with neutral NAE was 1.47 and this is also fairly similar to the ratio

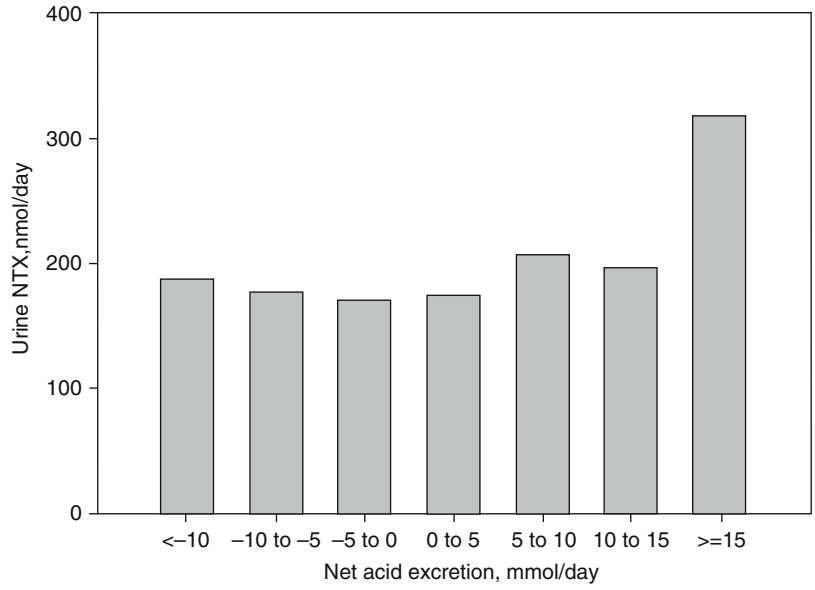

Fig. 9.3 Mean (± SEM) 24-h urinary N-telopeptide (NTX) by category of net acid excretion (NAE) after 3 months of treatment with placebo, or median doses of 81 mmol or 122 mmol per day of $KHCO_3$ (P for trend < 0.001) (From Dawson-Hughes et al. [26], reproduced with permission)

present in the DASH diet, which included 9.6 servings of F+V and 7.5 servings of grains per day [27]. Moreover, the reduction in urinary NTX in our dose-finding study was similar to that observed in the DASH study [28], suggesting that the acid-lowering impact of the DASH diet was responsible for its favorable effect in reducing bone resorption. As expected, subjects with higher NAE values at baseline were consuming fewer F+V and more grains. Those with NAE levels ≥15 mmol/day, for example, reported consuming an average of 5.5 servings per day of F+V and 7.2 servings of grains, giving a ratio of 0.77. It was notable that the protein intake of subjects in our dose-finding study was constant across the NAE categories and averaged about 86 g per day [26]. We had expected that the subjects with higher NAE levels would have had higher protein intakes. Dairy servings were also similar across the NAE categories, and averaged 2.1 servings per day.

To assess the effect of treatment on NAE levels in the 64 % of subjects in our dose-finding study who were consuming acid-producing diets, defined as NAE levels ≥5 mmol per day at entry, we determined the proportions who reached the optimal range, those who 'overshot' to alkaline levels, and those who continued to excrete an acid load in each treatment group [26]. As shown in Table 9.1, high dose treatment (median 122 mmol per day) reduced NAE to levels to below the

optimal NAE range of −5 to 5 mmol per day in 72.5 % of subjects, which may explain why this dose didn't cause a significant reduction in urinary NTX. Only 11.8 % of subjects treated with the high dose were in the neutral range; the remaining 15.7 % of subjects continued to excrete acid. The lower dose treatment (median 81 mmol per day) reduced NAE levels into the optimal −5 to 5 mmol per day range in 39.1 % of subjects, and the other subjects were fairly evenly distributed above (28.3 %) and below (32.5 %) that range. As expected, most subjects in the placebo group (76.5 %) continued to excrete acid.

Table 9.1 Urinary net acid excretion (NAE) levels after 84 days of treatment in the 148 older men and women with baseline NAE levels >5 mmol per day, by treatment group (placebo, low dose = 81 mmol of $KHCO_3$ per day, or high dose = 122 mmol of $KHCO_3$ per day)

NAE, mmol/day	Placebo (n = 51)	Low dose (n = 46)	High dose (n = 51)
< −5 n (%)	0 (0)	15 (32.6)	37 (72.5)
−5 to +5 n (%)	12 (23.5)	18 (39.1)	6 (11.8)
≥ +5 n (%)	39 (76.5)	13 (28.3)	8 (15.7)

Bone Loss and Fractures

Observational Studies

Several observational studies have identified inverse associations of dietary acid load with bone ultrasound and BMD in older adults [29–32]. A large observational study in France identified no overall association between renal net acid excretion (RNAE), calculated from a food frequency questionnaire, and fracture risk; however, there was a negative association in the subset of subjects with very low calcium intake (<400 mg per day) [33]. In a recent report, Jia et al. found no association between dietary acid load calculated from 7-day food records and risk of fractures in a large cohort of community dwelling adults age 70 years and older in Sweden [34].

Randomized Controlled Trials

Two 2-year, placebo-controlled, randomized trials have examined the effect of Kcitrate on rates of bone loss. Macdonald et al. found no significant effect of a low dose (18 mmol per day) or a moderate dose (55 mmol per day) of Kcitrate or of 3 servings of fruits and vegetables on rates of bone loss in postmenopausal women (70 per group) [25]. In contrast, Jehle et al. reported small but statistically significant increases in spinal and hip BMD in older men and women treated with 60 mmol/day of Kcitrate compared with placebo [23]. The Kcitrate treated subjects in the latter study also had improved trabecular density measured by high resolution peripheral quantitative computer tomography. It is unclear why the results of these trials differed. NAE was measured in one [23] but not the other [25], thus it is unclear how comparable the two study groups were with respect to dietary acid load at entry into the studies. More trials are needed to define the role of alkali supplementation on rates of bone loss and, if positive, also on fracture risk.

In conclusion, a large proportion of older adults are consuming acid-producing diets. These diets have been associated with increased bone resorption and calcium excretion. Short-term alkali intervention trials have demonstrated reductions in calcium excretion and biochemical markers of bone resorption whereas the two available placebo-controlled two-year trials have inconsistent results. Further work is needed to determine whether lowering the dietary acid load either through alkali supplements or diet modification will have a sustained favorable effect on bone mass and ultimately on fracture risk in older men and women.

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Vitamin E Homologues: Current Evidence

10

Tiffany C. Yang and Helen M. Macdonald

Abstract

Public health approaches to chronic disease prevention often advocates a healthy lifestyle, including a healthy dietary pattern and exercise regimen. Consumption of fruits, vegetables, and whole grains are promoted for their nutrient content and bioactive compounds such as carotenoids, polyphenols, and tocopherols. These compounds exhibit antioxidant and inflammatory properties and may therefore by beneficial to chronic diseases associated with low-grade chronic inflammation such as osteoporosis in postmenopausal women. The vitamin E homologue, α-tocopherol, has had significant interest due to its potent lipoperoxyl radical-scavenging capacity, leading to its use in food processing and supplementation. However, recent evidence has suggested that vitamin E homologues may have potentially different impact on bone indices and, though studies purport that vitamin E benefits bone through its anti-inflammatory properties, this relationship has not been shown in humans. In this chapter, we evaluate the current evidence on the two most prevalent vitamin E homologues, α- and γ-tocopherol, with bone indicators in peri-menopausal and postmenopausal women. We then present data on dietary sources of α- and γ-tocopherol in a population of postmenopausal women in northeast Scotland and examine whether there is a relationship between these homologues and four inflammatory markers.

Keywords

Tocopherols • Vitamin E • Antioxidant • Inflammation

T.C. Yang, PhD, MPH, RD (✉)
Rowett Institute for Nutrition and Health, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB21 9SB, UK
e-mail: tcyang@abdn.ac.uk

H.M. Macdonald, PhD
Department of Musculoskeletal Research, Health Sciences Building, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK
e-mail: hmacdonald@abdn.ac.uk

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Introduction

Osteoporosis is a personal and economic burden that disproportionately affects postmenopausal women who are at greater risk for osteoporosis due to waning levels of oestrogen; men are also at risk in later decades of life [1, 2]. Factors associated with an increased risk of osteoporosis include low peak bone mass, low body weight, smoking, low vitamin D and calcium intake, the use of certain drugs, hormonal status, low physical activity levels, and inflammation [3, 4]. The increase in oxidative stress and production of pro-inflammatory cytokines from the declining oestrogen levels after menopause are associated with bone loss [5]. Therefore, it would be beneficial to investigate the relationship between antioxidants and bone mineral density (BMD) and identifying factors that affect mineralization would have significant public health benefit. Few studies exist for dietary factors such as vitamin E on bone health [6].

Vitamin E is a potent antioxidant and anti-inflammatory fat-soluble vitamin that exists in eight naturally-occurring tocopherol (α, β, δ, γ) and tocotrienol (α, β, δ, γ) homologues [7, 8]. Discovered in 1922 as a micronutrient essential for reproduction, it is most known as a lipid-soluble antioxidant and anti-inflammatory agent, characteristics of which have been proposed to positively influence bone. Found in varying proportions in cereal grains, nuts, seeds, and foods that have been fortified with vitamin E, the greatest source of tocopherols come from vegetable oils [9, 10]. α- and γ-tocopherols are the most pervasive in diet. In Westernized countries such as the United States (US), the widespread use of soybean and corn oils have resulted in the majority of vitamin E dietary intake occurring in the form of γ-tocopherol, though α -tocopherol is the most prevalent form in the body due to selective transport from the liver into circulating lipoproteins by α-tocopherol transfer protein (α-TTP) [7, 10, 11]. There are differences between US and European concentrations of α- and γ-tocopherols in the blood due to differences in types of oils consumed, although the previous decades have seen an expansion in use of rapeseed/canola, sunflower, and soybean oils in northern Europe, which are frequently imported from the US [12].

Vitamin E supplements are typically in the form of α-tocopherol due to its higher antioxidant activity, selective uptake and distribution in the body, and ability to reverse vitamin E deficiency [13, 14]. This makes generalizations about the differential effects of the other homologues particularly challenging. While low levels of vitamin E in the form of α-tocopherol has been associated with diseases such as Alzheimer's [15], HIV [16], and others [17], many studies find no or negative effects from vitamin E supplementation [18–24]. α-tocopherol may exhibit a U-shaped curve [25, 26] whereby insufficient or excess amounts may be harmful [23, 27]. Several studies suggest that excess α-tocopherol may be damaging to bone through vitamin K interference [28] and exhibit pro-oxidant effects at high concentrations [27, 29]. γ-tocopherol has been associated with decreased prostate [30] and breast [31] cancer, but also with obesity [32] and elevated inflammation [33, 34].

Vitamin E Homologues and Bone Health

Animal studies have shown that the α-TTP deficient mice (α-TTP$^{-/-}$), a model of vitamin E deficiency, have reduced α-tocopherol concentrations, ataxia, infertility, and age-related behaviour issues; deficiencies that were ameliorated through α-tocopherol supplementation [35]. These animals also have an increased bone mass due to decreased bone resorption, shown by a decrease in the osteoclast surface and the bone resorption marker serum deoxypyridinoline; bone formation was not altered [35]. As α-tocopherol supplementation rescued the high bone mass shown by these mice, it was understood that α-tocopherol played a role in decreasing bone mass. In a separate experiment, when bone marrow cells of wild-type mice were cultured in the serum of α-TTP$^{-/-}$mice, it was observed that osteoclast formation was reduced [35]. This was also rescued in a dose-dependent manner when α-tocopherol was supplemented into the culture, suggesting that α-tocopherol affects bone through osteoclast

activity [23, 35]. The authors also showed that α-tocopherol stimulates osteoclast differentiation without affecting its antioxidant capability [35]. These authors suggest that α-tocopherol may have the ability to uncouple bone turnover.

γ-tocopherol is also hypothesized to uncouple bone turnover, through its role in the production of nitric oxide (NO) [36]. Reactive nitrogen species are increased during inflammation, and γ-tocopherol is a nucleophile capable of trapping these electrophiles and producing NO [37, 38]. NO can regulate osteoclasts and has been associated with reduced bone resorption and increased bone formation [39], though human studies have been inconclusive [40–42]. However, a high NO level in itself is damaging to bone, increasing osteoclast-mediated bone resorption and osteoblast apoptosis [36]. Moreover, γ-tocopherol elevates inflammation through increased leukocyte recruitment and cell signalling [43, 44]. While α-tocopherol is able to decrease the infiltration of leukocytes, this action was blocked by γ-tocopherol at levels 10% of α-tocopherol tissue concentrations, a ratio that is typically found in Western countries [33, 43]. γ-tocopherol has previously found to be positively associated with osteoarthritis, a disease marked by inflammation, whereas α-tocopherol was found to be negatively associated [45, 46]. These opposing functions of α- and γ-tocopherol on inflammation and their ability to uncouple turnover could influence their relationship with BMD.

The literature surrounding vitamin E and bone health in peri-menopausal and postmenopausal women are inconclusive. There are no randomized controlled trials assessing the effect of vitamin E on bone health. Human studies have focused on markers of bone turnover, BMD, or fracture risk (Table 10.1). Nine of the fourteen studies investigated dietary intakes of vitamin E [47–53], with only the study by [51] specifying dietary α-tocopherol. Some studies did not identify the food composition table used [48, 50]. Three studies focused on supplement use, either specifically vitamin E [54], a combination of vitamins E and C [54, 55], or supplements that contain vitamin E [56], while others took supplement use into account when assessing dietary vs. total (diet + supplement) intake [49, 57, 58]. Two studies measured only α-tocopherol in plasma [59] or serum [60], while α- and γ-tocopherol were both measured in serum in studies by Hamidi et al. (2012) and Wolf et al. (2005).

The intervention study by [55] does not provide strong support for the benefit of supplementing vitamin E. In that study, it was difficult to determine whether the proposed benefit in the study was due to vitamin E alone, or only in combination with vitamin C, while a study by [54], which did not differentiate results by sex, showed benefit of vitamin E supplementation on lowering concentrations of bone osteoclast markers, but not for osteoblast markers, in individuals who already had osteoporosis [54]. Cross-sectional studies of serum tocopherols are conflicting; studies have found positive associations between serum α-tocopherol and LS BMD [60], and γ-tocopherol with the osteoblast marker serum alkaline phsophatase [57], or no association of α-tocopherol or γ-tocopherol with bone turnover markers [57] or at any BMD site [58].

Study design differs across these studies, with dietary intakes assessed by 24-h recalls or food frequency questionnaires, some of which are validated. Vitamin E content of foods can vary depending on agricultural practices, seasons, weather, processing, etc. and therefore the vitamin E content of foods may be different in different food composition databases, as well as the method in which their content was measured. Dietary methods are subject to bias, but blood measurements may also not be precise or accurate in reporting biological value.

As α-tocopherol is the form of vitamin E selectively retained by the body, the use of only dietary measures to assess health endpoints should be cautioned against, as dietary intake does not reflect serum concentrations [61]. Additionally, blood may not be the best marker for endogenous biological value, as ingestion of tocopherols results in increasing levels in muscle and adipose tissue, as well as other organs, rather than in plasma [62]. As tissue sampling is difficult in human subjects, platelet and buccal cell vitamin E concentrations have been proposed as more valid indicators of biological status than the serum and plasma concentrations currently used [63].

Table 10.1 Human studies on vitamin E and bone health in peri- and postmenopausal women [57]

Author	Population characteristics	Vitamin E assessment, dose	Outcome measure	Results and comments
Sun et al. [47][a]	726 elderly Chinese with hip fracture matched with controls (n = 549 for women)	Food frequency questionnaire Tocopherol intakes based on Chinese Food Composition Table 2002 Dietary vitamin E (mg/d) intake (mean(SD)): Cases 7.71(4.11) Controls 10.07(4.46)	Risk of hip fracture	Dose-dependent inverse associations between dietary intake of vitamin E and risk of hip fracture (*P* for trend < =0.001 for women) OR of hip fracture for highest (v. lowest) quartile of intake 0.23 (0.16–0.33 95 %CI) for vitamin E for combined men and women Low vitamin E + low vitamin C in current smokers (v never smokers) have OR = 4.9 (2.2,11.0) in multivariable model, other combinations of E and C not significant
Melhus et al. [48][a]	Postmenopausal women (n = 205 cases; n = 746 controls)	Food frequency questionnaire Nutrient database not stated Dietary vitamin E (mg/d) (mean(SD)); Cases: Current smokers 5.6(2.7) Never smoker 6.5(2.6) Controls: Current smokers 6.1(2.2) Never smokers 6.3(2.3)	Hip fracture risk	OR for hip fracture among current smokers with low intake of vitamin E = 3.0(95 %CI 1.6, 5.4), OR = 1.1(0.5, 2.4) with high intakes of vitamin E among current smokers
Maggio et al. [59][a]	Postmenopausal women (n = 75 osteoporotic; n = 75 controls)	Plasma vitamin E (does not specify if α-) Plasma μmol/L mean(SD): Osteoporotics: 46.7(5) Controls: 62.8(8.76)	Bone health status (normal/ osteoporotic) and femoral neck BMD	Plasma α-tocopherol not correlated with FN BMD, lower in osteoporotics (*P* < 0.001)

Macdonald et al. [49][b]	891 perimenopausal women (aged 45–55 years at baseline, 50–59 years at follow up)	Food frequency questionnaire and supplementation questionnaire Nutrients from McCance and Widdowson's food composition tables Dietary vitamin E (mg) mean(SD): Baseline: 6.5(2.2) Follow-up: 6.6(2.2) Total vitamin E (diet + supplements) Baseline: 8.3(10.1) Follow-up: 13.3(32)	Femoral neck and lumbar spine BMD	Dietary vitamin E negatively correlated with change in FN BMD (r = –0.110) and LS BMD (r = –0.100) in adjusted models No findings with total vitamin E
Wolf et al. [58][c]	Postmenopausal women (n = 11,068)	Food frequency questionnaire and supplementation questionnaire Nutrients calculated using University of Minnesota Nutrition Coding Center version 30 Serum α-tocopherol and γ-tocopherol (n = 379) Dietary vitamin E(mg) mean(SD):7.8(3.8) Total vitamin E (diet + supplements) mean(SD): 28.9(49.4) Serum (μg/mL) mean(SD): α-tocopherol 15.4(6.3) γ-tocopherol 2.6(1.4)	Total body, L2–L4 lumbar spine, total hip (femoral neck and trochanter) BMD	Dietary intake of vitamin E positively associated with FN BMD when adjusted only for age (β = 0.011; p = 0.002); no significance after full adjustment. Total intake vitamin E negatively associated with FN BMD when only age-adjusted (β = –0.003; p < 0.0001), no significance after adjustment

(continued)

Table 10.1 (continued)

Author	Population characteristics	Vitamin E assessment, dose	Outcome measure	Results and comments
Zhang et al. [50][a]	Elderly Men and women. 1215 cases, 1349 control	Food frequency questionnaire Nutrient database not stated Vitamin E from food (mg α-TE/day) mean(SD): Never smokers: Cases: 10(6) Controls: 10(5) Ever smokers: Cases: 10(6) Controls: 11(6) Total vitamin E (diet+supplements) (mg α-TE/day) mean(SD): Never smokers: Cases: 127(194) Controls: 140(204) Ever smokers: Cases: 97(168) Controls: 146(195) Supplement use (%): Never smokers: Cases: 65.6 Controls: 66.9 Ever smokers: Cases: 59.6 Controls: 65.0	Hip fracture incidence	Among ever-smokers, those in highest quintiles of vitamin E intake (v. lowest) had OR 0.29 (95%CI 0.16, 0.52, p-trend <0.0001) of hip fracture. No associations in never-smokers
Hamidi et al. (2012)[c]	Postmenopausal women (n=497)	24-h dietary recall Serum α-tocopherol and γ-tocopherol	Serum bone alkaline phosphatase (BAP; a bone formation marker) and urinary *N*-telopeptides (uNTx/Creatinine; a bone resorption marker)	Adjusted vitamin E supplement use and total α-tocopherol intake negatively associated with increased BAP (β=–0.10; *P*=0.04; β=–0.04; *P*=0.01, respectively). Adjusted serum γ-tocopherol positively associated with BAP (β=0.10; *P*=<0.01); adjusted α:γ taio negatively associated with BAP (β=–0.08; *P*=<0.01). No associations with bone formation marker

Mata-Granados et al. [60][c]	Early postmenopausal Spanish Caucasian women (n = 232)	Serum α-tocopherol:lipid ratio Serum α-tocopherol (μmol/L) mean(SD): 25.6 (5.8) α-tocopherol:lipid ratio (μmol/L) mean(SD): 3.12 (0.64)	Femoral neck (FN) and lumbar spine (LS) BMD	α-tocopherol:lipid ratio positively associated with LS BMD (β = 0.255; *P* = 0.002); *P* for trend in tertiles is 0.03 for LS BMD. No significant results for α-tocopherol:lipid ratio with FN BMD
Michaëlsson et al. [51][b]	Postmenopausal women (n = 61,422 followed for 19 years)	Food frequency questionnaire Intake of dietary α-tocopherol calculated using Swedish National Food Administration Range: Quintile 1 (<4.5 mg/d α-tocopherol) to Quintile 5 (>6.0 mg/day) Supplement users: 25.8–29.6 % of population	Hip and other fracture risk	Compared with highest quintile of dietary intake (median: 6.8 mg/d), lowest quintile intake (median: 4.3 mg/d) had multivariable-adjusted hip fracture HR of 1.86 (95 %CI: 1.67, 2.06), HR of any fracture was 1.20 (95%CI: 1.14, 1.28) Vitamin-E containing supplement users in multivariable-adjusted HR of hip fracture was 0.78 (95 %CI: 0.65, 0.93), 0.86 (95 %CI: 0.78, 0.94) for any type of fracture for those in highest quintile (30 % of participants in highest quintile using supplements vs 26 % in lowest)
Sugiura et al. [52][a]	Postmenopausal women in Japan (n = 293)	Food frequency questionnaire and supplement questionnaire Nutrient intakes determined from the Standard Tables of Food Composition in Japan Vitamin E generated into dietary patterns along with six carotenoids, vitamins C and D; three patterns identified: "retinol" pattern characterized by high intakes of vitamin E Vitamin E values also generated into tertiles Vitamin E intake (mg/d) GM(95 %CI): 8.1(7.8–8.4)	Radial BMD	"Retinol" pattern marginally negatively associated with low BMD (P for trend = 0.059) in adjusted model No significant *P* for trend for increasing vitamin E with low radial BMD

(continued)

Table 10.1 (continued)

Author	Population characteristics	Vitamin E assessment, dose	Outcome measure	Results and comments
Farrell et al. [53][a]	Postmenopausal women (n = 244)	8 days of (3 at baseline, 2 at 6 months, 3 and 12 months) food records and food frequency questionnaire Analysis of nutrients using USDA food composition database in 2005 Vitamin E intake (mg/d) mean(SD): by diet record: 9(4) by FFQ: 8(5)	L2–L4 BMD, femur trochanter, femoral neck, Ward's triangle, total body	No association between vitamin E and BMD at any site
Chuin et al. [55][d]	Postmenopausal women (n = 34)	3-day food records Antioxidant supplements (600 mg vitamin E + 1000 mg vitamin C daily) vs resistance exercise (3×/week) for 6 months	FN and LS BMD	LS BMD remained stable whereas placebo group had decreased LS BMD
Pasco et al. [56][b]	Postmenopausal women (n = 533)	Antioxidant supplement use (vitamins C and E); dose unspecified though duration of use is known	Whole-body BMD, markers of bone turnover (C-telopeptide [CTx] and bone-specific alkaline phosphatase [BSAP])	Duration of vitamin C or E use negatively associated with CTx No associations for BSAP or whole-body BMD

[a]Case-control study
[b]Longitudinal study
[c]Cross-sectional study
[d]Intervention study

Sources of Tocopherols and Relationship with Inflammation in a Scottish Cohort

Utilizing the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort, a longitudinal study of women in northeast Scotland, we examined the dietary sources of α- and γ-tocopherol from the Scottish diet and the relationship between dietary and serum tocopherols with markers of inflammation.

Five thousand one hundred nineteen women aged 45–54 years were initially recruited between 1990 and 1994 using random selection from Community Health Index records [64, 65]. These baseline participants were originally recruited for a population-based screening programme for osteoporosis fracture risk where they completed bone densitometry scans and were given questionnaires for risk factor assessments. Follow-up visits occurred between 1997 and 1999 (n = 3883) and between 2007 and 2011 (n = 2130), where data on participants' blood samples (n = 2030) and dietary and physical activity data (n = 1682) were obtained. Participant characteristics are shown in Table 10.2.

Table 10.2 Patient characteristics

	N	Mean(SD)/%
Age (years)	2129	66.05(2.21)
Energy intake (kcal/day)	1681	2474.3(305.19)
BMI (kg/m^2)	2122	27.88(4.89)
Physical activity level	1681	1.72(0.21)
Alcohol consumption (g/D)	1681	1.59(3.55)
Plasma cholesterol total (mmol/L)	2034	5.65(1.12)
Smoking status		
Current	188	8.88
Nonsmoker	1929	91.12
National deprivation category		
1	578	27.28
2	895	42.24
3	170	8.02
4	288	13.59
5	143	6.75
6	45	2.12
Vitamin E supplementation		
Yes	13	0.61
No/Missing	2116	99.39
Average α-tocopherol intake mg/day	10	295
	N	Geometric Mean (SD)
Serum tocopherols (μmol/L)		
α-tocopherol	2029	12.25 (1.39)
γ-tocopherol	2029	0.66 (1.53)
Dietary intake (mg/d)		
α-tocopherol	1681	7.42 (1.47)
γ-tocopherol	1681	6.13 (1.67)
hs-CRP (mg/L)	2033	1.75 (2.89)
IL-6 (ng/mL)	2039	1.52 (2.05)
SAA (mg/L)	2039	36.33 (2.13)
E-selectin (ng/mL)	2018	36.30 (1.47)

For the third visit, we sought to determine the dietary sources of α- and γ-tocopherol. Semi-quantitative and validated 136-question food frequency questionnaires (FFQ) were collected, along with a questionnaire regarding supplement use [66, 67]. Thousand six hundred and one women provided FFQs at the third visit. We used the United States Department of Agriculture's food composition database (SR27) to calculate dietary intakes of α-and γ-tocopherol. While α-tocopherol concentrations were well-represented in the database, γ-tocopherol measures were not available for all food items and, in these instances, the foods were assumed to have null values in the analyses. The 136-questions were then reduced into 37 food groups, which were condensed into seven major groups to illustrate main sources of α- and γ-tocopherol sin the diet.

Fats and oils are main contributors to both α- and γ-tocopherol intake (Fig. 10.1) [68]. A difference then distinguishes the intakes of tocopherols, wherein the largest proportion of α-tocopherol comes from vegetables, biscuits, cakes, puddings, and sweets, fruits, and cereals. Dominant sources of γ-tocopherol biscuits, cakes, puddings, and sweets, sauces and condiments, and cereals. Thus, α-tocopherol intake may be seen as an indicator of better diet quality, while γ-tocopherol may be seen as a poorer one.

Next, we sought to examine the relationship between α- and γ-tocopherol with inflammatory markers, as most studies hypothesize that vitamin E benefits bone health by counteracting

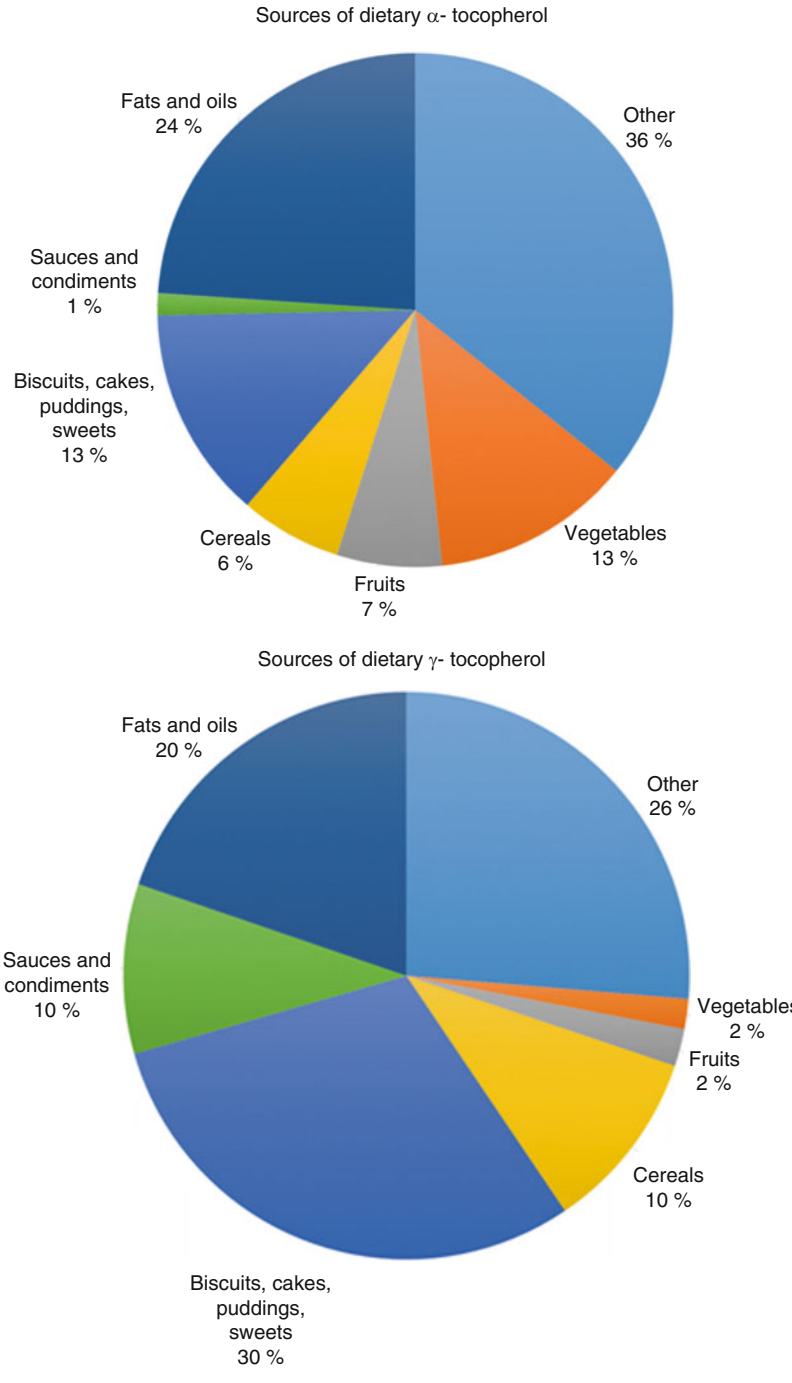

Fig. 10.1 Dietary sources of α- and γ-tocopherol in the Scottish diet. Other: red meat, processed meats, chicken, white fish, oily fish, other fish, eggs and egg dishes, dairy, bread, pulses, rice and pasta, soups, savoury snacks, coffee and teas, juices, fizzy drinks (diet/non-diet), alcohol

inflammation in the body, but the relationship between homologues and inflammation has not been examined in humans. In the process of bone remodelling, where a balance is maintained between osteoblastic bone formation and osteoclastic bone resorption, dysregulation could result in osteoporosis and an increase in fracture.

Fasted (12-h overnight or 4-h for afternoon study visits) blood samples were collected and serum prepared for storage (−80 °C). Samples were analysed in a single batch at the Department of Clinical Biochemistry, Aberdeen Royal Infirmary, Aberdeen, UK and tocopherols were analysed at the Rowett Institute of Nutrition and Health, University of Aberdeen, UK. Serum concentrations of α- and γ-tocopherol were determined by reversed-phase high performance liquid chromatography (HPLC) using fluorescence and visible detection after isohexane and chloroform extraction [69]. Total cholesterol, triglycerides, and high-sensitivity C-reactive protein (hs-CRP) serum concentrations were analysed with an automated assay (ADVIA 2400 Chemistry System; Siemens). Interleukin-6 (IL-6), serum amyloid A (SAA), and E-selectin concentrations were calculated using quantitative sandwich enzyme immunoassay kits (R&D systems). Inflammatory marker assays showed inter- and intra-assay CV values fewer than 5 %.

Using Spearman's correlations, we did not observe any association between dietary intakes of α- and γ-tocopherols with any inflammatory markers (Table 10.3). With serum concentrations, we observed significant positive correlations between γ-tocopherol with the inflammatory markers IL-6, hs-CRP, SAA, and e-selectin, but negative correlations for α-tocopherol with IL-6 and hs-CRP (Table 10.4). When we removed individuals taking vitamin E supplements, the same relationships persisted. These findings suggest that serum α- and γ-tocopherol are related

Table 10.3 Correlation between dietary intake and inflammatory makers

	Dietary intake[a]	
	α-tocopherol	γ-tocopherol
IL6	0.03[b]	0.04[b]
P-value	0.17	0.12
hs-CRP	−0.02[c]	−0.01[c]
P-value	0.43	0.66
SAA	−0.01[b]	−0.02[b]
P-value	0.55	0.53
E-selectin	0.02[d]	−0.002[d]
P-value	0.52	0.94

[a]Energy-adjusted residuals
[b]n = 1612
[c]n = 1614
[d]n = 1595

Table 10.4 Correlation between serum intake and inflammatory markers

	Serum concentration			
	All participants		Not taking vitamin E supplements	
	α-tocopherol	γ-tocopherol	α-tocopherol	γ-tocopherol
IL6	−0.20[a]	0.05[a]	−0.19[d]	0.04[d]
P-value	<0.0001	0.03	<0.0001	0.05
hs-CRP	−0.06[b]	0.15[b]	−0.05[e]	0.15[e]
P-value	0.01	<0.0001	0.01	<0.0001
SAA	−0.03[a]	0.13[a]	−0.03[d]	0.13[d]
P-value	0.13	<0.0001	0.19	<0.0001
E-selectin	−0.02[c]	0.14[c]	−0.01[f]	0.14[f]
P-value	0.38	<0.0001	0.50	<0.0001

[a]n = 2029
[b]n = 2010
[c]n = 2013
[d]n = 2016
[e]n = 1997
[f]n = 2000

to inflammation, but have different relationships with inflammatory markers.

Conclusions

The current literature does not support the use of vitamin E supplements and observational studies are contradictory in whether vitamin E in the diet or blood is beneficial. Using a population of postmenopausal women in northeast Scotland, we found that dietary sources of α- and γ-tocopherol may be an indicator of dietary quality. Dietary intakes of tocopherols were not correlated with inflammatory markers, but serum concentrations showed divergent relationships, with α-tocopherol associated with decreased, and γ-tocopherol with increased, correlations with inflammation.

Given the issues with the nutrient-approach, and that α- and γ-tocopherol may be indicators of dietary quality, future methods using a whole-diet approach may be more useful than single-nutrient approach.

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Part IV

Bioactives

Dietary Dried Plum Increases Peak Bone Mass

11

Mohammad Shahnazari and Bernard Halloran

Abstract

Dietary dried plum (DP) can increase bone volume in adult and old mice. To determine whether a DP diet can also increase bone volume in young growing mice and as such promote attainment of peak bone mass we fed young growing mice diets containing 5, 15 and 25 % DP or a control diet for 1 month. Bone volume increased progressively with increasing level of DP in the diet. DP supplementation (25 %) for 4 w increased bone volume in young growing (4–8 w old, +63 %), and young adult (8–12 w old, +94 %) mice. DP concentrations as low as 5 % significantly increased bone volume. In our adult mouse studies concentrations of 15 % were needed to see significant effects. Young growing and young adult mice are more sensitive to and have a greater overall skeletal response to DP than adults. Our findings suggest that consumption of DP in young growing and young adult mice can promote the attainment of peak bone mass. If this can be recapitulated in human, it may serve as an effective way of reducing osteoporotic fractures later in life.

Keywords

Dried plum • Peak bone mass • Fracture risk • Osteoporosis • Prevention

Introduction

Osteoporosis is a major medical health problem. Half of women and one-fourth of men may suffer an osteoporotic fracture in their lifetime. Annual care costs exceed $14 billion and are expected to increase dramatically as our population ages. Current available therapies are limited. Although

M. Shahnazari, PhD
Northern California Institute for Research and Education, 4130 Clement Street, San Francisco, CA 94121, USA
e-mail: shahmod@gmail.com

B. Halloran, BS, MDS, PhD (✉)
Department of Medicine, University of California, 1700 Owens Street, San Francisco, CA 94158, USA
e-mail: Bernard.halloran@ucsf.edu

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the pathogenesis of osteoporosis is still not well understood it involves a gradual decline in bone volume and structure. The bone becomes weak and little or no trauma can result in a fracture, most often of the hip or vertebrae. The amount or volume of bone in the osteoporotic patient reflects the rate of bone loss and the peak bone mass achieved during growth and development. Even though the rate of bone loss may be similar between two individuals the subject with a higher peak bone mass to begin with will have more bone later in life and be less likely to sustain an osteoporotic fracture. Attainment of peak bone mass is therefore critical for maintaining skeletal health later in life.

Effects of Dried Plum on Bone

We and others have shown that dietary supplementation with dried plum (DP) can increase bone volume and strength in normal adult and aged mice, and can prevent bone loss and restore bone already lost due to gonadal hormone deficiency [1–5]. Our previous studies have also shown that dietary DP can not only restore bone lost during aging but can increase bone mass above that seen in young adult mice [6–8]. This remarkable finding suggests that DP or its constituents may provide new and safe therapies for patients with osteoporosis.

To determine whether dietary consumption of (DP) can also promote attainment of peak bone mass during growth and development we supplemented the diet of young growing C57/B6 male mice (Jackson Laboratories, Sacramento, CA) with DP at levels of 0 % (control diet, AIN93G), 5, 15 and 25 % DP by weight. Diets were formulated in a pelleted form and provided equal amounts of energy, protein, fat, carbohydrate, calcium, and phosphorus [8, 9]. A careful assessment of macro- and micro-nutrients was made to ensure that all diets were equivalent. Food intake and body weight were recorded daily.

Diets containing 15 and 25 % have been shown previously to be effective in adults. Based on studies with other fruits we suspected that diets containing levels as low as 5 % may also be effective in young growing animals. Two age windows were studied that span the spectrum from young growing (group 1–2, one to two months of age, comparable to a human approximately 6–12 years old) to young adult (group 2–3, two to three months of age, comparable to a human approximately12–18 years old) mice. Group 1–2 mice were provided the control diet to 1 month of age, fed the control or test diet from 1 to 2 months and euthanized at 2 months of age. Group 2–3 mice were fed the control diet to 2 months of age, fed the control or test diet from 2 to 3 months of age and euthanized at 3 months of age. Each study group contained 8 mice and all animals were allowed to adapt to the vivarium for 1 week before experimentation. At the time of euthanasia, body composition including body fat mass, lean mass, free water, and total body water were assessed using a whole body NMR-MRI composition analyzer (EchoMRI, Houston, TX). After euthanization, the distal femoral metaphysis was scanned ex vivo with a Scanco *Viva*CT 40 (Scanco Medical, Basserdorf, Switzerland). Trabecular bone volume expressed as a percent of total volume (BV/TV), trabecular number (Tb.N; 1/mm), thickness (Tb.Th; μm), and connectivity density (Conn-dens; 1/mm^3), were measured.

Dietary Supplementation with DP Increases Peak Bone Mass

In our study, bone volume increased progressively with increasing level of DP in the diet. DP supplementation (25 %) for 4 w increased bone volume in young growing (4–8 w old, +63 %), and young adult (8–12 w old, +94 %) mice (Fig. 11.1). DP concentrations as low as 5 % significantly increased bone volume. In our adult mouse studies concentrations of 15 % were needed to see significant effects. Young growing and young adult mice are more sensitive to and have a greater overall skeletal response to DP than adults (Table 11.1).

The change in bone volume in young mice fed DP comes about as a consequence of increased

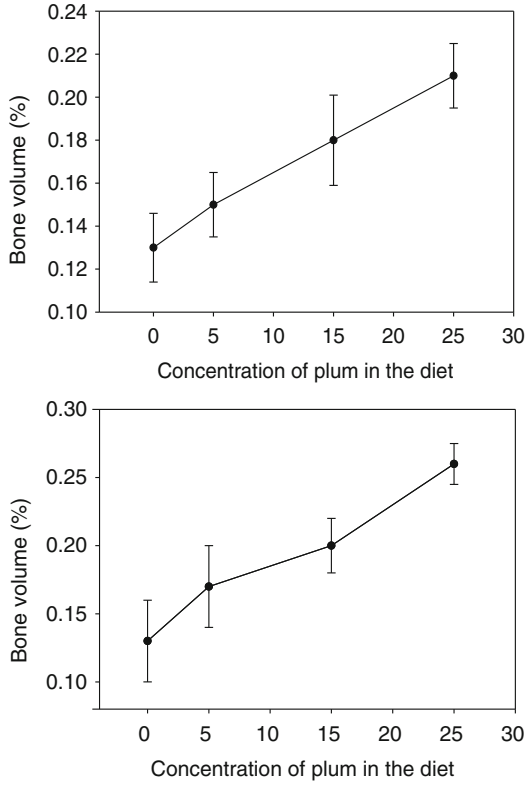

Fig. 11.1 Cancellous bone volume (BV/TV) in response to dietary supplementation with dried plum in 1–2 month old (*top*) and in 2–3 month old (*bottom*) mice. Concentratiopn of plum in the diet given in %

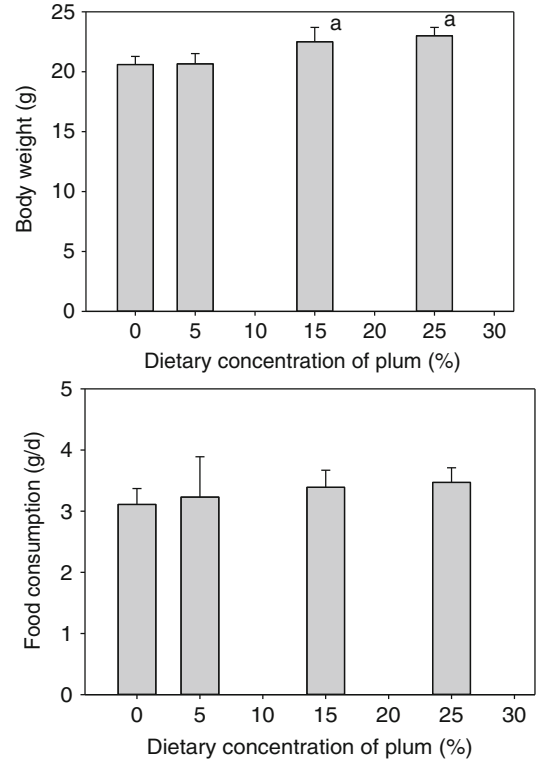

Fig. 11.2 Effect of dietary supplementation with dried plum on body weight and food consumption in 2 month old mice

Table 11.1 Effect of age on the response of cancellous bone (% increase) to dietary supplementation with dried plum in mice

Age (months)	% increase
1–2	64 %
2–3	94 %
3–4	64 %
6–12	78 %
18–24	33 %

All increases are significant, $p < 0.05$

trabecular thickness and number (Data no shown). Mass is being added to existing trabeculae and there is an increase in the bridging between trabeculae. As a consequence connectivity density increases by nearly 75 %.

The body weight of the 2 month old mice, but not 3 month old mice increased slightly. Food consumption was relatively unaffected by diet in both age groups (Fig. 11.2).

Combining data from these studies and earlier studies we calculated the effect of age on bone accrual in response to dietary DP and expressed the results as a percent increase over the control diet (Table 11.1). Bone volume in DP fed mice was greater at all ages but was highest in young adult mice (94 %) and lowest in the oldest mice (33 %).

Conclusions

Dietary supplementation with DP has an astonishing effect on bone at all ages.

Our findings suggest that consumption of DP in young growing and young adult mice can promote the attainment of peak bone mass and, if can be recapitulated in human, may

serve as an effective way of reducing osteoporotic fractures later in life.

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Transgenerational Benefits of Soy Isoflavones to Bone Structure in the CD-1 Mouse Model

12

Wendy E. Ward, Sandra M. Sacco, Elsa C. Dinsdale, and Jovana Kaludjerovic

Abstract

Diet during early life can set a lifelong trajectory for better bone health and is termed 'nutritional programming'. Using the CD-1 mouse model, exposure to soy isoflavones during neonatal life resulted in higher bone mineral density, improved bone structure, and greater bone strength of femurs and lumbar spine, and protected against ovariectomy-induced bone loss. The objective of this study was to determine if there are transgenerational benefits of isoflavones to bone health. Benefits to bone structure at femur neck but not at the femur midpoint, distal femur and LV3 were transferred to offspring. Understanding the mechanisms of this transgenerational inheritance requires further study.

Keywords

Bone structure • Isoflavones • Mice • Micro-computed tomography • Nutritional programming • Soy

W.E. Ward, MSc, PhD (✉) • S.M. Sacco, MSc, PhD
Department of Kinesiology, Faculty of Applied Health Sciences, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, ON L2S 3A1, Canada
e-mail: wward@brocku.ca; ssacco@brocku.ca

E.C. Dinsdale, MSc
Department of Nutritional Sciences, University of Toronto, 150 Colleage Street, Toronto, ON M5S3E2, Canada
e-mail: elsadinsdale@gmail.com

J. Kaludjerovic, MSc, PhD
Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, 188 Longwood Ave REB 315, Boston, MA 02115, USA
e-mail: Jovana_kaludjerovic@hsdm.harvard.edu

Introduction

Diet during early life can set a lifelong trajectory for better bone health. This concept is referred to as 'nutritional programming' and may occur by an epigenetic mechanism such as altered DNA methylation or histone modification, in which the expression of a given gene or group of genes, but not DNA sequence, is altered. Infants fed soy protein based infant formula are exposed to markedly higher levels of isoflavones (i.e., genistein, daidzein) than infants fed breast milk or cow's milk formula [1, 2]. Moreover, when isoflavone intake is calculated on a body weight

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basis, infants fed soy infant formula consume tenfold higher levels of isoflavones than adults who consume soy as a predominant protein source [3, 4]. Using the CD-1 mouse model, exposure to soy isoflavones with potential estrogen-like activity during the first 5, 10 or 21 days of life, resulted in higher bone mineral density, improved bone structure, and greater bone strength at young adulthood [5–8]. The effect is strongest in female mice. The improved bone status also protected against the deterioration of bone tissue that occurs after ovariectomy [9]. Altered DNA methylation is a possible mechanism as we have shown reduced expression of DNA methyltransferase 3a (Dnmt3a) in female mice exposed to isoflavones during the first 10 days of life using the CD-1 mouse model [10]. Dnmt3a is an enzyme that adds a methyl group to cytosine nucleotides and is involved in setting up DNA methylation patterns during development. Given there is some evidence for transgenerational epigenetic inheritance, the objective of this study was to determine if there are transgenerational benefits of soy isoflavones to bone structure in the subsequent generation of female mice (F2). We specifically chose to focus on bone structure in this preliminary study because it is a reliable functional measure of bone health.

Materials and Methods

Mouse Intervention

Male and female CD-1 mice were obtained at 6 weeks of age (Charles River Laboratories, Canada) and subsequently bred at 8 weeks of age. CD-1 mice were studied as this strain has been used in all our previous investigations studying effects of early life exposure to isoflavones in F1 generation [5–10]. Litters consisted of 8–12 mice and at birth pups were cross-fostered to minimize the risk that pups from within one litter are more similar to each other than among litters. Cross-fostered litters were randomized to one of two groups: corn oil control (CON) or isoflavone (ISO). The ISO intervention consisted of a combination of 2 mg daidzein/kg body weight/day + 5 mg genistein/kg body weight/day. Both corn oil and ISO was administered in a 20 µL volume once daily by subcutaneous injection for the first 10 days of life. This dose has been shown to result in similar total serum isoflavone level as infants fed soy based infant formula [11]. All offspring were weaned at age 21 days and were subsequently fed control diet (AIN93G) devoid of any isoflavones or other phytoestrogen for the remainder of the study. At 8 weeks of age, female offspring were bred with control males (that had not received any ISO) to obtain F2 generation females. F2 generation females were fed control diet throughout the study and were followed to 4 months of age to investigate potential transgenerational effects at a stage of life when peak bone mass is achieved in a mouse model [12]. Euthanasia was performed using carbon dioxide followed by cervical dislocation. One representative female from each litter, selected by average body weight for the group, was examined (n = 10 for CON; n = 8 for ISO). Lumbar spine and femurs were collected for structural analyses. All procedures were approved by the Animal Ethics Committee at the University of Toronto and followed the policies of the Canadian Council on Animal Care.

Micro-computed Tomography Analyses of Femurs and Lumbar Vertebrae

Scanning, Reconstruction and Reorientation

Bone structure of lumbar spine and femurs was performed using micro-computed tomography (Skyscan 1176, Bruker-microCT, Kontich, Belguim). Bones were wrapped in parafilm to prevent dehydration and placed axially in a foam holder for scanning. The scanning parameters for the femurs and lumber vertebrae were 9 µm pixel size, 45 kV, 545 uA, 0.25 mm aluminum filter, 850 ms integration time, 0.2° angle increment over a 180° scan and no frame averaging. Scanned images were reconstructed (NRECON version 1.6.6.0) using a Gaussian smoothing kernel and corrections for ring artifacts and beam hardening

were applied to reduce signal noise. The same reconstruction parameters and corrections were applied to all sample images within the study. Reconstructed images were then reoriented using DataViewer version 1.5.0 and transaxial images were saved.

Regions of Interest, Thresholding and Microstructural Analyses

The femur neck, distal metaphyseal region and midpoint of the femurs were analyzed to investigate various femoral sites that differ in the proportion of trabecular and cortical bone (Fig. 12.1). For analysis of trabecular bone microarchitecture at the femur neck, transverse slices were made from the largest circumference of the femoral head to halfway between the smallest circumference of the neck and its blending with the trochanters, resulting in a region of interest spanning 0.704–1.046 mm. For analysis of trabecular bone microarchitecture at the distal metaphyseal region, 100 axial slices (0.879 mm) starting 0.528 mm above the growth plate at the distal metaphysis and spanning toward the diaphysis of the femur were evaluated. For cortical bone, 51 axial-cut slices (0.448 mm) spanning above and below the midpoint of the femur were selected. At the lumbar vertebra, transverse slices were made along the whole bone excluding 50 slices (0.440 mm) above and below the proximal and distal growth plates, respectively, resulting in a region of interest spanning 1.645–2.595 mm (Fig. 12.2).

Manual contouring was used at all sites to separate the trabecular and cortical regions. Trabecular bone at the neck and distal metaphysis of the femur and at the lumbar vertebra were segmented using adaptive thresholding in three-dimensional space. For each voxel, the threshold was calculated as the mean of the minimum and maximum of all voxel grayscales within a radius of 8 voxels. A lower

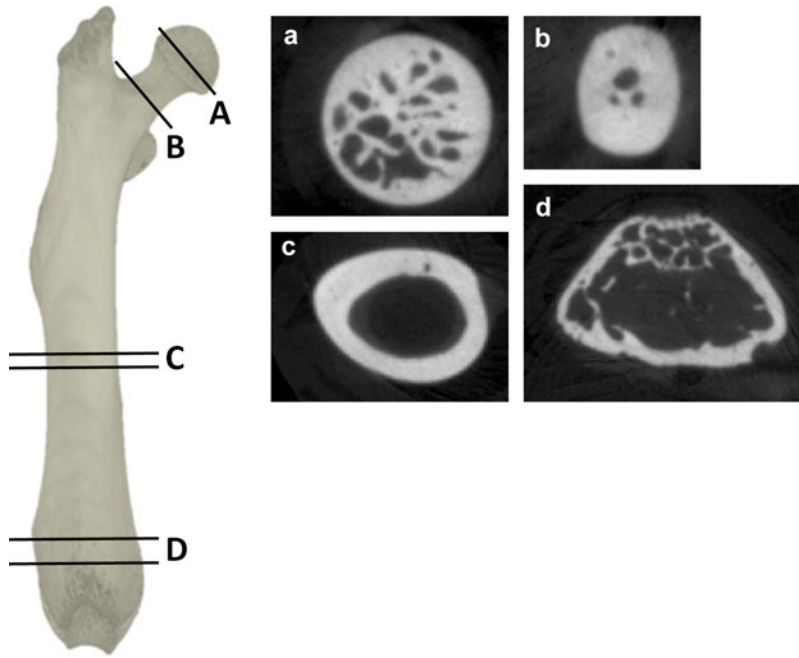

Fig. 12.1 Depictions of the regions of interest for femur analyses. At the femur neck, transverse slices were made from the largest circumference of the femoral head (**a**) to halfway between the smallest circumference of the neck and the blending with the trochanters (**b**). At the femur midpoint (**c**), a region consisting of 0.448 mm spanning above and below the midpoint of the femur was selected for cortical bone analyses. At the distal metaphysis (**d**), a region consisting of 0.879 mm starting 0.528 mm on the proximal end of the growth plate and spanning toward the diaphysis of the femur was evaluated

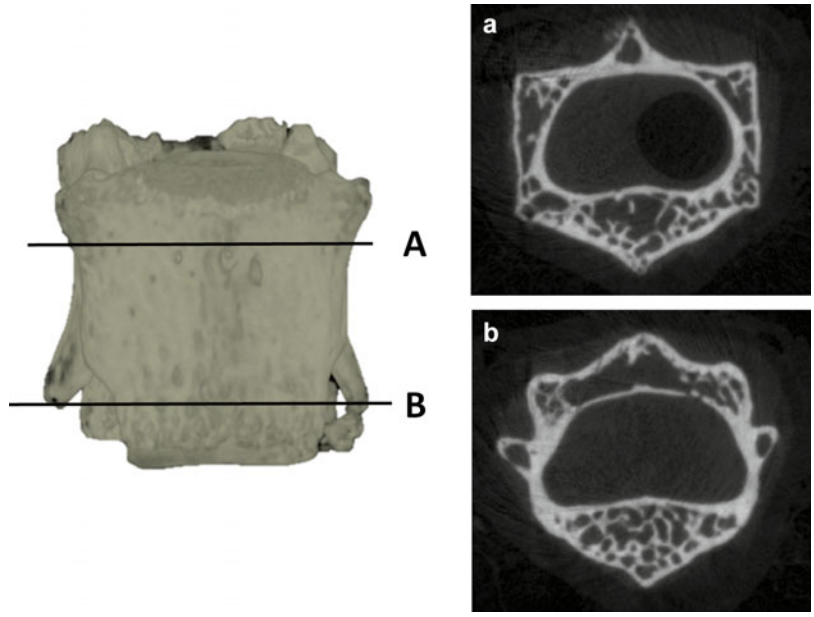

Fig. 12.2 Depiction of the region of interest selected for analysis at the lumbar vertebra. Transverse slices were made along the vertebra, excluding 0.440 mm above (**a**) and below (**b**) the proximal and distal growth plates, respectively

grey pre-threshold for the femur neck, distal metaphysis and lumbar vertebra were applied as 144, 82, 84, respectively, and the upper grey threshold at all sites was 255. A despeckling function was applied to all vertebral samples to remove any thresholded objects smaller than 10 voxels and the following three-dimensional trabecular bone properties were evaluated: bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, mm^{-1}), trabecular separation (Tb.Sp, mm), structure model index (SMI, 0 = parallel plates, 3 = cylindrical rods). Cortical bone at the femur midpoint was segmented using global thresholding (lower grey threshold = 120, upper grey threshold = 255). A shrink-wrap function in two-dimensional space was then applied to make the boundary of the region of interest to exactly follow the boundary of the periosteal envelope. Two-dimensional properties were then analyzed: total cross-sectional area inside the periosteal envelope (Tt.Ar, mm^2), cortical bone area (Ct.Ar, mm^2), cortical area fraction (Ct.Ar/Tt.Ar, %), and cortical thickness (Ct.Th, mm).

Statistical Analyses

Differences between CON and ISO groups were determined using unpaired t-tests (SigmaStat; Systat Software, Inc., Version 3.5, Chicago). Results are expressed as mean ± standard error of the mean (SEM). The significance level was set to $p < 0.05$.

Results

Improved structure at the femur neck was observed in F2 generation females whose mothers had received isoflavones during the first 10 days of life. This included higher BV/TV ($p = 0.006$), Tb.N ($p = 0.03$) and Tb.Th ($p = 0.03$) as well as lower Tb.Sp ($p = 0.02$) and SMI ($p = 0.04$) (Fig. 12.3a–e). At the distal femur, there were no differences in BV/TV, Tb.N, Tb.Th or Tb.Sp between groups (Fig. 12.4a–d).

At the lumbar vertebra, only Tb.Th was different between groups, with Tb.Th. greater among female offspring whose mothers were exposed to isoflavones in early life (Fig. 12.5a–e). There

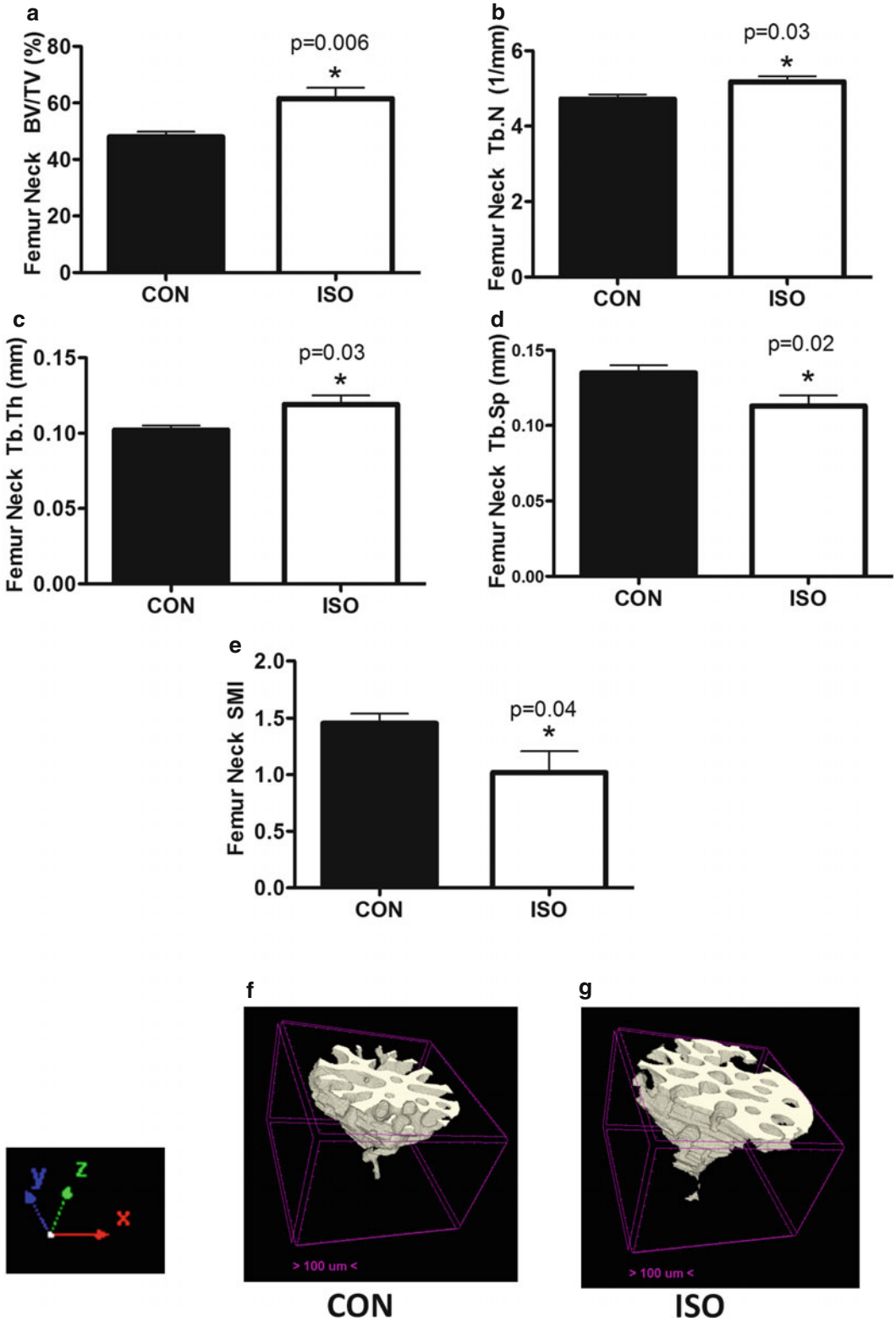

Fig. 12.3 Trabecular bone parameters of femur necks (**a**–**e**) and a representative micro-computed tomography image for the CON and ISO groups (**f**, **g**)

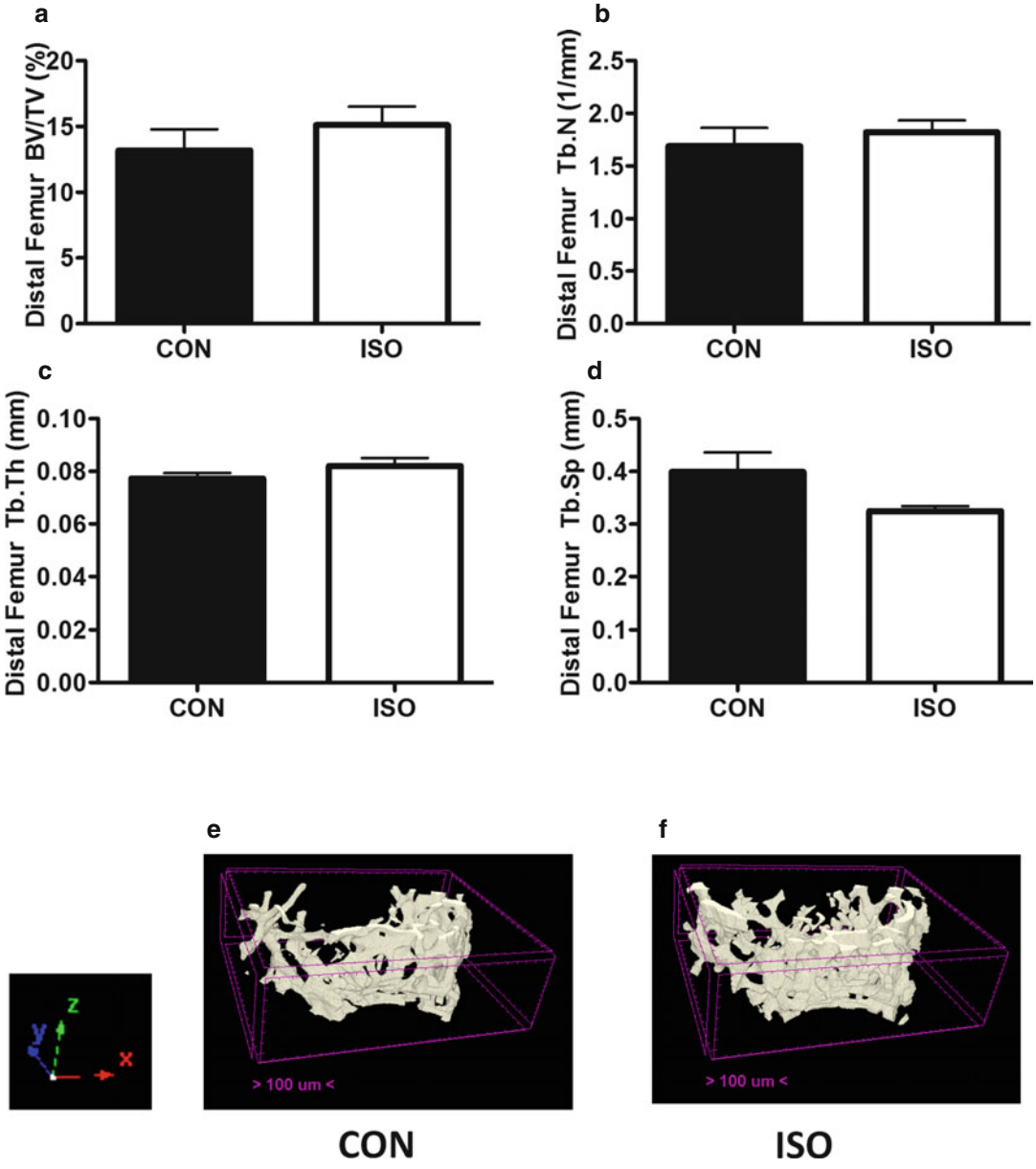

Fig. 12.4 Trabecular bone parameters of distal femurs (**a**–**d**) and a representative micro-computed tomography image for the CON and ISO groups (**e**, **f**)

were no differences in Ct.Ar/Tt.Ar or Cs.Th. at the femur midpoint (Fig. 12.6a, b). Representative images of trabecular structure at the femur neck (Fig. 12.3f, g), distal femur (Fig. 12.4e, f), lumbar vertebra (Fig. 12.5f, g), and cortical structure at femur midpoint (Fig. 12.6c, d) provide visual evidence that support the data.

Discussion

Our findings show that F2 generation females whose mothers were exposed to ISO during the first 10 days of life, exhibit some but not all of the benefits to bone health that are observed in the first generation. Because the F2 generation

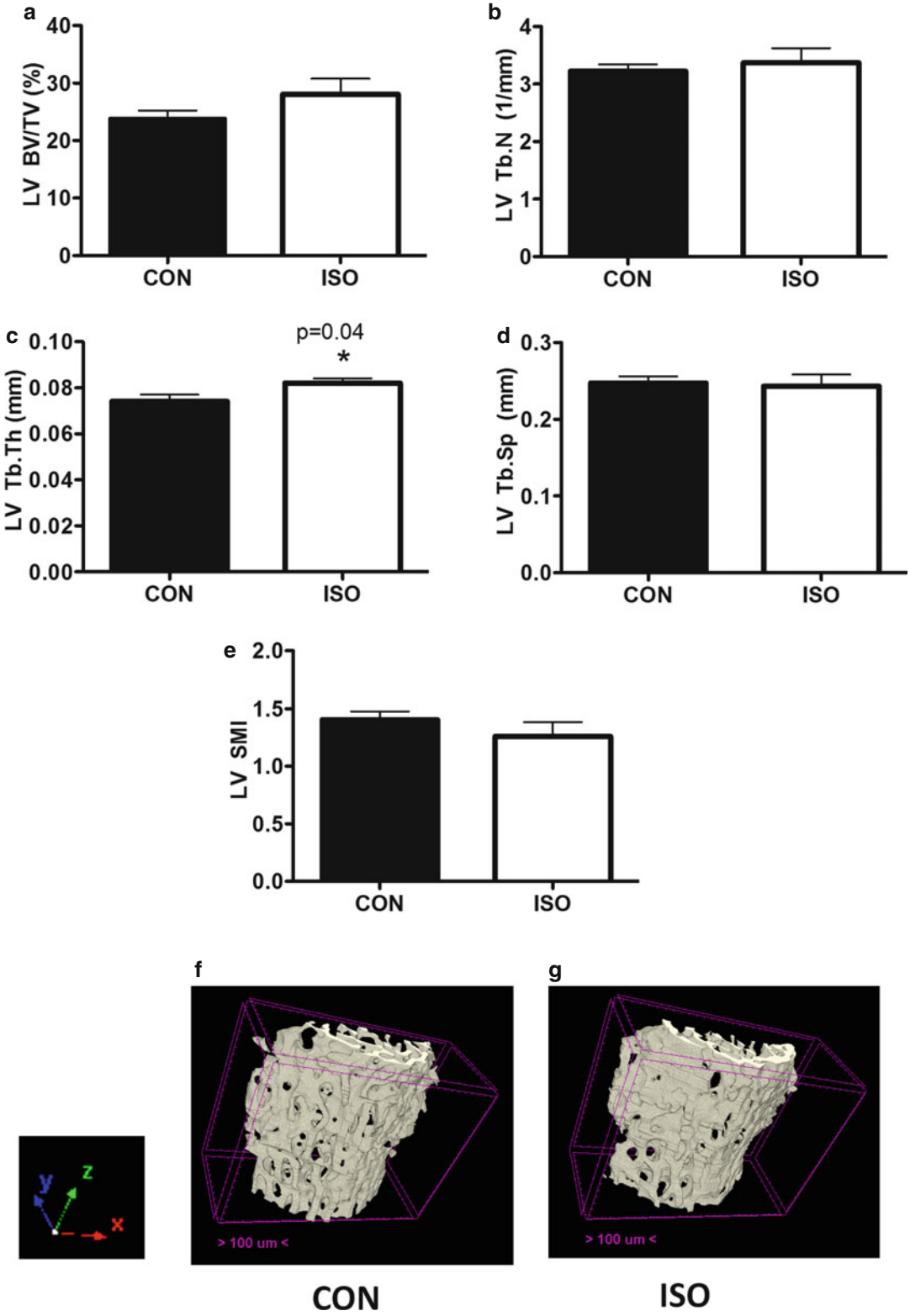

Fig. 12.5 Trabecular bone parameters of lumbar vertebrae (**a**–**e**) and a representative micro-computed tomography image for the CON and ISO groups (**f**, **g**)

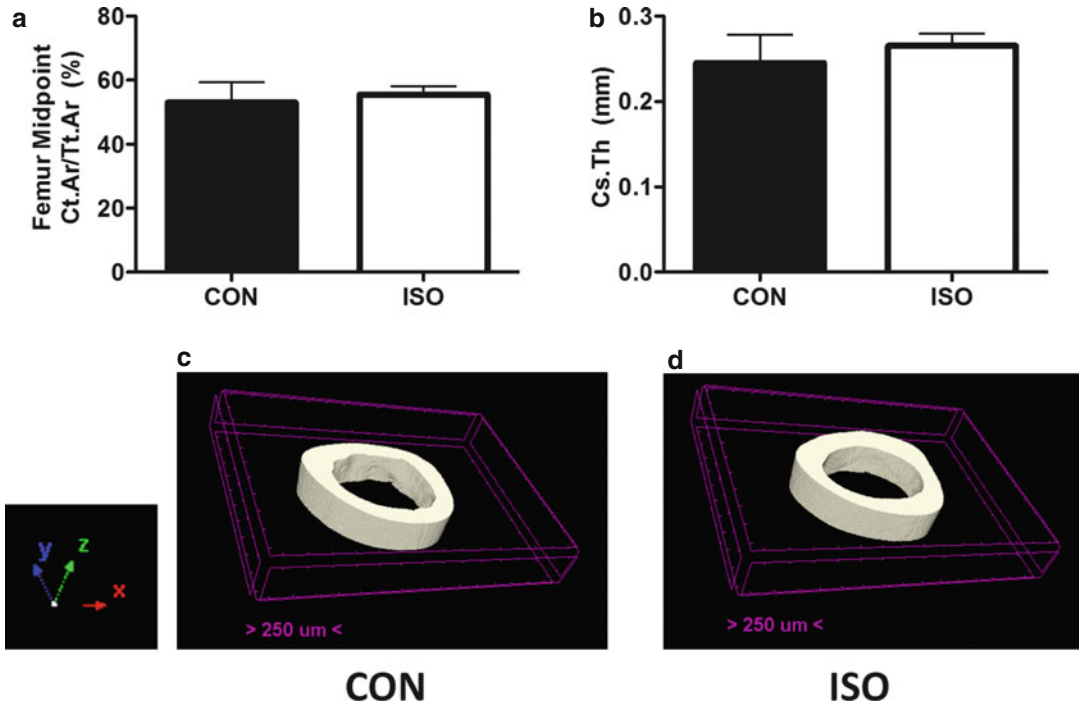

Fig. 12.6 Cortical bone parameters of femur midpoint (**a**, **b**) and a representative micro-computed tomography image for the CON and ISO groups (**c**, **d**)

females received no direct intervention, the observed benefits to this generation are likely due to transgenerational inheritance. Among F2 generation females, beneficial effects were strongest at the femur neck with a markedly lesser effect at lumbar spine and no benefit at the distal femur and femur midpoint. The improvements in trabecular thickness and connectivity resulted in higher bone volume at the femur neck, and suggest a strong and meaningful inheritance. Other sites such as the distal femur and lumbar vertebra – that have measurable amounts of trabecular bone – were not significantly improved but the images and data suggest that statistically significant differences may have emerged with a larger sample size. The exclusive presence of cortical bone at the femur midpoint indicates that inheritance did not occur in this bone type, although stronger and improved cortical structure has been observed in the F1 generation. The skeletal-site specific effects emphasize the importance of investigating multiple bone sites when assessing the impact of nutritional programming, and particularly the potential for transgenerational inheritance.

Present understanding of trangenerational epigenetic inheritance is complex and in its infancy. As articulated in a recent review [13], the fact that DNA methylation and other epigenetic changes are removed or introduced during development makes it difficult to understand how epigenetic traits are passed to subsequent generations. With an increasing number of studies, including the present study, providing evidence of transgenerational epigenetic inheritance, further investigation is required to understand the mechanism of action.

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13 A Lignan-Rich Bioactive Fraction of *Sambucus williamsii* Hance Exerts Oestrogen-Like Bone Protective Effects in Aged Ovariectomized Rats and Osteoblastic Cells

Hui-Hui Xiao, Man-Sau Wong, and Xin-Sheng Yao

Abstract

Sambucus williamsii Hance as a folk medicine has been used for the treatment of bone and joint diseases for thousands of years in China. The present study aims to examine the effects of the bioactive fraction (SWC) of *S. williamsii* on bone in aged OVX rats, identify the major bioactive components in SWC and circulation post-ingestion, and investigate the mechanism involved in its bone protective effects. The results indicated that the lignan-rich fraction SWC is effective in protection against ovariectomy induced bone loss in aged rats and its actions might be mediated by the direct oestrogen-like action of lignan on osteoblasts.

Keywords

Sambucus williamsii Hance • Chemicals • Lignan • Aged OVX rat • Osteoblasts • Nongenomic ER signaling pathway

H.-H. Xiao, PhD
State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Shenzhen Research Institute of The Hong Kong Polytechnic University, South High-Tech 7 Road, Shenzhen 518057, China
e-mail: irene.xiao@polyu.edu.hk

M.-S. Wong, PhD (✉)
Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon 999077, Hong Kong
e-mail: man-sau.wong@polyu.edu.hk

X.-S. Yao, PhD
Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Huangpu Road, Guangzhou 510632, China
e-mail: tyaoxs@jnu.edu.cn

Introduction

Sambucus willamsii Hance (SWH) (North China red elder), reclassified to Adoxaceae from Caprifoliaceae using new genetic evidence, is one of the five *Sambucus* species in China [1]. The dried stem and branches of SWH was first recorded in Tang Materia Medica (A.D. 659) for healing fractures and alleviating pain. It also has been used as a folk medicine for the treatment of joint disease, rheumatoid arthritis, gout, Kaschin-Beck disease, as well as wounds [2].

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Our previous study demonstrated that the ethanol extract of SWH exerted protective effects on trabecular bone and cortical bone in both ovariectomized (OVX) rats and mice [7, 9]. Its mechanism involved the stimulation of osteoblast differentiation, inhibition of osteoclastogenesis as well as osteoclast differentiation and maturation [7, 9]. However the high dose SWH stimulated the increase of uterus weight [9]. Subsequently, the extract of SWH was separated to four fractions using macroporous resin column in order to accumulate its active components and reduce its side effects. A fraction (SWC, 50 and 95 % eluates) of SWH was identified as the bioactive fraction for its preservation of bone mineral density, biomechanical strength and bone micro-architecture without uterus weight gain in OVX mice [3]. Our recent photochemical studies showed that the major components in SWC are lignans which account for the bone protective effects of SWC [4].

The present study was carried out to further investigate the potential of SWC to be an agent for osteoporosis treatment. The present study aims to examine the effects of bioactive fraction (SWC) of *S. williamsii* on bone in aged OVX rats, identify the major bioactive components in SWC and in the circulation post-ingestion, and investigate the mechanism involved in its bone protective effects.

Dose-Dependent Effects of SWC Extract on Bone Properties in Aged Ovariectomized Rats

A total of 96 specific-pathogen-free female rats, aged 9 months, were used in this study. All animals were allowed free access to distilled deionised water and OVX rats were pair-fed a phyto-oestrogen-free diet (D00031602; Research Diets, Inc., New Brunswick, NJ, USA). The rats were either sham-operated (Sham, *n* 18) or ovariectomized (OVX, *n* 78). Twelve weeks after ovariectomy, a subset of sham (*n* 6) and OVX rats (*n* 6) were sacrificed and scanned using Micro-CT to confirm the loss of bone. The remaining OVX rats were randomly divided into six groups: vehicle-treatment group (OVX, *n* 12); Premarin administration group (*n* 12; 0.13 mg/kg/d); XG (Xinling Gubao group; *n* 12; 320 mg/kg/d); SWCL (SWC low dose group; *n* 12; 57 mg/kg/d); SWCM (SWC mid dose group; *n* 12; 114 mg/kg/d) and SWCH (SWC high dose group, n 12; 228 mg/kg/d). Premarin, a commercial conjugate estrogen, and Xinling Gubao, a commercial traditional Chinese medicine formula, were used as positive controls. After oral administration for 12 weeks, the rats were sacrificed; blood, uterus and tibia were collected for further analysis. The animal study scheme was shown in Fig. 13.1. Uterus index was calculated as the ratio of uterus weight to body weight of each rat. The proximal metaphysic of tibia in the axial direction at a distance of 2.2 mm was scanned with a high-resolution micro viva CT 40 system (Scanco Medical, Brassdorf, Switzerland). All scans were done in 21 μm sections to a total of 100 slices per scan.

Twelve weeks after ovariectomy, in OVX rats, the uterus weight was significantly decreased and the bone properties was significantly deteriorated compared with Sham group, suggesting that the osteoporosis model was established successfully. The osteoporotic rats were then orally administrated with different treatment for 12 weeks. Body weight and uterus index were not altered in OVX rats in response to treatment with SWC extract. High dosages of SWC extract significantly suppressed urinary calcium (P <0.05) and phosphorous level (P <0.01) in OVX rats. Moreover, high dosages of SWC extract significantly suppressed bone loss (BMD, P <0.05), improved bone micro-architecture (Conn.D, P <0.05) and increased bone strength (Elastic modulus, P <0.05) on tibia (Fig. 13.2), while the mid dosages of SWC extract only exerted some beneficial trend on bone without any statistic significance. The potency of the protective effects of SWC extract on trabecular BMD, connectivity density (Conn. D) and cortical bone elastic modulus was comparable with those of Premarin (Fig. 13.2). The above results indicated that high dose SWC exerted bone protective effects on aged osteoporotic rat model without side effect on uterus index.

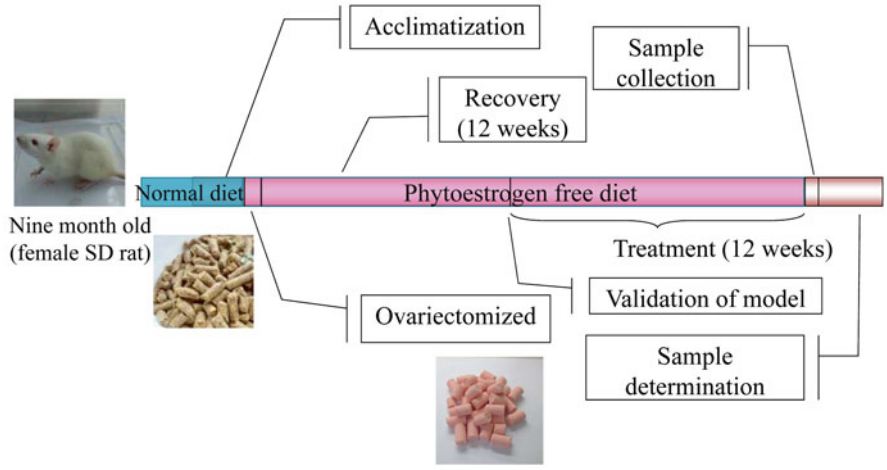

Fig. 13.1 Animal study design The rats were sham or ovariectomized operated after acclimatization for 1 week. After confirmation of the loss of bone mass at 12 weeks using the Micro-CT data of a subset of rats, the rest rats were treated with different extracts for 12 weeks. A phytoestrogen-free diet which was prepared with corn oil instead of soybean oil was used throughout the experiment to eliminate interactions between diet and extracts

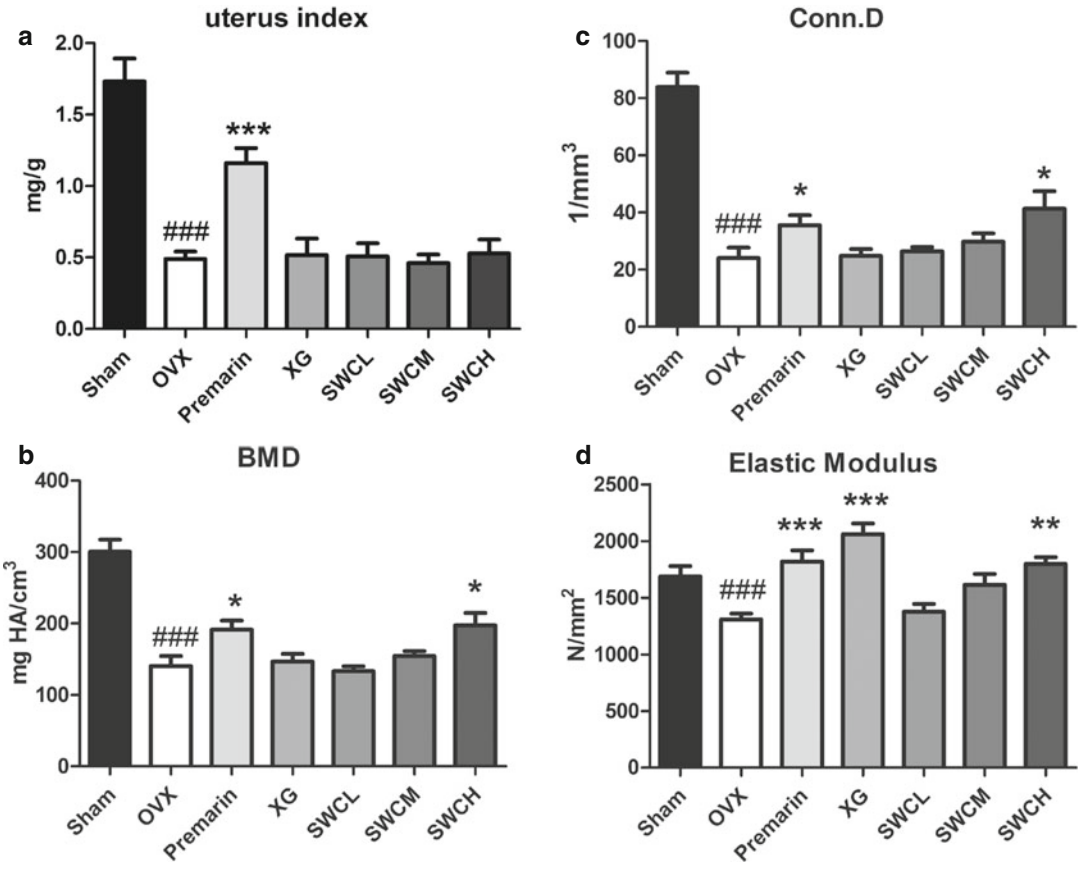

Fig. 13.2 Effects of the bioactive fraction of *Sambucus williamsii* Hance on uterus index and bone (**a**) Uterus index, the value was represented as uterine weight divided by body weight; (**b**) Bone mineral density (BMD); (**c**) Bone connectivity density (Conn. D); (**d**) Elastic Modulus. Data were expressed as mean ± SEM. ### P <0.001 compared to Sham group. * P <0.05, ** P <0.01, *** P <0.001 compared to OVX group

Bioactive Ingredients in SWC That Account for Its Bone Protective Effects

Although the beneficial effects of SWC on bone has been demonstrated repeatedly, the ingredients which are responsible for its bone-protective effects remain unclear. We systematically investigated the chemical compounds of SWC using various chromatographic techniques. Eighty-five compounds were isolated from SWC, including 55 lignans [4], 6 phenolic acids [5], 12 triterpenoids [8] and 12 other compounds (Fig. 13.3). Their structures were elucidated by spectroscopic analyses such as nuclear magnetic resonance (NMR), infrared spectra (IR), ultraviolet spectra (UV) and circular dichroism spectra (CD) (Fig. 13.4). The proliferation effects of all compounds were evaluated for their anti-osteoporosis potential on osteoblastic-like UMR 106 cells using MTS assay. 40 lignans, 6 phenolic acid, 9 tritepenoids, and one aliphatic compound exerted significant stimulatory effects on cell proliferation. The *in vitro* results suggested that lignans were the bioactive ingredients that account for the protective effects of SWC on bone.

To identify the bioactive ingredients that account for the protective effects in vivo, we aimed to characterize the chemical ingredients in blood obtained from animals treated with herbal extract. Ten weeks old C57BL/6 J mice were orally administrated with the simplified subfraction (50 % methanol-aqua eluate of ODS column) of SWC for 3 days. After the last administration, the mice plasma was collected at different time points of 0, 0.5, 1.0, 1.5 and 2.0 h. The plasma at same time point ($n=3$) were pooled together, deproteined and analyzed using UPLC/QTOF MS (Waters, Manchester, UK). The results showed that two lignans *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropanyl)-2-methoxy- phenoxyl]-1, 3-propanediol (PPD) and dihydrodehydrodiconiferyl alcohol DDA (Fig. 13.4) were identified in treated plasma and their maximum absorption was at 0.5 h. The results further suggested that lignans were the bioactive ingredients of SWC.

Mechanism Involved in Mediating the Bone Protective Action of the Identified Bioactive Ingredients in SWC

Although lignans were absorbed into blood, their actions and mechanism involved in bone anabolic effects are still unclear. The actions and mechanism of PPD, one of the absorbed components into blood, was characterized using osteoblasts. The protective actions of PPD were determined by proliferation assay, alkaline phosphatase (ALP) activity assay as well as calcium deposition. To determine if PPD activated ER and if its effects were ER dependent, estrogen receptor (ER) antagonist ICI182,780 and mitogen-activated protein kinase kinase (MEK) inhibitor U0126 blocking assay, competitive ER radioligand binding assay, ERE-dependent luciferase reporter assay and immunoblotting were used.

PPD at 10^{-12} to 10^{-8} M significantly increased cell proliferation in MC3T3-E1 cells and UMR 106 cells. It also significantly altered the differentiation potential of mesenchymal cells into osteoblasts. In addition, co-treatment of cells with ICI182,780 as well as U0126 completely abolished the stimulatory effects of PPD on cell proliferation and ALP activities. Further mechanism results showed that PPD failed to bind to either ERα or ERβ, and also failed to activate ERα or ERβ-medicated ERE-dependent luciferase activities in UMR 106 cells. However, PPD significantly induced ERK phosphorylation and Ser118 phospho-ERα to ERα protein expression at both 10^{-10} and 10^{-8} M, while the phosphorylation induced by PPD was abolished by U0126 (Fig. 13.5).

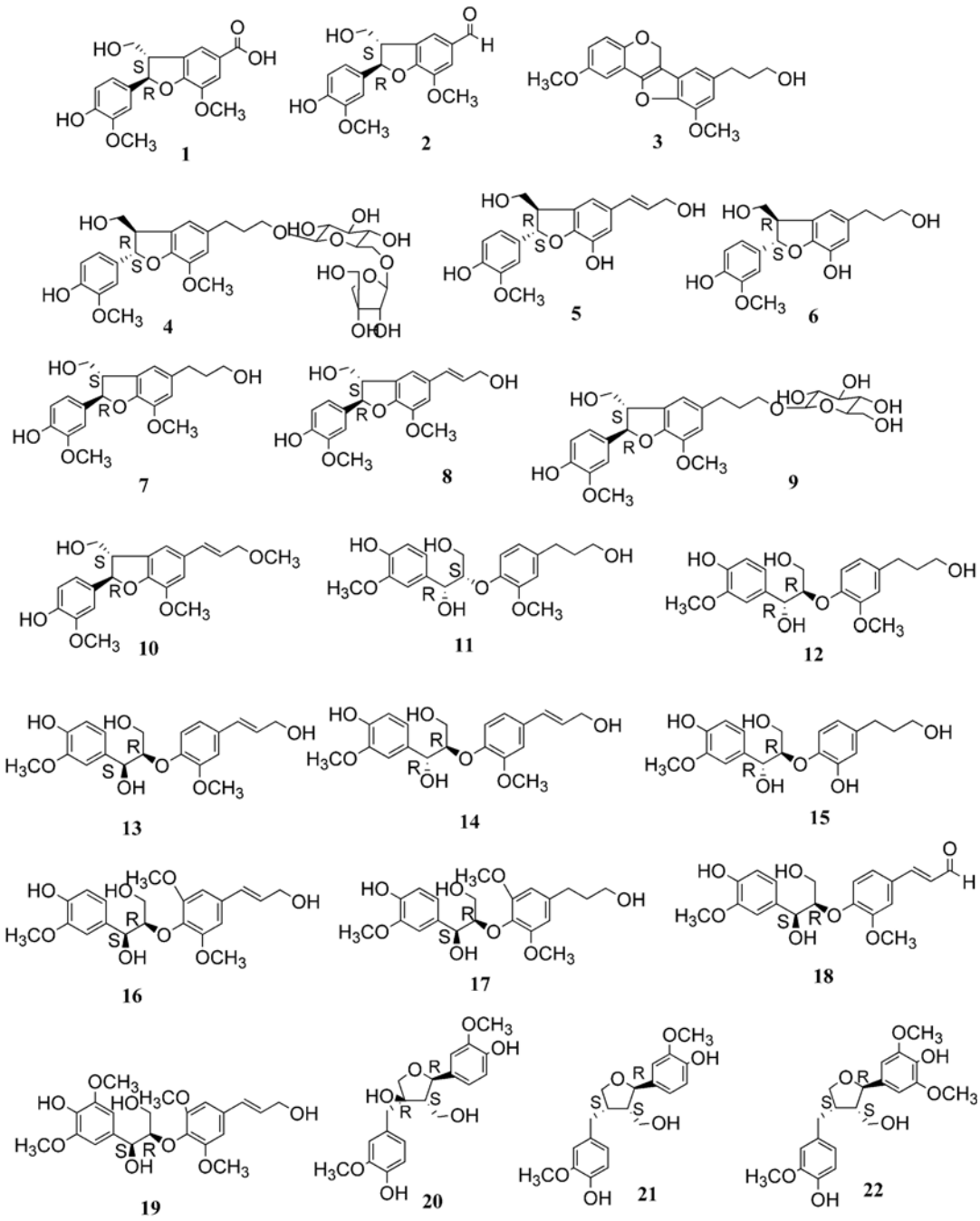

Fig. 13.3 Percentage and partial structures of isolated compounds in the bioactive fraction of *Sambucus williamsii* Hance 85 compounds were isolated from SWC, including 55 lignans, 6 phenolic acids, 12 triterpenoids and 12 other compounds

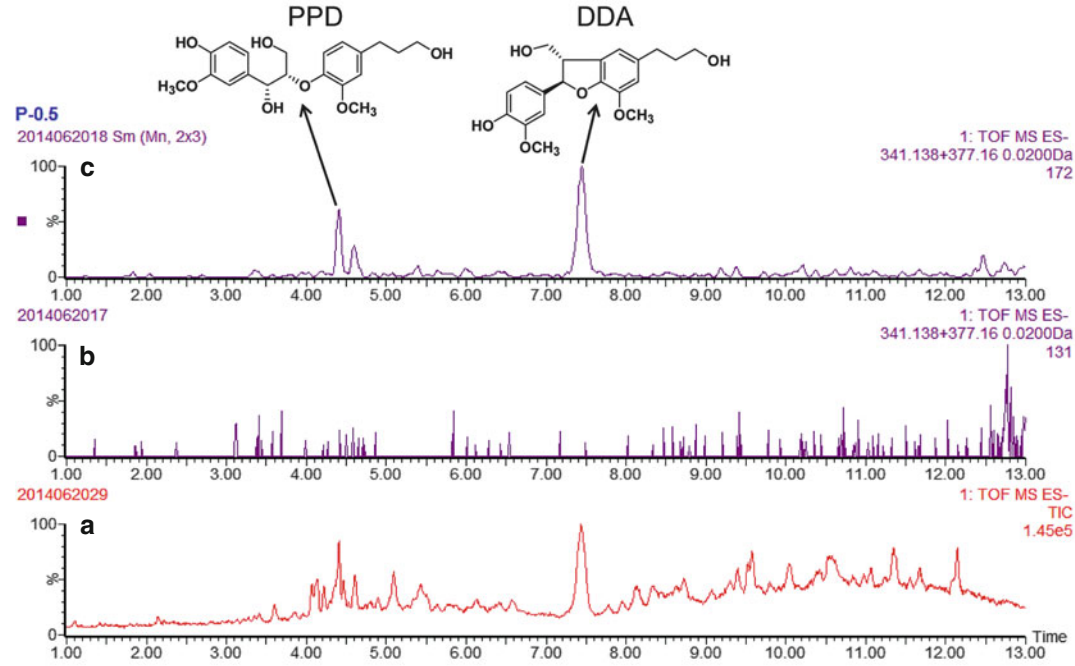

Fig. 13.4 Identified lignans in plasma treated with the bioactive fraction of *Sambucus williamsii* Hance (**a**) the bioactive fraction, (**b**) blank plasma, (**c**) plasma treated with the bioactive fraction. Sample analyses were performed using a ACQUITY™ UPLC I-Class system coupled to a quadrupole orthogonal time-flight (Q-TOF) tandem mass spectrometer on an Acquity HPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm) at a temperature of 35 °C

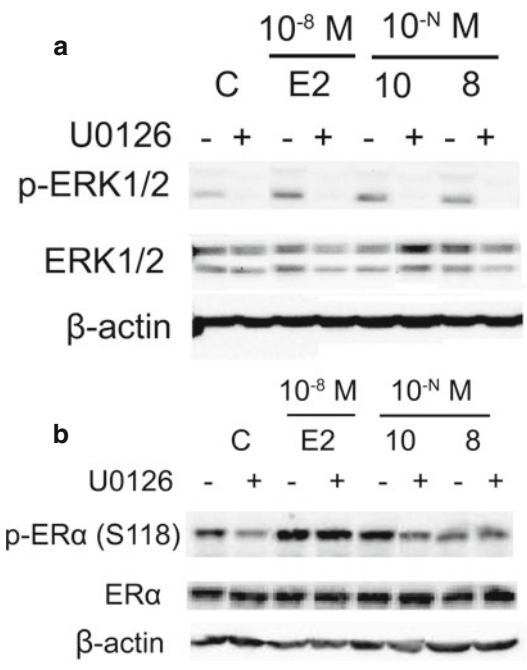

Fig. 13.5 Effects of PPD on phosphorylation of ERK and ERα in rat osteoblast-like UMR 106 cells. Cells were treated with vehicle, E2 (10^{-8} M) or PPD (10^{-8} M) for 10 min in the absence (−) or presence (+) of U0126. The western blot for ERK (**a**) and ERα (**b**) were shown. Results were obtained from three independent experiments

Taken together, all results indicated that PPD exerted oestrogen-like actions in osteoblasts via ligand-independent, ERE-independent and MAP Kinase-mediated rapid nongenomic ER signaling pathway [6].

Conclusion

In summary, the present study demonstrated that the lignan-rich fraction obtained from *S. williamsii* is effective in protection against ovariectomy induced bone loss in aged rats and its actions might be mediated by the direct oestrogen-like action of lignan on osteoblasts. Our results provide further support for the potential of SWC as a natural alternative on management of postmenopausal osteoporosis.

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81220108028) as well as the National Major Scientific and Program of Introducing Talents of Discipline to Universities (B13038).

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Prebiotics, Calcium Absorption, and Bone Health

14

Connie M. Weaver and Steven Jakeman

Abstract

The gut microbiome is emerging as a regulator of bone health through a gut-bone axis. This chapter reviews the associations between changes in gut microbiota and calcium utilization observed with feeding prebiotic fibers that are fermented in the lower gut in animal models and humans. Fermentation by-products are short chain fatty acids, but whether these compounds are directly responsible for improved calcium utilization or are simply concordant events is unclear. Here we describe an *in vivo* direct-to-cecum rat model developed to address this question.

Introduction

The gut microbiome is the new frontier for exploring inter-relationships among host characteristics, the environment, and health. This research has only recently become possible with the development of modern molecular biology tools to profile gut microbial communities, their structure, and function. Previously, identification of the presence of a particular microbial species required culturing of the bacteria, but most of the residents in the gut are anaerobic and cannot be cultured. Only when fecal DNA of dead bacteria could be characterized, did it become possible to link diet-induced shifts in gut microbiome to health.

C.M. Weaver, PhD (✉) • S. Jakeman, MS
Department of Nutrition Science, Purdue University, 700 W State Street, West Lafayette, IN 47907, USA
e-mail: weavercm@purdue.edu

The interest in impact on health by the gut microbiome extends to essentially all tissues and bone is no exception, but the impact on bone has been little studied. There has been a link made between the gut microbiome and immune function which affects bone [22]. Ohlsson and Sjögren propose that the gut microbiota regulate osteoclastogenesis through its effects on the immune system [16]. This hypothesis comes in part from their observation that colonization of gut microbiota in germ-free mice normalized bone and reduced TNF α expression and T cells.

One group has shown that feeding a probiotic, *Lactobacilles reuteri* (ATCC 6475), to 14-week old male mice for 4 weeks increased femoral and vertebral trabecular bone volume/total volume and bone mineral content (BMC) compared to non-treated males [13]. Surprisingly, there was no response to the probiotic in female mice. Other Lactobaccillus bacteria have also been

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reported to have beneficial effects on mineral utilization and bone health [6, 12, 20].

Our group and others have been studying the influence of prebiotics on mineral absorption and retention and bone properties, for many years (reviewed in Jakeman and Weaver [11], COR). Prebiotics are nondigestible fibers that serve as substrates for lower gut microbes to ferment as a source of energy and nutrients. Only recently have we evaluated the role of the gut microbiome for its influence on this relationship of prebiotics and bone health. Specifically, we have asked the question: Can diet, and specifically prebiotic fibers, alter the gut microbiome to improve calcium absorption and utilization?

Improving Calcium Utilization

Calcium is a shortfall nutrient as identified by the 2015 Dietary Guidelines for Americans [5]. Increasing calcium intake has been the traditional approach to improving calcium nutrition. Increasing calcium intake in adolescent girls from approximately 21.1 mmol/d to over 47.4 mmol/d results in increased calcium absorption by 11.6 mmol/d and decreased bone resorption by an equivalent amount [23]. Unfortunately, milk (the major source of dietary calcium) consumption has been declining as has the use of calcium supplementation due to concern over risk of cardiovascular disease [26]. An increase in peak bone mass by 10 % has been estimated to delay osteoporosis by 13 years [8]. This supports a public health effort to develop strategies to increase bone mass. Increasing calcium intake also suppresses bone resorption in postmenopausal women [18], and consequently, could lower bone loss.

An alternative strategy to improving calcium nutrition by increasing calcium intake is to improve calcium utilization efficiency. A variety of prebiotics have been shown to improve mineral absorption including disaccharides like lactulose, oligosaccharides like short chain fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), and polysaccharides like inulin (long chain FOS) and resistant starch (reviewed in [17, 27, 30]). Whether fiber fermentation in the lower gut stimulates active or passive calcium absorption pathways or both is unknown. If prebiotics work primarily on bone through increased mineral absorption, then it would be expected that calcium metabolism would be affected similarly to increasing calcium intake, i.e., bone balance would be increased through suppressing bone resorption. We demonstrated almost complete suppression of bone resorption with short and long chain FOS in an OVX rat model [32]. We also saw increased calcium absorption and decreased bone formation, and bone balance was improved by 89 %. Vitamin D was adequate in these studies

Prebiotic-Induced Shifts in Gut Microbiota

Recent progress has been made in our understanding of the type and dose of prebiotics that shift the gut microbiome and the genera affected. However, much more work is needed to understand the effects on physiological function.

All of the types of prebiotics, i.e., short, medium, and long chain, have been associated with increases in bifidobacteria, which are a group of anaerobic bacteria that normally reside in the intestines, as reviewed by Ariefdjohan et al. [2], and which function to regulate microbial homeostasis, inhibit pathogens, modulate immune responses, improve gut mucosal barrier, and importantly for this discussion, ferment carbohydrates such as prebiotics. For example, in our dose response study in growing male Sprague-Dawley rats evaluating a prebiotic, GOS, we used quantitative PCR of fecal DNA to show a dose-response increase in the proportion of bifidobacteria ($P=0.0001$) [24]. We then showed a dose-response increase in bifidobacteria with GOS feeding for 3-week periods in a cross-over study in adolescent girls using quantitative PCR with bifidobacteria-specific primers [29]. We have tested a variety of prebiotics in rats and found all to have some benefit to bone [25]. One of the most effective prebiotics was soluble corn fiber (SCF) which we have since studied extensively.

As methodology becomes more sophisticated, more comprehensive changes in microbial community structure and function is becoming possible. In adolescent boys and girls fed SCF vs. placebo for 3 weeks in a cross-over trial, we used 454 pyrosequencing of the PCR-amplified 16S ribosomal RNA gene to profile comprehensive changes in bacterial genera [28]. The phylum Bacteriodes was significantly greater after SCF feeding than during the control period. Similarly, both SCF and polydextrose feeding in healthy adult men resulted in shifts in Bacteriodes:Firmicutes ratios and they applied metagenomics to explore functional implications [9]. Prebiotic feeding altered several functional pathways paving the way for future endeavors linking diet, the gut microbiome, and bone.

Shifts in the microbial communities occur relatively quickly in response to dietary intervention and to its cessation. In postmenopausal women, feeding soy bars rich in isoflavones was associated with increases in *Bifidobacterium and Eulacterium* from DGGE profiles of PCR amplified 16S rRNA genes by 7 days [15]. Pyrosequencing of the 16S rRNA gene showed changes that were positively correlated with changes in soy metabolites.

In our controlled feeding SCF study in adolescents discussed above [28], daily changes in PCR-DGGE fingerprints from fecal DNA showed that community profiles stabilized after about 4 days. This time period is consistent with the 3.5 day average time needed to reach a new steady state on high fat diets in mice [3]. More extreme diet changes (herbivores and carnivorous) have resulted in detectable microbial shifts within 1 day [4] In our human study, microbial profiles were more similar in the later half of the 3-week controlled feeding periods than the first half regardless of the presence or absence of prebiotic consumption. However, intersubject variation was large. This suggests that controlled diets can reduce the heterogeneity of response to diet effects that work through changes in the gut microbiome, and that cross over designs where subjects are their own controls, are especially powerful.

Association of Prebiotic-Induced Shifts in the Gut Microbiome with Calcium Utilization

The effect of prebiotics on increasing fractional calcium absorption in adolescent studies in which gut microbiota were measured were 10–12 % regardless of type or dose of prebiotics. We showed GOS at 2.5 or 5 g/d or SCF at 10 or 20 g/d were similarly effective at increasing fractional calcium absorption and no dose response was observed [28, 29, 31]. Two RCTs of SCF in adolescents were conducted. Figure 14.1 shows the study designs of the two trials.

The two studies differ in important ways. Study 1 was an efficacy study to determine whether SCF should alter the gut microbiome and increase fractional calcium absorption on a controlled diet in a supervised setting of a

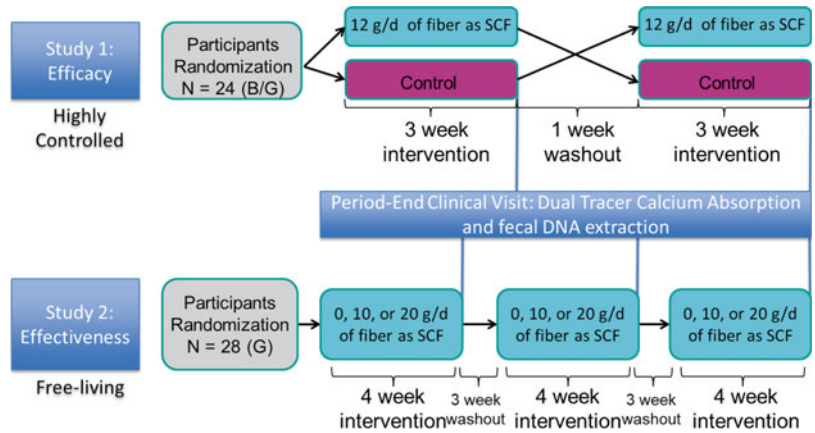

Fig. 14.1 Two randomized, double blind, crossover SCF studies in adolescents ([28, 31]; unpublished)

metabolic balance study of two- 3 week periods. Study 2 was an effectiveness study in which subjects were free living and their diet and activity was self-selected. Only at the end of each 4 week intervention did participants come into the clinic for fractional calcium absorption tests and fecal collections for subsequent gut microbiome profiling. In the Efficacy Study 1, racially diverse boys and girls were studied while only adolescent white girls were studied in the Effectiveness Study 2. Fractional calcium absorption was determined by the dual stable calcium isotope tracer method in both studies (Fig. 14.2)

Figure 14.2 illustrates that increased fractional calcium absorption due to prebiotic fiber feeding was not apparent in the first 24 h, but was in the second 24 h. This is consistent with lower gut absorption. There was no significant dose response effect though the trend was in the right direction in contrast to our results with GOS [29]. The magnitude of the effect on increasing fractional calcium absorption would increase bone accrual by 15.1 g or 1.8 % of BMC over a year if persistent. Abrams et al. have shown persistent effects of feeding inulin plus FOS in adolescent girls on fractional calcium absorption that resulted in increased whole body BMC and BMD [1]. The difference in bone mass accrual between the girls receiving the prebiotic and the placebo was 35 g or about, 2.6 % of whole body BMC.

It is difficult to compare shifts in gut microbiota across studies because of the rapidly changing molecular tools for profiling microbial communities and the changing microbial genome data bases. As gut microbial profiles are reported as proportions of the whole, discovery of new members shifts the profile whether or not the intervention perturbs the profile. In our first human study, GOS produced a dose response shift in bifidobacteria numbers as determined with specific primers [29]. In our next study (Efficacy Study 1 in Fig. 14.1) using 454 pyrosequencing of PCR-amplified 16S ribosomal RNA genes, fractional calcium absorption correlated with changes in *Bacteroides* in the phylum Bacteroidetes and Buctyricicoccus, Oscillibacter, and Dialister in the Phylum Firmicutes which are known fiber fermenters [28]. In our Effectiveness Study 2 (Fig. 14.1), we used a similar approach and found fractional calcium absorption significantly correlated with changes in recently discovered saccharolyic bacteria that were not in the database at the time Study 1 was analyzed.

It was surprising that the efficiency of Study 1 and effectiveness of Study 2 yielded similar increases in fractional calcium absorption with SCF. We speculate that the reduced variability in Study 1 with controlled diet and activity was offset by reduced variability due to a more homogeneous population in study 2.

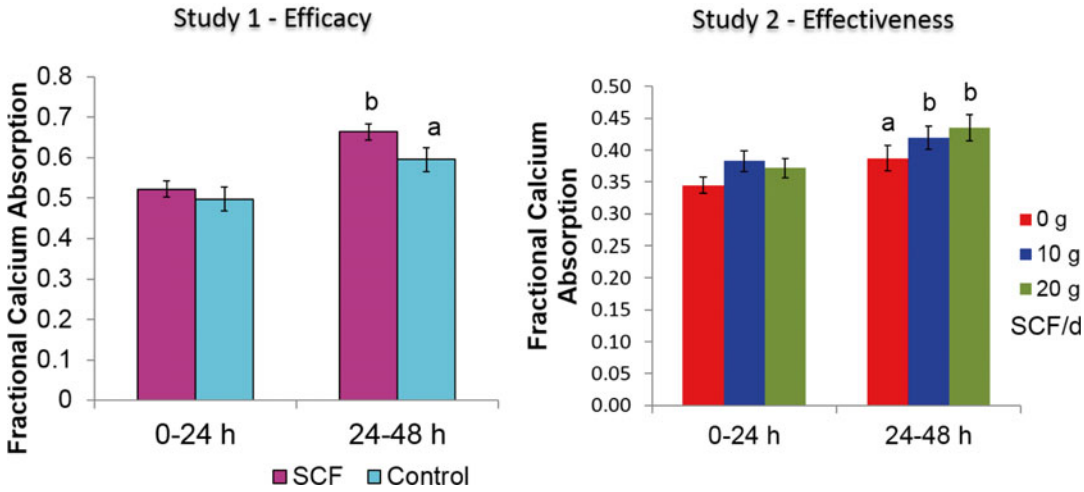

Fig. 14.2 SCF increases calcium absorption in the lower gut by ~12 % in adolescents (Whisner et al. unpublished). Different letters indicate significant differences at $P < 0.05$

The dose response benefit of SCF to bone calcium retention in postmenopausal women using a novel approach of urinary appearance of ^{41}Ca from labeled bone was recently demonstrated by our group [10]. Thus, the benefit of prebiotics is not limited to growing bone.

Mechanisms of Action of Fermenting Microbes

The most often proposed mechanism for the increase in mineral absorption with prebiotic fiber feeding is through production of short chain fatty acids (SCFA) which would produce an acidic environment ideal for increasing the solubility and transcellular absorption of mineral ions such as Ca^{2+}. On the other hand SCFA production was not associated with increased fractional calcium absorption when a variety of prebiotic fibers were fed to growing male rats [25]. Moreover, in isolated intestine in a Ussing Chamber study, reduced pH with HCl had no effect despite a dose-dependent increase in Ca^{2+} transport with SCFA [14]. Other mechanisms have been suggested with some support including increased intestinal surface area, i.e., crypt depth, epithelial cell density, and cecal vein flow [19, 21] and possibly through altered inflammation [22].

We developed an *in vivo* direct to cecum rat model to study the ability of SCFA to enhance calcium absorption (Fig. 14.3). The 3 month-old Sprague Dawley male rat model includes two surgeries. The first surgery involves implanting a cecal catheter (100% silicone Silastic® laboratory tubing; 0.51 mm I.D. × 0.94 mm O.D.) under anesthesia (isofluorane) to administer SCFA or other potential regulators of lower gut calcium absorption. The second surgery is to implant a jugular catheter for serial blood draws 2-days before ^{45}Ca kinetics studies. We chose to administer a mixture of SCFA salts (5:2:1 sodium acetate:proprionate:butyrate) twice a day for 11 days as 1 ml doses before performing the calcium kinetic study. The kinetic study involved a post-fasting cecal test dose of 0.8 mmol Ca as calcium ascorbate and 20 μCi ^{45}Ca. Serial blood draws were collected at 0, 50, 120, 180, 240, 330, 420, 540, 720, 900, 1250, and 1440 min after dosing and radioactivity was assessed with a liquid scintillation counter. Plasma ^{45}Ca kinetics was determined using the Win-SAAM (Simulation Analysis of Modeling) program as previously described [7].

Our pilot studies compared control, low dose (300 umol) SCFA and high dose (600 umol) SCFA after a 12 h fast. There were no differences in calcium absorption. There were significant differences in cecal wall weight, but not body

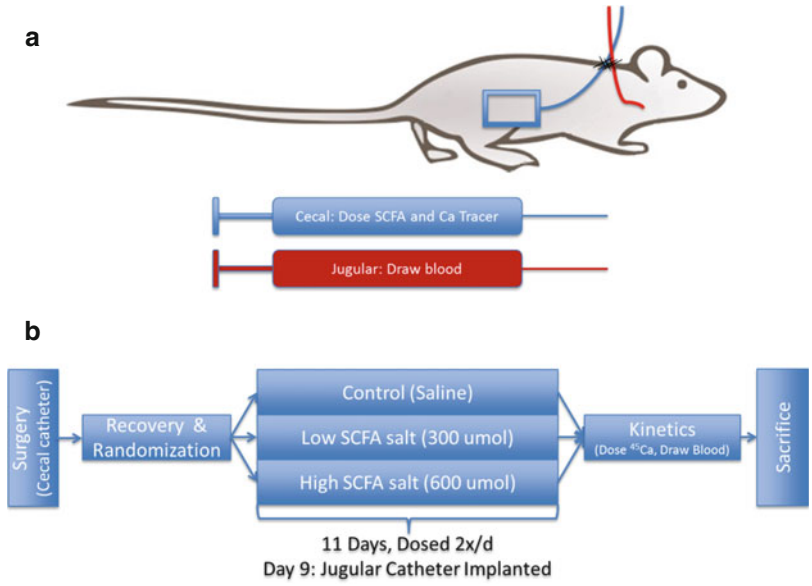

Fig. 14.3 Direct-to-cecum rat model for testing mechanisms for enhancing lower gut calcium absorption. (**a**) Shows location of catheters. (**b**) Study design

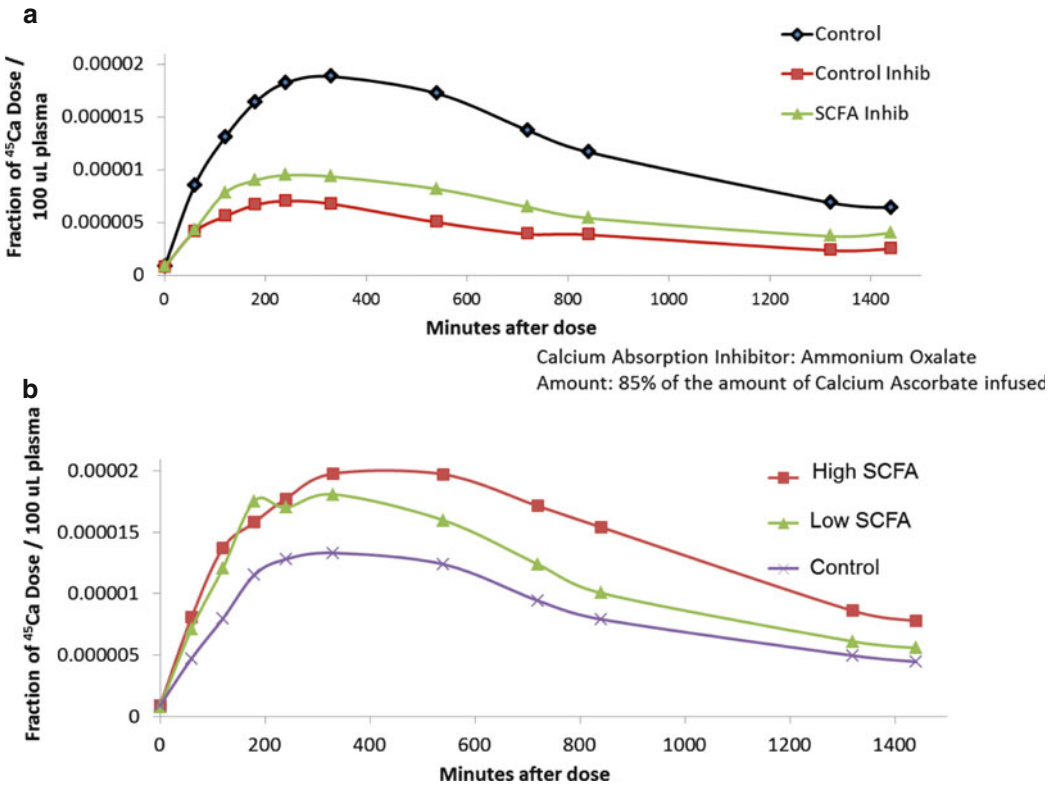

Fig. 14.4 SCFA effect on calcium absorption in direct-to-cecum rat model. (**a**) Shows addition of inhibitor and 12 hr fast conditions and (**b**) shows 30 minute fast conditions

weight, cecal pH, or cecal content weight in SCFA administered rats. Negative results for calcium absorption could have been due to too long of a fast period between the last SCFS dose and ^{45}Ca absorption test so that any effect was not captured or due to absence of inhibitors to calcium absorption to obviate an effect. Perhaps SCFA are beneficial to calcium absorption by preventing complex formation in the presence of inhibitors. Both of these changes in protocol appeared to improve the model (Fig. 14.4).

Conclusions and Next Steps

Several studies now show a correlation between prebiotic induced shifts in the gut microbiome and increases in fractional calcium absorption in life stages of growth and bone loss. We do not yet know the mechanisms, but newer methodology will help close the gap in our understanding.

The preliminary results from our direct-to cecum rat model suggest that a range of experimental conditions can be tested to determine factors that influence lower gut calcium absorption. Further investigation will include dose-range testing of SCFA and pH and ultimately associations with changes in gut microbiota and temporal changes.

Systems biology approaches will be needed to determine the multiple contributions of host and gut microbiota characteristics and metabolites involved in bone health.

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Prebiotics, Probiotics, Synbiotics and Foods with Regard to Bone Metabolism

15

Katharina E. Scholz-Ahrens

Abstract

Prebiotics beneficially affect the host's health, inter alia the mineral metabolism and bone health in experimental animals and humans. This is due to several factors, including the habitual diet. Differing beneficial effects on bone by specific prebiotics can be explained by their distinctive and characteristic fermentation profile, showing that e.g. a prebiotic mixture containing acacia gum leads to a more harmonized fermentation than oligofructose alone. The reason may be a higher and more consistent yield of energy and nutrients for the gut microbiota. Moreover, it has been shown that the antioxidative potential of prebiotics varies, and that several non-digestible oligosaccharides (NDOs) affect the relative bacterial proportion differently.

Apart from several reported health outcomes probiotics have been shown to support the host's bone health which seems plausible since microbiota is associated with bone mass. Probiotics target the intestine and its digestive function, immune regulation, and anti-inflammatory defense. The reduced expressions of bone resorbing cytokines after some defined probiotics contribute to alleviate osteoporosis in animal models showing that there exists a link between osteoporosis and inflammation. Inconsistency of in vivo effects under this aspect can be explained by differential innate microbiota and immune status of study participants. Recent investigations have shown that some selected probiotic bacteria are able to reduce osteoclastogenesis and inflammation, and to decrease osteoporosis and periodontitis. Effects of synbiotics on bone health are less frequent. Furthermore, it is more complex to conclude whether synbiotics have positive effects on bone health, because this has to be investigated individually for each synbiotic combination.

K.E. Scholz-Ahrens
Moorstuecken 6, Bad Bramstedt D-24576, Germany
e-mail: k.scholz.ahrens@gmail.com

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Background

It has been demonstrated that prebiotics have beneficial effects on diverse aspects of the host's health including bone health [26, 32, 35]. The visibility of such effects depends on several factors including the composition of the habitual diet. Probiotics have also been shown to act beneficially on the host's health. These effects were predominantly visible in the with respect to gastro-intestinal tract, digestion, inflammation and immunity, while the innate microbiota affected the outcomes. Synbiotics are a combination of prebiotics and probiotics. There has been little research on the effect of synbiotics on bone health, bone gain or bone preservation.

It is well known that microbiota is associated with bone mass, since studies in germ-free mice revealed that the absence of gut microbiota increased their bone mass [34]. According to measures by μCT mice had higher values of bone volume (BV/TV) in the distal metaphyseal region of the femur, compared to the conventionally raised control group.

Osteoporosis, obesity and inflammation have intersections [14], as there is reciprocity between adiposity and inflammatory processes, and since inflammation is a common link between obesity and osteoporosis [11]. Low-grade systemic inflammation (LGSI) is indicated by moderately elevated serum levels of high sensitivity C-reactive protein (HsCRP), and HsCRP is negatively correlated with bone mineral density (BMD) and positively with bone resorption and fractures [8, 9]. These observations reveal inflammation as risk factor for osteoporosis.

Diet, Gut Microbiota and Bone

The commensal bacteria that colonize specifically the intestine with trillions of bacteria since birth are considered a multicellular organ that communicates with and affects its host in numerous ways [22]. Diet is one factor that modulates the proportions of commensal bacteria, and treatment with antibiotics drastically interferes with the gut microbiome's composition as well. In health the microbiota is at equilibrium and contributes to a balanced immune system and gut permeability, protecting the host against invading pathogens. Temporary dysbiosis may be triggered by inappropriate meals [22], long-lasting dysbiosis by "unhealthy" dietary habits.

Probiotics, Prebiotics or Synbiotics and Gut Ecology

Gut ecology is affected by prebiotics [26] and probiotics [6], whereby the differentiation between a direct effect of prebiotics and a concomitant effect of probiotics is difficult. The mode of action of probiotics on gut ecology may be mediated by bacterial metabolites. For example, it was demonstrated that probiotic bifidobacteria produce exopolysaccharides. They are discussed to be responsible for distinct survival rates or metabolic activities of certain bacteria, based on observations of bacteria when they are in coculture instead of monoculture [25]. Synbiotics as a combination of probiotics and prebiotics may affect the intestinal ecosystem differently. An ideal combination would be advantageous for the host as it is supposed to improve the survival of the probiotic bacteria since their preferred substrate for fermentation is readily available.

Changes in the profile of intestinal bacterial metabolites were observed in humans after they had consumed synbiotic functional foods containing fructooligosaccharides and the probiotic strains *Lactobacillus helveticus Bar13* and *Bifidobacterium longum Bar33* [38]. The authors concluded that the consumption of synbiotics had a potential health promoting effect since they observed a significant increase of ketones, carbon disulfide methyl acetate and finally short chain fatty acids disulfide, methyl (SCFA) following the feeding period. Especially the latter is addressed to be a positive factor for the gut ecosystem.

In an experiment with ovariectomized rats the caecum chymus content as an indication for fermentation capacity was not affected in the group receiving probiotic bacteria [33]. Nevertheless, in the groups fed with prebiotics or synbiotics the cae-

cum chymus content was significantly or considerably higher [33]. The weight of the rats' large intestine as indicator of absorption surface was not affected by the probiotic bacteria whereas it was slightly higher after the administration of prebiotics. However, merely when both ingredients were combined (SYN; *Lactobacillus acidophilus NCC90* plus oligofructose + acacia gum) a statistically significant effect was observed, as the colon weight was 0.64 ± 0.07 g compared to 0.53 ± 0.11 g of animals on probiotics alone. Furthermore, the jejunal pH was significantly lower, and the microbiota composition was shifted towards higher counts of bifidobacteria in the short term and towards higher counts of bacteroides in the long-term [33].

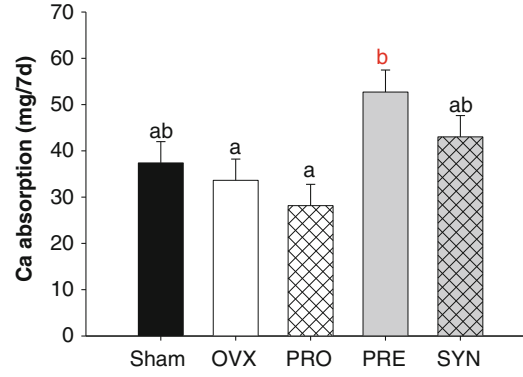

Fig. 15.1 Effect of probiotics (*PRO*), prebiotics (*PRE*) or synbiotics (*SYN*) on calcium absorption in ovariectomized adult rats after 16 weeks of dietary intervention (According to Scholz-Ahrens et al. [32])

Probiotics, Prebiotics or Synbiotics and Mineral Absorption – Acute Effects/Short-Term Effects

Defined prebiotics have been shown to improve calcium balance and diminish bone loss [28, 32], and specific probiotic bacteria were reported to increase the calcium availability from milk products in vitro [3, 4]. The combination of fermentable fibres with definded and characterized bacteria was investigated with respect to bioavailability of calcium, magnesium and phosphorus in repeated metabolic balances in rats [24], and synbiotics were found to increase mineral balances. In fact each bacterial strain has to be investigated in combination with its own preferred or most preferred substrate, because the outcomes of experiments with one microbial subspecies cannot be extrapolated to another species, in particular if combined with another prebiotic substrate. Evidently, it is more challenging to find synbiotics and prove their beneficial effects than doing it for each single component (probiotic or prebiotic).

In an experiment with aged ovariectomized rats it was explored whether a specific probiotic (*L. acidophilus NCC90*) or a specific prebiotic (oligofructose + acacia gum) or the combination of both in a synbiotic group improved calcium absorption persistently, when compared to an ovariectomized control group [32]. Metabolic balances were performed in week 5 or 16. In the short term there was no significant difference in calcium absorption between experimental groups and control group. After 16 weeks calcium absorption was slightly decreased in the ovariectomized control group (OVX) compared to sham-operated animals (SHAM) but was not improved by probiotics (Fig. 15.1). The prebiotics-fed animals had significantly higher calcium absorption values while in the synbiotic group calcium absorption tended to be higher. It has to be considered that balance studies only display short-term effects because they just allow a view through a narrow metabolic window. If this effect is not persistent no conclusions on health benefits with respect to bone or osteoporosis prevention can be drawn.

Probiotics, Prebiotics or Synbiotics and Bone Mineral – Long-Term Effects/Cumulative Effects

When bone mineral was analyzed in the axial skeleton at the end of the experiment after 16 weeks, bone ash of the lumbar vertebrae was significantly lower after ovariectomy, but tended to be higher after probiotics and prebiotics. It was significantly higher after synbiotics (Fig. 15.2). This demonstrates the advantage of the simultaneously offered prebiotics together with a beneficial bacterial strain.

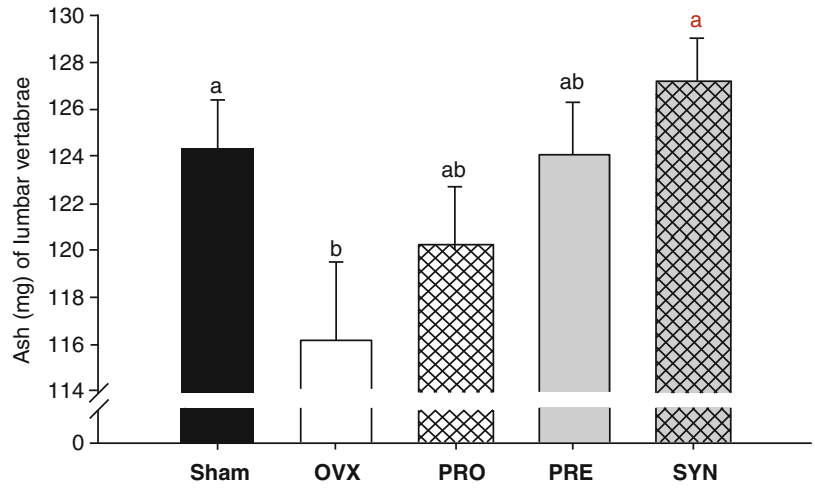

Fig. 15.2 Effect of probiotics (*PRO*), prebiotics (*PRE*) or synbiotics (*SYN*) on bone mineral content in the axial skeleton (lumbar vertebrae) after 16 weeks of dietary intervention in ovariectomized adult rats (According to Scholz-Ahrens et al. [32])

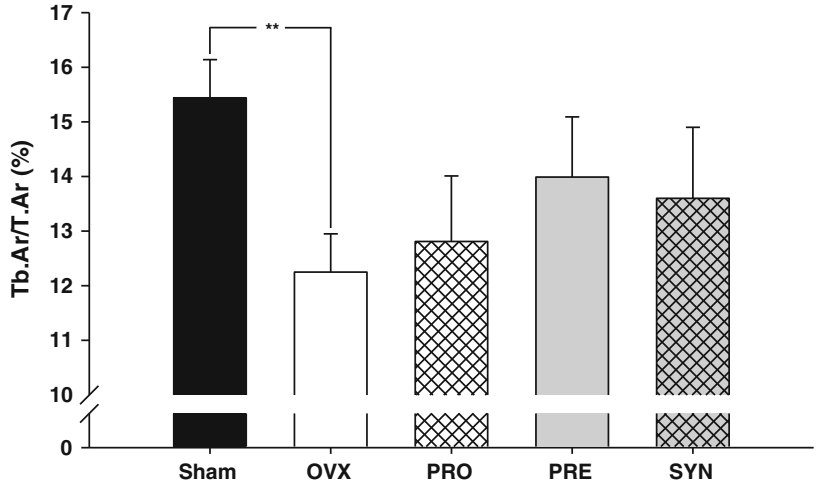

Fig. 15.3 Effect of probiotics (*PRO*), prebiotics (*PRE*) or synbiotics (*SYN*) on bone trabecular structure (TbAr./T. Ar) in the appendicular skeleton (tibia) after 16 weeks of dietary intervention in ovariectomized adult rats (According to Scholz-Ahrens et al. [32])

In the appendicular skeleton this effect was less prominent. Bone ash in the femur (not shown) was only slightly improved by the supplements, but mostly by synbiotics. The trabecular area in the tibia (Fig. 15.3) was also improved by the supplements, but the effect did not reach significance.

It may be that the beneficial effect after consuming synbiotics in the first instance affects the axial skeleton. However, since rats are quadrupeds it cannot be concluded whether these observations could be extrapolated to humans.

Prebiotics and Antibiotics

Low-dose antibiotics are known to be used preventively, and as growth promoters to increase

daily weight gain in farm animal and meat production. In the light of consumers' rejection of such kind of food production research is looking for alternatives, with probiotics and prebiotics as candidates among others [39]. Accordingly the mechanisms of action on how body composition is affected have to be investigated.

Recently it was demonstrated that low-dose penicillin given to mice early in life had effects on body composition and bone mineral content later in life [7]. These effects were mediated by the change in the microbiota through the low-dose penicillin, not by the antibiotic per se. The authors could demonstrate it by transferring the microbiota of low-dose penicillin-treated mice to germ-free hosts. The body composition of the hosts was more comparable to the low-dose penicillin-treated mice with increased body mass and fat mass than to germ-free controls. At the same time the relative abundance of Lactobacillus, Allobaculum, Rikenellaceae, and Candidatus Arthromitus decreased. Lactobacilli have been known for a long time as protective microorganisms, but the others have also been selected and investigated as protective ones [7]. Interestingly, in that experiment bone mineral content was decreased in male but increased in female mice after low-dose penicillin. Obviously one cannot generalize the effects of low-dose penicillin on bone and further research is required.

In a study with female rats bone mineralization, as indicated by bone structure analysis, decreased after ovariectomy, an effect that was partly prevented if the semipurified experimental diets contained oligofructose (Fig. 15.4, [32]). When the semipurified experimental diets contained antibiotics (neomycin/metronidazole) bone mineralization was not affected. After adding oligofructose to the antibiotics-containing diet loss of bone mineralization was completely prevented and the trabecular bone perimeter reached values of the sham operated control group. It may be concluded that the specific antibiotics used did not affect trabecular bone structure in either a harmful or beneficial way. But when certain bacterial strains had been erased by this antibiotic combination, the resistant remaining bacteria had a long-lasting profit from their substrate and consequently this combination significantly prevented ovariectomy-induced bone loss.

The effect of antibiotics on intestinal microbiome is varying. When comparing therapeutic-dose pulsed antibiotic treatment (PAT) with

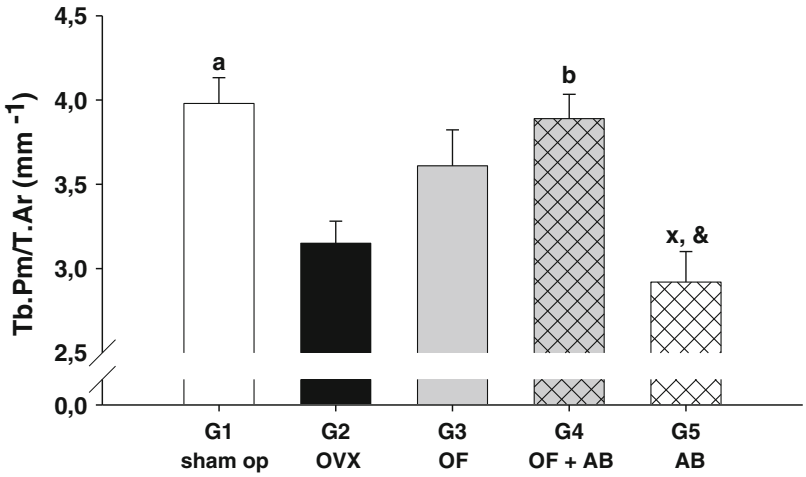

Fig. 15.4 Tibia trabecular perimeter in ovariectomized rats on long-term treatment with prebiotics (oligofructose, OF), antibiotics (neomycin/metronidazole, AB) or their combination (OF+AB). Mean, SEM, n = 14. G1 = sham operated, *OVX* ovariectomized control group. Significantly different from OVX with $P < 0.05$: (*a*), with $P < 0.01$ (*b*). Significantly different with $P < 0.05$ from OF: (*x*) and from OF+AB: (&) (Scholz-Ahrens et al. [32], with permission)

tylosin or amoxicillin in early life, PAT decreased the richness in diversity of the intestinal microbiome with sustained reductions during microbiota maturation [21]. This effect was more pronounced following tylosin exposure (macrolide) and milder following amoxicillin (β-Lactam), while the richness in diversity remained relatively constant in control mice [21]. To date there is not enough information to discuss the effects of early-life treatment with antibiotics and possible consequences for bone health in later life.

Conditions That Affect Experimental Outcomes

Quite a large number of publications have reported on effects of prebiotics on mineral metabolism and bone, while experiments on probiotics are rare. However, the outcomes and the drawn conclusions are controversial and remain unsettled in several points. Obviously, certain study conditions (Table 15.1) as implicated by the different experimental protocols affected the outcomes in different ways. Therefore, conclusions on the effects and actions of prebiotics or probiotics may be valid only for the defined experimental setup with respective extrinsic, intrinsic or experimental conditions.

For example, if the background diet or habitual diet contains higher amounts of calcium or if the calcium source is less bioavailable due to lower water solubility, prebiotics may be more effective compared to a condition with lower dietary calcium content, when absorbability is already higher [1, 31]. Comparably, the raise of dietary calcium from a certain level may not further increase the amount of bioavailable calcium in the lumen, because of a simultaneous decrease of luminal calcium solubility. In the presence of prebiotics or absorption enhancers, however, a further increase of bioavailability is possible [1, 31]. Furthermore, if the habitual diet already contains absorption enhancer prebiotics may be less efficient. The habitual diets in population-based studies may already contain quite an amount of foods rich in non-digestible oligosaccharides (NDO) with prebiotic activity like blueberry, pear, watermelon, and nectarine from the fruits, and garlic, leek, onions, scallion [12], roots and others from the vegetables (see section "Foods with prebiotic or antioxidative activity, "healthy diets" and practical consideration") or prebiotics-containing foods or functional foods like breakfast cereals.

In this case supplemented prebiotics may not be further effective. Apparently there is a threshold concentration of dietary prebiotics above which no further benefit occurs, at least with respect to some health-indicating variables like calcium absorption and bone mineralization.

Several intrinsic factors have an impact on mineral metabolism as well. Young individuals, especially in the phase of growth spurt, and pregnant or nursing women have a higher demand for calcium. At the same time these biological phases are characterized by a higher absorption capacity, in contrast to aged individuals or women after menopause [2]. Another important point is the vitamin D status which is an extrinsic and intrinsic factor since vitamin D status is determined by vitamin intake and dermal synthesis. It may be assumed that different vitamin D status as assessed by plasma level of 25-(OH) vitamin D unequally affect the calcium absorption efficiency. Therefore, vitamin D status should be assessed as baseline variable in human studies when short-term effects of probiotics, prebiotics or synbiotics on calcium absorption are investigated.

Animal experiments allow keeping all variables constant and varying only one or a defined

Table 15.1 Study conditions that affect experimental outcomes

Extrinsic	Intrinsic	Experimental
Habitual diet	Physiological age and status	Animal model
Vitamin D status	Vitamin D status	Duration of experiment
Physical activity	"Healthy"	Investigated skeletal site
Medication	Menopausal state	
	Ca absorption capacity	
	(Innate) Gut microbiota	

Adapted and modified from Scholz-Ahrens et al. [32]

number of study parameters. Nevertheless, the outcomes may differ depending on the duration of a study. They may be significant in a short-term observation study but may disappear in the long term, because the effects are not persistent or because the body adapts and counter-regulates initial effects. This has to be taken into consideration if conclusions with respect to bone health are drawn from single meal or short-term absorption studies. On the other side, differences in absorption may be small and not significant, but if this observation is persistent, the absorbed small amount may be large enough over time to create a cumulatively generated significant surplus of calcium ready for bone formation.

Apart from the duration of a study other experimental conditions are relevant, like the animal model, or its age. Mostly rats are used as animal models and ovariectomy is performed to simulate postmenopausal estrogen deficiency. The choice of the analyzed biological material or the investigated skeletal site is also of importance. For example, dietary prebiotics given with a diet that contains recommended calcium content for rats (5 g/kg diet) improved bone mineral in the appendicular skeleton provided that the amount of oligofructose was high. This effect was not observed in the axial skeleton [31]. On the other side, a medium dose oligofructose was sufficient to stimulate femur mineralization significantly in the presence of a high content of dietary calcium (10 g/kg diet). This effect was visible only in the axial skeleton and was not observed at the recommended calcium content [31]. In summary, animal experiments have helped to sort out conditions and situations when dietary interventions with prebiotics were most effective. Based on those findings human intervention studies can be designed more constructively.

Mechanisms How Probiotics, Prebiotics or Synbiotics Affect Bone

Several mechanisms have been proposed on how functional food ingredients affect bone accretion or prevent bone resorption, e.g. via luminal fermentation, solubilization of minerals in the gut, increasing the absorption surface, stimulation of the expression of calcium binding protein, improving gut health, increasing the immune defense, affecting bone modulating factors, stabilizing the intestinal microbiota, modulating growth factors, suppression of bone resorption over formation, degradation of mineral-complexing phytic acid, and stimulation of calcium uptake by enterocytes (for review see Scholz-Ahrens et al. [32]). Meantime studies have shown that anti-inflammatory effects are involved as well and may play an important role next to luminal fermentation [22]

Prebiotics, Luminal Fermentation and Intestinal Flora

Several fermentable substances show diverging fermentation profiles as a consequence of their chemical structure and characteristics. This could be demonstrated *in vitro* using a digestion simulation technique [Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)], when two blends of prebiotics a) FOS + inulin (blended in a proportion of 70:30 %) or b) FOS + inulin + acacia gum (41 % FOS, 41 % acacia gum, 18 % inulin) were compared [17]. In both cases the total amount of short chain fatty acids production was increased and growth of bifidobacteria stimulated. However, when blend b) was fermented the speed of fermentation was slowed and the fermentation profile, expressed as pH profile, was shifted towards a more harmonized profile along the different parts of the large intestine (ascending, transverse, and descending part). The more harmonized profile was flatter and had less peaks. Such a fermentation profile may indicate a more constant substrate yield for the microbes with fewer gas production and by this a better tolerable fermentation process for the consumer (Fig. 15.5). It becomes obvious that each potential prebiotic substance has to be tested individually, like each potential probiotic bacteria or each synbiotic mixture.

The bacterial proportion was also affected showing that blend 2 induced a significant increase in bacteroidetes in the simulated descending colon [17]. We made a concordant observation in faeces of ovariectomized rats after consumption of a

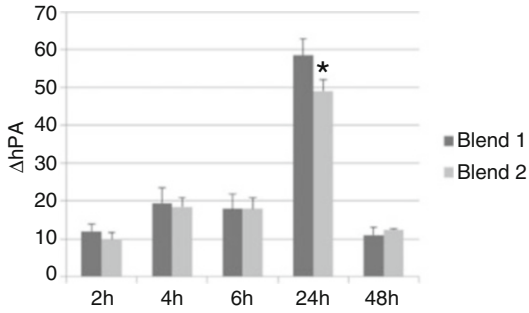

Fig. 15.5 Δ Total gas production in the SHIME® systems after Blend 1 = FOS + inulin in a proportion of 70:30 %; and Blend 2 = FOS + inulin + acacia gum in a proportion of 41 % FOS, 41 % acacia gum, 18 % inulin) after different times. Differences between the two blends by Student's two-tailed *t*-test with * for P = 0.06 (According to Marzorati et al. [17], with permission)

blend of oligofructose+acacia gum. The proportion of bacteroides had not changed after 6 weeks but was increased at the end of the experiment after 16 weeks, when lumbar vertebra mineral content was higher compared to ovariectomized controls [33]. Interestingly the genus bacteroides was also increased in ovariectomized rats after the consumption of a potential prebiotic (soluble maize fibre) and was positively correlated with calcium absorption [43], showing that bacteroides may be involved in the beneficial effect of fermentable carbohydrates on bone mineral accretion. Bacteroides and bifidobacteria were also elevated in aged volunteers after consuming milk-derived prebiotics [41]. At the same time several markers of immune function were affected, showing that milk-derived prebiotics may have a bone-preserving potential as well, which has to be proven. Galacto-oligosaccharides increased calcium absorption in young girls and thus demonstrated at least a short-term effect [44]. Several novel fibers have been investigated in growing rats and had positive effects on bone properties including breaking strength [42], but the size of effects was partly mediated by other factors than efficiency of intestinal fermentation [33, 42].

Prebiotics, Bone and Inflammation

Inflammation is negatively associated with osteoprotection. The origin of the beneficial effect of prebiotics on inflammation was accredited to their probiotic-stimulating effect. Others stated that this effect might be independent of a change or shift of the bacterial proportion of the microflora. In a cell culture experiment it was shown that oligosaccharides affected the intestinal immunity via activation of peptidoglycan recognition protein 3 (PGlyRP3) and increasing PPARγ [45]. In Caco-2 cells, when treated with the oligosaccharides α-3-sialyllactose or fructooligosaccharides (Raftilose P95), the secretion or gene expression of the pro-inflammatory cytokines of IL-12, IL-12p35, IL-8, NF-kB, and TNF-α was reduced (Fig. 15.6). These results demonstrate that oligosaccharides, like oligofructose P95, exert an antiinflammatory effect *per se* [45].

Probiotics, Bone and Inflammation

Certain specific bacteria, lactobacilli in particular, have been investigated for their bone preserving potential. It was reported that *L. reuteri* significantly prevented the ovariectomy-induced loss of cortical bone volume in the femur and tended to prevent it in lumbar vertebra ([5], Fig. 15.7). The authors could demonstrate a lower number of osteoclasts which indicates a depression of bone resorption after administration of *L. reuteri*.

Ohlsson et al. [23] demonstrated in mice after 6 weeks of dietary intervention that both, a single probiotic lactobacillus strain (L. *paracasei DSM13434*) or a mixture of three strains of lactobacilli *(L. paracasei DSM13434, L. plantarum DSM 15312 and DSM 15313*) prevented the loss of cortical bone mineral content. However, at the cortical area the single strain was more effective than the three strains combined (Fig. 15.8). Bone resorption markers were increased following ovariectomy but were normalized after treatment with single strain lactobacilli as well as with the lactobacilli mixture. It was suggested that probiotic lactobacilli prevented cortical bone loss in that bone resorption was reduced.

Probiotic bacteria have been introduced as functional food ingredients. They have been shown to affect gut health [40]. Meantime probiotic effects of lactobacilli on inflammation have been reported [23]. In ovariectomized mice, the

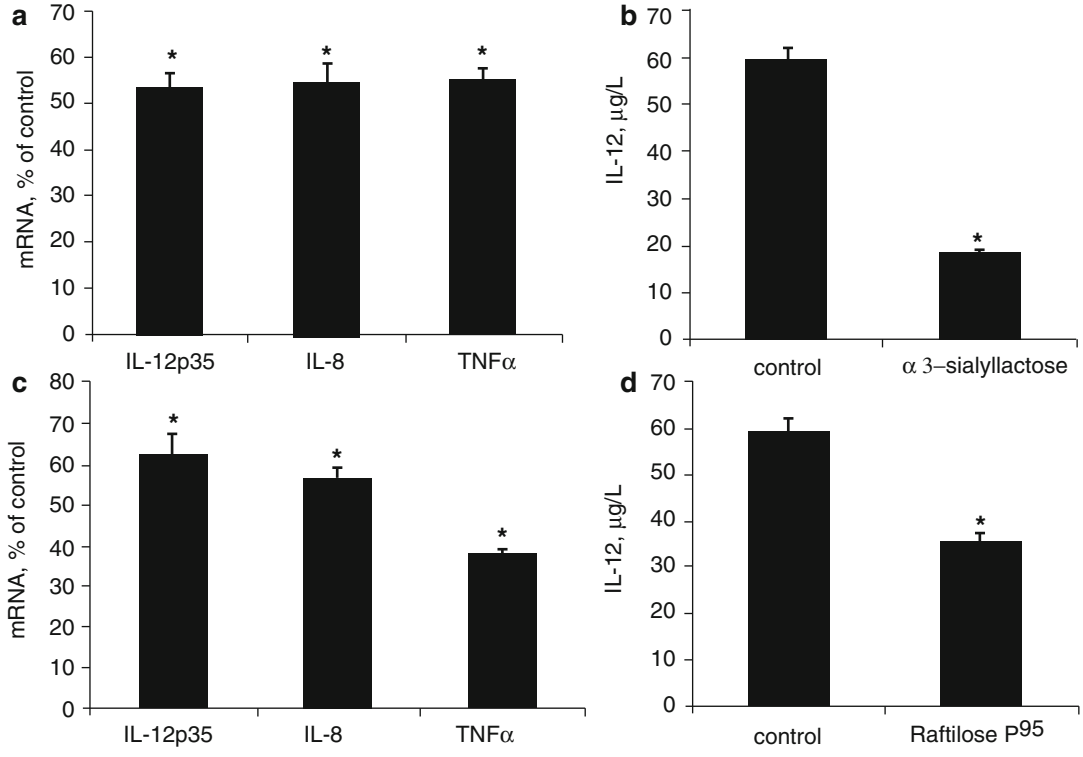

Fig. 15.6 Inhibition of proinflammatory cytokines in Caco-2cells treated with α-3-sialyllactose (**a**, **b**) or Raftilose P95 (**c**, **d**) (According to Zenhom et al. [45], with permission)

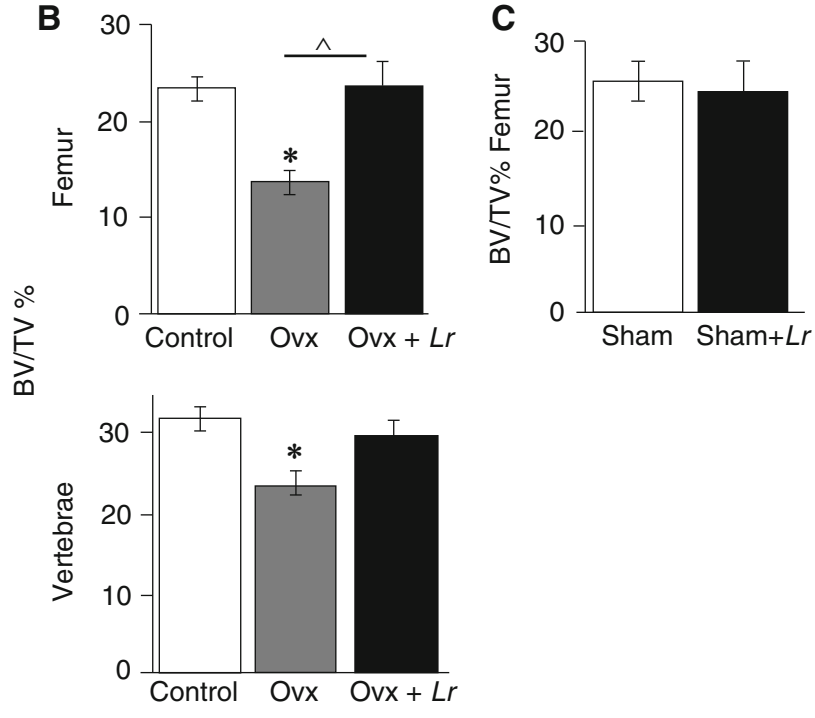

Fig. 15.7 Probiotic L. reuteri (*Lr*) prevented bone loss in ovariectomized mice (According to Britton et al. [5], with permission)

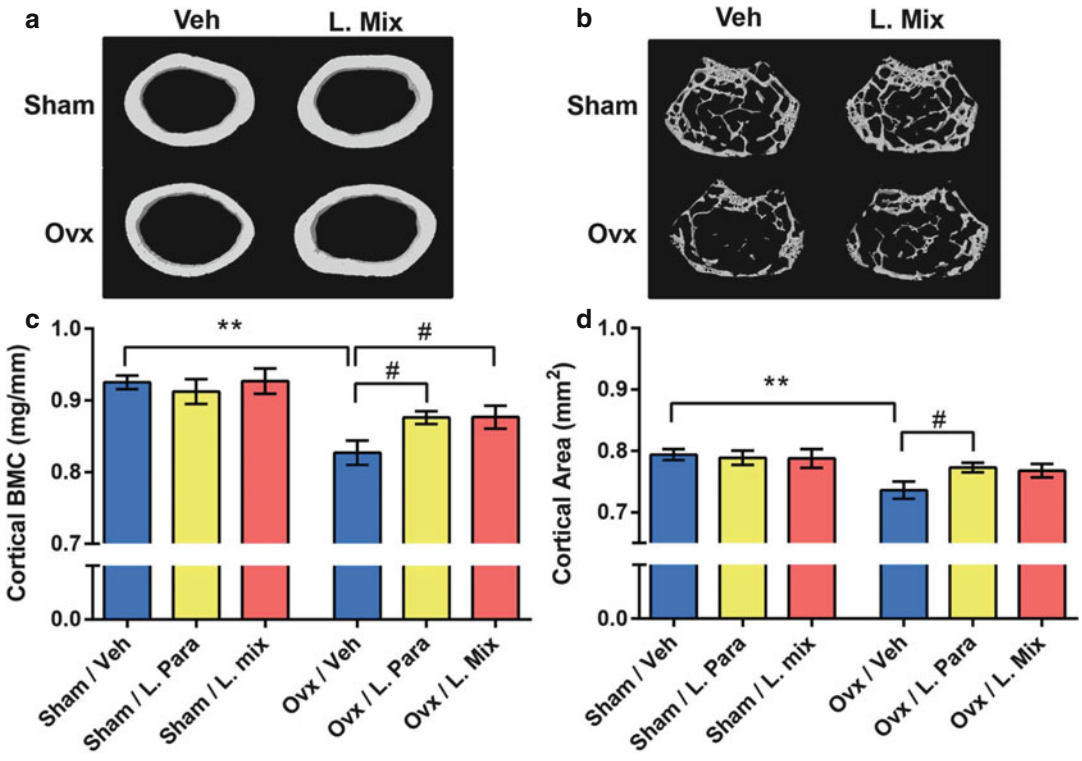

Fig. 15.8 Probiotics Protect Mice from Ovx Induced Cortical Bone-loss. Eight-week-old mice were treated with either vehicle (veh), a single Lactobacillus (L) strain (L. para) or a mixture of three strains (L. mix) during 6 weeks, starting two weeks before ovx or sham surgery to study the preventive effect of probiotic treatment on ovx induced bone-loss. At the end of the experiment, dissected femurs were analysed with highresolution mCT and peripheral quantitative computed tomography (pQCT). Representative mCT images of one cortical section from the veh and L. mix treated sham and ovx groups (A). Representative images of the trabecular bone volume (cortical bone excluded) from the distal metaphyseal region of femur (B). Cortical bone mineral content (BMC) (C) and cortical area (D) were measured by pQCT in the mid-diaphyseal region of femur. Values are given as mean 6SEM, (n = 9–10). ** p#0.01, *p#0.05. Students *t* test ovx vs. sham. # p#0.05, ANOVA followed by Dunnett's post hoc test within the groups, ovx L. Para and L. mix vs. ovx veh. (According to Ohlsson et al. [23], with permission)

pro- inflammatory cytokines TNFα and IL-1 β decreased after consumption of a mix of probiotic lactobacilli. At the same time the expression of osteoprotegerin, an inhibitor of osteoclastogenesis, increased (Fig. 15.9). This observation was in line with an effect on regulatory T cells (CD4+CD25+Foxp3 +) in bone marrow: After ovariectomy their frequency had decreased but if ovariectomized mice had consumed probiotics the decrease of regulatory T cells was less pronounced [23]. Regulatory T cells are important for maintaining immune response. They regulate inflammation and depend on TGFβ for their induction and maintenance. The authors suggested that probiotic lactobacilli preserved ovariectomy-induced cortical bone loss in that they normalize the suppression of regulatory T cells via the stimulation of TGFβ. This signaling cytokine was increased in bone marrow of mice that were fed probiotics and was closer to values of control mice.

Foods, functional foods or whole diets affect nutritional physiology and metabolism with consequences for health including immune system. Ilich et al. [11]) discussed that consuming a "modern diet" means that more than 70 % of energy intake is covered from refined sugars, refined vegetable oils, highly processed cereals and dairy products. Furthermore, modern diets are associated with an over-consumption of n-6 PUFA coupled with under-consumption of n-3 PUFA, which contributes to low-grade chronic inflammation and by this triggers the development of chronic inflam-

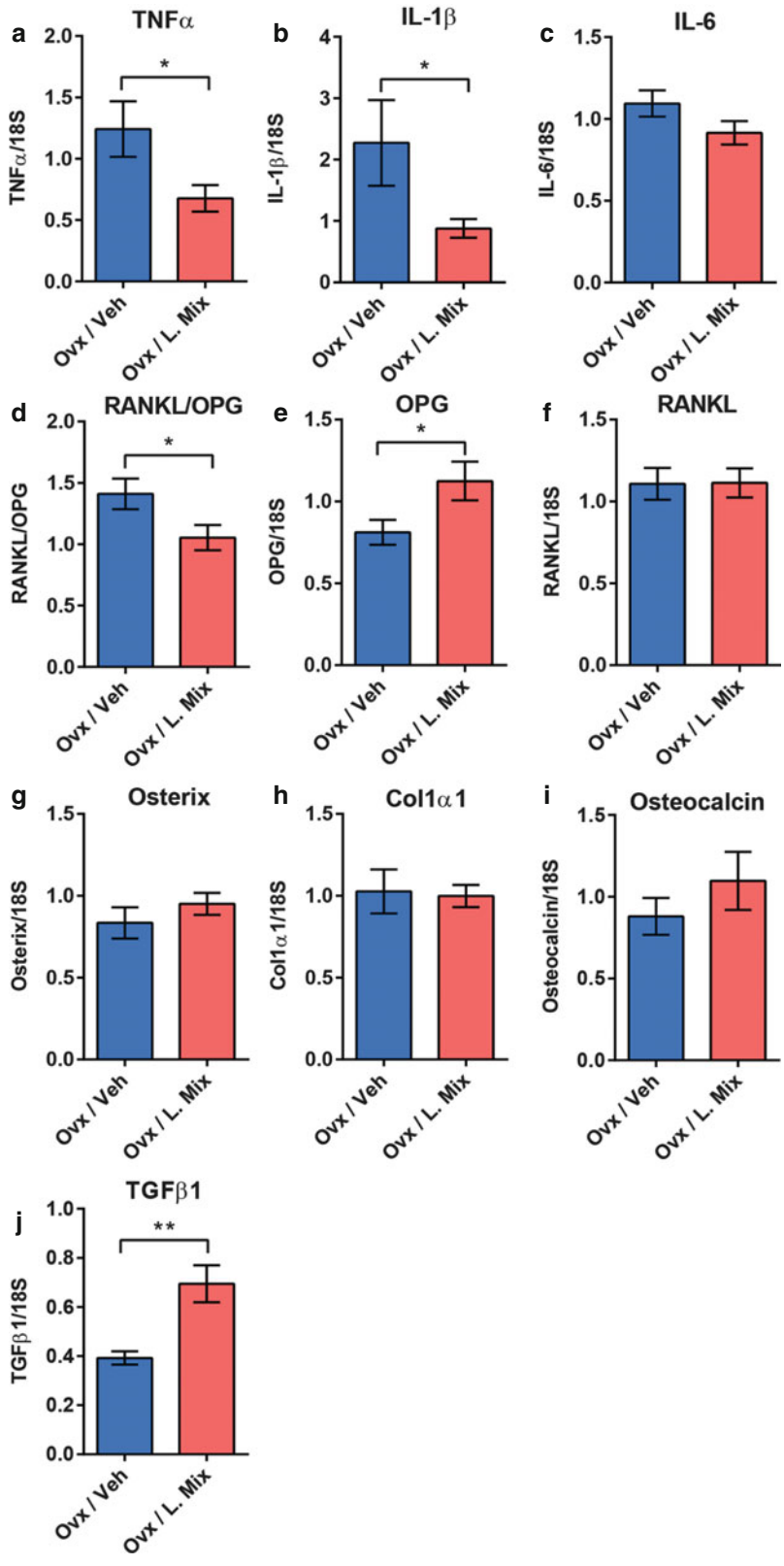

Fig. 15.9 Probiotics reduces expression of inflammatory cytokines and the RANKL/OPG ratio in cortical bone and increases expression of TGFβ in bone marrow. QRT-PCR analysis of the expression of genes known to promote bone resorption; (**a**) Tumor Necrosis Factor alpha (TNFα), (**b**) Interleukin-1β (IL-1 β), (**c**) Interleukin-6 (IL-6), (**d**) Ratio of Receptor activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegerin (OPG), and individual graphs for (**e**) OPG, (**f**) RANKL and genes known to promote bone formation; (**g**) Osterix, (**h**) Collagen, type I, α1 (Col1α1) and (**i**) osteocalcin in cortical bone and (**j**) transforming growth factor β (TGFβ) in bone marrow from 14-week-old ovariectomized mice treated with either vehicle (veh) or a mixture of three probiotic Lactobacillus strains (L. mix). Values are given as mean ± SEM, n = 9–10. ** $p \leq 0.01$, * $p \leq 0.05$ versus veh treatment, Student's *t*-test (Ohlsson et al. [23], with permission)

mation-favored diseases, including osteoporosis. The differentiation of the mesenchymal stem cells lineage toward either adipogenesis or osteoblastogenesis depends on specific cytokines that can be triggered by dietary or environmental means. If such chronic inflammation-supporting "modern diet" has become the habitual diet, especially if this dietary change goes along with the observed increased presence of reactive oxygen species, the stem cell differentiation favors adipogenesis and diminishes osteoblastogenesis [11].

Osteoporosis and periodontitis have intersections because a general skeletal loss of bone mineral affects the interdental bone of the periodontium as well, and both diseases are triggered by inflammation. Specific probiotic bacteria have shown health beneficial effects with regard to periodontitis. In mice an experimental periodontitis was induced mechanically by placing a silk ligature around a maxillary molar. When the animals were treated with *Lactobacillus brevis CD2* bone loss was significantly lower compared to placebo-treated animals [15]. Furthermore, probiotic-treatment decreased the expression of anti-inflammatory cytokines like tumor necrosis factor, and interleukin-1β, -6 and -17A (Fig. 15.10). In addition the probiotics modulated the composition of the oral flora when the animals showed higher counts of aerobic bacteria and lower counts of anaerobic bacteria than placebo-treated mice [15]. These results demonstrate a probiotic potential of *Lactobacillus brevis CD2* with respect to dental health.

Foods with Prebiotic or Antioxidative Activity, "Healthy Diets" and Practical Consideration

General loss of bone mineral and bone density includes the loss of oral bone (Fig. 15.11). In the following, periodontitis, agumphiasis and loss of teeth occur and lead to increasing costs for dental prosthesis. Subsequently, problems in chewing make people avoiding hard foods like some fruits and vegetables that, besides delivering minerals, trace elements and vitamins, put mechanical load on the periodontal tissue, which supports bone formation and saliva flux. Avoiding also nuts, seeds and grains because of tooth problems further leads to less healthy dietary habits and bone health-supporting foods may be missed [29, 30]. Saliva flux is important for oral hygiene, and at unfavorable oral conditions some bacteria trigger

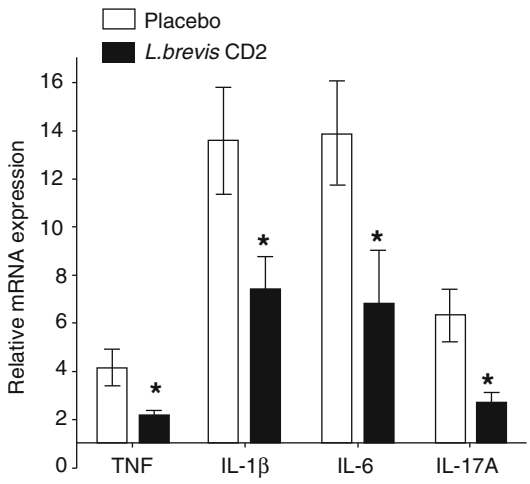

Fig. 15.10 Probiotic L.brevis CD2 inhibits mRNA expression of proinflammatory cytokines. Significantly different with *p<0.01 between the indicated groups. *IL* interleukin, *TNF* tumor necrosis factor (According to Maekawa and Hajishengallis [15], with permission)

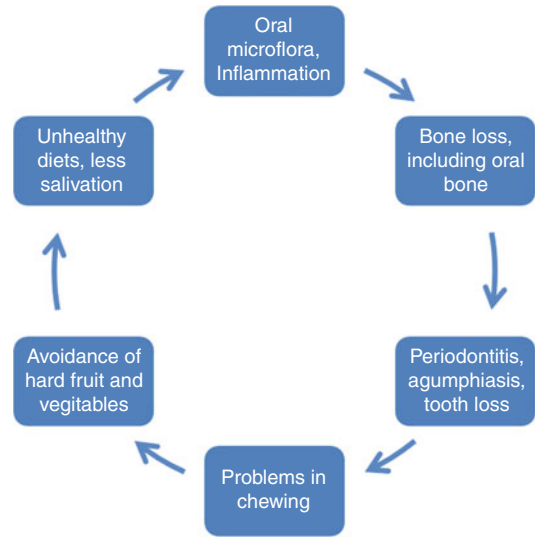

Fig. 15.11 Habitual diets, choice of foods and consequences for bone and teeth health (Adapted from Scholz-Ahrens et al. [30])

inflammatory processes which may also lead to general bone loss.

Osteoporosis is an age-dependent disease and is considered a chronic disease with an imbalance in reactive oxygen species (ROS) being a risk factor [27]. Therefore ROS should be in homeostasis to avoid cell damage with subsequent chronic ROS-related diseases including loss of bone mineral and osteoporosis [13, 27]. In sex-steroid deficiency the bone-turnover stimulating effect with a dominance of osteoclast activity is based on the physiologic aging processes when oxidative stress is increasing. Manolagas [16] therefore proposed a "paradigm shift from the estrogen-centric account of the pathogenesis of involutional osteoporosis to one in which age-related mechanisms intrinsic to bone and oxidative stress are protagonists". Natural antioxidants and ROS scavenging processes are ubiquitous. They occur in plants and microbes [37] and thus in foods like fermented foods, vegetables, herbs ~~herbes~~ and spices. In the gastrointestinal tract prevention of oxidative stress also counteracts a dominance of pathogen microbes. NDO of the fructan family including inulin and levans are accumulated as storage carbohydrate up to approximately 15 % in several plants [37]. Since they exerted anti-oxidative effects [36] and acted as important ROS scavengers in plants, this group of prebiotics might be of interest with respect to functional food ingredients with anti-oxidative potential.

NDO with prebiotic activity are inulin, oligofructose, and some Low Molecular Weight Carbohydrates (LMWC) such as glucooligosaccharides, maltooligosaccharides, xylooligosaccharides, stachyose, lactulose, and raffinose. They occur naturally in plant foods such as allium varieties, legumes, globe artichoke (Cynara cardunculus), and Jerusalem artichoke (Helianthus tuberosus) [10, 12, 18–20]. Moongngarm et al. have demonstrated a range of contents of inulin, FOS and LMWC in plant foods commonly used in Thailand. Bulbs, tuber/roots or rice samples were investigated. Garlic contained about 10 % more inulin + FOS than shallot and about 50 % more than onions; from a practical point of view onions are a good source of NDO due to their higher amount present in cooking recipes than garlic. Tuber and roots contained only about 10 % of prebiotics or less of that of onions, but in Thailand these are staple foods and thus may contribute comparable amounts of prebiotics or even more to their daily intake.

The content of NDO in foods is one characteristic, but the prebiotic activity or probiotic stimulating capacity is a different one. Therefore the authors also investigated the bacterial growth of *Lactobacillus acidophilus* on extracts from these plant foods. The prebiotic activity score of onion, shallot and garlic was higher than that of FOS and almost reached the one of inulin, which was the highest, while germinated rice cultivar was only about 10 % of that of onions. The prebiotic action or the probiotic stimulating capacity of these prebiotic ingredients on other bacterial strains remain to be investigated.

Summary and Conclusion

A substantial number of publications in cell culture, animal and human studies have dealt with the effect of prebiotics on mineral metabolism and bone health. Several mechanisms on the action of prebiotics are proposed. Reports on probiotics and bone are comparatively scarce and even fewer are studies on synbiotics in this respect. Probiotics came into focus few years ago. Specific bacteria, lactobacilli in particular, have shown effects on immune modulation, on expression of inflammatory cytokines that affect bone turnover, and on bone preservation in ovariectomized rodents. Probiotics also affected the periodontium and tooth health. Synbiotics have revealed bone preserving effects in animals. Probiotics and prebiotics have been investigated for their potential as alternatives for low-dose antibiotic utilization but studies are rare. A variety of study conditions affected the experimental outcomes, and the number of studies is too low to draw robust conclusions from these observations. Recent analyses of naturally occurring prebiotics in foods have shown that some fruit and several vegetables contribute to the amount of prebiotics in meals. Again, until now, there is too little information. Nevertheless, it may be concluded

that prebiotics, probiotics and synbiotics, either as natural occurring food or as supplemented functional food may contribute to make a "modern diet" healthier with respect to mineral bioavailability, immune response and bone preservation.

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Part V

Vitamin D and Calcium

Predicting Calcium Requirements in Children

16

Connie M. Weaver, Michael Lawlor, and George P. McCabe

Abstract

Calcium requirements can be set on the basis of intake for maximal retention as was done for most age groups by the Institutes of Medicine in 1997 or by the factorial method as was done in 2011. This chapter explores the possibility of a personalized calcium recommendation for calcium recommendations by age, sex, and ancestral group using adolescents aged 9–18 as an example. Data are available for most subpopulations for the maximal retention approach to nutrient requirements. As a step toward personalizing recommendations using the factorial approach, we developed a parametric model for determining average and peak bone calcium accrual by sex and age based on longitudinal data in white boys and girls.

Keywords

Calcium • Requirement • Adolescents • Maximal retention • Factorial approach

Determining Calcium Requirements

The Food and Nutrition Board of the Institute of Medicine (IOM) sets Dietary Recommended Intakes (DRIs) of North America [1]. Since 1997, DRIs have included where appropriate the Estimate Average Requirement (EAR) where 50 % of the population is adequate, the Recommended Dietary Allowance (RDA) which includes a margin of safety (upper 97.5 percentile of the requirement distribution), and the Upper Level (UL) to indicate a level not to exceed to reduce risk of excess. When the IOM deems an

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C.M. Weaver (✉)
Department of Nutrition Science, Purdue University, West Lafayette, IN 47907-2059, USA
e-mail: weavercm@purdue.edu

M. Lawlor • G.P. McCabe
Department of Statistics, Purdue University, West Lafayette, IN 47907-2059, USA

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EAR cannot be determined, an Adequate Intake (AI) is set to indicate intakes consistent with a healthy population. EARs have been determined using the factorial method, intake for maximal retention, and intake for maximizing a functional indicator. The factorial method is the most often used approach for mineral requirements which sets intakes based on adding daily needs for growth and losses adjusted for absorption efficiency. For calcium, the 1997 panel had sufficient data to determine intakes for maximal retention for most age and sex groups [2]. The data came from metabolic balance studies over a range of calcium intakes when calcium retention balance was plotted against calcium intakes that included sufficiently high intakes, a plateau was observed above which further increases in calcium retention were not statistically significant. The panel reasoned that the plateau intake would be a reasonable value for calcium recommendations because it would represent maximal bone accrual or retentions since >99 % of the body's calcium is in the skeleton. As there is a linear relationship between bone mass or more specifically bone mineral density (BMD) and reduced fracture risk [3], optimizing bone calcium retention is a functional indicator. The Food and Nutrition Board conservatively chose to use these threshold intakes to set AIs rather than EARs because it was a new approach. The next panel converted these AIs to the 2011 EARs and RDAs but used the factorial approach to determine the EARs [4]. For adolescents, the EAR and component values are shown in Table 16.1.

Strengths and Limitations of Maximal Retention and Factorial Approaches to Determining Calcium Requirements

Estimates of calcium retention on a range of calcium intakes are needed to find the intake where the plateau occurs. Theoretically, calcium retention could be determined by bone mineral content (BMC) from DXA measures as calcium is a constant fraction of BMC. However, it is practically difficult to control calcium intake for sufficiently long periods to determine change in BMC exception in animal models. Thus, calcium balance studies have been used to understand the relationship between calcium intake and calcium retention.

The 2011 IOM calcium recommendation for adolescents had access to longitudinal BMD (and therefore, bone calcium) data through the pubertal growth spurt for the first time. These data came from the Saskatchewan Bone Mineral Accrual Study [6]. The IOM panel reasoned that DXA is much more precise than balance (1–2 % vs. 10–15 % precision) without the systematic error of balance. Further, the intake for maximal retention approach used by the 1997 panel placed too much emphasis on high calcium intakes to determine the threshold. Balance studies do have systematic errors because under-collection of excretion is a problem, but not over-collection. However, systematic errors only influence the actual values for retention, not the intake where the plateau occurs. The 2011 IOM panel decided to use average calcium accrual values from the Saskatchewan study [5] for growth needs in factorial equations to determine calcium requirements for adolescents. Using average calcium accretion values blunts the demand for calcium at the peak.

The factorial approach used by the 2011 IOM panel for determining calcium requirements for adolescents has many limitations. Calcium accrual determined by average DXA data taken longitudinally in the Saskatchewan Growth Accrual Study was not in children consuming optimal calcium intakes, i.e. less than the threshold intake, so that calcium accrual may have been less than their genetic potential. Actual calcium intakes are not well known without controlling the diet and other dietary constituents, some of which influence calcium absorption and excretion. These confounding constituents can vary widely on a self-selected diet. In longitudinal DXA studies, calcium intake is estimated by diet assessment methods. Actual calcium intake cannot be validated as for energy intakes. We have shown that diet assessment using 6–9 day diet records under reports energy intake determined by objective methods (double labeled

Table 16.1 2011 IOM factorial approach to determining EAR in adolescents

Study Author	Gender age(y)	Average calcium age/accretion (mg/day)	Urinary losses	Endogenous fecal calcium losses (mg/day)	Sweat losses (mg/day)	Total needed (mg/day)	Absorption (%)	Estimated total intake (adjusted for absorption)
Vatanparast [5]	Female 9–13	151	106	112	55	424	38.0	1116
	Male 9–13	141	127	108	55	465	38.0	1224
	Female 14–18	92	106	112	55	365	38.0	961
	Male 14–18	210	127	105	55	500	38.0	1316
	Female 9–18	121	106	112	55	394	38.0	1037
	Male 9–18	175	127	108	55	465	38.0	1224

Table 16.2 Subgroup components of calcium components on adequate calcium intakes for factorial approach to determining EAR in adolescents

Race	Urinary losses gender	Endogenous fecal calcium losses (mg/day)	Absorption (mg/day)	(%)	Reference
White	Female	100	112	38	[9]
	Male	127	108	38	[5]
Black	Female	46	109	54	[9]
Asian	Female	87	104	55	[10]
	Male	79	154	54	[10]
Hispanic	Female	75	ND	39	[11]
	Male	100	ND	39	[11]

water and metabolizable energy intake) by an average of ~35% in adolescent boys and girls [7]. Inaccurate assessment of calcium intakes can alter interpretation of the amount of calcium that is required for maximal bone accrual. Not only does calcium accrual vary with calcium intake up to the threshold intake, but other components of the factorial equation do as well, especially calcium absorption. The 2011 IOM panel did not factor in these components in determining requirements.

The nutrition scientific community should have a serious discussion about which deserves more emphasis, nutrient intake or the outcome variable, i.e. retention, functional indicator, health benefit. The current trend is to strive for more rigorous outcome measures and settle for weak measures of independent variables, namely diet assessment methods of nutrient intake. This trend comes from adopting an evidence-based medicine approach, but for nutrient requirements, not controlling or knowing nutrient intakes accurately may be unwise [8]. When the outcome measures of interest are disease outcomes (for bone this means fracture), it is impractical to control nutrient intakes for sufficiently long. Furthermore, chronic disease outcomes are rarely the result of a single nutrient deficiency.

Personalizing Calcium Requirements in Adolescents

We have sufficient data for determining recommended calcium intakes for adolescents for many of the major subgroups of sex, age, and ancestry using the intake for maximal retention approach (Table 16.2).

These values come from highly controlled and supervised feeding studies of 3 week duration for each intake using the second 2 weeks for balance to determine average daily retention values. The appropriate indicators for compliance with collections were used, i.e. PEG, a nonabsorbable fecal marker and urinary creatinine.

Separate subgroup recommendations have not been determined using calcium accrual by DXA and the factorial method. The Saskatchewan Bone Accrual Study was only in white boys and girls. Our goal is to develop subgroup accrual data in various subgroups to later incorporate into subgroup appropriate factorial equations.

Determining DXA-derived Bone Calcium Accrual

The Saskatchewan Pediatric Bone Mineral Accrual Study used 6 year longitudinal DXA data to determine calcium accrual in 60 white boys and 53 girls. Bailey et al. [12] used schematics to illustrate the fact that if individual peak accretion rates were averaged, the peak was greatly blunted compared with determining average peak BMC accrual rates [13] when peaks were aligned (407 vs 320 g/y for boys (Fig. 16.1) and 322 vs 240 for girls). The average values shown in Fig 16.1 with boys is only for the 5 representative boys. The 2011 IOM panel used calcium accrual rates calculated from BMD accrual rates as visualized in panel

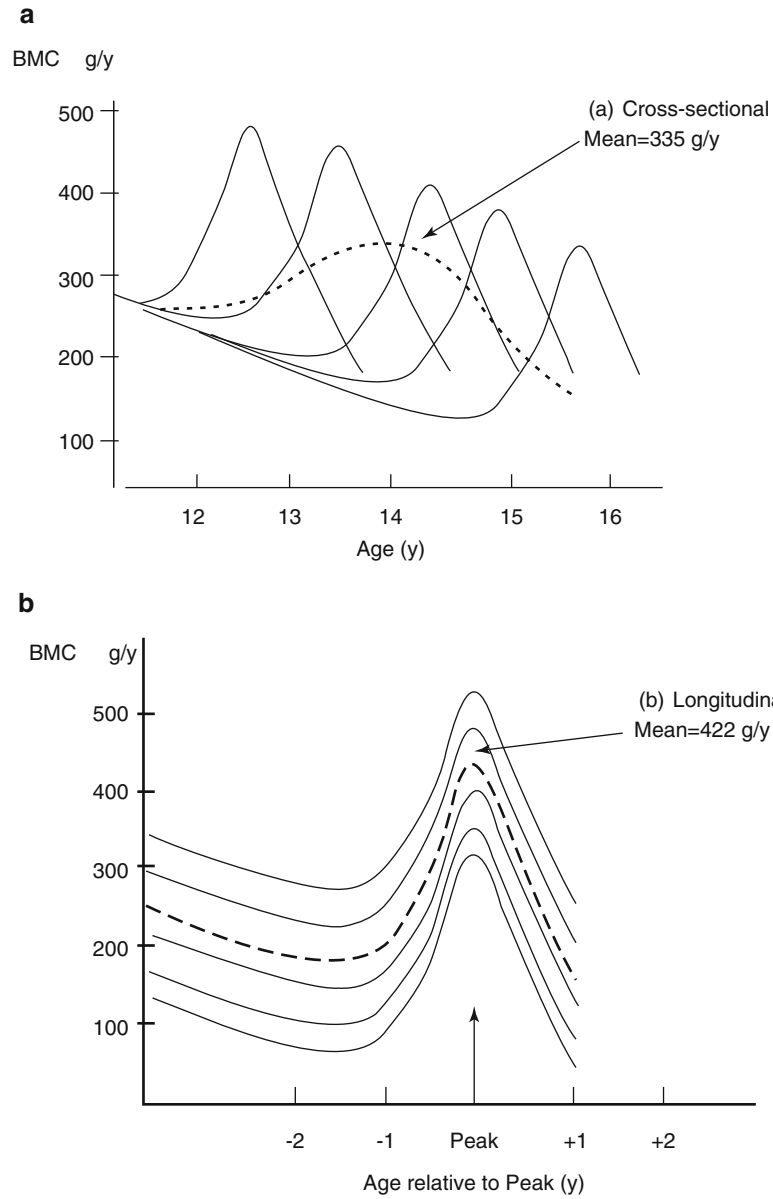

Fig 16.1 Bone accrual from Saskatchewan Bone Mineral Accrual Study in boys [6]

A of Fig. 16.1 rather than at peak demand as visualized in Panel B. We developed a parametric model to estimate the joint distribution of the age and the accrual at the peak. We then used this distribution to determine calcium demand for bone accrual both as an average over age (as in Panel A) and at the peak (as in Panel B). To do that, we received data for BMC from the Saskatchewan Bone Mineral Accrual Study from Adam Baxter-Jones.

We substituted our model-derived bone calcium accrual averaged over the age ranges of 9–13 and 14–18 y for calcium accretion, age ranges used by the IOM, but we retained all of the other components of the factorial method as shown in Table 16.1. Next we substituted our model-derived peak calcium accrual for calcium accretion into the factorial equation. A comparison of the calcium EARs determined using the three approaches for adolescents is

Table 16.3 Adolescent calcium DRIs using three approaches for determining calcium accrual needs

Age	DRI	Gender	IOM	Our model average	Our model peak
9–13	EAR	Female	1100	1203	1274
		Male	1100	1326	1484
14–18		Female	1100	876	1274
		Male	1100	1176	1484
9–13	RDA	Female	1300	1375	1482
		Male	1300	1628	1834
14–18		Female	1300	1068	1482
		Male	1300	1572	1834

shown in Table 16.3. The RDAs were determined from the EAR plus 2 SD. Using our model-derived peak calcium accrual estimates, we determined that the percent of individuals not meeting their peak demand would be 95.1 % for girls and 98.6 % for boys using the current IOM EAR.

Obviously, meeting the peak calcium intake demand is not necessary throughout adolescence. The risk of not meeting the peak needs at this narrow window is unknown, but inadequate intakes may be costly for ensuring the strongest peak bone mass possible to reduce risk of osteoporosis. Even if catch up growth occurs, as suggested by Matkovic et al. [14], fracture risk is higher during the period of delayed BMC accrual. There is no correct measure to determine age of PBM accretion velocity for the individual to intervene proactively. However, the investigators of the Saskatchewan Bone Mineral Accrual Study found a 0.5 y lag between peak height and PBM accrual velocity [15]. Consequently, when adolescents begin their rapid growth in height, there is time to adjust calcium intakes for the next approximately 2 years. Perhaps there should be two recommended calcium intakes during adolescence, one for peak growth and one for the rest of adolescence. There is a precedence for life stage adjustments in recommended intakes as iron in women over 50 y who have gone through menopause vs. those who have not or pregnant and lactating women aged 19–30 y.

Next Steps

Our parametric model determined the distribution of peak calcium accrual in white adolescent boys and girls. The model will be validated using another longitudinal data set of white adolescent boys and girls from the Iowa Bone Development Study.

Toward our goal of developing subgroup calcium recommendations, we will calculate bone calcium accrual from NHANES data. Then we can adjust calcium losses and fractional calcium absorption using data from our controlled feeding studies to determine calcium recommendations by sex and race/ethnicity. This has promise to be an advancement toward personalized nutrition.

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Assessment of Vitamin D Status

17

Paul Lips, Natasja M. van Schoor,
and Renate T. de Jongh

Abstract

Vitamin D status is usually assessed by the measurement of the serum 25-hydroxyvitamin D (25(OH)D) concentration. Serum 25(OH)D can be measured by immunoassays, HPLC and mass spectrometry, the latter being the gold standard. A high variability between methods and laboratories exists, leading to problems when comparing vitamin D status between countries and diagnosing vitamin D deficiency or inadequacy. Quality control and standardization programs are decreasing the variation. The measurement of serum 25(OH)D is influenced by vitamin D binding protein (DBP), which is in its turn influenced by pregnancy and disease. Genetic variants of DBP influence the binding to 25(OH)D and thereby the concentration. The measurement of serum 25(OH)D can also be influenced by other metabolites such as 24,25-dihydroxyvitamin D and the 3-epimer of 25(OH)D. It has become possible to measure the free (unbound) serum 25(OH)D, but the clinical consequences still are uncertain.

Introduction

Vitamin D status is usually assessed by the measurement of serum 25-hydroxyvitamin D (25(OH)D). While 25(OH)D has been measured for more than 30 years, a high variability between methods and laboratories still exists [1–3]. This leads to problems when comparing vitamin D status between different countries or between different observational studies and clinical trials, and in clinical decision making [1, 3, 4]. The number of serum 25(OH)D measurements has been increasing very rapidly over the last 10 years in most laboratories. It is questionable

P. Lips, MD, PhD (✉) • R.T. de Jongh, MD, PhD
Department of Internal Medicine, Endocrine Section,
VU University Medical Center,
1007 MB, P.O. Box 7057, Amsterdam,
The Netherlands
e-mail: p.lips@vumc.nl

N.M. van Schoor, PhD
EMGO Institute for Health and Care Research,
VU University Medical Center,
Amsterdam, The Netherlands

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whether a 25(OH)D measurement always is necessary. Sometimes a vitamin D deficient or replete state can be easily predicted and some prediction models have been developed [5].

Serum 25(OH)D can be assessed by immune-based and mass- based techniques, the latter becoming the gold standard. Quality control programs have been initiated many years ago, but real standardization has been started more recently [3].

The measurement of serum 25(OH)D can be hampered by vitamin D binding protein (DBP), the lower the DBP, the lower the serum 25(OH)D level [6, 7]. The DBP concentration is influenced by physiological and pathological states. Genetic variants of DBP also influence binding, and different DBP assays may give highly different results [8].

Assays for serum 25(OH)D may be influenced by other vitamin D metabolites. Part of the problems may be overcome by measurement of the free (unbound) 25(OH)D concentration. These points will be the subjects of the current review.

Prediction of vitamin D Status

Vitamin D deficiency is defined as serum 25(OH)D < 30 nmol/l (12 ng/ml) according to the Institute of Medicine [9]. Serum 25(OH)D between 30 and 50 nmol/l (12–20 ng/ml) is inadequate for some persons. Almost everybody is vitamin D sufficient with a serum 25(OH)D of at least 50 nmol/l (20 ng/ml). A serum 25(OH)D above 75 nmol/l (30 ng/ml) is not consistently associated with increased benefit [9]. The risk for vitamin D deficiency in residents of nursing homes may be over 80 % [1], while vitamin D inadequacy (serum 25(OH)D 30–50 nmol/l) may exist in the remainder. One may wonder whether measurement of 25(OH)D is necessary, because supplementation with vitamin D is indicated in most nursing home residents anyhow. On the other side of the spectrum, outside workers, farmers or life guards usually have a very high vitamin D status, making measurement of 25(OH)D not a priority. Prediction instruments have been developed to estimate vitamin D status with easily assessable variables. Such an instrument was developed in the Longitudinal Aging Study Amsterdam, an ongoing epidemiological study on the determinants of healthy aging [5]. In this study, simple predictors were chosen such as age, sex, season, bicycling, gardening, transport use and medication. These are indicators of general health and sunshine exposure. A low score indicated a low risk for vitamin D deficiency, while a high score indicated a high risk. Season is a very important predictor. The mean serum 25(OH)D increased 22 nmol/l from winter to summer in people aged 55–65 years and 14 nmol/l in persons of 65 years and older in the Longitudinal Aging Study Amsterdam [10].

Assessment of Vitamin D Status: Laboratory Methods

Assays for 25(OH)D have shown a steady evolution from precise, laborious methods allowing the measurement of few samples in one run [11] to high throughput methods allowing the measurement of hundreds of samples per day [12]. Initially we used HPLC for separation of metabolites followed by three competitive protein binding assays to analyze 25(OH)D, 24,25(OH)2D and 1,25(OH)2D in the same procedure, which was very precise, but very laborious and costly [11, 13]. This assay has been replaced by radioimmunoassays (RIA) and high throughput methods. The types of assay are summarized in Tables 17.1 and 17.2. Currently, liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) has been developed as the gold standard.

Two major characteristics for every assay are precision and accuracy. The precision is the ability to reproduce the same results on the same sample within the same run (intra-assay) or between different runs (interassay). It is expressed as coefficient of variation, which is for RIA typically around 10 % and for LC-MS/MS near 5 %. The accuracy is the ability to obtain the "true" value, i.e. the value defined by an international standard. The error may be 10–20 %. The LC-MS/MS should perform the best in this respect.

Table 17.1 Methods for measuring serum/plasma 25-hydroxyvitamin D concentration

Antibody based methods using kits
Rapid high output automated immunoassays
HPLC + UV detection
Mass spectrometry: LC-MS/MS (currently gold standard)
Free 25(OH)D assay

Table 17.2 Variations in the 25-hydroxyvitamin D assay and impact on clinical decision making

Classification	IDS	Diasorin
25(OH)D		
<25 nmol/l	6 %	17 %
25–50 nmol/l	29 %	34 %
>50 nmol/l	65 %	49 %
Needing treatment: (<50 nmol/l = 20 ng/ml)	35 %	51 %

Adapted from Barake et al. [3]

Standardization

International comparison of serum 25(OH)D measurements showed poor results with differences between laboratories of 30 % or more [2]. This may partly explain the high variability in surveys of vitamin D status and different outcomes in supplementation studies.

The Vitamin D External Quality Assessment Scheme (DEQAS) was established in 1989 in order to compare laboratory results on a panel of serum samples. As result the participating laboratories receive their laboratory performance compared to the mean of the participating laboratories. A study on DEQAS results classified according to the laboratory method showed large systematic differences between radioimmunoassays (e.g. between Diasorin and IDS). HPLC and LC-MS/MS showed a positive bias with regard to the mean of about 10 % and 5 % respectively [3]. The high variation in the results of RIAs had a large impact on clinical decision making. The percentage of serum 25(OH)D < 25 nmol/l was 6 % according to one and 17 % according to another RIA. The percentage needing treatment (serum 25(OH)D < 50 nmol/l) was 35 % according to one and 51 % according to the other RIA [3]. This shows that further standardization is highly necessary.

The Vitamin D Metabolites Quality Assurance Program of the National Institute of Health and the National Institute of Standardization provides standard sera to improve accuracy. The Vitamin D Standardization Program (VDSP) is the most recent step to standardize assays, cross-calibrate different study centers and to enable comparison between epidemiological studies and clinical trials and to pool data from different studies [14]. The European study ODIN (Food based solutions for optimal vitamin D nutrition and health through the life cycle) uses the VDSP standardization to compare serum 25(OH)D between 14 population studies and to perform individual patient based meta-analyses of cohort studies and clinical trials.

Influence of Vitamin D Binding Protein

The 25(OH)D in the circulation is bound to vitamin D binding protein (DBP) or Group specific Component (Gc) for 85 %. About 15 % is loosely bound to albumin. The free (unbound) fraction is less than 1 % and only this fraction is thought to be metabolically active.

The DBP concentration may influence the measured total serum 25(OH)D concentration, the lower the serum DBP, the lower the serum 25(OH)D concentration. DBP is influenced by physiological and pathological states. It increases during pregnancy [6], and decreases after trauma [7]. It is also decreased in hemodialysis patients to a variable degree. The change in DBP is associated with similar changes in serum 25(OH)D. The "free 25(OH)D index", i.e. serum 25(OH)D/serum DBP may adjust for changes in serum 25(OH)D in pregnancy or after trauma. Recently, the influence of serum DBP on the measurement of 25(OH)D by automated assays, radioimmunoassay and LC-MS/MS was studied in pregnant women, dialysis patients, intensive care patients and a healthy control group [12]. The LC-MS/MS and the RIA performed best and were not influenced by the concentration of serum DBP, while most automated assays were more or less influenced, making the results less accurate.

The DBP concentration is different according to naturally occurring DBP (Gc) variants. The most common genotypes are Gc 1f, Gc 1 s and Gc 2, all characterized by one aminoacid change. The Gc 2 genotype is associated with a lower DBP concentration and a lower serum total 25(OH)D concentration with a dose-allele effect. The homozygote Gc 2 is associated with the lowest serum DBP, followed by heterozygote Gc 2 while the other genotypes are associated with higher DBP concentrations [8]. In addition, the Gc subtypes vary by country and race. The Gc 1f is the commonest in African countries, Afro-Americans and some Asian countries, while Gc 1 s and Gc 2 are more common in European countries and white Americans.

Recently, the serum DBP concentration, DBP (Gc) subtypes and serum 25(OH)D were studied in American blacks and whites [15]. The Gc 1f homozygotes and heterozygotes were very common in blacks, as was known, but the DBP concentration was also much lower (about half) in blacks than in whites. As in other surveys, serum 25(OH)D was much lower in blacks than in whites, and the investigators calculated the bioavailable 25(OH)D, which was similar in blacks and whites. This might be an explanation why American blacks do not show signs of vitamin D deficiency, according to the authors. However, the paper was much criticized because of the used methods. The authors had used a monoclonal antibody assay for DBP measurement, while in other studies polyclonal assays were used. It appeared that the monoclonal assay did not measure serum DBP adequately, because results for serum DBP with this monoclonal assay were consistently lower in blacks, than serum DBP measured with a polyclonal antibody [16, 17]. This is a clear illustration how assay methods can lead to erratic conclusions.

Interaction with Other Vitamin D Metabolites

Assay results for serum 25(OH)D can be influenced by other vitamin D metabolites. Not all immunoassays will recognize 25(OH)D2 to a similar degree as 25(OH)D3. Both metabolites are well separated by LC-MS/MS because the masses are different [18]. Recognition of D2 metabolites is less important in most European countries because vitamin D2 is not naturally occurring and vitamin D2 supplements are uncommon in Europe.

Overestimation of serum 25(OH)D is possible by interaction with 24,25-dihydroxyvitamin D (24,25(OH)2D), which can cross-react with 25(OH)D in immunoassays. The percentage bias may be more than 10 %, even after adjustment [19]. HPLC and LC-MS/MS are less prone to bias caused by 24,25(OH)2D.

The 3-epimer of 25(OH)D is mainly observed in neonates, but it may also occur in adults. As a stereo-isomer it only differs from 25(OH)D in stereological arrangement and cannot be distinguished by usual immunological or mass-based methods. The mean serum concentration of the 3-epimer is around 5 % but it may vary considerably [20].

Measurement of Free 25(OH)D

According to the free hormone hypothesis, only the free hormone is metabolically active. An analogy exists with thyroxine, T4, which is bound to a specific binding protein to a large degree. Currently, free T4 is measured as it better reflects disease states.

Measurement of the free serum 25(OH)D concentration avoids problems with DBP. Recently, it has become possible to measure free 25(OH)D with a commercially available kit. The measured free 25(OH)D concentration correlated only moderately with the calculated free 25(OH)D [21]. The correlation was worse in patients with liver failure. The correlation of free 25(OH)D with total 25(OH)D was reasonable, but the regression line was very different in patients with liver failure due to a much lower DBP [21]. It is not yet known whether the free 25(OH)D assay will better predict clinical outcomes than the total serum 25(OH)D.

Conclusion

Last years the number of serum 25(OH)D measurements has exponentially increased. Prediction instruments based on simple

predictors may decrease the necessity for serum 25(OH)D measurements. Vitamin D status is poor in nearly all nursing home residents, so measurement of serum 25(OH)D is not necessary in this group, because almost all need vitamin D supplements. Many antibody based 25(OH)D assays still have an important measurement error leading to misclassification of vitamin D status in 10–20 %. The LC-MS/MS method is more precise than immunoassays and currently the gold standard. Standardization programmes can considerably decrease the measurement error. Official standard serum samples can improve accuracy.

Immunoassays for 25(OH)D measurement perform differently according to vitamin D binding protein status. DBP concentrations are influenced by pregnancy, diseases and trauma, and genetic variants, and thereby also influence total serum 25(OH)D. DBP variants influence DBP concentration and total serum 25(OH)D. Measurement of DBP by monoclonal assay yields much lower DBP results in blacks than those obtained by polyclonal assays. This may lead to erroneous conclusions regarding bioavailable 25(OH)D.

Other vitamin D metabolites, such as 24,25(OH)2D, or variants, such as the 3-epimer of 25(OH)D may influence the measured serum 25(OH)D concentration. Measurement of free 25(OH)D may partly solve measurement problems, especially those caused by DBP.

Challenges

Standardization of serum 25(OH)D measurement is urgent in order to obtain the same classification of vitamin D status (deficient, inadequate, sufficient) at different laboratories. The problems of measurement of serum DBP variants by monoclonal vs polyclonal assays should be solved. The influence of other vitamin D metabolites and variants on serum 25(OH)D measurement should be minimized. The usefulness of the measurement of free 25(OH)D in clinical situations, e.g. pregnancy, trauma, liver disease, and as predictor for vitamin D-related outcomes should be further investigated.

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Vitamin D in Obesity and Weight Loss

18

Sue A. Shapses, L. Claudia Pop, and Stephen H. Schneider

Abstract

Vitamin D maintains calcium and phosphate homeostasis, is essential for bone development and maintenance, and also has non-skeletal effects [1]. Severe vitamin D deficiency causes rickets in children and osteomalacia in adults, while less severe vitamin D deficiency is associated with secondary hyperparathyroidism and increased bone turnover causing bone loss and fracture, especially in older populations [2]. Vitamin D deficiency has also been related to numerous diseases including diabetes and metabolic syndrome [3]. The obesity epidemic represents a public health concern worldwide and is associated with multiple comorbidities [4–7]. Vitamin D deficiency is also prevalent in many populations, including the obese [8–11]. Accumulating evidence shows that adiposity negatively influences vitamin D metabolism, storage, and action, and may influence the health risks associated with obesity. This chapter focuses on how obesity and body weight affect vitamin D metabolism and levels.

Introduction

Vitamin D maintains calcium and phosphate homeostasis, is essential for bone development and maintenance, and also has non-skeletal effects [1]. Severe vitamin D deficiency causes rickets in children and osteomalacia in adults, while less severe vitamin D deficiency is associated with secondary hyperparathyroidism and increased bone turnover causing bone loss and fracture, especially in older populations [2]. Vitamin D deficiency has also been related to numerous diseases including

S.A. Shapses, PhD (✉) • L.C. Pop, MD
Department of Nutritional Sciences, Rutgers University, 96 Lipman Drive, New Brunswick, NJ 08901-0231, USA
e-mail: shapses@aesop.rutgers.edu; claudiapop01@gmail.com

S.H. Schneider, MD
Division of Endocrinology, Metabolism and Nutrition, Rutgers-Robert Wood Johnson Medical School, 125 Paterson Street, Suite 5100A, New Brunswick, NJ 08901, USA
e-mail: steve.schneide@gmail.com

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diabetes and metabolic syndrome [3]. The obesity epidemic represents a public health concern worldwide and is associated with multiple comorbidities [4–7]. Vitamin D deficiency is also prevalent in many populations, including the obese [8–11]. Accumulating evidence shows that adiposity negatively influences vitamin D metabolism, storage, and action, and may influence the health risks associated with obesity. This chapter focuses on how obesity and body weight affect vitamin D metabolism and levels.

Recommended Intakes and Serum Levels in Healthy Populations and Obesity

In 2011, the Institute of Medicine (IOM) published the Recommended Dietary Allowances (RDAs) for vitamin D (600 IU/d for ages 1–70 year and 800 IU/d for ages 71 year and older). These RDA's meet the requirements of at least 97.5 % of the population and correspond to a serum 25-hydroxyviatmin D (25OHD) concentration of at least 20 ng/ml (50 nmol/liter) [1]. IOM recommendations were based on data regarding the relationship between vitamin D and skeletal health (including bone mineral content and density, fracture risk, and rickets/osteomalacia), calcium absorption and biomarkers such as serum 25OHD and parathyroid hormone (PTH), but not the extra-skeletal conditions [1]. However, bone health studies used in the IOM report did not include studies with vitamin D intakes at higher levels than 1000 IU/d. As a result, studies continue to be conducted to address the optimal dose for bone health and falls prevention with particular attention to populations at higher risk of vitamin D deficiency including the obese and the institutionalized elderly. Because excess adiposity attenuates the rise in 25OHD in response to vitamin D supplementation, the recommended intake remains unclear and is discussed in further detail below.

Body Weight, Adiposity and Vitamin D

The majority of fat-soluble vitamin D is stored in the adipose tissue [12]. Over the past decade, several studies have reported lower serum 25OHD concentrations in the obese compared to lean individuals [13]. The negative relationship between vitamin D status and anthropometric measurements of obesity has been observed across all ages and ethnic groups and has been confirmed by several meta-analyses [14, 15].

Lower serum 25OHD concentrations in the obese compared to lean subjects is attributed to differences in volume dilution between normal and higher body weight individuals [15–17]. Alternatively, fat-soluble vitamin D compounds can be stored in adipose tissue, leading to a reduced serum concentration [16, 18, 19], yet can be released into the circulation with lipolysis. This is further supported by evidence that only the obese have lower 25OHD whereas in larger non-obese persons (n=489), height and body surface area are positively associated with 25OHD [20]. This relationship may be attributed to an increased synthesis of vitamin D in the skin as a result of increasing body surface area without excess adiposity [20].

A recent study used genetic variants to better understand the potential causal relationship between BMI and 25OHD (using related single nucleotide polymorphisms-SNPs). This study found an association between obesity–related SNPs and low concentrations of 25OHD [21]. However, low serum 25OHD was not a determinant for higher BMI, and there was no relationship between vitamin D–related SNPs and obesity-traits [21, 22]. Previously, clinical trials with vitamin D supplementation for weight loss in overweight/obese individuals yielded inconsistent results [23–25]. There was weak support for an inverse association between BMI with calcium and vitamin D (or dairy) in epidemiological studies [26]. In the agouti obese mouse, high calcium diets have been shown to reduce lipogenesis, increase lipolysis and suppress body fat and weight gain [26], but subsequent clinical trials studies including RCTs were unable to find such a

relationship for Ca or with vitamin D [23–25, 27, 28]. Overall, the mechanistic basis for by which higher intakes of Ca and vitamin D (or serum levels) significantly affects adipose tissue and body weight is not supported by clinical evidence. In contrast, there is strong evidence that BMI or fat mass is negatively associated with 25OHD concentrations.

Whether a specific fat depot influences serum 25OHD has also been studied. In a study examining three cohorts from two large epidemiological studies (SHIP-Trend and Health2006), serum 25OHD was negatively associated with abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (Fig. 18.1) [29].

This study confirmed previous observations from other large epidemiological studies [30–32] generally showing a similar inverse association between total fat, VAT and SAT with serum 25OHD concentrations in adult populations. In some studies, after further statistical adjustments in the analysis, only VAT remained associated with 25OHD [32, 33]. Although Hannemann et al. specifically reported a 0.64 ng/mL decrease in serum 25OHD concentration for each additional kg of VAT (n = 4000 individuals), the strength of the association between VAT or SAT and 25OHD was similar [29]. A major limitation of most of these cross-sectional studies is that dietary vitamin D intake is almost never reported, and measurements are performed only on a single occasion and taken at different times throughout the year. Nevertheless, this association remains significant even when controlled for season of the year.

Mechanisms Explaining a Low Serum 25OHD in Obesity

Several studies have explored possible mechanisms underlying the association between obesity and vitamin D deficiency. Data showing similar levels of serum 25OHD between obese and normal weight individuals, when controlling for body size, would suggest that greater fat mass offers a larger distribution volume and explains lower 25OHD in larger body size individuals [16]. Lower 25OHD concentrations in obesity may also occur due to reduced synthesis of vitamin D_3 in the skin since the response to a given ultraviolet (UV) B radiation dose is attenuated in obese subjects compared to lean individuals [34]. Moreover, the ability of skin to release vitamin D into the circulation may be altered in obesity [34].

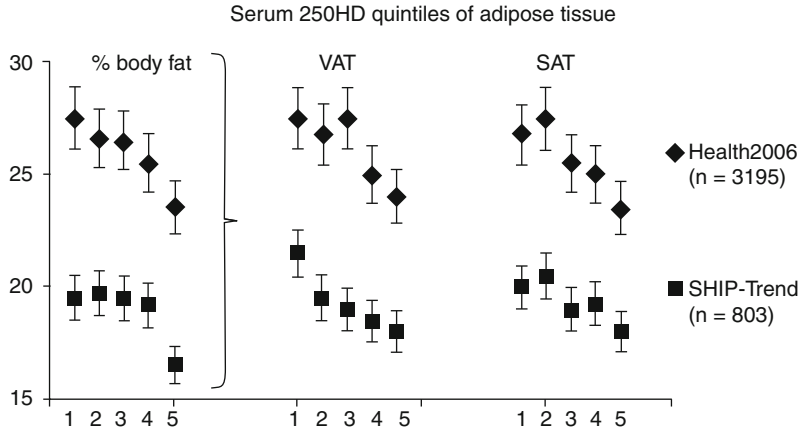

Fig 18.1 Serum 25OHD and quintiles of adipose tissue. Adjusted mean serum 25-hydroxy vitamin D (25OHD) concentrations with 95% confidence intervals according to sex-specific quintiles of visceral and subcutaneous adipose tissue (VAT and SAT) measured by MRI in SHIP-Trend and by ultrasound in Health2006 and of % body fat in SHIP-Trend (N = 803) and Health2006 (N = 3195) (Reproduced from Hanneman et al. [29] Original publisher: BioMed Central Ltd. Image reproduced in accordance with open- access policy of BioMed Central)

Consistent with these findings, Macdonald et al. reported lower mean vitamin D status in women in the highest compared to the lowest BMI quartile, despite greater body surface area and similar sunlight exposure in the higher BMI group, over 15 months [35]. This suggests that lower serum 25OHD levels in the obese compared to lean individuals are not due to insufficient sunlight exposure. In general the obese have reduced skin area exposure to sunlight due to wearing excess clothing and a lack of outdoor activity [18, 36]. This may further diminish vitamin D production in the skin and aggravate other obesity-related comorbidities.

Interestingly, in diet-induced obese C57BL/6 male mice, UV radiation exposure suppressed weight gain, glucose intolerance, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) measures [37]. However, many of the benefits of UV light were not reproduced by vitamin D supplementation. It is possible that the improvement of the metabolic syndrome occurs independently of vitamin D and is dependent on other UV radiation induced mediators such as nitric oxide.

In addition, hepatic biosynthesis of the vitamin D pathway might be altered in the obese in the presence of NAFLD. Nonalcoholic fatty liver disease and vitamin D deficiency are often found together, and while this is not unexpected, given that both are associated with obesity, there is growing evidence suggesting a closely linked and potentially causative relationship [38]. In support of this, there are studies in Chinese populations with and without diabetes showing an association between NAFLD and low concentrations of 25OHD that was independent of visceral adiposity [39, 40]. This association was also found in adolescents. Specifically, a lower seasonally adjusted serum 25OHD concentration was associated with NAFLD, independent of adiposity and insulin resistance in 994 adolescents living in Australia [41]. The inverse association between 25OHD with nonalcoholic steatohepatitis (NASH) and fibrosis was also shown in an Italian study in 73 children (8–18 year old) with NAFLD with a confirmed histological diagnosis in the liver [42]. These findings could imply an altered hepatic synthesis of 25OHD in the presence of fatty liver disease that is contributing to low serum levels in obese adults and adolescents [40, 41]. However, a recent study conducted in 239 overweight/obese individuals (mostly men) matched for BMI and adiposity found no relationship between vitamin D status or 25OHD levels and the amount of liver fat, the severity of NASH or insulin resistance [43]. This well designed study used magnetic resonance spectroscopy and liver biopsy to characterize the presence of liver fat and NASH [43]. Overall, the relationship between NAFLD and vitamin D status in obesity may differ in specific subpopulations and should continue to be explored.

Other possible causes for lower serum 25OHD have been attributed to a greater vitamin D catabolism in obesity due to elevated degrading enzyme CYP24A1 found in the obese compared to lean subjects [19]. Low serum 25OHD in obesity has also been attributed to the negative feedback control of hepatic 25OHD synthesis exerted by their elevated 1,25-dihydroxyvitamin D and parathyroid hormone concentrations [16]. In addition, because there is a higher prevalence of obesity in minority groups, it is possible that the ethnic/racial differences in vitamin D binding protein (DBP) and vitamin D receptor (VDR) genetic polymorphisms is contributing to the lower circulating 25OHD in many obese individuals [44].

Implications of low serum 25OHD in the obese The negative health consequences of low vitamin D levels may be further exacerbated by the excess adiposity in obesity [45–48]. For example, both adiposity and vitamin D deficiency are associated with insulin resistance. Cardiovascular disease is also associated with both obesity and vitamin D deficiency. Additionally, obesity in combination with vitamin D deficiency is associated with risk for several cancers that may be due to increased inflammation. Some of these disease states have been attributed to the chronic low-grade inflammation in the obese and it is possible that inflammatory markers are influenced by

vitamin D. Significant associations between low vitamin D status and markers of inflammation (including the ratio of IL-6 to IL-10) in overweight/obese elderly adults have been reported in the literature [49]. The fact that adipose tissue regulates and can be regulated by vitamin D suggests there may be clinical implications for the prevention of CVD, diabetes or cancer in the obese, but long-term and carefully designed studies are needed to confirm this. While the obese have higher prevalence of low vitamin D levels, it is still unclear what dose of vitamin D should be used in these individuals in order to reach vitamin D sufficiency and how to maintain a normal vitamin D status. In addition, vitamin D requirements in the obese may differ depending on the treatment targets including bone health or other health conditions.

Response to Vitamin D Supplementation in the Obese

Studies addressing serum 25OHD response to vitamin D supplementation in normal to overweight populations (BMI < 30 kg/m^2) estimated the rise in serum 25OHD to ~0.28 ng/mL per µg of vitamin D intake/d [50] or greater, ranging from 0.40 to 0.79 ng/mL/µg [17, 51]. These differences were attributed to differences in the type of vitamin D used, or varying characteristics of the subjects (age, BMI, etc.), compliance issues, or assay variability [51]. Since the response to vitamin D supplementation is greatly influenced by body weight, more recent studies have addressed the dose response relationship between serum 25OHD and vitamin D intake in obese populations.

In healthy, middle-aged adults (BMI < 27 kg/m^2), the combined increase in serum 25OHD at two levels of vitamin D intake (2000 IU and 4000 IU) was 0.26 ng/mL/µg of vitamin D/d [52]. In a similar study design in obese individuals (BMI ≥ 35 kg/m^2), the rise in serum 25OHD was 0.16 ng/mL/µg/d, which is lower compared to the rise in the non-obese [53]. Also, in two separate studies (ViDOS and STOP IT) conducted in normal weight and obese older Caucasian women, consuming a daily dose of vitamin D ranging from 400 to 4800 IU, the difference in dose response curves was 7 ng/mL higher on average for normal-weight (BMI < 25 kg/m^2) compared to the obese women (BMI > 35 kg/m^2) [54]. A more recent study, conducted in middle-aged and older adults, included a wide range of BMIs and characterized the dose–response relationship between vitamin D supplementation and serum 25OHD according to body weight. [55] On average, serum 25OHD increase per 1,000 IU vitamin D intake/day was 5.2, 4.6 and 3.4 ng/mL/µg/d in normal weight, overweight and obese individuals, respectively. Similarly, an attenuated serum 25OHD response to vitamin D supplementation due to high BMI and excess adiposity has been found in younger adults [56, 57] and children [58].

The influence of body weight on serum 25OHD response to vitamin D intake was also examined in a systematic review [15]. The review included 94 independent studies, published from 1990 to November 2012, examining 9,766 control group subjects and 11,566 intervention group subjects (placebo vs vitamin D doses ranging from 200 to 10,000 IU). The results confirmed that body weight was an important predictor for the rise in 25OHD for a given dose of vitamin D. Besides body weight, other important predictors included the type of supplement (vitamin D_2 or D_3), age, use of calcium supplements and baseline 25OHD levels. Based on these findings, the authors suggested a formula using body weight, age and baseline 25OHD levels to calculate the increase in circulating 25OHD in response to vitamin D dose [Incremental change in nmol/l = 49.4 + 16.03 × Ln vitamin D dose (µg per kg body weight per day) + 0.22 × age (years) − 0.13 × baseline 25OHD (nmol/l)]. (Fig. 18.2).

However, there remains variability in vitamin D response even when correcting for body weight, and this might be partially attributed to genetic factors, such as variance in the genotypes of VDR, DBP and CYP enzymes (vitamin D hydroxylases) [59]. Overall, body weight specific vitamin D intakes are needed to achieve serum 25OHD targets.

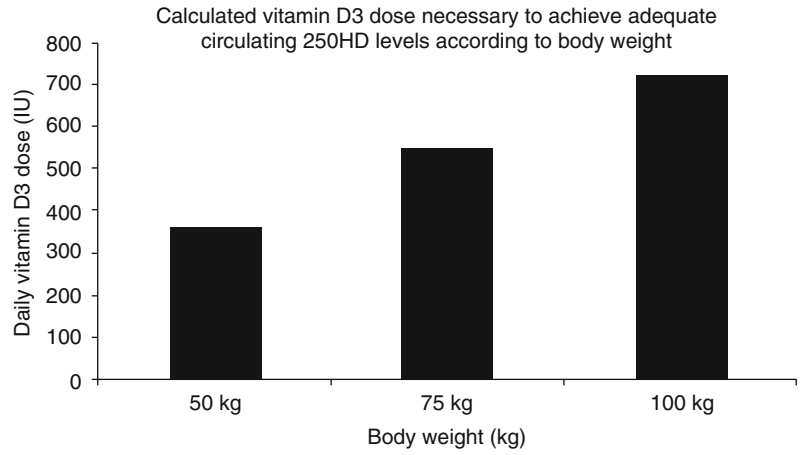

Fig 18.2 The vitamin D supplementation needed for a given body weight (Data from Zittermann et al. [15])

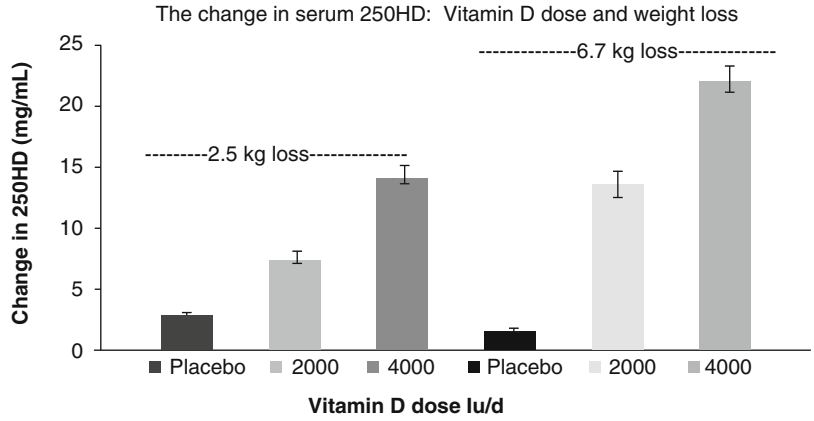

Fig 18.3 Rise in serum 25OHD at various levels of vitamin D supplementation during weight loss (Data for 2.5 kg loss is preliminary data from Shapses lab 3-dose vitamin D study (3DD), and data for 6.7 kg loss is taken from Mason et al. [63] and Zitterman et al. [24])

Serum 25OHD with Weight Loss

Most moderate weight-loss studies in overweight/obese individuals are accompanied by a rise in vitamin D status, and it could be hypothesized that this may contribute to the beneficial health consequences of reducing body weight. Data from a 2-year weight-loss clinical trial including 383 overweight or obese women reported a 1.9, 2.7 and 5.0 ng/mL increase in serum 25OHD for those who did not lose weight, those who lost 5–10 % and those who lost >10 % body weight, respectively [60]. In addition, 83 % of those who achieved a normal BMI after 24 months met the recommended serum concentrations (>20 ng/ml). A smaller study (n = 26) conducted in obese women (BMI 37 kg/m^2) reported a 2.9 ng/mL increase (from 15.4 to 18.3 ng/mL) in serum 25OHD concentration with 10 % weight reduction after a 20 week intervention [61]. A similar 3 ng/mL increase (from 30.3 to 33.2 ng/mL) in serum 25OHD concentration in 43 perimenopausal women was found after 11.5 % weight loss after a 12-week weight loss study [62]. Consistent with these studies, a larger study conducted in overweight and obese postmenopausal women (n = 439) randomly assigned to diet, exercise, diet + exercise intervention, or control found greater increase in serum 25OHD with greater weight loss, independent of baseline vitamin D status or changes in dietary vitamin D intake (Fig. 18.3) [63]. In the subgroup of women who lost ≥15 % body weight the nearly 8 ng/mL (or 20 nmol/L) rise in serum 25OHD concentration is significant in terms of vitamin D status. Moreover, overweight/obese children who

reduced their BMI from 27 to 26 kg/m [2] using a 12-month lifestyle intervention program also show an increase (+5 ng/mL) in serum 25OHD concentration [64]. In weight loss studies conducted in our lab, we have shown a rise in 25OHD levels with weight loss that is greater than individuals who were weight stable [65].

In patients undergoing bariatric surgery, alterations in serum 25OHD concentrations with weight loss have shown mixed results [66, 67]. The extent of weight loss post-surgery and malabsorption differs with type of surgery and individual surgical techniques can all influence serum 25OHD. Serum levels generally remain the same or rise slightly with the large weight loss associated with bariatric surgery, but the variability is large [68, 69].

Vitamin D Supplementation During Weight Loss

Weight loss has beneficial health consequences; however, it may also decrease calcium absorption and negatively affect bone [70]. Therefore, supplemental intake during weight loss interventions may attenuate bone loss. In studies where vitamin D supplementation is given during weight loss in randomized controlled trials, individuals who lose weight increase serum levels of 25OHD to a greater extent than those who lose less weight [24, 71, 72]. For example, a study in our laboratory in women given 2500 IU/d vitamin D3 intake who lost even a modest amount of weight (~3 %) compared to those who didn't lose weight showed a 20 % and 9 % rise in serum 25OHD, respectively [71]. In addition, poor vitamin D status has been linked to metabolic disorders and elevated chronic inflammation in both obese and normal weight individuals [73, 74]. Hence, additional vitamin D is expected to improve metabolic health independent of weight loss. Several randomized controlled trials showed a beneficial effect of vitamin D on lipid profile and inflammation as well as insulin sensitivity and insulin resistance [24, 75, 76]. The effect of vitamin D supplementation on inflammation was addressed in a randomized placebo controlled weight loss trial comparing a daily dose of 2000 IU vitamin D3 vs placebo in 218 overweight/obese postmenopausal women with serum 25OHD levels between 10 and 32 ng/mL. The vitamin D supplement was effective at reducing IL-6 levels, but not other cytokines (TNFα, IL1β, IL8, and IL10) or adipokines (adiponectin, leptin) [77].

The question of whether vitamin D supplementation can promote weight loss has also been addressed. While one study reported that vitamin D supplementation could potentiate fat loss [78], most studies show that achieving a serum 25OHD concentration greater than 34 ng/mL with vitamin D supplementation does not affect body weight and measures of adiposity [23, 24]. Specifically, a study by Zittermann et al. showed no difference in weight change in 200 overweight subjects who received 3320 IU vitamin D3 vs placebo after a 12 month-weight loss intervention [24]. Similarly, a more recent study [72] found no effect of 2000 IU vitamin D at improving weight loss in 218 overweight/obese women on reduced-calorie diets for 12 months. Weight loss has clear beneficial effects on health outcomes, including improvements in vitamin D status, but currently there is no evidence that vitamin D supplementation enhances the health outcomes associated with weight loss.

Preliminary Findings

In a 12-month study, we examined 58 women (age, 58 ± 6 years; BMI, 30.2 ± 3.8 kg/m^2, serum 25OHD, 27.3 ± 4.4 ng/ml) who were randomly assigned to one of 3 daily doses of vitamin D3 (600 IU; 2000 IU; 4000 IU) and 1.2 g Ca/day while counseled for moderate weight loss (3DD trial). Serum was analyzed for total 25OHD, parathyroid hormone (PTH), DBP and albumin. Free and bioavailable 25OHD (free + albumin-bound) were calculated from total 25OHD, DBP, and serum albumin levels. Bone was measured by dual energy x-ray absorptiometry and peripheral quantitative computed tomography. After 1 year, serum 25OHD levels increased to 30.5 ± 5.0, 36.0 ± 4.2 and 40.8 ± 7.4 ng/ml, in the 600, 2000 and 4000 IU groups, respectively, and differed

significantly between groups ($p<0.01$). In this healthy population, serum PTH decreased to a similar extent between groups. Weight change did not differ with treatment (−3.0 ± 4.1 %). Free and bioavailable 25OHD between groups increased significantly with vitamin D treatment ($p<0.01$) whereas serum DBP did not change. Also, the percentages of free and bioavailable 25OHD at 0.02 % and 7 % of total 25OHD concentrations remained stable from baseline to final measurements and was independent of the dose of vitamin D supplementation. We also found that the relationship between total, free and bioavailable vitamin D with BMI and fat mass did not differ. There was a trend for trochanter BMD to decrease in women who took 600 IU/d compared to those taking the higher D doses ($p<0.06$). In addition, cortical thickness increased more at higher levels of intake compared to the lowest dose ($p<0.02$; mixed models ANCOVA). Multiple regression analysis showed that total, free and bioavailable 25OHD were equally efficient at predicting bone changes.

As expected, the rise in serum 25OHD in response to vitamin D supplementation was attenuated in these obese individuals. The rise in serum 25OHD concentration of 3.6 ng/mL for 1000 vitamin D3 IU/day intake was similar to that seen in weight stable obese subjects [54, 55] and was less compared to studies with >8 % weight loss [24, 65, 72]. Moreover, moderate weight loss has a minor contribution to serum 25OHD levels without noticeable effects on the kinetics of change in free and bioavailable 25OHD relative to changes in total 25OHD. In addition, there is little effect on bone in older women other than a trend to attenuate loss of trochanter BMD and an increase in cortical thickness. Compared to serum 25OHD, neither free nor bioavailable 25OHD were better predictors of bone or PTH due to obesity or weight loss. This is in contrast to a previous study in a leaner population who were not taking supplements. In this study of 265 postmenopausal women with low BMD, total 25OHD correlated with PTH, whereas only free and bio-available 25OHD was associated with BMD [79]. It is possible that adjusting for DBP gene polymorphism could help clarify how free and bioavailable 25OHD influence the relationship with bone and other outcomes [79]. In addition, the interaction with other factors including the type of adiposity, glomerular filtration rate or dietary intake, should also be considered.

Conclusion

In summary, obesity negatively affects serum levels of total 25OHD. To our knowledge, the influence of obesity on circulating free and bioavailable 25OHD is not known and was not possible to address in our small study with only overweight and obese women. Excess adiposity results in negative health consequences that may be further aggravated by vitamin D deficiency. The rise in serum 25OHD with vitamin D supplementation is attenuated in the obese and exacerbated in those individuals losing body weight. Achieving targeted serum 25OHD levels may need to require body weight specific recommendations. The mechanism by which supplemental vitamin D affects obesity-related health outcomes with and without weight reduction still requires additional investigation.

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Vitamin D and Fall Prevention: An Update

19

Heike A. Bischoff-Ferrari
and Bess Dawson-Hughes

Abstract

This chapter will summarize evidence from clinical trials that tested vitamin D supplementation for the prevention of falls. Next to its established benefits on calcium metabolism, several lines of evidence support a direct benefit of vitamin D on muscle. In fact, it has been proposed that fracture prevention observed with vitamin D supplementation among the senior population may in part be explained by its effects on muscle strength and fall prevention. In this chapter, we also discuss conflicting data proposed from recent comprehensive meta-analyses.

Keywords

Vitamin D • Falls • Muscle strength • Bone density • Fractures

Background and Current Guidelines

Seventy-five percent of hip and non-hip fractures occur among seniors age 65 and older [1], and over 90 % of all fractures occur after a fall [2]. Remarkably, the primary risk factor for a hip fracture is a fall [2]. Thus, critical for the understanding and prevention of fractures at later age is their close relationship with muscle weakness [3] and falling [4, 5]. Vitamin D has been proposed as a strategy to prevent both falls and resulting fractures among the senior population due to its documented direct benefits on muscle [6, 7] in addition to its established role in calcium metabolism [8]. In fact, several recent guidelines extended the basis of their recommendation on vitamin D supplementation from bone health to fall prevention. These include the Agency for Healthcare Research and Quality (AHRQ), the U.S. Preventive Services Task Force [9], the US endocrine society [10], the international osteoporosis foundation [11], and the American and British Geriatric Society [12].

H.A. Bischoff-Ferrari, MD, DrPH (✉)
Department of Geriatrics and Aging Research, University of Zurich and University Hospital of Zurich, Raemistrasse 100, Zurich, Switzerland
e-mail: Heike.Bischoff@usz.ch

B. Dawson-Hughes, MD
Jean Mayer UDSA Human Nutrition Research Centre on Aging, Tufts University, Boston, MA, USA
e-mail: Bess.dawson-hughes@tufts.edu

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Mechanistic Data that Link Vitamin D to Muscle Health

Mechanistically, several lines of evidence link vitamin D to muscle strength and falling [7]. Proximal muscle weakness is a feature of clinical vitamin D deficiency [13]. Also, the vitamin D receptor (VDR) is expressed in human muscle tissue, as documented in all [14–19] but one [20] investigation. Further, several studies among older individuals suggest that its activation promotes de novo protein synthesis preferentially in type II fast twitch muscle fibers relevant for fall prevention [21–23].

Clinical Trials on Vitamin D and Fall Prevention

At the clinical level, eight individual double-blind randomized-controlled trials with prospective fall records throughout the trial period were summarized in a meta-analysis [24]. The results suggested an overall benefit at a vitamin D dose of 700 to 1000 IU per day in both community-dwelling [25–28] and institutionalized seniors [29–31]. A lower dose did not appear to be effective, and data on higher doses were limited [24, 32, 33]. Extending to more comprehensive meta-analyses of any vitamin D dose, route of application (oral daily, bolus, intra-muscular) and trial quality with respect to both study design (blinded and open designs) and fall ascertainment (any interval, prospective and retrospective), vitamin D supplementation still reduced fall risk significantly in most peer-reviewed meta-analyses (see Table 19.1), including those that focused on trials that tested active vitamin D metabolites. The most recent 2014 comprehensive meta-analysis by Bolland and Reid included 25 clinical trials and documented only a small non-significant 5 % benefit on fall prevention with vitamin D [34].

We suggest that the main inconsistency in the most recent meta-analysis with the trials presented in Table 19.1 is based on the inclusion of several trials in the Bolland and Reid 2014 meta-analysis that cannot be considered reliable indicators of true treatment efficacy. The authors included several open-design trials [35–39] and trials that did not define how falls were assessed and/or did not assess falls prospectively [40–42] or falls were imputed from fracture data [43]. Also, the authors included trials where vitamin D was a component of a multi-factorial intervention [44], or was applied to seniors with unstable health such as those in acute hospital care [44, 45] or those who had suffered a stroke [46] or heart failure [40]. Further, they included several trials with oral [45, 47, 48] or injected [49] unphysiologically large bolus doses of 300,000 IU vitamin D or more, which may not be considered equivalent to daily or lower bolus dosing. In summary, the latest meta-analysis of Bolland and Reid does not invalidate the overall finding that vitamin D at a daily dose between 700 and 1000 IU per day reduces the risk of falling in seniors at risk for vitamin D deficiency.

Which Target Population Benefits Most from Vitamin D Supplementation and Fall Prevention?

It has been suggested that vitamin D supplementation may only be beneficial in frail and institutionalized seniors [50]. This hypothesis was tested in the 2009 meta-analysis of double-blind RCTs. The results suggested an overall benefit independent of type of dwelling at a vitamin D dose of 700 to 1000 IU per day. Fall reduction at the 700 to 1000 IU dose per day was found in both community-dwelling [25–28] (RR = 0.77; 0.65–0.93) and in institutionalized (RR = 0.86; 0.70–1.07) seniors [29–31] with no significant difference between groups ($p = 0.46$).

Alternatively, a key requirement for a detectable benefit of vitamin D supplementation and fall prevention may not be type of dwelling but baseline vitamin D status. All trials included in the 2009 meta-analysis targeted seniors at risk of vitamin D deficiency. In contrast, a 2-year placebo-controlled trial published in 2015 by Uusi-Rasi et al. from Finland tested 800 IU vitamin D per day in vitamin D replete community-dwelling women age 70–80 years old with a fall

Table 19.1 Meta-analyses of clinical trials testing vitamin D on fall prevention

Year	Author; Journal	% Fall reduction with vitamin D	Effect size 95 % CI
2004	Bischoff-Ferrari HA et al.; *JAMA* [32]	–22 % With supplemental and active vitamin D (*only double-blind RCTs*)	OR = 0.78 (0.64–0.92)
2007	Jackson C et al; *QiM* [67]	–12 % With vitamin D3 supplementation	RR = 0.88 (0.78–1.00)
2008	O'Donnell S et al.; *J Bone Miner Metab* [68]	–34 % With active vitamin D	OR = 0.66 (0.44–0.98)
2008	Richy F et al.; *Calcif Tissue Int* [69]	–21 % With active vitamin D	RR = 0.79 (0.64–0.96)
2009	Bischoff-Ferrari HA et al.; *BMJ* [24]	–19 % with 700–1000 IU/d +10 % with 200 to 600 IU/d Of vitamin D3/2 supplementation (*only double-blind RCTs*)	RR = 0.81 (0.71–0.92) RR = 1.10 (0.89–1.35)
2010	Kalyani RR et al.; *J Am Ger Soc* [70]	–14 % with 200–100 IU/d	RR = 0.86 (0.79–0.93)
2010	Cameron ID et al.; *Cochrane Syst Rev* [71]	–28 % With vitamin D supplementation	RaR = 0.72 (0.55–0.95) *rate*
2011	Michael YL et al.; *Ann Intern Med* [9]	–17 % With vitamin D supplementation	RR = 0.83 (0.77–0.89)
2011	Murad MH et al.; *J Clin Endocrinol Metab* [72]	–14 % With vitamin D supplementation	OR = 0.86 (0.77–0.96)
2014	Bolland M et al.; *Lancet Diabetes Endocrinology* [34]	–5 % With vitamin D supplementation	RR = 0.95 (0.90–1.00) *Any quality trial*

OR odds ratio, *RR* relative risk, *RaR* rate ratio

in the previous year [51]. In the intent-to-treat analyses vitamin D maintained femoral neck bone mineral density and increased tibial trabecular density marginally, but did not reduce falls. The lack of benefit in the vitamin D group may be explained by the fact that none of the participants were vitamin D deficient at baseline. The placebo group started with mean 25(OH)D levels of 27.5 ng/ml and stayed at 27.5 ng/ml at the 2-year follow-up exam. The group that received vitamin D started at 25.1 ng/ml and reached 37.0 ng/ml at the 2-year follow-up. In contrast, 160 Brazilian postmenopausal women with a fall in the past year and low 25(OH)D levels, were treated with placebo or 1000 IU of vitamin D3 for 9 months [52]. The vitamin D3 group started at a 25(OH)D level of 15.0 ng/ml and reached 27.5 ng/ml on treatment. The placebo group started at 16.9 ng/ml and dropped to 13.8 ng/ml during the trial. Over the 9 month intervention period, the risk of falling in the placebo group was almost twice that in the vitamin D treated group (RR 1.95 [1.23–3.08]).

Supporting a concept that fall prevention benefits of vitamin D supplementations are primarily seen in vitamin D deficient seniors, a recent meta-analysis of 17 RCTs suggested that a benefit of vitamin D on lower extremity strength may be seen primarily among those with vitamin D deficiency, defined as 25(OH)D < 10 ng/ml [53].

Is There a Therapeutic Range for Vitamin D and Fall Prevention?

In two meta-analyses, low dose vitamin D did not appear to be effective compared with a standard dose of 800 IU/day [24, 32, 33]. If and to what extend higher doses than the current

recommendation of 800 IU/day provide additional benefits needs further investigation.

In one trial among 173 frail seniors after acute hip fracture, 2000 IU/day vitamin D versus 800 IU/day did not reduce falling over a 12 month follow-up (+28 %; 95 % CI: −4 %, +68 %) [54]. Notably, the achieved mean 25(OH)D level at 12 months was 44.6 ng/ml in the 2000 IU/day group compared with 35.4 ng/ml in the 800 IU/day group [54]. In another trial by Sanders et al. with 2256 senior women at high risk for hip fracture, an annual bolus of 500,000 IU vitamin D versus placebo increased risk of falling significantly (RR = 1.15; 95 % CI: 1.02 – 1.30) [47]. The bolus achieved a 25(OH)D level of 48 ng/ml at 1 month and 36 ng/ml at 3 months follow-up, the timeframe where most falls and resulting fractures occurred in the trial [47]. Both trials provide a signal that reaching 25(OH)D levels beyond 40 ng/ml among frail seniors may be detrimental for fall prevention.

This signal received further support by a trial published in 2016 [55]. This trial tested 3 monthly doses of vitamin D (a standard dose of 24,000 IU per month and two higher monthly doses with 60,000 IU and 24,000 IU plus calcifediol) among 200 community-dwelling seniors aged 70 and older. Entering the trial, all 200 participants had fallen in the prior year and 58 % were vitamin D deficient (25(OH)D < 20 ng/ml). During the 12 month follow-up under treatment, 61 % (121 of 200) fell, 48 % in the standard dose (24,000 IU vitamin D) group, 67 % in the 60,000 IU group, and 66 % in the 24,000 IU plus calcifediol group (see Fig. 19.1). While a benefit of the standard vitamin D dose was likely even in the absence of a true placebo group as a comparator, both higher monthly doses had no benefit on lower extremity function and had higher percentages of participants who fell, when compared with the standard dose group. The fall reduction benefit in the 24,000 IU group is further supported by our finding that lower extremity function improved significantly over time in this group. Notably, a consistent pattern was seen by achieved 25(OH)D blood levels. The best functional improvement and fewest falls were observed at the lower replete 25(OH)D range of 21 to 30 ng/ml, while no functional benefit and more falls were observed at levels of 45 ng/ml and higher. Notably, the 24,000 IU monthly dose was most effective in shifting participants into the most desirable 25(OH)D range, independent of their baseline starting level of 25(OH)D. Even among participants who started the trial in the replete range for 25(OH)D (>20 ng/ml) the monthly dose of 24,000 IU was safe and did not raise 25(OH)D levels above 45 ng/ml and higher (where fall risk was increased) in any participants.

The authors of the most recent trial had expected that higher monthly doses would be superior at reducing falls compared to the current recommendation of 24,000 IU vitamin D, which is equivalent to 800 IU/day. One explanation for these unexpected finding may be that there is a therapeutic range for vitamin D with respect to fall prevention among seniors who had a prior fall. In fact, the study points to the range between 21 to 30 ng/ml as optimal while both serum 25(OH)D levels below 21 ng/ml (vitamin D deficiency) and above 45 ng/ml were associated with increased risk of falling. This is consistent with the findings from the earlier meta-analysis from 8 double-blind RCTs where fall prevention started at achieved levels above 20 ng/ml [24]. However, at that time, trials that achieved 25(OH)D levels greater than 38 ng/ml were missing [24].

An alternative explanation may be that monthly doses of vitamin D are not advantageous. In two prior studies testing 500,000 IU vitamin D annually [47] or 100,000 IU every 4 months [41], fall risk increased significantly by 15 % or declined non-significantly by 7 %. Notably, the physiology behind a possible detrimental effect of a high bolus dose of vitamin D on muscle function and falls remains unclear and needs further investigation. Notably, the 2016 trial assessed function and muscle mass and could not detect toxic effects of the two high dose monthly vitamin D applications [55].

Further, there is the possibility that high dose monthly vitamin D may have increased physical activity in seniors at risk of falling and thereby provided the opportunity to fall more often. The authors of the most recent trial explored this hypothesis but could not confirm it based on the physical activity

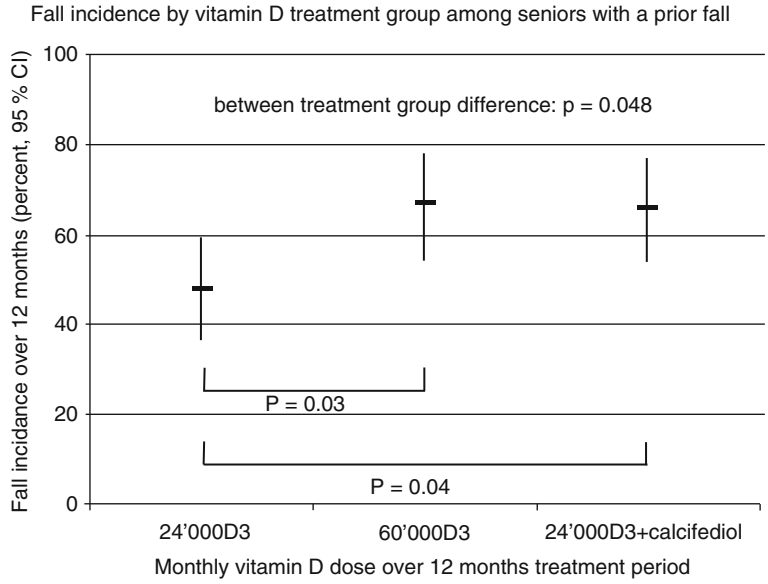

Fig. 19.1 This shows fall incidence from the Zurich Disability Prevention Trial testing 3 monthly doses of vitamin D among community-dwelling seniors age 70 and older [55]. Entering the trial, all 200 participants had fallen in the prior year and 58 % were vitamin D deficient (25(OH) D < 20 ng/ml). During the 12 month follow-up under treatment, 61 % (121 of 200) fell, 48 % in the standard dose control group with 24,000 IU vitamin D group, 67 % in the 60,000 IU group, and 66 % in the 24,000 IU plus calcifediol group

measures assessed in this trial [55]. It also has been hypothesized that a higher bolus dose may trigger the active vitamin D metabolite (1,25(OH)2D) to be catabolized more rapidly as a protective mechanism [56]. This has been suggested in a rat model [57], but could not be confirmed at the human level, but so far could not been confirmed after bolus treatment in humans [55, 58]. Finally, a possible explanation involves the effect of vitamin D on vision. In a study of aged mice, administration of Vitamin D for 6 weeks resulted in a significant improvement in visual function, resulting from significant reductions in retinal inflammation, amyloid beta accumulation, and retinal macrophage numbers [59]. Thus, participants in the higher bolus groups may have experienced improvements in vision, and perhaps the mis-match between their improved vision and their current eyeglass prescription could have caused them to stumble.

Is There a Therapeutic Range of Vitamin D for Fall-Related Fractures?

For fracture prevention, a similar pattern as outlined above for falls has been emerging [60–62]. Two meta-analyses of double-blind RCTs support a daily dose of 800 IU for fracture prevention at any non-vertebral site (−14 % [62] to −20 % [61]) and at the hip (−18 % [61] to −30 % [62]), and a lack of benefit at lower doses. The benefit for fracture prevention was observed in trials that achieved mean 25(OH)D levels of 30 to 44.8 ng/ml [61] in their treatment groups. Higher doses of vitamin D were explored in three large trials with respect to fracture prevention, one with 100,000 IU vitamin D3 (Trivedi et al. [41]) given every 4 months orally, one with 300,000 IU vitamin D2 (Smith et al. [49]) given intra-muscularly and one with 500,000 IU vitamin D3 (Sanders et al. [47]) given orally in a yearly bolus. While in the Trivedi trial fracture risk was reduced in the Trivedi trial with treatment (RR = 0.78 (95 % CI 0.61 to 0.99) for any first fracture), bolus vitamin D increased fracture risk in the other two trials (Smith [49]: RR = 1.09 (95 % CI 0.93–1.28) for any first fracture; Sanders [47]: RR = 1.26 (95 % CI 1.00-1.59; P = .047) for any first fracture). With respect to achieved 25(OH)D levels in the treatment groups, the Trivedi trial reached mean 25(OH)D levels of 30 ng/ml [41], while the Sanders trial achieved a 25(OH)D level of 48 ng/ml at 1 month and 36 ng/ml at 3 months follow-up [47]. In the Smith trial, a small group of participants were investigated for 25(OH)D levels and had mean levels of 56.5 ng/ml at baseline with a non-significant increase of 21 % in the treatment group at 4 months [49].

Two ongoing trials (VITAL and DO-HEALTH) are testing 2000 IU vitamin D3 per day for fall and fracture reduction, but the patient characteristics differ from those of the prior studies in targeting relatively healthy older adults and seniors. Nevertheless, these trials will provide important opportunities to verify the potential signal (or threshold effect) of a possible therapeutic range of vitamin D with respect to fall and fracture prevention.

Summary

In this update on vitamin D the authors conclude that available data to date support current recommendations of 800 IU vitamin D per day for fall and fracture prevention. The benefit of 800 IU vitamin D per day on fall and fracture prevention may be most pronounced among vitamin D deficient seniors, independent of their living situation. Based on the most recent trial with monthly doses of vitamin D that is equivalent to 800 IU/day, 24,000 IU/month, is safe and effective for reducing fall risk, independent of 25(OH)D starting level, and the most desirable "therapeutic range" for vitamin D might be 21 to 30 ng/ml. In contrast, levels on either the deficient range (<20 ng/ml) or above 45 ng/ml may not be warranted in seniors at risk of falling. Notably, vitamin D deficiency is still highly prevalent. About 50 % of the world population are expected to be vitamin D deficient [63]. In the latest US National Health and Nutrition Examination Survey 2005 to 2006 data, the overall adult prevalence rate of vitamin D deficiency (25(OH)D) concentrations ≤20 ng/ml), was 41.6 %, with the highest rate seen in blacks (82.1 %), followed by Hispanics (69.2 %) [64]. Among seniors at risk of hip fracture prevalence of vitamin D deficiency has been reported as high as 80 % [65, 66].

Given the high prevalence of vitamin D deficiency and demonstrated safety of 800 IU per day and 24,000 IU vitamin D per month among those who start below or above 20 ng/ml, current recommendations of general supplementation without prior measurement of 25-hydroxyvitamin D hold. Notably, getting the recommended level of vitamin D of 800 IU daily from natural food sources is virtually impossible to do without consuming an extreme amount of fish, and almost no one in the US or Europe achieves this intake. Thus, it is reasonable to ensure vitamin D sufficiency with vitamin D supplementation to prevent both falls and fall-related fractures.

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20 After Vitamin D Supplementation There Is an Increase in Serum 25 Hydroxyvitamin D but No Evidence of a Threshold Response in Calcium Absorption

J. Christopher Gallagher and Lynette M. Smith

Introduction

Vitamin D enters the body as a nutrient from dietary sources and as a pre-vitamin that is activated by sunlight in skin. Circulating vitamin D undergoes further activation in the liver to 25 hydroxyvitamin D (25OHD) and then in the kidney to the active metabolite 1,25 dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol) that functions as a hormone. There is positive stimulation of 1,25 dihydroxyvitamin D secretion by parathyroid hormone (PTH) mediated by changes in serum calcium [1] and negative feedback on 1,25 dihydroxyvitamin D secretion by Fibroblast growth factor 23 (FGF23) a hormone derived mainly from osteocytes that is stimulated by and regulates changes in serum phosphorus [2]. 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) binds to the vitamin D receptor (VDR) in the intestine and controls the efficiency of calcium absorption by determining the amount of calcium absorbed from the diet [3]. When calcium intake is low there is increased PTH secretion, increased 1,25 dihydroxyvitamin D and increased calcium absorption and when calcium intake is high the mechanism is reversed [4]. This adaptive process was first described by Nicolaysen in 1953 long before the discovery of 1,25 dihydroxyvitamin D [5].

Calcium absorption in the intestine occurs by an active transport system that can be saturated and which is under the control of $1,25(OH)_2D$ [6], there is also a passive transport system that depends on a diffusion gradient from a higher calcium concentration in the gut to a lower one in the extracellular fluid compartment. With aging there is a gradual decline in intestinal absorption starting around age 70 years [6]. Later in the eighth decade there is also a decrease in renal production of $1,25(OH)_2D$ by the kidney associated with declining renal function [7, 8] and there is an impaired response in $1,25(OH)_2$ D production by the kidney to parathyroid hormone stimulation suggesting that the decrease in renal mass is the cause [8].

In postmenopausal osteoporosis there is a marked decrease in the active transport in the intestine of calcium [4] and a decrease in serum $1,25(OH)_2D$ levels[4].

However, the lower $1,25(OH)_2D$ does not fully explain the decrease in calcium absorption and resistance by the intestine to circulating 1,25(OH)2D may be part of the problem [4, 9]. In subsequent studies we did not find any decrease in intestinal VDR with age in biopsy specimens taken from the duodenum [10]. In previous studies of osteoporotic patients and normal aging subjects treated with oral synthetic 1,25

J.C. Gallagher
Endocrinology, Creighton University Medical School, Omaha, NE 68131, USA
e-mail: jcg@creighton.edu

L.M. Smith
Statistics, College of Public Health, University Nebraska Medical Center, Omaha, NE 68131, USA

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dihydroxyvitamin D 0.25 mcg twice daily there was a marked increase in calcium absorption measured both by radioactive calcium studies and metabolic balance studies [11].

Serum $1,25(OH)_2D$ levels can decrease depending on the substrate supply of 25 hydroxyvitamin D (25(OH)D. Evidence suggests that this is not critical until serum 25(OH)D decreases below 10 ng/ml [12] and in another study data showed that once the serum 25(OH)D level decreased below 4 ng/ml there were decreases in serum $1,25(OH)_2D$ and a decrease in calcium absorption [13].

The Effect of Vitamin on Calcium Absorption

The measurement of calcium absorption after plain vitamin D is not well studied. Most data has been derived from cross sectional studies. We conducted 2 prospective studies in women, one in older women aged 65–80 years and the other in younger women aged 25–45 years. These studies have been described in detail in previous publications [14–16]. In subsequent papers we showed the effect of 1 year of vitamin D supplementation on calcium absorption and serum 25(OH)D in an older group 163 Caucasian women ranging from age 57–90 years [17] and in a younger group 198 healthy young women, 119 white and 79 African American that were recruited from general population [18]. As a major inclusion criterion all women had baseline serum 25 hydroxyvitamin D (25OHD)$\leq$20 ng/ml (50 nmol/L). In the study of older women, they were randomly assigned to one of eight groups -, 400, 800, 1600, 2400, 3200, 4000 or 4800 IU of vitamin D3 per day or matching placebo. In the younger group women were randomly assigned to one of four groups -, 400, 800, 1600, 2400 or matching placebo. Advice on increasing dietary calcium was given to women to reach an RDA of 1200 mg/day in the older group and 1000 mg/day in the younger group. If that were not possible then women were given Calcium supplements to achieve the RDA. In the older group baseline dietary calcium was 685 mg/day and 12-month total calcium intake was 1280 mg/day. In the younger group baseline dietary calcium was 757 mg/day in white and 502 mg in black women and 12-month total calcium intake was 1031 mg in white and 1002 mg in black women.

Calcium absorption was measured using a single isotope radioactive calcium test. In this technique 2–3 microcuries of 45 Ca is equilibrated with elemental calcium 100 mg as the carrier. The amount of calcium carrier determines the percent of calcium absorbed [19]. When a small carrier is used as in this study the fractional absorption is higher (~50 %) compared to studies with carrier loads of 250 mg (~25 %). A single isotope study is highly correlated with results from double isotope tests [4].

Results in the Older Women

Although vitamin D doses increased from 400 to 4800 IU the unadjusted change in the mean percent calcium absorbed per 100 mg dose was small from 52.4 to 55.2 % [17] (Fig. 20.1). In a multiple regression model only baseline absorption was significantly related to the final value. Baseline serum $1,25(OH)_2D$, baseline serum 25(OH)D, age, weight and dietary calcium intake were not related to the change in calcium absorption but baseline serum $1,25(OH)_2D$ was correlated with calcium absorption.

Because the change in serum 25(OH)D level on increasing doses of vitamin D shows considerable variance [14], the data was compared to the 12-month serum 25(OH)D level at 12 months. There was a small increase in the unadjusted percent of calcium absorbed as serum 25(OH)D increased (Fig. 20.2). The group with lowest serum 25(OH)D$<$20 ng/ml had the lowest calcium absorption and this may be is due to the effect of the calcium supplement (average 580 mg) depressing active transport of calcium.

The mean percent of calcium absorbed increased by 7.6 % from 52.4 to 60% over a serum 25(OH)D range of 20–66 ng/ml. Because the calcium load in the test is 100 mg, this corresponds to an increase in the amount of calcium absorbed of 7–8 mg. If the calcium intake were

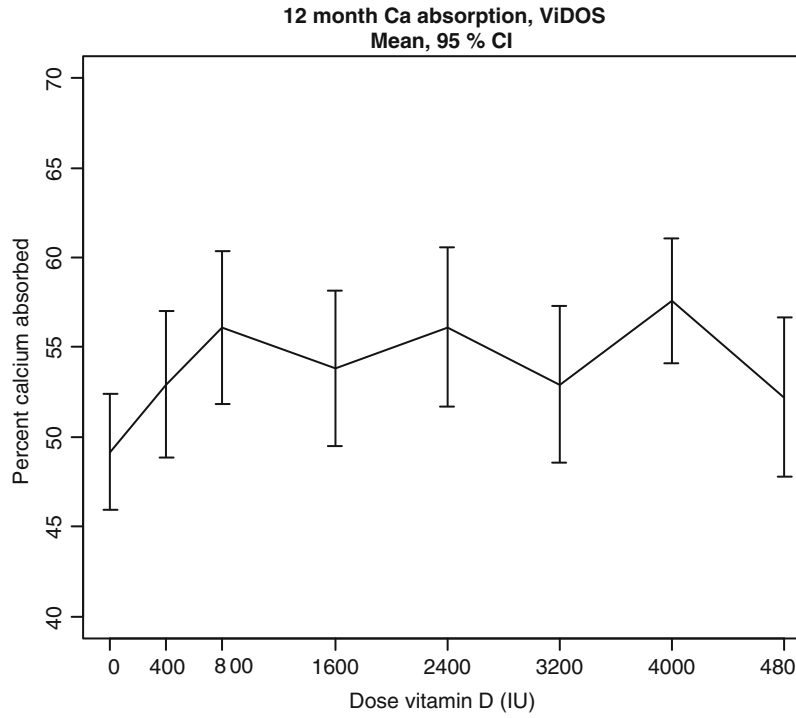

Fig. 20.1 Mean and 95 % CL (Confidence limits) for unadjusted calcium absorption versus vitamin D dose (Adapted from Ref. [17])

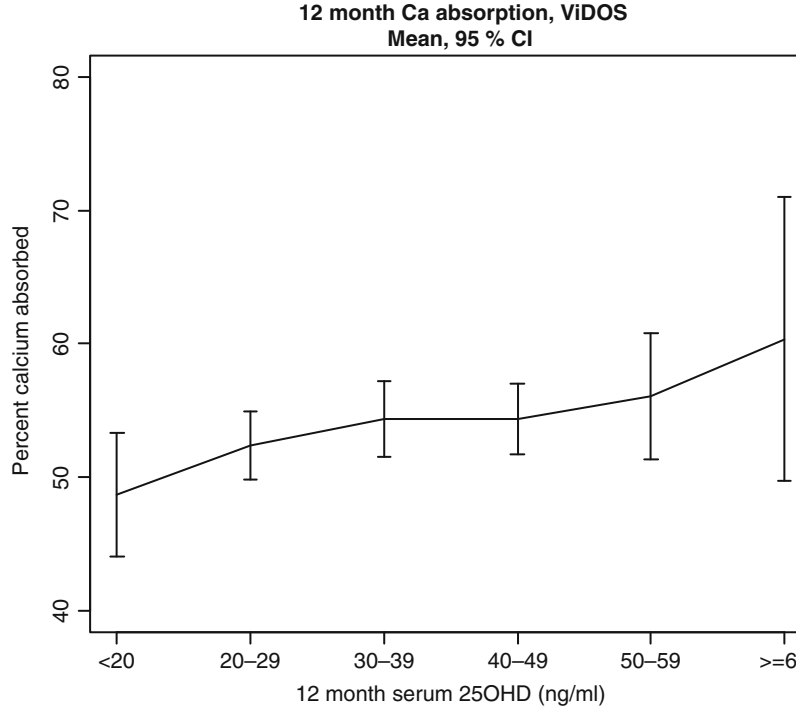

Fig. 20.2 Mean and 95 % CL (Confidence limits) for unadjusted 12-month calcium absorption versus 12-month serum 25OHD sub groups. There are only 6 subjects in the group >60 ng/ml (Adapted from Ref. [17])

800 mg daily, then the estimated increase would be 48 mg that is equivalent to about a third of a 6-ounce cup of milk.

By comparison, in a study in osteoporotic women of similar age and a mean serum 25(OH)D level of 30 ng/ml synthetic $1,25(OH)_2D$ 0.5 mcg daily increased calcium absorption by 17 % over 1 year using a double isotope test and the same calcium carrier of 100 mg [4, 11]. In those patients that underwent metabolic calcium balance studies there was a similar increase in calcium absorption of 20 % [11]. Because of these contrasting results between vitamin D and $1,25(OH)_2D$ 0.5 mcg daily it suggests that doses of vitamin D up to 4800 IU daily may produce only very small increases in serum 1,25 $(OH)_2D$ and that is why there were minimal increases in calcium absorption.

Results in the Younger Women

In this group the doses of vitamin D used ranged from 400 to 2400 IU showed no effect on the percent of calcium absorbed [18]. The mean baseline was 55.9 % and the mean 12-month value was 56.2 % (Fig. 20.3). In the multivariate analysis Vitamin D dose was not predictive of the final 12 month calcium absorption. Race was marginally predictive ($p<0.053$) with African American women tending to have lower (4 %) 12 month calcium absorption than whites. Total calcium intake ($p<0.009$), weight ($p<0.019$), and baseline calcium absorption ($p<0.012$) were predictive of 12-month absorption. Baseline serum 25(OH)D and $1,25(OH)_2D$ were not predictive. Another study showed that ^{47}Calcium retention used as a surrogate for calcium absorption was similar in younger white and black women but serum 1,25 $(OH)_2D$ levels were higher in blacks suggesting some resistance to 1,25 $(OH)_2D$ in blacks [20].

Because of the large variance in serum 25(OH)D response to vitamin D we looked at the effect of serum 25(OH)D on unadjusted mean 12 month calcium absorption. In the group with serum 25(OH)D <20 ng/ml baseline mean calcium absorbed was 52.8 % and final mean absorption was 56.3 % in the group with serum

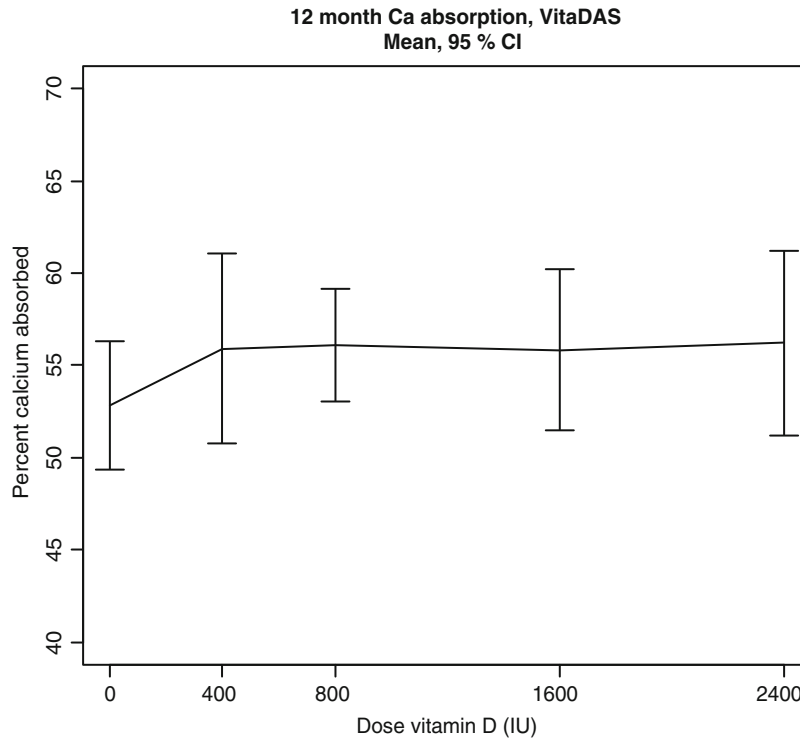

Fig. 20.3 Mean and 95 % CL (Confidence limits) for unadjusted calcium absorption versus vitamin D dose (Adapted from Ref. [18])

25(OH)D <50 ng/ml (Fig. 20.4). A multivariate analysis showed that final serum 25(OH)D was not a significant predictor of 12 month calcium absorbed. African American race was a predictor of lower absorption ($p<0.05$) as was total calcium intake ($p<0.01$), weight ($p<0.018$), and baseline absorption ($p<0.011$).

Comparing absorption results with younger women the older women have lower calcium absorption at every level of serum 25OHD.

The slightly larger increase in calcium absorption in the elderly compared to the younger group may be due to secondary hyperparathyroidism in the elderly, thus parathyroid hormone is driving the conversion of 25(OH)D to 1,25 dihydroxyvitamin D in the kidney, however at this time we do not have the evidence that serum 1,25(OH) 2D are higher in the elderly.

Baseline Cross Sectional Analysis

To see if we could reproduce the results from Australia [13] that calcium absorption really declines at very low levels of serum 25(OH)D due to lower serum 1,25(OH)2D levels we examined the baseline data in the elderly group before vitamin D supplementation. The inclusion criteria specified that all subjects had serum 25(OH)D levels <20 ng/ml before calcium absorption was measured. We compared serum 25(OH)D in subgroups 5–10 ng/ml, 11–15 ng/ml and 16–20 ng/ml. There was no significant difference in calcium absorption although the numbers are small in the lowest group although serum 1,25(OH)2D levels were significantly lower in the 5–10 ng/ml subgroup (Figs. 20.5 and 20.6). In the older women there was a significant correlation between baseline calcium absorption and serum 1,25(OH)2D but not with serum 25(OH)D (Figs. 20.7 and 20.8) There is no corresponding data for younger women.

Summary

In these prospective studies of vitamin D supplementation in young and older women there is no evidence of a threshold increase in absorption of calcium associated with increasing serum 25(OH)

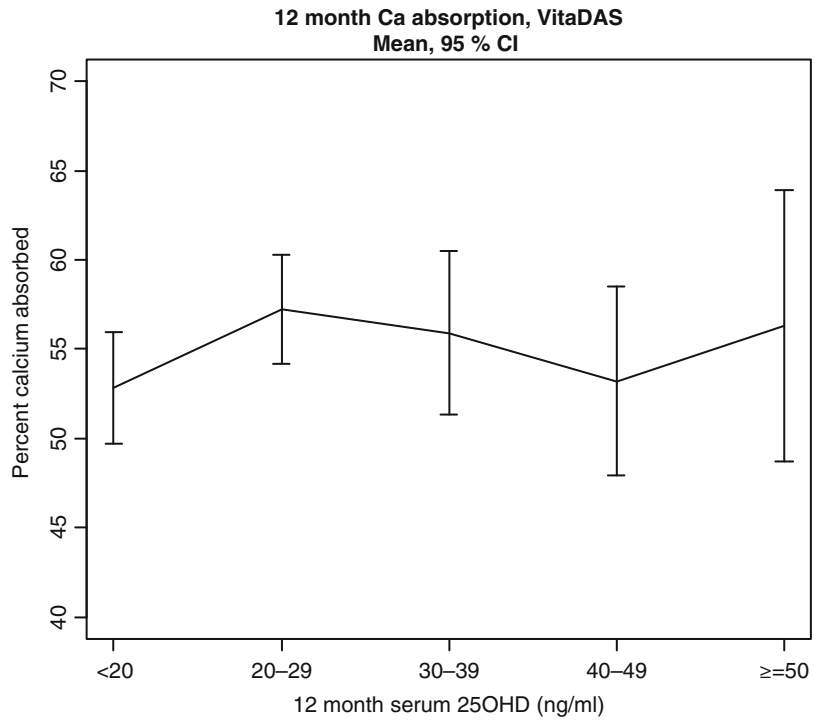

Fig. 20.4 Mean and 95 % CL (Confidence limits) for unadjusted 12-month calcium absorption versus 12-month serum 25OHD sub groups (Adapted from Ref. [18]

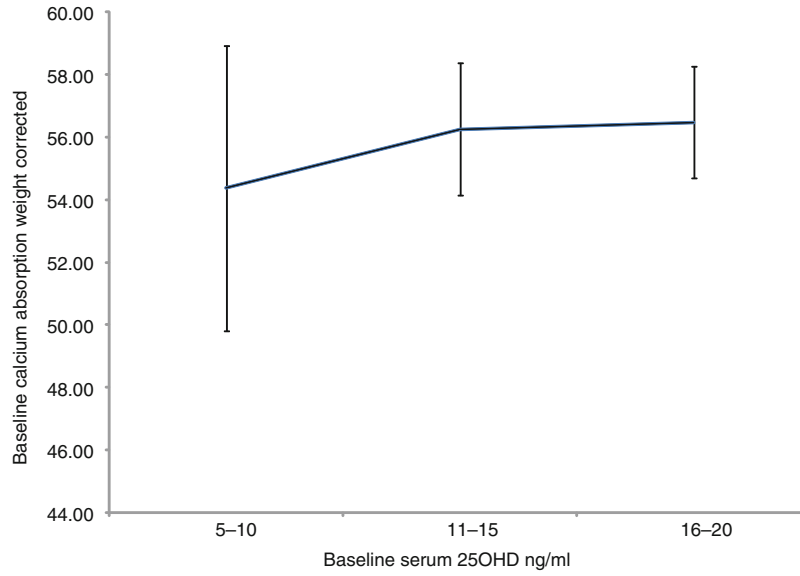

Fig. 20.5 Calcium absorption versus serum 25OHD subgroups from baseline data from study (Ref. [17])

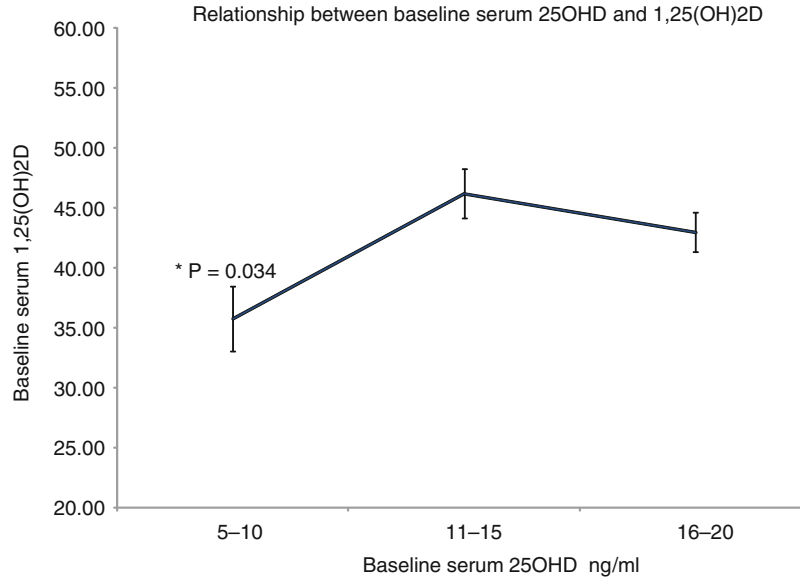

Fig. 20.6 Serum 1,25(OH)2D versus serum 25OHD sub groups from baseline data from study. *Sub group 5–10 ng/ml significantly lower than others (Ref. [17])

D levels even though on vitamin D the increase in 25(OH)D extended from a baseline of 13–15 ng/ml to an upper limit of 65 ng/ml.

We know from calcium balance studies that malabsorption of calcium occurs with severe vitamin D deficiency and osteomalacia. In a previous paper from Australia [13] that had more subjects with serum 25(OH)D levels <10 ng/ml cross sectional analysis of calcium absorption and serum 25(OH)D levels showed that calcium absorption decreased when serum 25(OH)D levels were less than 4 ng/ml and was accompanied be a decrease in serum 1,25(OH)2D levels.

We found lower serum 1,25(OH)2D levels in those with serum 25OHD 5–10 ng/ml but no significant difference in calcium absorption but that might be due to small numbers of subjects in that subgroup. In the 163 older women at baseline Calcium absorption was significantly correlated with serum 1,25(OH)2D but not with 25(OH)

D. In a previous study of 489 elderly women with serum 25(OH)D levels averaging 30 ng/ml there was a highly significant correlation between calcium absorption and serum 1,25(OH)2D but not serum 25OHD [21] and this has been confirmed in yet another larger study [22]. Taken together, these results suggest that in the normal range of serum 25(OH)D, serum 1,25(OH)2D is the important vitamin D metabolite controlling calcium absorption. Once serum 25(OH)D levels decrease below 5 ng/ml there is a significant decrease in serum 1,25(OH)2D and this is responsible for the significant decrease in absorption of calcium and probably phosphorus in severe vitamin D deficiency. This data makes sense teleologically. In the old world an important protection against low vitamin D levels would be to allow calcium and phosphorus absorption at very low levels of serum 25(OH)D in order to protect the skeleton from rickets, osteomalacia and osteoporosis. It is known that in an animal model knock out (KO) the vitamin D receptor (VDR) in the intestine leads to development of rickets, however if a rescue diet of high calcium and phosphorus intake is given to these VDRKO animals then rickets is prevented suggesting that the most important function of the VDR in the intestine is to increase calcium and phosphorus absorption in the intestine and prevent demineralization of bone [23].

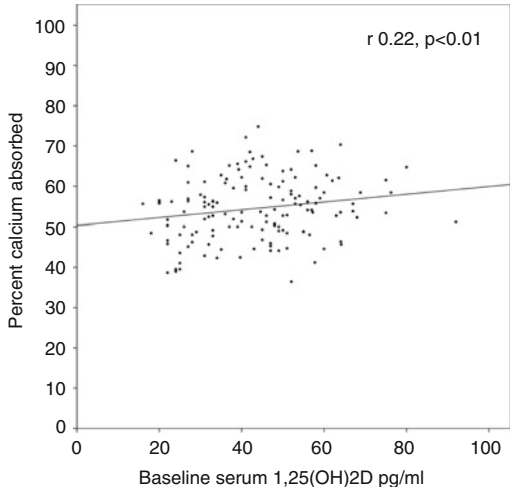

Fig. 20.7 Calcium absorption is significantly correlated with serum 1,25(OH)2D, r 0.22, $p<0.01$ from baseline data from study [17]

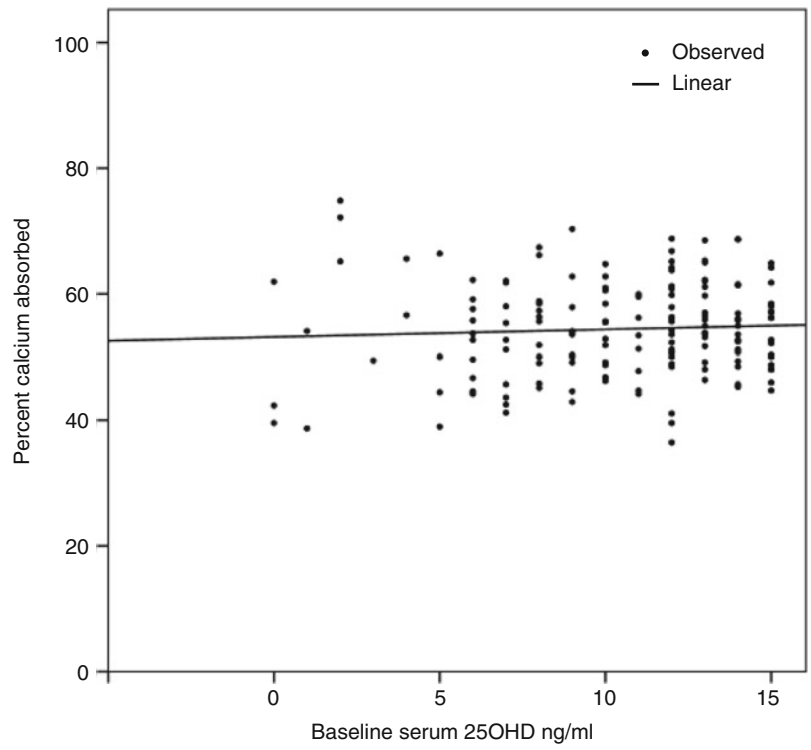

Fig. 20.8 Calcium absorption is not correlated with serum 25OHD from baseline data from study [17]

These human studies together with the VDRKO experiments in animals have taught us much about the physiology of calcium absorption and the interaction of calcium absorption, vitamin D metabolism and the prevention of osteomalacia and osteoporosis.

The studies suggest that there is minimal role for using vitamin D in prevention of osteoporosis since calcium absorption is maximized above very low levels of serum 25(OH)D (5 ng/ml) and the concept that vitamin D increases calcium absorption is only valid for subjects with serum 25 OHD levels < 5 ng/ml or that have significant secondary hyperparathyroidism For these individuals one can recommend vitamin D 200–400 IU daily. Our dose response studies [14] show that vitamin D 400 IU daily increases serum 25(OH)D by about 10 ng/ml when serum 25(OH)D levels start between 10–15 ng/ml. For those subjects or patients with disease that have calcium malabsorption such as severe osteoporosis, corticosteroid treatment or malabsorption syndromes the use of active forms of vitamin D such as 1,25dihydroxyvitamin D, 0.25 mcg twice daily is more rational. Because absorption of calcium is generally good in most normal subjects, increasing calcium intake for those who are deficient is recommended.

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Vitamin D and Omega-3 Fatty Acids and Bone Health: Ancillary Studies in the VITAL Randomized Controlled Trial

21

Meryl S. LeBoff, Amy Y. Yue, Nancy Cook, Julie Buring, and JoAnn E. Manson

Abstract

Vitamin D is widely used to support skeletal health, but results of its effects on bone are inconsistent. The VITamin D and OmegA-3 TriaL (VITAL): Effects on Fractures and Bone Structure and Architecture are 2 ancillary studies to VITAL, which are testing effects of daily vitamin D on fracture risk and bone health measures. Fractures are adjudicated through medical record review in the VITAL cohort and bone health measures assessed in a sub-cohort. VITAL will clarify the relationship between supplemental vitamin D and bone health, and inform public health guidelines on use of vitamin D for the prevention of fractures.

Keywords

Vitamin D • Omega-3 fatty acids • Bone density • Randomized controlled trial • Bone health

Rationale

Osteoporosis is a major public health problem that results in an estimated two million fractures each year in the United States (U.S.) and more than nine million fractures worldwide [1–3]. Among adults 50 years and older, 50 % of women and 25 % of men in the U.S. will suffer an osteoporotic fracture in their remaining lifetime [4]. Hip and other fractures are associated with excess morbidity, mortality, and healthcare expenditures, which are projected to reach more than $25.3 billion/year in the U.S. by 2025 [1, 3, 5]. Primary prevention strategies are needed to reduce the burden of fractures.

Disclosure The authors have nothing to disclose.

M.S. LeBoff, MD (✉) • A.Y. Yue
Endocrine, Diabetes and Hypertension Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
e-mail: MLEBOFF@PARTNERS.ORG; ayue@partners.org

N. Cook • J. Buring • J.E. Manson
Division of Preventative Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
e-mail: ncook@rics.bwh.harvard.edu; jburing@rics.bwh.harvard.edu; jmanson@rics.bwh.harvard.edu

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Low vitamin D levels have been associated with decreased bone density and an increased risk for fractures. According to the National Health and Nutrition Examination Survey (NHANES), recent estimates of vitamin status among adults in North America show that vitamin D deficiency (i.e., 25-hydroxyvitamin D [25(OH)D] levels <20 ng/ml) is present in: 39 % of females and 31 % of males and 73 % of black adults [6, 7]. In postmenopausal females enrolled in osteoporosis studies and males in the Osteoporotic Fractures in Men Study (MrOS), >50 % had 25(OH)D levels <30 ng/ml (75 nmol/L) [8, 9]. Markedly higher proportions of deficient 25(OH)D levels have been reported among black adults due to reduced cutaneous vitamin D synthesis and vitamin D intakes, and other mechanisms; there is an increased interest and conflicting data on whether free vitamin D levels are similar between black and white adults [10–15]. Deficient vitamin D levels have also been widely reported among obese adults (due to vitamin D sequestration in fat, increased volume distribution) [16], elders, and those with hip fractures [17–21].

Although vitamin D supplements are widely used to support bone health, this dogma has been challenged by inconsistent data from systematic reviews and meta-analyses. Results from large randomized controlled trials (RCTs) on the effects of supplemental vitamin D alone *without* added calcium are conflicting (Table 21.1) [22–24]. While the 2011 U.S. Preventive Services Task Force (USPSTF) summary of five RCTs concluded that combined calcium and vitamin D supplementation decreased the risk of fractures by 12 % in older adults, vitamin D supplementation alone was *not associated* with a reduction in fractures [25]. In a group pooled analysis of seven RCTs from the U.S. and Europe including 68,500 older adults, the "Vitamin D Individual Patient Analysis of Randomized Trials" (DIPART) found that while vitamin D supplementation alone (400 and 800 IU/day) did not prevent fractures, combined calcium and vitamin D supplementation did reduce hip and total fractures [26]. A recent meta-analysis has also reported beneficial effect of supplemental calcium plus vitamin D on fracture outcomes [27]. In another pooled analysis of 11 RCTs including 31,022 adults (91 % females), those assigned to vitamin D had a 7 % reduction in non-vertebral fractures, without a significant effect on the risk of hip fractures. However, stratification of the data according to actual intake of vitamin D, rather than supplementation dose, revealed that those in the highest quartile of total, actual vitamin D intake (median 800 IU/day) had a reduction in hip fracture risk by 30 % and non-vertebral fracture by 14 % [28]. In light of these conflicting results, a 2014 systematic review for the Agency for Healthcare Research and Quality (AHRQ) concluded that effects of supplemental vitamin D on fracture reduction are inconsistent [29]. Lastly, the extensive review by the Institute of Medicine committee (IOM) emphasized the need for more research from large randomized clinical trials to "test the effects of vitamin D on skeletal and non-skeletal outcomes" and "identify the threshold effects and possible adverse effects where present" [30].

Omega-3 Fatty Acids and Bone

Studies of the effects of omega-3 fatty acids on health are sparse, but suggest potential benefits. A review of a few studies conducted in animals supported the skeletal benefits of omega-3 on bones, possibly through reductions in inflammation [31]. In ovariectomized mice and older rats, dietary fish oil prevented bone loss possibly by inhibiting osteoclasts or increasing mineral apposition rate. Eicosapentaenoic acid (EPA) improved structural and mechanical properties of cortical bone in rodent femurs. Recent increases in use of essential fatty acids supplements in the U.S. have prompted concerns about the clinical effects of essential fatty acids on skeletal health. In a nested case–control study (n = 400) in the Women's Health Initiative of participants with incident hip fractures and controls, measurement of polyunsaturated fatty acid levels in red blood cells showed that women with the highest tertile of omega-6/omega-3 fatty acids

Table 21.1 Randomized controlled studies of supplemental vitamin D without added calcium

Placebo-controlled, randomized controlled trials of supplemental vitamin D					
Study	Vitamin D supplement	Length of study	N	Study population	Results
Lips et al. [22]	400 IU/day	3.5 years	2578	Dutch women and men, aged 70 years or older	**No effect** On hip or vertebral fractures
Trivedi et al. [23].	100,000 IU every 4 months	5 years	2686	Community-dwelling British men and women, aged 65–85 years	**Reduced risk** 33 % reduction in the relative risk of the first hip, wrist/forearm or spine fracture
Sanders et al. [24]	500,000 units once a year	3 years	2256	Community-dwelling Australian women, aged 70 years or older	**Increased risk** Increased risk of fractures and falls in older women treated for 3 years

ratio had nearly twice the fracture risk as those in the lowest tertile [32]. A second observational study of 854 men and women from the Framingham Osteoporosis Study found that individuals with the highest intakes of the omega-3 linolenic acid compared with the low intakes (but not EPA and/or docosahexaenoic acid [DHA]) had a 54 % lower hip fracture risk [33]. A recent analysis of data from NHANES showed that older adults who used omega-3 supplements have significantly higher lumbar spine bone mineral density (BMD) [34]. While these epidemiological data and results from observational studies are promising, RCTs are needed to clarify the role of omega-3 fatty acids on bone and non-skeletal outcomes in women and men.

Design

The *VIT*amin D and Omeg*A*-3 Tria*L* (*VITAL*) is a population based, double blind, placebo-controlled primary prevention study among men 50 years of age or older and women 55 years of age or older from 50 states in the U.S. investigating whether daily supplemental vitamin D_3 (cholecalciferol, 2000 IU) and/or marine omega-3 fatty acids (Omacor® fish oil, a 1 g/day capsule containing EPA [465 mg] + DHA [375 mg]) supplements (or placebos) reduces the risk for developing cancer, heart disease, and stroke in people who do not have a prior history of these illnesses; the mean length of the treatment period will be 5 years.

Eligible participants at study entry had no history of cancer (except non-melanoma skin cancer), myocardial infarction (MI), stroke, transient ischemic attack (TIA), coronary artery bypass graft (CABG), or percutaneous coronary intervention (PCI). Enrollment in VITAL was contingent upon participants: (a) limiting consumption of supplemental vitamin D and calcium to no more than 800 IU/day and 1200 mg/day respectively, from all sources combined, and (b) forgoing use of supplemental fish-oil during the trial. Persons were excluded from the study if they had renal failure or dialysis, history of hypercalcemia, hypo- or hyperparathyroidism, severe liver disease (cirrhosis), or sarcoidosis or other granulomatous diseases such as active chronic tuberculosis or Wegener's granulomatosis, allergy to soy (in the vitamin D placebo pill) or fish, or other serious illness that would preclude participation. Individuals who met these criteria and demonstrated good pill-taking compliance (taking ≥2/3 of the study pills during the 3-month run-in phase) were randomized into the trial. Among the approximately 40,000 individuals who entered the run-in phase, 25,874 men 50 years or older and women 55 years or older were enrolled at the close of randomization on March 24, 2014.

Baseline Characteristics

VITAL Parent Study

The parent VITAL study exceeded the total randomization goal by enrolling 25,874 participants and also surpassing the projected goals for minority enrollment, with 5107 African Americans enrolled in the trial. Baseline blood samples have been collected in 16,956 participants. Among the final cohort enrolled in the parent VITAL study, 15 % participants are aged 50–59 years, 55 % are aged 60–69 years, 25 % are aged 70–79 years, and 5 % of participants were aged 80+ at baseline. The mean age at baseline for the overall VITAL cohort was 67.1 years (Table 21.2) [35].

VITAL has a broad geographic distribution, with participants enrolled from all 50 states. In this cohort, 27.7 % of participants are from the Northeast, 24 % are from the Midwest/Mountain region, 20.3 % are from the West/Southwest which includes Hawaii and Alaska, and 28 % are from the Southeast including Puerto Rico (Table 21.3) [35].

Table 21.2 Baseline characteristics of overall VITAL cohort

Characteristic	Percent/mean
Female %	50.6 %
Mean age ± SD, years	67.1 ± 7.1
Age ≥ 65, %	61.9 %
African American, %	20.2 %
Current smoker, %	7.1 %
Mean body mass index (BMI), kg/m^2	28.1
Obesity (BMI ≥ 30 kg/m^2), %	28.8 %
History of diabetes, %	13.1 %
History of hypertension, %	53.0 %
Current use of cholesterol-lowering medication, %	36.8 %
Current use of multivitamins, %	44.8 %
Current use of supplemental vitamin D (≤800 IU/day), %	42.6 %
Current use of supplemental calcium (≤1200 mg/day), %	26.4 %

Table 21.3 Geographical distribution of VITAL cohort

Region	Percent
Northeast	27.7
Midwest/Mountain	24.0
West/Southwest*	20.3
Southeast**	28.0

*Includes Hawaii and Alaska

**Includes Puerto Rico

VITAL-Bone Health

To comprehensively assess effects of supplemental vitamin D and omega-3 fatty acids on bone in terms of fracture risk and bone health outcomes, two concurrent and complementary VITAL bone health ancillary studies are ongoing. There are several mechanisms through which supplemental vitamin D may reduce fracture risk and increase bone mass. These mechanisms include increasing intestinal calcium absorption, improving bone mineralization, decreasing parathyroid hormone (PTH) levels, reducing bone resorption, and enhancing bone structure and architecture (Fig. 21.1) [36]. Additional studies are needed to fully understand the mechanisms through which high doses of daily supplemental vitamin D may affect bone health in women and men.

VITAL Fracture

Among the overall VITAL cohort of 25,874 participants across the U.S., VITAL: Effects on Fractures is being conducted to evaluate the effects of supplemental vitamin D and/or omega-3 fatty acids on fracture outcomes. Participants were asked to self-report incident fractures. When a fracture event is self-reported, participants approve the release of medical records and fractures are adjudicated by study investigators who are blinded to treatment. Hip and pelvic fractures are further adjudicated by review with a musculoskeletal radiologist using x-ray, CT, and MRI images. VITAL participants with self-reported fractures thus far are 66.2 % female, with the average age of 68.7 years (Table 21.4).

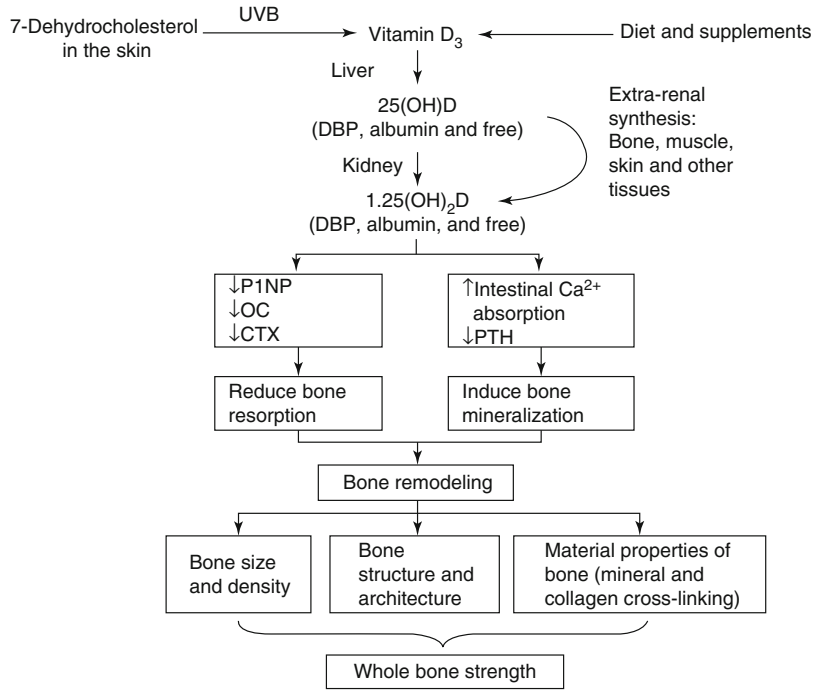

Fig. 21.1 Mechanisms through which high dose vitamin D may benefit bone health

Table 21.4 Preliminary characteristics of participants with self-reported fractures (2 years randomization; n = 829)

Characteristics of VITAL participants with self-reported fractures (Preliminary) (2 years randomization; n = 829)	
Characteristic	Percent/mean
Female,%	66.2
Mean age ± SD, years	68.7 ± 7.4
Age, %	
<60	12.4
60 to <70	49.6
≥70	38.0
Mean body mass index (BM), kg/m^2	27.5
History of diabetes, %	13.3
Geographic region, %	
West/Southwest	21.0
Midwest/mountain	27.3
Southeast	23.4
Northeast	28.3

VITAL Bone Health Sub-cohort

In a second ancillary study titled VITAL: Effects on Bone Structure and Architecture, the mechanisms through which supplemental vitamin D and/or omega-3 fatty acids have effects on bone health outcomes are being evaluated in a sub-cohort of 776 VITAL participants including pQCT and DXA [36]. Participants in the VITAL bone health sub-cohort are from the New England area and are part of a sub-group of VITAL participants that completed in-person visits at the Harvard Catalyst Clinical and Translational Science Center (CTSC) in Boston. Participants in this subgroup underwent at baseline detailed phenotyping (weight, height, and other anthropomorphic measurements) and bone health assessments at baseline and 2-years post-randomization as detailed below. The baseline characteristics of participants in the VITAL CTSC bone health sub-cohort are comparable to those of participants in the overall VITAL cohort (Table 21.5). At baseline, women and men weighed an average of 74.1 and 88.1 kg, were 162.2 and 176.0 cm tall, and had an average body mass index (BMI) of 28.2 and 28.4 kg/m^2, respectively. In this sub-cohort, 33.7% of the women were obese while 27.0% of the men were obese (BMI >30 kg/m^2) (Table 21.5).

Table 21.5 Preliminary baseline characteristics of the VITAL CTSC bone sub-cohort (N = 773)*

Preliminary baseline characteristics of the VITAL CTSC bone health sub-cohort with bone density and body composition (N = 773)[a]		
Baseline characteristics	All participants	
Female, %	46.8 %	
Age (years), %		
50–59	28.3 %	
60–69	56.9 %	
70–79	13.5 %	
≥80	1.3 %	
	Women	**Men**
Weight (kg)	74.1	88.1
Height (cm)	162.2	176.0
Mean body mass index (BMI), (kg/m^2)	28.2	28.4
Obesity (BMI ≥ 30 kg/m^2), %	33.7	27.0

[a]N = 776; three participants had pQCT but not DXA (DXA being serviced)

Randomization

All participants in VITAL were randomized at the start of the study to vitamin D or placebo groups (n = 12,927/12,947 in each group). After being assigned to either group, the vitamin D and placebo groups were then randomized into omega-3 fatty acid and placebo groups (Fig. 21.2). Marine omega-3 fatty acids supplements in VITAL contain two omega-3 fatty acids EPA and DHA. As VITAL is a large 2 × 2 factorial, double blind, placebo controlled trial, the VITAL cohort is randomized equally into vitamin D or placebo and between omega-3 fatty acids and placebo (Fig. 21.2).

Quality Control

VITAL is using Centers for Disease Control (CDC) reference material for quality control (QC) material, going beyond routine QC procedures. VITAL is also working closely with the CDC to evaluate and calibrate the assays for serum 25(OH) D at Quest Diagnostics (liquid chormatography-tandem mass spectrometry [LC-MS/MS]) and ABBOTT Atherotech Diagnostics (chemiluminescent micro particle immunoassay [CMIA]).

Hybrid Design of VITAL Bone Health Studies

In this hybrid design, we are testing whether vitamin D_3 and/or EPA + DHA supplementation reduces incident, total, hip and non-vertebral fractures in the overall VITAL cohort of 25,874 women and men over an average treatment period of five years. Among a total sub-cohort of 776 participants in VITAL Bone Health, detailed phenotyping is also underway to assess bone density, bone turnover biomarkers, bone structure, body composition, and physical performance measures at baseline and 2-years post-randomization and high-resolution pQCT (HR-pQCT) is being carried out in year 2 post-randomization. pQCT measures vBMD at the distal radius and tibia, distinguishes trabecular and cortical bone, and measures bone strength indices [37]. HR-pQCT measures volumetric bone density (total, cortical, and trabecular) at the radius and tibia, bone microarchitecture, and trabecular morphology (thickness, number, separation) [37, 38].

At baseline, we completed 773 DXA scans, and 2-year post-randomization scans are in progress. Measurement of volumetric BMD (vBMD) at the distal radius and tibia have also been completed among approximately 600 participants at baseline using pQCT scans, and 2-year post randomization scans are also in progress. Bone microarchitecture assessments at the distal radius and tibia by HR-pQCT are also being completed at 2-years post-randomization. The hybrid design of the VITAL bone health studies allows for comprehensive testing of the effects of the study interventions on bone, both through fracture outcomes and comprehensive in-clinic, bone health measurements.

Bone Imaging Technologies

A variety of bone imaging technologies are used in the VITAL bone health examinations. DXA measures spine, hip, total body, and forearm bone density as well as body composition and trabecular bone score (TBS) [36]. TBS predicts fractures risk independent of BMD and can be used to in

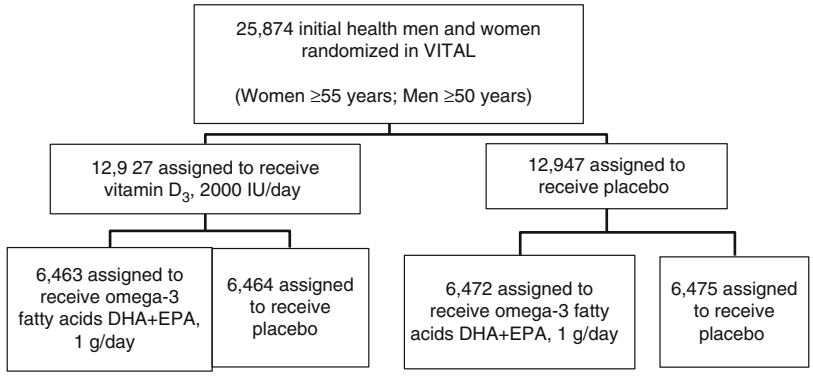

Fig. 21.2 VITAL randomization design

addition to BMD and FRAX to improve fracture risk assessments. TBS scores decrease with advancing age and have been shown to identify 66–70 % of women who had fragility fractures [39, 40]. TBS captures a larger portion of diabetes-associated fracture risk than does BMD alone [41]. TBS scores are lower in secondary causes of osteoporosis and increase in response to bone active medications over several years [42–45].

DXA can also distinguish fat and lean tissue and provide measurements of total adiposity including fat mass index (FMI-fat mass/height^2) and total percent body fat as well as regional obesity including visceral adipose tissue (VAT). VAT thresholds (normal 10–100) are associated with metabolic risk factors for coronary heart disease. Additional regional adiposity measurements include trunkal and regional fat distribution and muscle including lean mass index (LMI; lean mass/height^2) and appendicular (limb) lean mass/height^2 (appendicular LMI). Appendicular LMI has been used to determine sarcopenia, which is defined in men as LMI of <7.00 kg/m^2 and in women <5.25 kg/m^2. Other definitions for sarcopenia have been proposed by various scientific groups. Recently, the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium recommended use of appendicular lean mass adjusted for BMI (ALM_{BMI}) to determine weakness and low lean mass, with ALM_{BMI} cutpoints of <0.789 for men and <0.512 for women [46].

We predict that high-dose of supplemental vitamin D will result in (a) reduced risk of total, hip, and non-vertebral fractures; (b) small increases or reduced loss of areal bone mass density (aBMD) in the spine, hip, and total body; (c) reduced bone turnover as assessed by markers of bone resorption (c-telopeptide [CTX]) and formation (amino-terminal propeptide of type 1 collagen [P1NP]); and (d) improved vBMD, cortical thickness, and trabecular number.

Other Hypotheses

Other questions of interest include testing whether these outcomes vary according to sex, age, and/or race/ethnicity; how adiposity influences vitamin D needs in the population [7]; and whether high dose supplemental vitamin D improves physical performance measures. Additional assessments will be carried out to investigate the relationship between these outcomes and omega-3 fatty acids. The VITAL study will also make it possible to determine whether supplemental vitamin D and/or omega-3 fatty acids confer other health benefits. There are 18 VITAL ancillary studies, funded by the National Institutes of Health and one funded by the American Heart Association, investigating whether these supplements confer additional health benefits such as lowering the risk for diabetes, high blood pressure, asthma, depression, chronic knee pain symptoms, and physical disability and falls, autoimmune conditions, impaired kidney function, among other health

conditions. Thus, it will also be possible in VITAL to determine whether there are any associations between bone and a number of medical conditions such as cancer and cardiovascular health as well as many other conditions, and whether these medical conditions affect fracture risk among older adults. Thus, the VITAL database is an invaluable resource to investigate relationships between vitamin D and omega-3 fatty acids and many other outcomes.

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Preventive Medicine

JoAnn Manson, M.D., DrPH; Julie Buring, Ph.D.; Nancy Cook, Ph.D.; Trish Copeland, MS, RD, LDN; Joseph Walter

Radiology BWH

Bharti Khurana, M.D.

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Vitamin D, Exercise, and Health

22

Kirsti Uusi-Rasi, Radhika Patil,
and Christel Lamberg-Allardt

Abstract

Targeted exercise training and sufficient vitamin D intake may significantly improve muscle performance and balance and thus reduce the risk of falling among older people.

A 2-year randomized controlled vitamin D and exercise trial of 409 home-dwelling women aged 70–80 years included four study arms: (1) exercise + vitamin D (800 IU/day), (2) exercise + placebo, (3) no exercise + vitamin D (800 IU/day), (4) no exercise + placebo. Primary outcomes were falls. Injurious falls, bone health, physical functioning, quality of life and fear of falling were also assessed.

Neither vitamin D nor exercise reduced falls. However, exercise more than halved injurious falls with and without vitamin D. Vitamin D maintained femur BMC and increased tibial trabecular density slightly, while only exercise improved physical functioning, but neither treatment improved quality of life, nor reduced fear of falling. Participants were largely well-functioning and vitamin D replete.

Future research is needed to elaborate the effects of interventions on people with varying baseline levels of vitamin D and physical functioning.

Keywords

Falls • Injurious falls • Vitamin D • Exercise • Physical function • Older women

K. Uusi-Rasi, PhD (✉) • R. Patil, PhD
Bone Research Group, UKK Institute for Health Promotion Research,
Kaupinpuistonkatu 1, P.O. Box 30, Tampere
FI-33501, Finland
e-mail: kirsti.uusi-rasi@uta.fi

C. Lamberg-Allardt
Department of Food and Environmental Sciences,
University of Helsinki, Helsinki, Finland

Falls in older adults are a major health concern in ageing societies. They often result in severe injuries, functional limitations, long-term care and reduced quality of life. Although it is important to prevent falls in general, it is particularly important to prevent injurious falls; the most serious

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consequences of falls being fractures and head injuries. Moreover, if ageing is combined with extended years of healthy life it could also produce desirable social, economic and health benefits. Health maintenance for people throughout life, via actions such as exercise and proper nutrition, contribute to lifelong wellbeing [1].

Sustaining a fracture depends on the force of impact of the fall event and the strength of bone. Osteoporosis is a multifactorial disease in older adults characterized by low bone mineral density (BMD) and decreased bone strength, which predisposes to fractures. However, a majority of all fractures occur as the result of a fall. Older persons are often susceptible to falls, which are the leading cause of unintentional injuries and death among this population. Approximately 30 % of community living people aged 65 years or older fall each year, the number being even higher in institutions. Although only 5–10 % of falls result in serious injuries [2, 3], fall prevention is widely seen as the most essential element in the planning of effective injury and fracture prevention among any elderly population.

In addition to physical injuries, falls may increase fear of falling, which further may lead to a loss of confidence in the ability to ambulate safely, and mobility restrictions and functional decline, depression, social isolation and reduced quality of life (QoL) [4]. Although cross-sectional and prospective studies in the general population have found positive associations between physical activity and QoL, evidence from randomized trials is limited [5]. A recent Cochrane review showed positive, but temporary effects of exercise interventions on fear of falling [6].

Preventing falls and injuries among older adults is challenging, even promoting physical activity is a major challenge because our lifestyle is designed to reduce, or even eliminate physical activity at every opportunity [7]. However, there is strong evidence from randomized controlled trials and subsequent systematic reviews and meta-analyses that regular strength and balance training among community-living older adults can reduce the risk of both noninjurious and injurious falls by 15–50 % [8, 9]. Randomized controlled trials indicate that not only individually tailored training but also more untargeted group exercise programs are effective in preventing falls [10, 11]. However, all exercise is not equally effective. The most effective exercise approach for the prevention of falls and fractures in community-dwelling older adults is a combination of balance and strength training. To be effective, multifactorial preventive programs should include an exercise component accompanied by individually tailored measures focused on high-risk populations [12].

Vitamin D is known to be vital for bone metabolism and health; insufficient serum 25-hydroxivitamin D levels [25(OH)D] are associated with increased bone loss and risk of fractures, and even with increased fall rates. Furthermore, people with low 25(OH)D levels have been suggested to have lower physical functioning [13, 14]. However, systematic reviews and meta-analyses of clinical trials exploring the role of vitamin D in reducing falls and fractures and improving physical functioning in community dwelling older people, are inconclusive [15–19]. In addition, vitamin D supplementation is suggested to be related with improved psychosocial functions, including quality of life, self-rated health, and mood [20, 21].

In randomized controlled trials the incidence of falls was almost halved and musculoskeletal function improved among older people with a combination of vitamin D and calcium compared with calcium supplement alone [22, 23]. Falling may, at least partly, be a consequence of impaired neuromuscular function associated with vitamin D deficiency, since abnormal motor performance, increased body sway, and quadriceps weakness have been reported in those with low vitamin D status [13]. The dose of vitamin D in falls prevention efficacy is probably more important than the type of vitamin D, duration of therapy, and sex. Participants receiving 20 μg/day (800 IU) seem to have lower risk of falls, while 10 μg (400 IU) was insufficient for reducing falls [24].

Although a recently published meta-analysis found insufficient evidence that current multifactorial falls prevention programs could prevent falls and related injuries [25], it must be kept in mind that great majority of fall-prone older adults

has more than one risk factor for falls. Randomized controlled trials suggest that exercise may effectively improve many risk factors of falling, such as muscle strength, flexibility, balance, coordination, proprioception, reaction time and gait [26–28] and prevent falls and fractures [29–31].

In this study we evaluated the separate and combined effects of multimodal exercise training and vitamin D supplementation in reducing falls and injurious falls, and in improving bone mass and physical functioning among older women at risk for falling. In addition, we evaluated the effects of both factors on quality of life and fear of falling.

Design

This study was a 2-year double-blind placebo-controlled vitamin D and open exercise intervention trial with four arms (Fig. 22.1). The trial was done between April 2010 and March 2013. Eligibility criteria and recruitment of participants have been described in detail previously [32, 33]. Briefly, 70–80-year old home-dwelling women living in the City of Tampere, Finland, were eligible if they had fallen at least once during the previous 12 months, did not use vitamin D supplements, and had no contraindications to exercise. The criterion for exclusion was participation in moderate to vigorous exercise more than 2 h per week.

The study protocol was approved by the Ethics Committee of the Tampere University Hospital, Finland (R09090). Each participant provided her written informed consent prior to randomization. The study protocol is registered at ClinicalTrial.gov (NCT00986466).

Participants

Four-hundred and nine participants were randomly assigned to one of four groups using a computer-generated list based on simple randomization with random allocation sequence to ensure equal group sizes: (1) vitamin D 800 IU/day and exercise (D^+Ex^+) (2) placebo and exercise (D^-Ex^+) (3) vitamin D 800 IU/day without exercise (D^+Ex^-) (4) placebo without exercise (D^-Ex^-).

Participants received one daily pill containing either 800 IU (20 μg) vitamin D_3 or placebo for 24 months [32]. All tablets were provided by Oy Verman Ab (Kerava, Finland) and were similar in size, appearance and taste. Each participant received a pack of pills for 6 months at a time. Used packs were returned at the time of laboratory measurements every 6 months, and new full packs were given. The compliance was confirmed by pill counts.

Exercise consisted of supervised, progressive group training classes two times a week for the first 12 months, and once a week for the remaining 12 months of the 24-month intervention [32]. Training sessions were carried out in 8-week periods, alternating between the exercise hall and gym by physiotherapists who also monitored attendance. Sessions in the exercise hall focused on balance challenging, weight bearing, strengthening, agility, and functional exercises. Gym sessions included a combination of pin-loaded weight machines, pulleys, and free weights; beginning with 30–60 % of one repetition maximum (1RM), progressing to a target level of 60–75 % of 1RM. The non-exercising groups were asked to maintain their pre-study level of physical activity.

Outcome Measures

The primary outcome was falls, and injurious falls, fallers and injured fallers were analyzed as secondary outcomes. In addition, changes in bone mass, physical functioning (muscle strength, balance and mobility), quality of life (QoL) and fear of falling were evaluated.

The number of falls was obtained from prospective fall diaries returned monthly via mail, and details of each registered fall were ascertained by a telephone call. A fall was defined "an unexpected event in which the participant comes to rest on the ground, floor or lower level" [34]. Injurious falls were those for which participants sought medical care (nurse, physician, or hospital). These included injuries, such

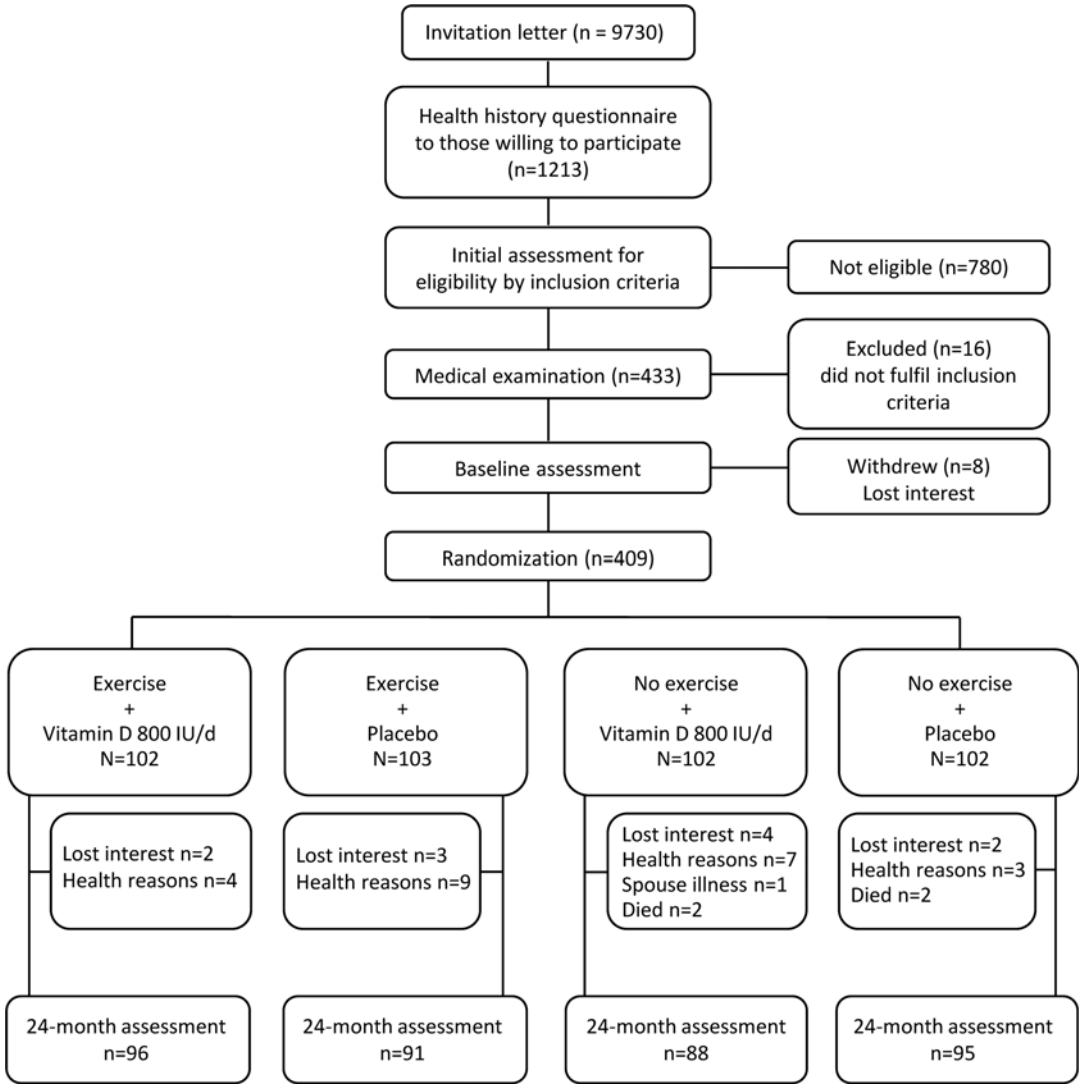

Fig. 22.1 Flowchart of the study

as bruises, abrasions, contusions, sprains, fractures, and head injuries.

Data Collection

All measurements were done at baseline, 12-, and 24-months. Blood samples and physical functioning were also assessed at 6-, and 18-months.

Height and weight were monitored with standard methods. Body composition and bone mineral content (BMC, g) of the total body and left proximal femur were assessed using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Lunar, Madison, WI, USA) [35]. Distal site (5 %) and the mid-diaphysis (50 %) of the tibia were assessed with peripheral quantitative computed tomography (pQCT) (Norland/Stratec XCT 3000, Pforzheim, Germany) [36].

Disability scores in activities of daily living (ADL) (range 6–36), instrumental ADL (IADL) (range 8–48) and mobility (range 4–24) were calculated using validated questionnaires [37]. Physical functioning was assessed by the Short Physical

Performance Battery (SPPB) [38], which comprised static balance, 4 m normal walking speed and five-time chair stand tests, and by the Timed up and go (TUG) test [39]. Dynamic balance was assessed using backwards walking [40]. Maximal isometric leg-extensor strength at a knee angle 110° was measured by a strain gauge dynamometer (Tamtron, Tampere, Finland). Each participant recorded her daily steps with a pedometer (Omron HJ-112-E) over the entire 24-month study period.

Fasting Serum 25-hydroxy-vitamin D [(25OH) D] was measured as a marker of vitamin D metabolism with OCTEIA immunoenzymometric assay (IDS, Bolton, UK). Reproducibility was ensured by adhering to the Vitamin D External Quality Assessment Scheme, DEQAS (deqas.kpmd.co.uk). Interassay variation was avoided by measuring all samples from the same subject in the same series. Serum intact parathyroid hormone (S-iPTH) was measured with an Immulite 1000 automated immunoassay (Siemens Healthcare Diagnostics, Malvern, PA). Dietary intake of calcium and vitamin D were assessed with a validated food frequency questionnaire [41].

Quality of Life (QoL)

The Leipad questionnaire was used to assess QoL [42]. The core instrument constructed for use with older people has six subscales: physical function (5 items), self-care (6 items), depression and anxiety (4 items), cognitive functioning (5 items), social relations (3 items), and life satisfaction (6 items). Items are scored on a 4-point scale ranging from 0 to 3, with 0 indicating the best condition and 3 the worst. Two questions about sexual functioning were excluded, which reduced the maximum possible total score by six points. A summed index of all items was used as an indicator of overall QoL, higher scores indicating worse QoL (range 0–87).

Fear of Falling

Fear of falling was assessed with the Falls Efficacy Scale International (FES-I) questionnaire, which contains items scored on a 4-point scale (1 = not at all concerned to 4 = very concerned). It assesses concern about falling, a term that is closely related to fear, but is less intense and emotional, and therefore may be more socially acceptable for older people to disclose [43]. Higher scores indicate worse outcome (range 16–64).

Statistical Methods

Power calculations indicated that a sample size of 260 (130 in exercisers and non-exercisers) would have an 80 % power to detect a 30 % between-group difference in the rate of falls during the two-year intervention with a significance level of $\alpha = 0.05$. However, in order to eliminate the role of chance in detecting the possible interaction of vitamin D and exercise, a total number of 400 participants (100 participants in each group) were recruited into the study.

All data were analyzed on an intention-to-treat basis. Follow-up time for falls and fallers were calculated from the beginning to the end of the 24-month intervention unless the participant withdrew from the study or died. Falls incidence rates were calculated as the total number of falls divided by the time over which falls were monitored (100 person-years) in each group. Negative binomial regression was used to estimate the incidence rate ratios (IRR) for falls and injurious falls. Cox-regression models were used to calculate hazard ratios (HR) for fallers and injured fallers in each group, with the $D^{-}Ex^{-}$ group as reference.

For physical functioning and bone traits, between-group differences in time were estimated by linear mixed models for normally distributed outcomes, and generalized linear mixed models with gamma distribution and log link function for non-normally distributed outcomes using age, height, weight and use of hormone therapy as covariates. Pairwise multiple comparisons with Sidak adjustment were used to test the differences among treatment groups and interaction between vitamin D and exercise. All statistical analyses were conducted using IBM SPSS statistics software version 22 (Chicago, IL).

Table 22.1 Baseline characteristics of the study groups, mean (SD)

Characteristic	D^-Ex^- N = 102	D^+Ex^- N = 102	D^-Ex^+ N = 103	D^+Ex^+ N = 102
Age, years	73.8 (3.1)	74.1 (3.0)	74.8 (2.9)	74.1 (2.9)
Height, cm	160.7 (5.4)	159.2 (5.8)	159.4 (6.1)	159.7 (5.9)
Weight, kg	72.0 (12.4)	73.0 (13.1)	70.9 (10.6)	73.2 (10.5)
Fat, %	41.1 (6.7)	42.0 (7.2)	41.3 (6.4)	42.8 (5.3)
Dietary Calcium intake, mg/day	1040 (345)	1125 (420)	1119 (346)	1109 (385)
Dietary Vitamin D intake, µg/day	10.2 (4.1)	10.9 (4.2)	10.3 (3.6)	10.4 (3.9)
S-25(OH)D, nmol/L	67.6 (18.8)	65.8 (17.1)	69.5 (18.0)	65.5 (17.5)
S-PTH, pmol/L	5.0 (2.7)	5.0 (2.2)	4.7 (2.0)	5.2 (2)
Disability scores[a], (range)				
ADL score (6–36)	6.7 (1.6)	7.0 (2.2)	6.8 (1.9)	6.9 (1.8)
IADL score (8–48)	9.8 (2.6)	10.7 (4.8)	9.9 (3.8)	10.3 (4.0)
Mobility score (4–24)	4.76 (1.85)	5.11 (2.58)	4.71 (1.79)	4.78 (1.63)
SPPS, range (0–12)[b]	10.6 (3–12)	10.7 (1–12)	10.9 (7–12)	10.8 (5–12)
Isometric leg extensor muscle strength, N/kg	23.1 (6.1)	23.4 (7.7)	23.6 (6.0)	22.2 (6.6)
Normal walking speed, m/s	1.04 (0.21)	1.00 (0.21)	1.01 (0.19)	1.03 (0.20)
5*Chair-stand, s	12.6 (2.4)	12.6 (3.3)	12.5 (2.8)	12.4 (2.5)
TUG-time, s	9.3 (2.1)	9.7 (6.4)	8.9 (1.9)	8.9 (1.6)
Backwards walking, proportion of those able to do 6.1 m, %	41.8	30.3	45.8	51.0
Quality of life, Leipad[a]				
Total score, (0–87)	14.9 (7.0)	16.6 (8.1)	15.8 (7.7)	15.7 (8.5)
Fear of falling, FES-I[a] (16–64)	22.9 (5.7)	24.2 (6.7)	22.7 (6.4)	23.4 (6.0)

Note: D^-Ex^- placebo, D^+Ex^- 800 IU vitamin D daily, D^-Ex^+ placebo + exercise, D^+Ex^+ 800 IU vitamin D daily + exercise

[a]Lower score indicates better functioning

[b]Higher score indicates better functioning

Results

The main results are previously reported [33]. There were no clinically relevant between-group differences in age or anthropometry at baseline (Table 22.1). Body composition or disability scores did not change significantly during the trial. Mean (SD) daily dietary calcium intake was sufficient, being 1098 (378) mg at baseline and 1212 (400) mg at 24 months, while respective values for dietary vitamin D intake were 10.4 (3.9) µg and 10.5 (4.3) µg. Of the participants 356 women (87 %) had at least one diagnosed chronic disease, most commonly cardiovascular, and 79 (19 %) used hormone therapy currently.

Thirty-nine women (9.5 %) did not complete the end point measurements, most of them due to health reasons, and four died (Fig. 22.1). Mean pill compliance (range) was 98 % (42–100 %), while mean exercise compliance measured as attendance at all offered training sessions for group and home training was 73 % (0–97 %) and 66 % (0–100 %), respectively. In general, the training program was well tolerated. There were no severe adverse effects or injuries due to the training. Twenty-two participants from the Ex^+ and one from the Ex^- groups had musculoskeletal complaints, mainly mild overuse symptoms, and three injuries occurred during the gym training.

Vitamin D concentrations differed significantly between D^- and D^+ groups. Mean S-25(OH)D levels remained stable in the D^- groups, being 68.6 (18.4) nmol/L at baseline and 68.6 (17.2) nmol/L at 24 months with small seasonal variations,

Table 22.2 Rate of falls per 100 person-years in each study group during the 24-month intervention, and incidence rate ratios (IRR) (95 % CI) for falls and injurious falls

Outcome	D^-Ex^-	D^+Ex^-	D^-Ex^+	D^+Ex^+
Falls, all	118.2	132.1	120.7	113.1
Injurious falls	13.2	12.9	6.5	5.0
Falls with fractures	2.9	3.0	2.4	1.5
IRR				
All falls	1	1.08 (0.78–1.52)	1.07 (0.77–1.45)	0.99 (0.72–1.39)
Injurious falls	1	0.84 (0.45–1.57)	0.46 (0.22–0.95)	0.38 (0.17–0.81)
Multiple falls	1	1.05 (0.60–1.86)	1.11 (0.63–1.94)	1.14 (0.65–1.99)
Multiple injurious falls	1	1.04 (0.56–1.96)	1.10 (0.59–2.05)	1.54 (0.84–2.81)

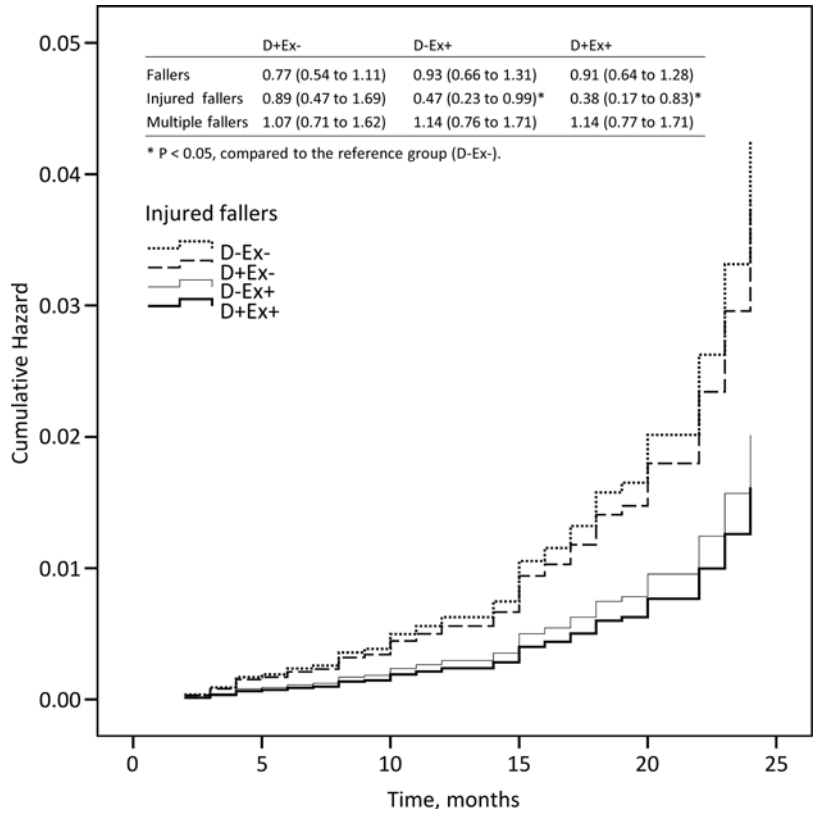

Fig. 22.2 Hazard ratios (95 % CIs) for fallers, injured fallers, and multiple fallers using the D^-Ex^- group as a reference

whereas mean levels increased in the D^+ groups from 65.7 (17.3) nmol/L at baseline to 92.3 (18.5) nmol/L at 24 months. There were small seasonal variations in S-PTH in the D^- groups, while in the D^+ groups PTH declined slightly from baseline, remaining stable thereafter.

In total, there were 928 falls and 281 fallers with no between-group differences and no interaction between vitamin D and exercise (rate of falls given in Table 22.2). However, hazard ratios (HR; 95 % CI) for injured fallers were lower in both Ex^+ groups compared with D^-Ex^- group: 0.47 (0.23–0.99) for the D^-Ex^+ group, and 0.38 (0.17–0.83) for the D^+Ex^+ group. The D^+Ex^- group did not differ from D^-Ex^- group (Fig. 22.2). Results were similar concerning the IRRs of injurious falls (Table 22.2). The number of multiple fallers (190) and multiple injured

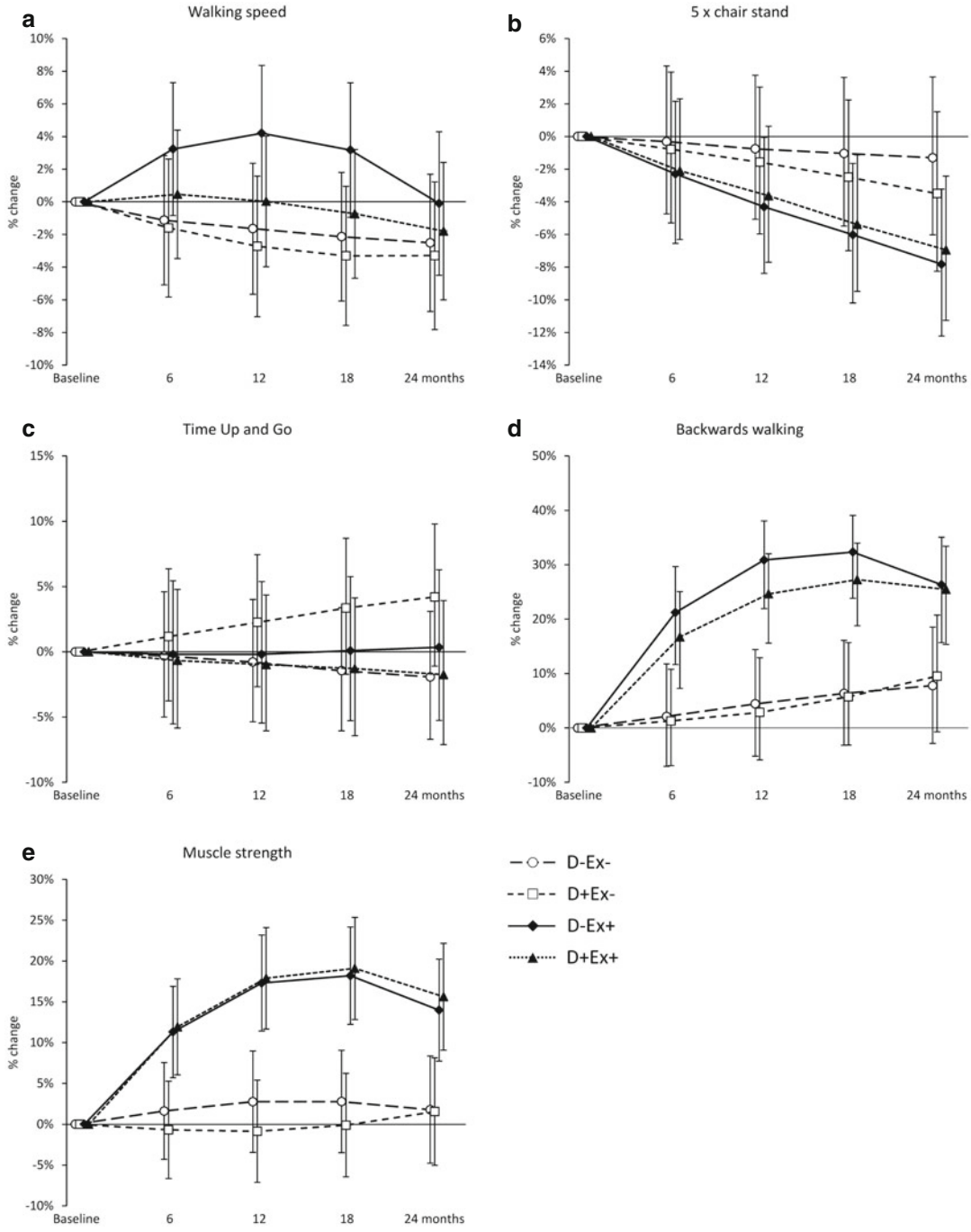

Fig. 22.3 Mean percentage changes (95 % CI) from baseline for normal walking speed (**a**), chair-stand test (**b**), Timed up and go (TUG) (**c**), Backwards walking (**d**), and lower limb extension muscle strength (**e**). In backwards walking, the %-change describes the change in proportion of those women able to walk 6.1 m. Figure legend: Placebo without exercise ($D^{-}Ex^{-}$); Vitamin D without exercise ($D^{+}Ex^{-}$); Placebo and exercise ($D^{-}Ex^{+}$); and vitamin D with exercise ($D^{+}Ex^{+}$)

Table 22.3 Baseline values (SD) and mean changes (95 % CI) in bone traits, quality of life and fear of falling in each study group

Outcome	Absolute value, mean (SD)	Change at 12 months, %	Change at 24 months, %	P compared with D^-Ex^-
Total body BMC, g/cm^2				
D^-Ex^-	2395 (410)	−0.37 (−2.33 to 1.58)	−0.59 (−2.58 to 1.41)	
D^+Ex^-	2323 (371)	−0.77 (−2.91 to 1.37)	−1.27 (−3.43 to 0.88)	0.38
D^-Ex^+	2352 (403)	−1.07 (−2.98 to 0.83)	−1.44 (−3.40 to 0.51)	0.56
D^+Ex^+	2400 (363)	−0.59 (−2.40 to 1.23)	−0.80 (−2.61 to 1.02)	0.10
Femur BMC, g/cm^2				
D^-Ex^-	32.10 (5.43)	0.33 (−0.81 to 1.48)	−0.05 (−1.22 to 1.11)	
D^+Ex^-	30.59 (4.53)	0.22 (−1.16 to 1.61)	−0.27 (−1.65 to 1.11)	0.30
D^-Ex^+	31.28 (4.99)	0.05 (−1.11 to 1.20)	−0.19 (−1.38 to 1.00)	0.49
D^+Ex^+	32.02 (4.94)	0.42 (−0.71 to 1.54)	0.20 (−0.92 to 1.32)	0.06
Distal tibia TrD, mg/cm^3				
D^-Ex^-	220.3 (34.8)	−0.25 (−3.54 to 3.03)	−0.49 (−3.77 to 2.80)	
D^+Ex^-	217.9 (28.6)	−0.09 (−3.50 to 3.32)	0.03 (−3.36 to 3.42)	0.12
D^-Ex^+	224.5 (29.4)	−0.13 (−3.39 to 3.13)	−0.16 (−3.43 to 3.11)	0.41
D^+Ex^+	224.4 (31.4)	0.17 (−3.05 to 3.39)	0.19 (−3.04 to 3.42)	*0.021*
Tibial shaft CoD, mg/cm^3				
D^-Ex^-	1104.6 (37.0)	−0.07 (−0.78 to 0.64)	−0.13 (−0.85 to 0.58)	
D^+Ex^-	1106.3 (38.0)	0.05 (−0.65 to 0.75)	0.11 (−0.60 to 0.82)	0.08
D^-Ex^+	1106.5 (33.6)	−0.04 (−0.72 to 0.65)	−0.08 (−0.77 to 0.62)	0.68
D^+Ex^+	1114.4 (34.0)	−0.06 (−0.75 to 0.63)	−0.12 (−0.82 to 0.58)	0.93
Total Leipad score (scale 0–87)				
D^-Ex^-	14.9 (7.0)	−1.8 (−12.1 to 9.7)	−0.9 (−11.7 to 11.2)	
D^+Ex^-	16.6 (8.1)	1.2 (−9.4 to 13.2)	5.3 (−6.3 to 18.3)	0.30
D^-Ex^+	15.8 (7.7)	−0.2 (−10.4 to 11.3)	2.4 (−8.6 to 14.8)	0.58
D^+Ex^+	15.7 (8.5)	−1.4 (−11.6 to 9.9)	−0.2 (−10.9 to 11.8)	0.95
FES-I score (scale 16–64)				
D^-Ex^-	22.9 (5.7)	2.0 (−4.1 to 8.5)	−0.7 (−6.8 to 5.9)	
D^+Ex^-	24.2 (6.7)	−4.9 (−10.7 to 1.3)	−1.2 (−7.4 to 5.4)	*0.02*
D^-Ex^+	22.7 (6.4)	−4.2 (−9.9 to 2.0)	0.9 (−5.3 to 7.5)	*0.02*
D^+Ex^+	23.4 (6.0)	−4.4 (−10.0 to 1.7)	−1.3 (−7.3 to 5.1)	*0.03*

fallers (117) were distributed similarly between the groups.

During the intervention, total body BMC declined slightly in all groups, but remained stable at the femur with no between-group differences (Table 22.3). There were also no statistically significant between-group differences in changes at tibial shaft cortical density. Vitamin D increased trabecular bone density at the distal tibia slightly, the mean difference being statistically significant between D^+Ex^+ and D^-Ex^- ($p=0.021$) (Table 22.3).

Changes in physical functioning are presented in Fig. 22.3. Normal walking speed was maintained in D^-Ex^+ compared to D^-Ex^- ($p=0.007$), while declined similarly in other groups. Chair-stand time improved in both Ex^+ groups, showing over 6 % improvement compared to D^-Ex^- but reaching statistical significance only in D^-Ex^+ ($p=0.027$). TUG time deteriorated significantly more in D^+Ex^- ($p=0.011$) compared to D^-Ex^-, while other groups did not differ from D^-Ex^-. Backwards walking improved significantly among exercisers; the difference compared to D^-Ex^- was statistically significant in D^-Ex^+ ($p=0.001$) and D^+Ex^+ ($p=0.026$). Irrespective of vitamin D, exercise increased muscle strength, while vitamin D alone had no effect. The predicted

mean increase in the lower limb extension strength was nearly 15 % in both Ex^+ groups and differed significantly from D^-Ex^- ($p<0.001$).

There was a small but insignificant improvement in perceived QoL during the first 6 months in all four groups. However, when comparing with the D^-Ex^- group, there were no statistically significant between-group differences in changes in QoL. The improvement seen at 6 months was no longer apparent at 12 months (Table 22.3). The median FES-I score at baseline was 21 suggesting moderate fear about falling. However, according to the FES-I, 16 (3.9 %) women reported no fear of falling at all at baseline, 89 (21.9 %) women had high (FES-I score 28–64), and 186 (45.8 %) had moderate (FES-I score 20–27) fear for falling, respectively. All three intervention groups (D^+Ex^-, D^-Ex^+ and D^+Ex^+) showed reduced fear for falling during the first 12 months, and differed significantly compared with the D^-Ex^- control group (Table 22.3). However, this reduction in fear of falling was not maintained at the end of the intervention.

Discussion

This large randomized controlled clinical trial of vitamin D and exercise showed that exercise training reduced injurious falls among home-dwelling older women, while the rate of falls was not affected by either treatment. Exercise improved physical functioning, but against initial expectations vitamin D did not. However, vitamin D increased trabecular density at the distal tibia slightly.

Current evidence for the effectiveness of vitamin D in falls prevention is inconsistent, most likely because of varying study designs and baseline 25(OH)D concentrations, and different doses or type of vitamin D supplement used. According to some experts, adequate serum 25(OH)D concentration should be ≥75 nmol/L [44], while many guidelines consider 50 nmol/L to be sufficient [45]. A recent paper showed that although very high vitamin D doses increased calcium absorption, benefits for bones, physical functioning, or falls were small [46].

There is also inconclusive evidence on whether vitamin D could modulate physical functioning [9, 47]. A recent meta-analysis suggested that vitamin D had no effect on muscle strength with vitamin D levels over 25 nmol/L [19]. In a population-based survey of men and women aged 60 year or older, a higher concentration of 25(OH)D was associated with better neuromuscular function [14]. Surprisingly, chair-stand test performance appeared to decline at the highest 25(OH)D concentrations (>120 nmol/L), possibly due a small number of observations in the highest category [14]. This finding needs confirmation, since in trials using high doses of vitamin D, high 25(OH)D concentrations have increased the risk of falls and fractures suggesting negative influences on balance and mobility [48, 49]. Very little is known about the combined effects of vitamin D and exercise, and two pilot studies have suggested a positive influence [50, 51].

In the present trial, exercise training, irrespective of vitamin D supplementation, reduced injurious falls and injured fallers. Apparently this effect was attributable to improved physical functioning. Muscle strength and balance training have earlier been shown to prevent falls in community-dwelling older adults, and recent meta-analyses have confirmed that such exercise programs are effective in preventing fall-induced injuries, too [8, 52].

Our results indicated that vitamin D may not improve neuromuscular function, at least when vitamin D intake is sufficient at baseline. Exercise improved muscle strength, balance, and mobility, except the TUG test time. Similarly, though normal walking speed was maintained in both exercise groups with or without vitamin D, the vitamin D-treated group without exercise (D^+Ex^-) showed the greatest decline. Exercisers on vitamin D supplementation showed consistently smaller benefits than placebo-treated exercisers.

Despite improved physical performance exercise conferred no effect on the overall fall rate. Some recent studies have also provided similar results [53, 54]. More falls have been reported among exercisers despite improved muscle strength and physical performance [53]. Nevertheless, in our study the rate of injurious falls and injured fallers

more than halved among exercisers. Good physical condition may help prevent injuries when falling, perhaps via better and safer landing techniques. This trend fully concurs with recent meta-analyses [8, 52].

The exercise training was primarily intended to improve balance, muscle strength and mobility rather than bone density, since limited physical capacity or co-morbidities of participants were likely to restrict the intensity or type of loading needed to strengthen bone. Nevertheless both vitamin D groups showed increased trabecular bone density in the tibia suggesting that mean 25(OH)D levels were sufficient for a skeletal effect (85 % of the participants in both vitamin D groups exceeded the target level ≥75 nmol/L) [44].

There is inconsistent evidence about the association between low vitamin D levels and low quality of life among generally healthy populations [21, 55, 56]. Positive associations found between vitamin D concentrations and physical functioning could explain better QoL. In a large Dutch cohort, physical performance explained the largest part of the association between lower 25(OH)D and lower QoL scores [21]. However, in our study QoL scores reacted similarly in all four groups despite clear positive changes in physical functioning among exercise groups with or without vitamin D. Minor but short-term benefits in change in QoL in the exercise groups compared with non-exercisers are in accordance with previous results of similarly aged women [57]. In our study, QoL seemed to improve slightly during the first 6 months, but the trend was similar in all four groups suggesting an attention effect, rather than the effect of added vitamin D or exercise. It is also not known which particular time point during training would be optimal for the perception of changes [58], and it is possible that the participants adapted to successive minor changes due to the treatments (vitamin D or exercise) well before the first follow-up measurement which did not reflect these adequately.

While it has been suggested that multifactorial programs, community-based Tai Chi and home-based exercise interventions are effective in reducing fear of falling in community-dwelling older people [59], our result supports the recent Cochrane review (30 studies, n = 2878), which found that exercise interventions regardless of type, frequency or duration probably reduce fear of falling only to a limited extent immediately after the intervention, with insufficient evidence to determine whether this reduction lasts beyond the intervention [6]. We found a slight but temporary reduction in the mean FES-I score in exercisers compared with controls (3 % net difference in mean values) at 12 months, which was no longer apparent at 24 months.

To our knowledge, there are no RCTs assessing effects of vitamin D supplementation on fear of falling. It may be speculated that if vitamin D affects mood or anxiety, it may also have an influence on fear of falling.

The strength of this study is the randomized controlled design with two years' duration. While it was not possible to blind for exercise, the study was double-blinded for vitamin D. Assessors of physical functioning and falls were, however, blinded to exercise group allocation. The trial was population-based, and the drop-out percent was very low (9.5 %) with good participation in exercise training and excellent pill compliance. Falls were collected monthly using falls diaries, and all reported falls were scrutinized by the investigators. Also, physical functioning and bone traits were comprehensively assessed.

There are also some limitations. Very few participants had insufficient vitamin D levels (S-25OHD < 50 nmol/L), and none were deficient (<25 nmol/L) at baseline. Vitamin D may have a threshold over which supplementation does not give an additional benefit, and therefore these results may not account for possible benefits in those with vitamin D deficiency. It may also be possible that the measured outcomes were not sufficiently sensitive to changes in mental well-being, and minute changes may not be easily recognized by healthy, community-living participants in their daily lives. Although our trial is population-based, it is possible that the women willing to participate in the study may have been more physically active and motivated to exercise than the general population of older women, and our findings may overestimate the effects of the exercise

intervention. Also, our findings cannot be generalized to institutionalized people or men.

Conclusions

Given the fact that fall risk is multifactorial, exercise may be the most effective and feasible strategy for preventing injurious falls in community-dwelling older adults replete with vitamin D. Vitamin D increased tibial trabecular density slightly and exercise clearly improved physical functioning. While neither treatment reduced the rate of falling, injurious falls more than halved among exercisers with or without vitamin D. Vitamin D or exercise neither improved quality of life, nor reduced fear of falling. Our participants were vitamin D replete with sufficient calcium intake. Future research is needed to elaborate the effects of interventions on people with varying baseline levels of vitamin D and physical functioning, especially when changes in wellbeing parameters are subtle.

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Part VI

Dairy

The Potential Role of Dairy Foods in Fracture Prevention in Elderly in Aged-Care

23

Sandra Iuliano

Abstract

The global population is both expanding and aging, resulting in a rise in the burden of fragility fractures. The highest fracture rates occur in the oldest old, or those greater than 80 years of age. The majority of institutionalised elderly are women aged 80 years and older, so a substantial fracture burden arises from the aged-care sector. Malnutrition, and more specifically deficiencies of protein, calcium and vitamin D contribute to fracture risk, and are common in elderly in aged-care. While drug therapy is not an option to reduce the public health burden of fractures, correction of these deficiencies may be a safe, accessible and cost saving approach to fracture prevention.

The nutritional benefits of oral nutritional supplements in relation to weight gain and fewer health complications have been demonstrated in the short term, but limited compliance and efficacy have been observed for interventions greater than 6 months. Significant improvements in bone metabolism in elderly aged-care residents have been observed with fortification of foods, especially dairy foods fortified with calcium and vitamin D. However, fortified products may not be available to all elderly. A food-based approach that warrants attention is improving the dairy content of the food supply in aged-care, as dairy is a good source of protein, calcium (and vitamin D) and this approach satisfies the requirements of safe, low cost and accessible. Feasibility studies of added dairy foods indicate significant improvements in nutritional status in elderly aged-care residents using this method. To advocate for a dairy-based approach to fracture prevention, quality studies demonstrating anti-fracture efficacy are required.

Keywords

Aged-care • Calcium • Dairy • Elderly • Fractures • Malnutrition • Protein

S. Iuliano, PhD
Department of Endocrinology, Level 2 Centaur Building, Heidelberg Repatriation Hospital, West Heidelberg 3081, Australia
e-mail: sandraib@unimelb.edu.au

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The Aging Population: A Global Phenomena

Globally, the population is expanding, and life expectancy lengthening resulting in the those older than 65 years becoming one of the fastest growing population groups [1]. Within the elderly population, the oldest old, or those over 80 years of age is expanding rapidly. 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. In developed countries one in ten people will be 80 years or older [1]. The trend of reduced mortality (deaths) and fertility (births) is leading to the population distribution shifting from a large base of younger people supporting a proportionally smaller number of elderly, to a more cylinder like shape; fewer children and younger productive people (aged 15–64 years) supporting a larger number of elderly (>65 years of age). It is projected that by 2047 the number of people aged >60 years will outnumber children [1]. It is often questioned if a longer lifespan is associated with more years of good health, or a longer period of morbidity and so prolonged disability and dependency. If the later ensues the sustainability of the aging population may be challenged. Falls, fractures and immobility combine to form one of the four non-communicable 'diseases' that will afflict the elderly and contribute to the health care costs of the aging population [1].

The Growing Fracture Burden

As the population ages, the burden of fractures also increases. Globally, nearly 9 million fragility fractures occur each year [2]. It is estimated that 1⁄3 of women and 1⁄5 of men aged 50 years and over will suffer a fragility fracture [3]. For hip fractures alone, it is projected that by 2050, the worldwide incidence of hip fractures, will increase by ~300 % in males, ~200 % in females, and will total over 6 million hip fractures annually [4]. The highest fracture risk is observed in women over the age of 80 years, and they will make up the majority of the oldest old in the population [1]. Hip fractures are common in the very old and are a major contributor to loss of mobility, and requirement for institutionalised care. To curb the contribution of the elderly to the total fracture burden, effective strategies to reduce fracture risk are needed.

The Role of Institutionalized Care for the Elderly

The aging of the population creates specific demands at all levels of society. While in developed countries, the majority of elderly people (>60 years of age) live independently with or without a spouse, approximately 10 % of elderly people live in some form of institutionalised care [5]. The level of dependency and need will determine the type of care required. Elderly that are semi-independent but require assistance with basic care needs are accommodated in 'hostels', or assisted (USA) or residential (UK) care facilities, while those with greater disability and more severe and chronic illnesses that require higher care, are accommodated in 'nursing homes'. Of those living in institutionalised care, which will be collectively termed 'aged-care', over three quarters are aged 80 years and over, and the majority are women. As women aged over 80 years have the highest fracture rates in the community, fracture rates in aged-care are about three times higher than for elderly living in the community [6].

A considerable portion of the total fracture burden comes from elderly in aged-care [7, 8]. Thirty percent of all hip fractures and 25 % of all fragility fractures arise from elderly in aged-care [9, 10]. At a population level drug therapy is not an option to prevent fractures, so safe, accessible and cost effective strategies 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, similar to that seen

with reducing sodium intake at a population level to lower blood pressure and risk of cardiovascular disease [11–14]. In aged-care malnutrition rates are high, with up to 89 % of residents being malnourished or at risk of malnutrition, and deficiencies of protein, calcium and vitamin D are common. For example in a sample of elderly aged-care residents in Australia, 30 % consumed below the estimate average requirement for protein, mean calcium intakes was approximate 600 mg/day and over half of residents had serum 25(OH)D levels below 50 nmol/L [15, 16]. Given these high rates of malnutrition, and the high fracture risk in aged-care residents this high-risk group is likely amenable to improved intakes of these (and other) nutrients as a strategy to reduce fracture risk. The cost-benefit analysis of a nutrition-based fracture prevention program in aged-care has not been reported but the cost-effectiveness of dairy consumption on fracture risk reduction in the community has been modelled [17]. However, in aged-care the cost of identifying, treating and monitoring malnutrition is estimated at €8,000 (~$9,000USD) per resident per year for those at risk of malnutrition, and €12,000 (~$13,000USD) per resident per year for malnourished residents, so reducing malnutrition alone will likely have cost-saving benefits as well as potentially reducing fracture risk.

Dietary Factors That Contribute to Fracture Risk in Elderly in Aged-Care

Malnutrition in Elderly Aged-Care Residents

Malnutrition results from an imbalance in the diet or to compromised absorption of nutrients. However, in the elderly, acute or chronic inflammation also contributes to malnutrition. Inflammation, associated with acute illness, injury or disease increases resting energy requirement, impairs protein utilization and increases nitrogen excretion, heightening nutrient requirements [18]. During this acute inflammatory phase, it has been suggested that nutritional support alone may not be sufficient to prevent a decline in lean mass reserves [18]. Jensen et al. (2010) defines the point at which lean mass declines and functional status is impaired as 'disease-related malnutrition' [19]. In the aged-care setting mild to moderate inflammation may be present in some residents due to chronic disease or illness. For example, Van Nie-Visser et al. (2014) observed in a sample of nearly 20,000 aged-care residents across three European countries that the mean number of diseases, and the presence of specific diseases (e.g. CVD, diabetes mellitus, respiratory disease) was associated with malnutrition [20]. However, malnutrition occurs with or without the presence of inflammation, so in part relates to insufficient intake and/or absorption relative to need. Insufficient nutrients in the absence of inflammation may be successfully treated with nutrition interventions.

Malnutrition in institutionalized elderly is endemic, with up to 89 % of residents reported to be malnourished or at risk of malnutrition [15]. However, inconsistencies in defining malnutrition contribute to a lack of consensus on the extent of malnutrition in institutionalised elderly. Amongst aged-care residents, age and sex (female) are recognised risk factors for malnutrition, however, the majority of residents are elderly women >80 years of age [5, 20–22]. Other contributors to malnutrition include cognitive impairment, poor dentition, level of dependency, stroke, immobility, swallowing difficulties, infrequent weight checks, consuming ≤ half of meals provided and not eating snacks [21–24].

Malnutrition is associated with increased falls, fractures, greater morbidity, impaired immunity, pressure ulcers, mortality and reduced quality of life [25–30]. Neyen et al. (2013) investigate the relationship between nutritionals status (assessed by BMI, nutrient intake and unintentional weight loss) and risk of falls over 30 days in a cohort of over 6700 aged-care residents. Malnourished residents were 1.7 times more likely to fall (95 % CI: 1.4–2.1, $p < 0.01$), with a trend observed for greater falls risk for residents at risk of malnutrition (OR 1.2; 95 % CI: 1.0–1.4, $p = 0.084$) compared to residents with normal nutritionals status. Over 90 % of fractures result from falls, so

reducing falls will likely benefit fracture risk reduction [25].

Staff at aged-care facilities are pivotal in ensuring nutritional adequacy of residents. Low staff numbers is identified as a risk factor for malnutrition in elderly residents [31]. Simmons et al. (2006) observed that even with feeding assistance, nursing home residents did not achieve desired levels for energy intake. Staff assisted each resident with eating at meal times for approximately 6 min; which is less than the 30+ min estimated to ensure an adequate intake [32]. In a sample of 42 aged-care institutes in France, the risk of malnutrition was less in facilities where a higher number of staff were trained in nutrition screening (OR 0.54; 95 % CI: 0.32–0.91, $p<0.05$), managing malnutrition (OR 0.53; 95 % CI 0.31–0.92, $p<0.05$) and managing swallowing difficulties (OR 0.33; 95 % CI: 0.34–0.95, $p<0.05$) [33]. While malnutrition is common in elderly in aged-care, specific deficiencies of protein, calcium and vitamin D are also frequent, and contribute to fracture risk [34–37].

Protein Deficiency and Fracture Risk in the Elderly

Epidemiological studies in community-based older adults indicate no clear relationship between protein intake and fracture risk, likely because few elderly in these studies are truly protein deficient [38–44]. Relative to recommended protein levels, protein deficiency is more common in institutionalised elderly in whom rates of malnutrition are high [6, 15, 45, 46]. In some cases the provision of food to residents do not meet recommended protein intake levels [16].

Protein deficiency is associated with accelerated bone resorption, impaired bone formation and sarcopenia, so may affect both falls and fracture risk [47, 48]. Wengreen et al. (2004) compared a cohort of 1167 female and male cases to 1334 controls and observed a reduced risk of hip fracture with increasing quartiles for protein intake; 1st quartile OR = 1.0 (reference), 2nd quartile (OR = 0.51; 95 % CI: 0.30–0.87), 3rd quartile (OR = 0.53; 95 % CI: 0.31–0.89), 4th quartile (OR = 0.35; 95 % CI: 0.21–0.59, $p<0.001$ for trend) for those aged 50–69 years, but not for those aged 70–89 years [40]. Protein deficiency reduces IGF-1 levels, and low IGF-1 levels were associated with increased fracture risk in postmenopausal women independent of BMD [49, 50]. The mechanisms for the effect of protein deficiency on bone have been explored using the animal model. In aging male rats, protein deficiency was associated with reduced bone formation, with minor reductions in bone resorption, while in aging female rats, bone resorption was elevated, with limited formation, resulting in cortical thinning and loss of bone strength. Reduced IGF-1 was also observed [51–53].

It has been suggested that an adequate protein intake increases IGF-1, promotes calcium absorption, and stimulate muscle protein synthesis, potentially slowing bone and muscle loss [54]. Recommended daily protein intakes in the elderly range between 0.75 and 1.0 g/kg body weight (BW) however, these recommendations were based on nitrogen balance studies in adults in whom protein needs may differ to the elderly [54, 55]. Some authors have suggested that daily protein intakes of between 1.0 and 1.3 g/kg BW are required to maintain nitrogen balance in the elderly, due to observed reductions in protein synthesis efficiency and insulin action in the elderly [56]. Recommendations by the PROT-AGE study group suggest for elderly people with acute or chronic diseases, daily protein intakes of 1.2–1.5 g/kg BW are well tolerated, expect for those with severe kidney disease or on dialysis [57].

Some authors have suggested that to maximise muscle protein synthesis and prevent sarcopenia, in addition to consuming sufficient protein, each meal throughout the day should also contain between 25 and 30 g of high quality protein, while others have indicated that muscle protein synthesis can also be enhanced when consuming up to 80 % of total protein intake in a single meal [58–60]. Increases in IGF-1, muscle protein synthesis and lean muscle mass have been observed in older women in some, but not all studies, when supplemented with the branch chain amino acid leucine, [61, 62]. A recent meta-analysis of the effects of leucine-rich protein supplements in the elderly

reported beneficial effects on gains in weight (1.02 kg; 95% CI: 0.19–1.85, p=0.02) and lean mass (0.99 kg; 95% CI: 0.43–1.55, p=0.0005), but not muscle strength, and the benefits most evident in those with sarcopenia [63]. However the authors, and others have noted, that the differences in study design make direct comparisons between studies problematic. Dairy foods and various meats are good natural sources of leucine so investigating the benefits of a leucine-rich diet on muscle and bone is worthy of consideration.

Calcium Deficiency and Fracture Risk in the Elderly

No clear relationship between calcium intake (with or without supplementation) and fracture risk in the elderly has been observed [64, 65]. Nieves et al. (2008) observed no relationship between calcium intake and osteoporotic fractures over 3 years in over 70,000 postmenopausal women [65]. Analysis of the Swedish Mammography study cohort involving over 60,000 women followed for more than 19 years, indicated that after adjusting for various confounders, 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 women in the lowest quintile for calcium intake (<751 mg/day) compared to the reference intake (882–996 mg/day). However, a 15–17% increased risk of hip fracture was observed in the highest quintile for calcium intake (>1184 mg/day; dietary plus supplemental calcium) compared to the reference intake [66]. Bischoff-Ferrari et al. (2007) did not observe a 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) or for non-vertebral fractures and calcium intake (+ supplementation) in women (RR=0.92: 95% CI: 0.81–1.05) [64]. Warenjo et al. (2011) observed an increased risk of hip fracture in women with high calcium intakes, when it included supplementary calcium (RR=1.64, 95% CI: 1.02–2.64) [64]. Whether the observed increase in fracture risk in women in the highest intakes for calcium is due to those with a propensity for fractures supplementing their intake or due to suppression of bone turnover delaying fatigue repair of bone is unclear [67].

Low calcium intake is reported to increase bone remodelling intensity, and result in an apparent reduction in BMD, and bone loss if balance in the basic multi-cellular unit (BMU) is negative i.e. more bone is resorbed than formed in the individual BMU [68]. Correction of calcium (and vitamin D) deficiency likely contributed to the fracture risk reduction reported by Chapuy et al. (1992) who randomised over 3000 elderly female aged-care residents to 1200 mg calcium and 800 IU vitamin D daily or placebo for 18 months. Overall 42% fewer hip fractures (p=0.043) and 32% fewer non-vertebral fractures (p=0.015) were observed in those receiving supplementation. PTH levels were significantly lower, and serum 25(OH)D more than doubled in the supplemented women [69]. Markers of bone turnover are lowered with calcium supplementation, but a reversal of skeletal benefits are observed after calcium (and vitamin D) supplementation is ceased likely resulting from remodelling intensity returning to pre-supplemented levels [70].

Calcium intakes below recommended levels are common in elderly in aged-care, with intakes of less than 600 mg/day observed in elderly women in aged-care [71–74]. The FAO/WHO report higher fracture risk is in those consuming <500 mg/day of calcium [75]. However, there is lack of agreement on the required calcium intake to reduce fracture risk [76]. Despite this the FAO/WHO recommended a calcium intake of 1300 mg/day for postmenopausal women and men >65 years of age [55]. While the evidence for post-menopausal women is based on calcium balance studies, less data are available for elderly males so recommendations for men were increased as a precaution [75].

Vitamin D Deficiency and Fracture Risk in the Elderly

An association between low 25(OH)D levels and increased fracture risk has been observed in some

but not all studies, but the 25(OH)D level at which fracture risk increases in not well defined [35–37]. Case-controlled and prospective studies involving over 20,000 elderly men and women have observed increased fracture risk for those with serum 25(OH)D levels ranging from <42.2 nmol/L to <64 nmol/L [77–79]. De Koning et al. (2013) observed that hip fracture risk was only related to serum 25(OH)D when levels were <70 nmol/L [80]. While this observation may suggest that fracture risk reduction is no greater when serum 25(OH)D levels are >70 nmol/L, defining the 25(OH)D level at which fracture risk is increased requires further investigation.

Vitamin D deficiency may be exacerbated in institutionalized elderly due to reduced mobility and limiting time outdoors [81]. For example 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 remained low (29.9 ± 13.1 nmol/L) [82]. Mean serum 25(OH)D levels <50 nmol/L have been observed in up to 94 % of institutionalized elderly, and is observed in countries both with (e.g. Germany and the USA) and without (e.g. Spain, Greece and Australia) limited seasonal cutaneous production [83–86]. Vitamin D supplementation may be required to maintain vitamin D sufficiency in institutionalized elderly [87].

Dairy Intake and Fracture Risk in the Elderly

No consistent relationship between the intake of milk or dairy produce and fractures has been observed [88–91]. Various methodological issues add to the complexity of attempting to determine relationships between a particular food or food group and fracture outcomes, in the context of a mixed diet. For example, accuracy in measuring food intake may obscure results. Sahni et al. (2013) observed that in a validation study of a version of the FFQ used in their assessment of dairy intake, the correlation with dairy intake determined using a 7-day food record was between 0.57 and 0.94 [92]. Furthermore, fracture outcomes need to be relevant and clearly defined. Participants in the study cohort reported by Sahni et al. (2013) were aged 26–85 years; hip fractures increase with age, fragility fractures are uncommon in young adults, fractures were not exclusively validated by hospital radiology reports, and all fractures were included, so degree of trauma associated with fractures not reported [93].

Inconsistencies in the definition of dairy foods (i.e. some include ice-cream as a dairy food, which is not a substantial source of protein or calcium), the size of a standard serve, and the choice of foods for analysis further add to the difficulty of determining a relationship between dairy intake and fracture risk [89, 94]. For example, using data from the Swedish mammography study Michaelsson et al. (2014) observed a negative relationship between milk intake and hip fracture risk in women (HR 1.09; 95 % CI: 1.05–1.13) however, the opposite relationship was observed for intake of cheese and fermented milk products, so likely negating any relationship when dairy as a food group is analysed [89].

Strategies to Reduce Fracture Risk in Elderly Aged-Care Residents

Oral Nutritional Supplements and Fracture Risk Reduction in Elderly Aged-Care Residents

Most studies demonstrating benefits of oral nutritional supplements in the elderly are hospital-based, and report minor weight gain, improved health outcomes and reduced mortality, mostly in malnourished patients [47, 95]. Delmi et al. (1990) randomised 59 malnourished hip fracture patients (mean age 82 years) to protein supplementation (20 g protein) and observed more favourable outcomes (56 vs. 13 %), fewer complications and death during hospital stay (44 vs. 87 %) and at 6 months after discharge (40 vs. 74 %) and shorter hospital stay (24 vs. 40 days) compared to 32 controls consuming only hospital food [96].

Neelemaat et al. (2012) randomised 210 elderly malnourished patients (mean age 74 years)

to intervention or normal care (control) and reported a significant reduction in patients who fell within 3 months after discharge in those in the intervention group [97]. Intervention patients were provided a protein-energy enriched diet during hospital stay, oral nutritional supplements plus vitamin D and calcium supplementation post discharge, and support by telephone counselling by dieticians. Both protein intake (+11 g/day, $p<0.05$) and serum 25(OH)D (+11 nmol/L, $p<0.01$) were increased with intervention compared to controls. The basis for the benefits is unclear as neither fat free mass, hand-grip strength or physical performance were improved with supplementation. No benefit of a protein-based ONS on falls risk was observed over 16-weeks in 253 malnourished elderly patients (mean age 82 years), despite improved hand-grip strength in supplemented patients. Adherence to supplementation was <40 %, and no post-discharge counselling or support was provided [98].

As the majority of studies of oral nutritional supplements focussed on weight gain (to prevent or overcome malnutrition) with supplements admitted during hospitalization, the sustainability of oral nutritional supplement use in the elderly outside of the acute hospital setting is uncertain. Compliance with, and acceptance of oral nutritional supplements by elderly people is often poor, especially in longer-term trials (>6 months), and gastro-intestinal disturbances are frequently reported, so raising doubt about the long-term use of ONS as a strategy for falls and fracture prevention [98, 99].

Despite frequent prescription of ONS for elderly in aged-care, limited data are available to support the use of ONS as a strategy for fracture risk reduction [99]. Meta-analyses indicate weight gains of ~2.5 % (95 % CI: 1.7–2.7 %), but few studies demonstrate favourable changes to lean mass and physical function, both of which may influence falls and fracture risk [99]. Bonneyfoy et al. (2003) did not observe 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 % so effectively half of that prescribed [100]. Fiatarone 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 ONS daily that provided 360 kcal of energy and 18 g of protein. The supplement was administered by nursing staff, and compliance was 99 % [101]. Gains of ~0.8 kg in fat free mass were observed in elderly Alzheimer disease patients when randomised to receive a choice of oral nutritional supplements (soups, desserts & drinks; protein content 10–12 g) over a 3-month period. Supplement administration and monitoring was performed by care-givers [102].

In the aged-care setting oral nutritional supplements are often not delivered according to treatment plans and significant waste is observed [103, 104]. Kayser-Jones et al. (1998) reported that less than 1/3 of nursing home residents were provided with the correct type and dosage of oral nutritional supplements and mean intake was approximately 55 % [105]. 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 randomised 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 [106]. Given the reported effectiveness of ONS in the short, but not long-term, investigating alternative strategies to reduce fracture risk in the aged-care setting are warranted.

Fortification of Dairy Produce and Fracture Risk Reduction in Elderly Aged-Care Residents

Fortification of the food supply represents one approach that may ensure nutritional adequacy

for particular nutrients in certain at risk populations. For example in Australia bread-making flour is fortified with folic acid to reduce the risk of neural tube defects in new born babies, and iodised salt is used in bread due to the re-emergence of iodine deficiency in the population [107]. Countries such the United States of American and Canada fortify dairy foods with vitamin D, which may be effective in reducing the prevalence of vitamin D deficiency. For example, Shakur et al. (2014) used the food intake (24 h recall) in over 34,000 Canadians as part of the 2004 Canadian Community Health Survey 2.2 and observed that vitamin D fortification of milk, cheese and yoghurt a 270 IU/serving effectively doubled vitamin D intake and the proportion with inadequate intake was reduced from >80 to <50 %, with no intakes approaching the upper limit for vitamin D intake [108]. However, some authors have argued that fortification of only dairy produce may be insufficient to reduce the incidence of vitamin D insufficiency, and other food staples such as wheat flour, may also require fortification with vitamin D [109, 110]. For the elderly fortification may also serve to increase nutrient density, so even with limited appetite, nutrient adequacy may be achieved.

Using bread as the vehicle for nutrient delivery, Monacu et al. (2009) undertook a 1-year intervention providing daily buns fortified with vitamin D (5000 IU/day) and calcium (320 mg/day) to 45 elderly nursing home residents. Serum 25(OH)D levels increased from 29 ± 11 nmol/L to 126 ± 38 nmol/L; serum PTH levels more than halved from a baseline value of 59.3 ± 38.3 pg/ml to 19.0 ± 16.0 pg/ml ($p < 0.001$). Osteocalcin levels were lowered by ~27 % (20.1 ± 10.3 ng/ml to 14.7 ± 9.0 ng/ml, $p < 0.001$) but no significant difference was observed for serum C-telopeptide. BMD at both the total hip and lumbar spine significantly increased [111].

Dairy foods are a good source of protein, so fortification can improve nutrient density (e.g. calcium), or serves as a vehicle to provide bone-related nutrients (e.g. vitamin D). In the aged-care setting malnutrition is common, so providing fortified dairy products may improve nutrient intake and could potentially reduce fracture risk. Etjahad et al. (2105) developed a hypothetical model of vitamin D fortification of milk (42 IU/100 g) and yoghurt (89 IU/100 g), based on vitamin D intakes of over 5000 adults in a population at high-risk of vitamin D deficiency, and observed that nearly 80 % of the population could achieve the RDA for vitamin D, without any exceeding the upper limit of intake [112]. As aged-care residents are at high risk for vitamin D deficiency this may be an effective strategy to improve bone health.

Bonjour et al. (2009) observed a 12.3 % decrease in PTH and a 16.9 % increase in IGF-1 in elderly institutionalised 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 [113]. Bonjour et al. (2013) also 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 institutionalised women, and observed 25(OH)D was approximately 20 nmol/L higher ($p < 0.0001$) and PTH and CTX, 20 % ($p < 0.001$) and 8 % ($p < 0.05$) lower with fortification [114]. Residents consumed two 125 g serves daily, with two flavours provided. Products were rated as satisfactory, so long term adherence was uncertain. In a similar design, Bonjour et al. (2015) undertook a subsequent randomised study comparing fortified to regular yoghurt involving 48 women (mean age 73 years) in a community home and observed after 84 days of intervention a significant increase in serum 25(OH)D in both the fortified (34.3 ± 2.4–56.3 ± 2.4 nmol/L) and non-fortified yoghurt groups (35.0 ± 2.5–41.3 ± 3.0 nmol/L), while reductions in PTH (63.5 ± 4.6–60.7 ± 4.2 ng/L, $p < 0.01$), and bone resorption ($p < 0.05$) were observed in the fortified yoghurt consumers [115].

Iuliano-Burns et al. (2012) conducted a 2-year randomised cluster intervention using a dairy-based protein, calcium and vitamin D supplement in 813 ambulant elderly in aged-care (mean age 85.5 years). Supplementation reduced the number of residents who fell by 42 % (OR = 0.58, 95 % CI: 0.44–0.78, $p < 0.001$), 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% ($p<0.05$). Falls reduction was observed for those who did not fall in the prior year, or fell once or twice, but no benefit of supplementation was observed for frequent fallers (Fig. 23.1) [116].

Issue observed in the aged-care setting that hinder the implementation of nutrition-based interventions, are high staff turnover, and limited time and resources for staff to support these initiatives. In the previously mentioned study, Iuliano et al. (2013) reported that food service staff had difficulties mixing the supplement into the foods, with the problem being resolved only when the foods products were provided pre-fortified [116]. Staffing issues were also identified as a limitation to implementation by Grieger et al. (2009) who tested the provision of calcium and vitamin D fortified milk to elderly in aged-care. The authors observed that without supervision and guidance, food service staff did not make sufficient modifications to the menu to increase milk intake, so 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 [117]. A dietary approach to fracture prevention, using the established dietary guidelines as the foundations, relies less on staff involvement in preparing specialised food products, so may be an approach that may fulfil the requirements of being a safe, accessible and cost effective approach to fracture prevention.

Food-Based Approaches to Improving Nutrient Intake in Elderly in Aged-Care

Feeding Assistance

Of the methods explored to enhance nutritional intake in institutionalized elderly such as enhancing food flavours, provision of snacks, staff education, and altering dining environment, the greatest improvements are observed when residents are assisted with eating [118]. Feeding assistance is particularly pertinent for residents with dementia, who are also at high risk for falls, so ensuring an adequate nutrient intake in this group has even greater importance [119]. Simmons et al. (2008) undertook a 24-week cross over intervention in nursing home residents at risk of malnutrition who were assisted with feeding at meals and snacks. Sixty residents completed the trial. Energy intake increased by approximately 300 kcal/day, and weight was maintained or increased during the intervention, but lost during the control period. Staff assisted each resident for 42 min during meals and 13 min during snacks compared to the usual care of 5 min per resident for meals and less than 1 min per resident for snacks [120]. Whether these changes to

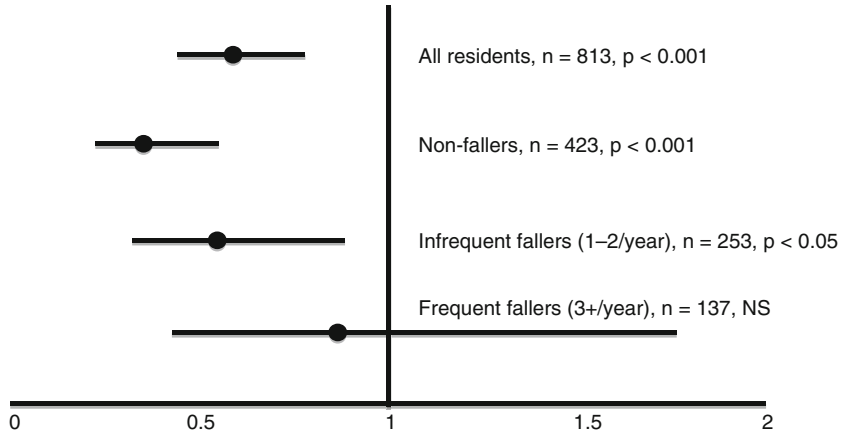

Fig. 23.1 Odds ratio (95% CI) for elderly aged-care residents receiving 8 months of dairy-based supplementation relative to non-supplemented control residents [116]

food intake translate to falls and fracture risk reduction require further investigation.

Are Residents Provided with Enough Food?

Institutionalized elderly are predominantly reliant on aged-care providers to meet their nutritional needs, with most meals and snacks prepared and provided through the facility's food service. Nutritional guidelines have been established to guide menu planning in aged-care to ensure nutrient needs of residents are met. Despite these guidelines, it has been observed in some cases that the actual foods provided in aged-care do not meet nutritional requirements [16, 121]. Protein and/or calcium intakes are frequently below recommended [122–125]. For example, in relation to calcium intake, Baric et al. (2006) observed from a random sample of 44 nursing home meals that women, but not men consumed sufficient energy (106 % vs. 88 % of the RDI), protein intake was adequate (>100 % of RDI for both), but calcium intake was well below recommended levels at 64 % of the RDI [126]. Food policies differ between aged-care providers with some prohibiting food prepared by others outside of the facility to be brought into facilities. However, it was observed that receiving additional meals provided by family members was negatively associated with malnutrition, which may indicate that some of the shortfall in food provision was being made up by this additional food [127].

Are Elderly in Aged-Care Provided the Right Types of Foods?

The cornerstone of the dietary guidelines is to choose a variety of foods from each of the food groups, which assists towards obtaining all required nutrients. Mila et al. (2012) used the double weighed method to record food intake over 21 days in 62 Spanish nursing home residents from various facilities. The authors observed that the highest intake was for milk (median intake 376 g/day), meat and meat alternatives were consumed in sufficient amounts (median intake ~ 150 g/day), but the consumption of cereals, fruit and vegetables were below recommended. With the high consumption of dairy (376 g of milk and 130 g of other dairy products) and meats, protein needs were met [128].

In contrast, Iuliano et al. (2013) observed in a sample of 199 elderly from 18 aged-care facilities, inadequate serving of dairy, vegetables and grain foods were consumed, and discretionary foods such as cakes and biscuits were consumed in excess of recommended, resulting in inadequate intakes of protein (61 % of residents below RDI) and calcium (98 % of residents below RDI). Meats and meat alternatives and fruit were consumed in recommended quantities [73]. In countries that do not have mandatory vitamin D fortification of foods, dietary intake of vitamin D is also marginal, amd supplementation may be necessary [73].

Both meat and meat alternatives (eg eggs) and dairy produce (milk, cheese, yoghurt) are sources of quality protein so adequate intakes may help ensure an adequate protein intake. In a sample of 215 aged-care residents (70 % females, mean age 85.8 ± 7.5 years) from 21 aged-care facilities both meat and dairy servings were not consumed in sufficient amounts, and 68 % were malnourished, or at risk of malnutrition based on mini nutrition assessment (MNA) scores. Protein intake was 87 ± 28 % of recommended and mean calcium (622 ± 263 mg/day) was well below recommended (1300 mg/day). Residents consumed on average 0.9 ± 0.5 servings of meat daily and 1.1 ± 0.7 servings/day of dairy. Using multivariate analysis, number of dairy and meat servings were related to proportion of recommended protein intake; one more dairy serve would contribute ~14 % to recommended protein intake levels, so on average, residents would meet protein needs (100 % of RDI), while an additional meat serving would contribute ~10 % to protein requirement. Number of dairy ($p < 0.001$) but not meat ($p = 0.4$) serving contributed to MNA score; one additional dairy serving would contribute four points on the MNA, so on average residents

would achieve normal nutrition status (minimum MNA score of 24) with the addition of one dairy serving daily. The provision of at least one additional serving of dairy daily could ensure protein intake meets requirements and reduce malnutrition in residents (Iuliano et al. unpublished).

Provision of Dairy Produce as a Food-Based Approach to Fracture Prevention

For an anti-fracture intervention to have widespread application it needs to be safe, effective and easily accessible. Food fortification laws differs between countries so fortified products may not be readily available to all. In some cases fortified foods are considered nutritional supplements, so would not be regarded or administered as part of the food supply. An important feature of a food-based approach is it can be implemented unimpeded through the food supply so is available to all residents. Using a dairy-based approach, Iuliano et al. (2013) undertook a feasibility study to determine if improving dairy intake from the two serving per day currently consumed, to the recommended four serving per day could be a potential anti-fracture strategy in institutionalised elderly [129]. 130 residents (mean age 86.5 years) in four aged-care facilities participated in the 4-week trial. Two facilities underwent intervention while two facilities served as controls so residents consumed from their normal menu. Following the 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 residents in control facilities mean energy intake remained below the EER, and protein 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 with intervention, 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 in other studies which used supplements (6 g/day) or food fortification/snacks (12 g/day) [106, 130, 131]. In addition, this level of protein supplementation is similar to amounts suggested to maximize muscle protein synthesis and prevent sarcopenia [58]. 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, so is a potential strategy to improve muscle as well as functioning to reduce fracture risk, and most likely beneficial in those with protein intakes below recommended [61, 62].

Why Target Anti-fracture Interventions at Elderly People in Aged Care?

Targeting institutionalised elderly is likely cost effective given that falls and fracture risk is highest in this group, so fewer require treatment to prevent a fracture, and many of the factors that compromise study design are overcome [7, 69, 132, 133]. Most notably in aged-care, rates of nutritional deficiency are high, so residents are more likely to benefit from treatment, food intake (compliance) can be closely monitored and outcome measures more readily quantified as the setting is well controlled. The demand on institutionalised care will increase as the aging population grows, so reducing fractures in this high-risk group will help reduce care and medical costs in this setting.

With the availability of more sensitive imaging techniques (e.g. High resolution micro pQCT), closer examination of bone structure and its response to nutrition interventions is achievable. If bone deterioration is too advanced in the very old, but in whom fracture rates are the highest, then other components of dairy may exert a benefit, such as protein influencing IGF-1, and so benefit both muscle and bone. The same scien-

tific rigour applied to clinical trials also needs to be applied to food-based intervention, to quantify the improvements achievable with enhanced dairy intake. The findings from these trials will help provide the evidence to shape future policies to ensure nutritional adequacy in elderly in aged-care, and the best time to intervene to achieve the optimal musculoskeletal benefits from consuming sufficient dairy foods. Such a large randomised controlled trial is warranted to evaluate the effectiveness of a dairy-based intervention on fracture prevention in high-risk elderly in aged-care. With the aging of the population, the sustainability of institutionalised care to cater for the growing number of elderly may depend on the efficacy and cost-benefit of such a food-based approach.

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Clinical Trial of Dairy in Adolescent Girls: Effect on Bone Accrual

24

Joan M. Lappe, Margaret A. Begley, Jean-Claude Des Mangles, Ann Laughlin, Donald J. McMahon, and Misty Schwartz

Abstract

Peak bone mass is acknowledged to be the best predictor of osteoporotic fracture in older adults. Thus, it is critical that children maximize their potential for bone mass accrual. Evidence shows that providing calcium supplementation to children with low calcium intake will increase bone mass accrual. However, few studies have evaluated the effect of increasing dairy foods on bone mass accrual in children. This chapter describes outcomes of a randomized clinical trial that examined the effects of increases in dairy food on bone accrual in adolescent girls who had low baseline dairy and calcium intake.

Keywords

Peak bone mass • Bone accrual • Dairy intake • Adolescent girls • Clinical trial

J.M. Lappe, PhD, RN, FAAN
Nursing and Medicine, Osteoporosis Research Center, Creighton University, 601 N. 30th Street, Suite 4820, Omaha, NE 68131, USA
e-mail: jmlappe@creighton.edu

M.A. Begley, BA, BSN, RN
Clinical Research Nurse, Osteoporosis Research Center, Creighton University, 601 N. 30th Street, Suite 4820, Omaha, NE 68131, USA
e-mail: margaretbegley@creighton.edu

J.-C. D. Mangles, MD
Pediatric Endocrinology, Lourdes Hospital, 169 Riverside Drive, Binghamton, NY 13905, USA
e-mail: Jeanclaude.desmangles@lourdes.com

A. Laughlin, PhD, RN • M. Schwartz, PhD, RN
College of Nursing, Creighton University, 2500 California Plaza, Omaha, NE 68124, USA
e-mail: alaugh@creighton.edu; mistyschwartz@creighton.edu

D.J. McMahon, BS, MS
Department of Medicine and Endocrinology, Columbian University Medical Center, 630 W. 168th Street PH8-864, New York, NY 10032, USA
e-mail: djm6@cumc.columbia.edu

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Introduction

Achievement of the full genetic potential for peak bone mass protects against osteoporotic fracture. The relative risk of fracture increases by as much as 2.6 for each decrease in bone mass of one standard deviation [1]. It has been estimated that increasing the bone mass of the elderly population by 5% would decrease hip fracture incidence by 50% [2]. Although peak mass is not achieved until about age 25, at least 90% of the adult mass is accrued by the age of 19 or 20 [3, 4]. Studies also show that children and adolescents with low bone mass have greater risk of childhood fractures than their peers with higher bone mass [5–7].

Numerous studies of calcium supplementation in children with low calcium intake show a positive impact on bone mass accrual [3, 8–21]. Supplementing with dairy food rather than calcium pills would be expected to have an even greater positive impact on bone accrual since dairy food contains additional nutrients known to be beneficial to bone, such as protein. However, few studies have evaluated the effect of dairy food on bone accrual.

The purpose of the analysis reported here was to determine whether increasing dairy food intake in post-menarcheal adolescent girls with habitual low calcium intake resulted in greater bone mass accrual over 1 year of study compared to girls who continued with low calcium intake.

Methods

Design

This was a 12-month randomized controlled trial with the primary aim to determine the effects of increasing dairy food intake on changes in percent body fat. A secondary outcome was change in bone mass (total body minus head bone mineral content; TBMH BMC). Eligible girls were randomly assigned in a 1:1 ratio to one of two groups within each of three body mass index (BMI) percentile categories: (1) Treatment group, asked to consume dairy providing at least 1200 mg of calcium/day (d); and (2) Control group, asked to continue their usual diet containing ≤600 mg of calcium/d and ≤ one glass of milk/d. The BMI percentile categories were: 50th to <70th; 70th to <85th; and ≥85th.

Participants

The convenience sample included 274 healthy females ages 13 or 14 and at least 1.5 years post menarche at enrollment. Inclusion criteria included: habitual dairy intake ≤ one glass milk/d and dietary calcium intake of ≤600 mg/d; willingness to increase dairy intake for 1 year; and body mass index (BMI) >50th and <98th percentile for age and sex based on Center for Disease Control Growth curves. Exclusion criteria included: menarche prior to age 10; history of lactose intolerance or milk allergy; dieting behavior with weight loss >10 lb in the last 3 months; weight >300 lb or metal in the skeleton (pins, rods) due to dual energy absorptiometry (DXA) limitations; current pregnancy; chronic disease or disorder such diabetes, polycystic ovarian syndrome, thyroid disease, eating disorder, seizures or cancer; and use of steroids, contraceptives, calcium supplements, anti-depressants, high dose Vitamin A, or weight reducing or seizure medications. A total body BMC Z-score below −2.0 measured by DXA was exclusionary as was having a sibling who had been on a dietary study in the last 5 years.

The Creighton University Institutional Review Board approved the protocol. Written informed assent and consent were obtained from the participants and parents/guardians, respectively.

Assessments

Girls were evaluated at baseline and every 3 months for 1 year (five visits). At each visit the following were completed:

- Dietary intake – multiple-pass 3-day recall diary with 2 weekdays and 1 weekend day using the Nutrition Data System for Research (NDS-R) software (Nutrition Coordinating Center, U. of Minnesota).

- Adherence to treatment – Dietary recalls and daily calcium intake checklists
- Physical activity – Modifiable Activity Questionnaire for Adolescents (MAQ-A) [22, 23]. Average Metabolic Equivalent of Task (MET) was calculated.

At baseline, 6 months, and end of study, the following assessments were made:

- Total body minus head bone mineral content – Hologic 4500
- Height – Harpenden stadiometer
- Weight – Scale-tronix Stand-on Scale (Scale-tronix, White Plains NY)
- Pubertal status – Tanner staging performed by an endocrinologist and a registered nurse with extensive training and experience. The Tanner stages range from 1 to 5, with one being no pubertal development and five being the adult-level of development.

Intervention

The Dairy group was asked to consume dairy foods (limited to low-fat yogurt and milk [skim, 1 or 2 %]) that provided at least 1200 mg/d of calcium. At randomization, the research dietician met with girls in each group and reviewed the USDA Food Guidance System "MyPyramid". The grant paid for the dairy foods purchased by the Dairy group. Families submitted itemized receipts for reimbursement.

Statistical Approach

Data were examined against assumptions of normality and transformations, usually log-transformed, when necessary; all results are presented in original measurement scale units. Univariable differences between groups were assessed with T-tests for continuous variables and chi-square or Fisher's Exact test for categorical variables. Change from baseline to 12-month endpoints was calculated for each variable. Multiple regression was used to model change in BMC as a function of the changes in the components of the average daily number of dairy servings and intake of dietary calcium. We used two-way ANOVA to assess whether increasing tertiles of components of the dietary intervention showed different levels of mean 12-month change in BMC. Change in average daily energy expenditure, change in height and change in lean mass were evaluated as confounders in both regression and ANOVA models. Data are presented as means ± SD or count and percent. A Scheffé adjustment for post-hoc comparison between tertile means was applied. No adjustment was taken for multiple comparisons. P-values less than 0.05 were used as the criterion for statistical significance.

Results

Baseline characteristics by randomization group are shown in Table 24.1. There were no statistically significant differences between groups. Racial distribution of the cohort is shown in Table 24.2. Seven (5.1 %) of the Control Group and 12 (8.8 %) of the Treatment Group reported Hispanic ancestry. At 12 months, 136/138 (99 %) of the Control group and 132/136 (97 %) of the Dairy group provided BMC accrual data. Five participants dropped from study, four from the Dairy group and one from the Control group, and one Control did not have a final DXA because of pregnancy. In the Controls, 11/138 (8 %) started the study at Tanner stage 4, and all but one advanced to Tanner stage five by the 12-month evaluation. In the Dairy group, 20/136 (15 %) started at Tanner stage 4, and all but one advanced to Tanner stage 5.

Mean values of selected nutrient intake during study are shown in Table 24.3. The Dairy group significantly increased intake of dairy and calcium ($p < 0.0001$). All but 13 of the girls who completed 1 year of dairy intervention averaged at least 1200 mg of calcium per day. Those below 1200 mg/d all averaged more than 1000 mg/d except for three. The two groups did not differ significantly in self-reported activity during the study.

Table 24.1 Baseline descriptives by treatment group

Variable	Control group (N = 138)	Dairy group (N = 136)
Age (yrs)	13.5 + 0.5	13.5 + 0.5
Height (cm)	162.2 + 6.1	162.6 + 5.5
Weight (kg)	60.41 + 9.0	61.20 + 8.5
Dairy intake (servings/d)	0.61 ± 0.45	0.63 ± 0.50
Calcium intake (mg/d)	531 ± 156	552 ± 173
Protein intake (g/d)	51.1 ± 14.5	49.7 ± 12.5
Vitamin D intake (mcg/d)	2.42 ± 1.5	2.42 ± 1.5
Energy intake (kCalories/d)	1441 ± 358	1390 ± 340
Physical activity (MET's/d)	44.7 ± 14.0	44.9 ± 15.1
Total body minus head BMC (g)	1575 ± 223	1567 ± 200

Yrs years, *cm* centimeters, *kg* kilograms, *d* day, *mg* milligrams, *g* grams, *mcg* micrograms, *kCalories* kilocalories, *MET's* metabolic equivalents

Table 24.2 Racial distribution by treatment group

Race	Control group	Dairy group
	N (%)	N (%)
White	104 (75.36)	119 (87.50)
Black	19 (13.77)	13 (9.56)
Native American	1 (0.72)	1 (0.74)
Asian	5 (3.62)	3 (2.21)
Other	9 (6.52)	0 (0.00)

Table 24.3 Average nutrient and activity values during study

Variable	Control group	Dairy group
Dairy intake (servings/d)	0.79 ± 0.71	3.3 ± 1.2
Calcium intake (mg/d)	735 ± 310	1526 ± 410
Physical activity (METS/d)	43 ± 15	42 ± 13

The Control group gained 53.73 ± 37.82 g in total body minus head BMC while the Dairy group gained 59.12 ± 40.59 g. The difference of 5.39 ± 39.21 g represents a +10.0 % difference in Dairy vs Control. This difference was not statistically significant. However, when BMC accrual was compared using ANOVA among tertiles of specific nutrient intake (dairy and calcium), statistically significant differences were found ($p < 0.05$). See Figs. 24.1 and 24.2 Differences were similar after adjusting for baseline TBMH BMC and change in height.

Discussion

This analysis showed that intake of dairy food is positively associated with BMC accrual over 1 year of follow up in post-menarcheal adolescent girls. The average total body minus head BMC accrual in the Dairy group was 10.0 % greater than in the Controls. Although this difference is not statistically significant, it has practical significance because estimates are that increasing the bone mass of the elderly population by 5 % would decrease hip fracture incidence by 50 % [2]. Adolescent girls who regularly include dairy in their diets would be predicted to have a greater peak bone mass and a decreased risk of fracture as they age.

The Dairy group significantly increased intake of dairy, as well as intake of calcium, protein, and vitamin D ($p < 0.0001$). This was to be expected since dairy foods are rich in calcium and protein, and milk in the U.S. is fortified with vitamin D. In addition, dairy foods contain colloidal calcium-phosphate-protein complexes that contain minerals and nutrients needed for bone growth.

When we evaluated BMC accrual by tertile of dairy food intake, the mean increase in BMC during 1 year was 22.4 % (8.5 g) higher in the third tertile than in the first. In addition, intake of calcium was independently associated with BMC accrual. Difference in gain in BMC between the first and third tertiles of calcium was 19.8 % (6 g).

This study is congruent with others that found increased gains with increased dairy food consumption [24–26]. Strong evidence for a dairy food effect on bone mass accrual is provided by a randomized controlled trial that supplemented 94 healthy 15–18 year-old adolescent females for 2 years with dairy foods providing 1000 mg calcium/day [24]. Baseline calcium intake was 765 ± 55 mg/d in the controls and 744 ± 44 mg/d

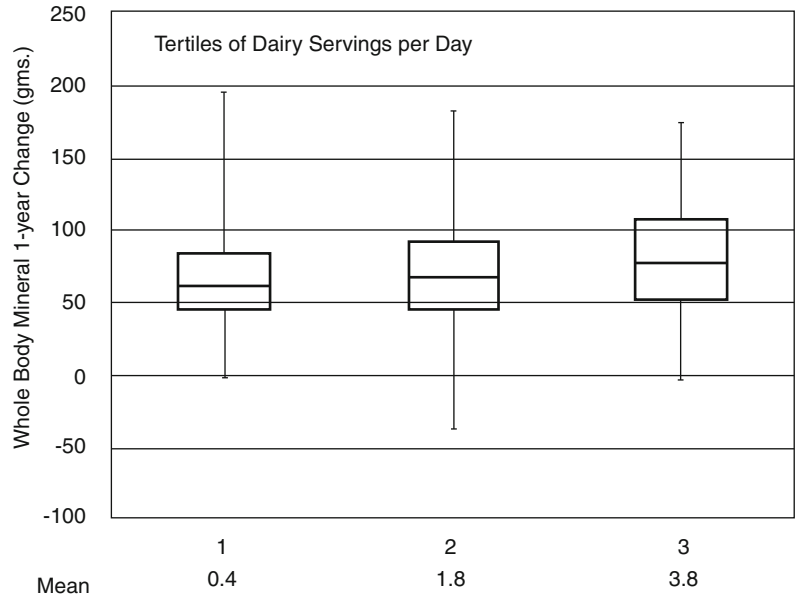

Fig. 24.1 Change in total body minus head bone mineral content during 1 year by tertile of servings of dairy food per day. Mean intake of each tertile is indicated at the *bottom* of the figure

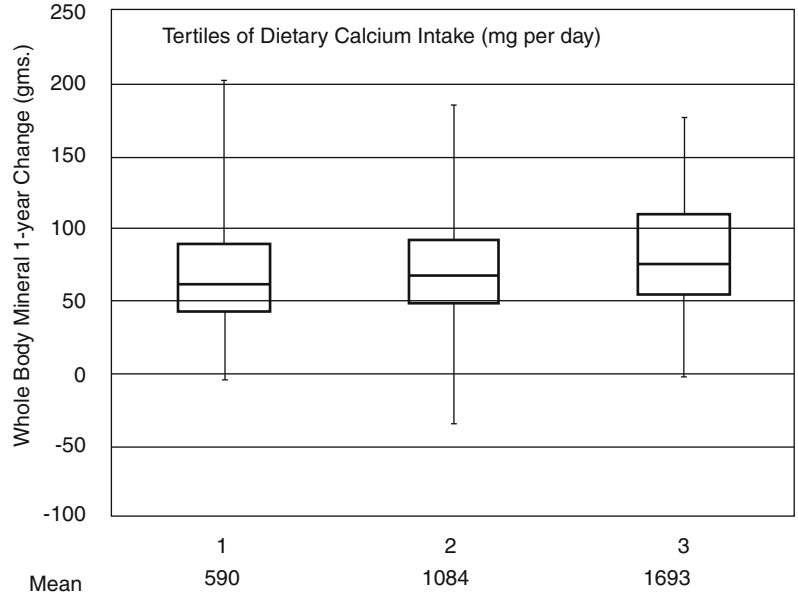

Fig. 24.2 Change in total body minus head bone mineral content during 1 year by tertile of calcium intake (mg) per day. Mean intake of each tertile is indicated at the *bottom* of the figure

in the supplemented group. Calcium intake at the end of study was 684 ± 47 in controls and 1155 ± 52 in the supplemented girls. Number of servings of dairy food was not reported. After 2 years, there was a significantly greater increase in trochanter (4.6 %), lumbar spine (1.5 %) and femoral neck (4.8 %) bone mineral density (BMD) ($p < 0.05$) in the high calcium group compared to controls. There was also a greater increase in BMC at the trochanter ($p < 0.05$). Our study supports findings of this RCT and provides evidence of an effect in early adolescent girls.

Greater than 99 % of the body's calcium is stored in the skeleton where it serves as a functional reserve to offset shortages of calcium in the bloodstream needed for homeostasis. The newborn's skeleton contains only about 2–3 % of the total adult skeletal calcium. Therefore, the need

for dietary calcium during the first decades of life is driven by skeletal growth. Our findings that early adolescent girls in the highest tertile of dietary calcium intake had significantly greater increases in BMC accrual than those in the lowest tertile are consistent with numerous studies showing that interventions with calcium supplement pills lead to a small, but consistent, positive effect on BMD and/or BMC accrual [3, 8–21].

The major limitation of this study is that it is a secondary analysis of a study designed to find possible effects of dairy food intake on change in body fat. However, studies of nutrient intake on bone accrual in children are somewhat limited, and our findings provide additional insight into the benefits of dairy and calcium for bone accrual. The strengths of our study are that baseline intake of dairy and calcium was below recommended levels and that the intervention significantly increased intake of dietary variables that are critical for bone health.

Optimizing bone accrual in children is key to preventing low trauma fractures in older adults and likely contributes to lower risk of fractures during childhood. Our findings support the importance of several nutrients for increasing bone accrual in early adolescent girls.

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Part VII

Nutrition, Bone and Special Conditions

Intestinal Calcium Absorption and Skeletal Health After Bariatric Surgery

25

Anne L. Schafer

Abstract

Because obesity has reached epidemic proportions, there has been escalating interest in surgical weight loss (bariatric surgery). Bariatric surgery produces durable weight loss, improves comorbidities, and decreases mortality. However, those who undergo bariatric surgery are at risk for long-term complications including nutritional deficiencies. Roux-en-Y gastric bypass, the most common bariatric procedure in the US in recent years, is known to have negative effects on bone health, with increases in bone turnover, substantial decreases in bone mineral density, and increased fracture incidence. This chapter reviews the effects of gastric bypass surgery on intestinal calcium absorption. It then describes the need for investigation about intestinal calcium absorption after other bariatric surgical procedures.

Bariatric Surgery and Skeletal Health

Obesity now plagues 34 % of US adults [>72 million, with body mass index (BMI) ≥30 kg/m^2], and 6 % of US adults are extremely obese (BMI ≥40 kg/m^2) [1]. Unfortunately, diet and exercise usually fail to produce weight loss of the magnitude so often required. As a result, bariatric surgery is increasingly used to treat obesity.

Bariatric surgery produces long-term weight loss, improves comorbidities, and decreases mortality [2–5]. Bariatric surgery results in marked weight loss (typically 60–100 lbs.), shown to persist through 15 years of follow-up [2, 6]. The estimated annual number of bariatric operations performed in the US rose almost tenfold from

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A.L. Schafer, MD (✉)
Endocrine Research Unit, San Francisco Veterans Affairs Medical Center,
San Francisco, CA 94121, USA

The Departments of Medicine and of Epidemiology & Biostatistics, University of California, San Francisco, 1700 Owens Street, Room 367,
San Francisco, CA 94158, USA
e-mail: anne.schafer@ucsf.edu

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1998 to 2009 (from 25,000 to 220,000) [7], and the popularity of surgery has persisted since. Roux-en-Y gastric bypass (RYGB) has been the most common bariatric procedure in the US [8].

Despite its metabolic benefits, RYGB surgery negatively impacts bone metabolism. It results in increases in bone turnover, decreases in BMD, and weakened bone microstructure [9–13]. For example, we have demonstrated a nearly threefold increase in the bone resorption marker serum C-telopeptide (CTX) and a 6.5 % decline in spinal volumetric bone mineral density (BMD) in the 6 months after surgery [9].

There is a concern that those who undergo RYGB, and possibly other bariatric surgical procedures, may be at increased risk for fracture-related morbidity and mortality, particularly as they age. Indeed, it was reported that bariatric surgery (mostly RYGB) patients were at higher risk of subsequent fracture than age- and sex-matched controls [14]. This is of great concern considering the tremendous medical and economic impact of fracture: Mortality 1 year after hip fracture approaches 25 % in older adults, and of those who survive, only half recover their pre-fracture functional status [15]. As a result, professional organizations now recommend calcium and vitamin D supplementation and laboratory monitoring after bariatric surgery [16–18], and some recommend consideration of BMD testing before and 1–2 years after RYGB and other malabsorptive procedures [16, 17].

Multiple candidate mechanisms exist for the skeletal effects of bariatric surgery. Potential contributing factors are decreased skeletal loading, changes in levels of fat-secreted hormones or estrogen, relative changes in body composition, and nutritional factors, including vitamin D deficiency, inadequate calcium intake, and calcium malabsorption [19, 20].

Intestinal Calcium Absorption in Obesity and with Nonsurgical Weight Loss

Intestinal calcium absorption is central to the maintenance of calcium and bone homeostasis. Ninety percent of absorbed calcium is usually absorbed by active transcellular transport in the duodenum and jejunum, with additional calcium absorption in the distal intestine by active transport and, when calcium intake is high, by passive paracellular transport. Vitamin D is critical for active transport, because its metabolite 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] acts on the intestine to stimulate calcium absorption.

There is a high prevalence of vitamin D insufficiency or deficiency in obesity, which is thought due in part to sequestration of the vitamin in adipose tissue [21–23], and dietary restriction during weight loss efforts may also threaten vitamin D status. As a result, some morbidly patients may have vitamin D-mediated impairment of intestinal calcium absorption before and/or after weight loss.

Dietary restriction during weight loss efforts may also result in inadequate calcium intake. Further, it has been shown that nonsurgical weight loss results in a decline in intestinal calcium absorption capacity [24, 25]. This phenomenon is not understood, but it could be mediated by decreased estradiol levels or dietary protein or fat intake during weight loss. In one trial, robust vitamin D supplementation attenuated the decline in fractional calcium absorption seen with weight loss [25].

Intestinal Calcium Absorption After Roux-en-Y Gastric Bypass

The RYGB procedure involves the creation of a small gastric pouch, division of the intestine at the midjejunum, and anastomosis of the distal portion to the gastric pouch. As a result, the procedure can cause vitamin D malabsorption, resulting in a high incidence of post-operative vitamin D deficiency and even in osteomalacia in the most severe cases [26, 27]. Additionally, the bypassed duodenum and proximal jejunum, which no longer come into contact with food, are usually the predominant sites of active, transcellular, 1,25$(OH)_2$D-mediated calcium uptake. However, calcium absorption does occur throughout the intestine [28], and so one can imagine that those who have undergone RYGB might theoretically maintain sufficient calcium

absorption, particularly if vitamin D status and calcium intake are robust.

Riedt et al. measured fractional calcium absorption in 21 women before and 6 months after RYGB [29]. The women lost a mean 39 kg during the study period. Fractional calcium absorption decreased post-operatively from 36 ± 8 % to 24 ± 9 %. At the 6-month post-operative time point, women with higher estradiol levels had higher fractional calcium absorption. This exciting study was important not only for documenting a decline in intestinal calcium absorption capacity but also for examining potential causes for the decline with its nutritional and hormonal assessments. However, over half of the women had 25-hydroxyvitamin D (25[OH]D) levels <25 ng/mL, and mean post-operative calcium intake was under 1000 mg with large variability. Thus, the study could not fully determine to what extent the portion of the gastrointestinal tract still in contact with food and supplements can participate in active, transcellular, $1.25(OH)_2D$-mediated calcium absorption and passive, paracellular calcium transport.

We determined the effects of RYGB on intestinal fractional calcium absorption in a cohort of 33 severely obese women and men, in the setting of a target 25(OH)D level of at least 30 ng/mL and calcium intake of 1200 mg daily [9]. We hypothesized that maintaining the robust vitamin D status and controlled calcium intake would mitigate post-operative declines in calcium absorption capacity.

We enrolled women and men ages 25–70 years who were scheduled for RYGB. Upon enrollment, low 25(OH)D levels were repleted to a target level ≥30 ng/mL, and total daily calcium intake was brought to 1200 mg through individualized dosing of a chewable calcium citrate supplement, based on dietary intake. Post-operatively, we monitored 25(OH)D levels and dietary calcium intake and adjusted each participant's supplement doses to maintain our goals. Like Riedt et al., we measured fractional calcium absorption pre-operatively and 6 months post-operatively with a dual stable isotope technique, with one stable calcium isotope administered orally to label dietary calcium and the other intravenously to measure calcium removal from the blood [30]. In our approach, a 10 mg dose of oral calcium–44 was administered in the middle of a standardized test breakfast consisting of 300 mg calcium. The nutrient composition of the breakfast was identical for the pre-op and post-op tests, and all participants consumed all of each meal. A 3 mg dose of calcium-43 was infused IV 1 h later. Blood was drawn 24 h later, when the orally and IV administered isotopes would be tracking together. Isotope enrichment was determined by mass spectrometry, and fractional calcium absorption was determined as the ratio of oral to IV isotope in the blood sample, adjusted for the dose of each isotope. Other study measures included calciotropic hormones, bone turnover markers, and BMD by dual-energy X-ray absorptiometry (DXA) and quantitative computed tomography (QCT) (Fig. 25.1).

As reported previously, mean 6-month weight loss in our study was 33 kg (26 % of pre-operative weight). We found that mean pre-operative fractional calcium absorption was 33 ± 14 %, which was within the range expected for this mostly female, middle-aged group and on par with the pre-operative mean of the Riedt et al. study [29, 31, 32]. Post-operatively, fractional calcium absorption decreased dramatically to a mean of 7 ± 4 %. This was despite median (interquartile range) 25(OH)D levels of 41 (33–49) ng/mL pre-operatively and 37 (29–40) ng/mL post-operatively. Consistent with the decline in calcium absorption, 24-h urinary calcium level decreased, PTH increased, and $1.25(OH)_2D$ increased (Table. 25.1).

Why did we observe a more dramatic decline in fractional calcium absorption than Riedt et al. did? The reason for the difference in findings is unclear and could include differences in surgical approach or isotope methodology. Regardless, we can certainly conclude that maintaining robust vitamin D status and calcium intake does not mitigate the fractional calcium absorption decline after RYGB.

Both studies employed a rigorous dual stable isotope technique that assesses fractional calcium absorption over 24 h, a time period ample for the complete absorption of calcium in the normal gastrointestinal tract (small intestinal and colonic) [28]. One might wonder whether the complete

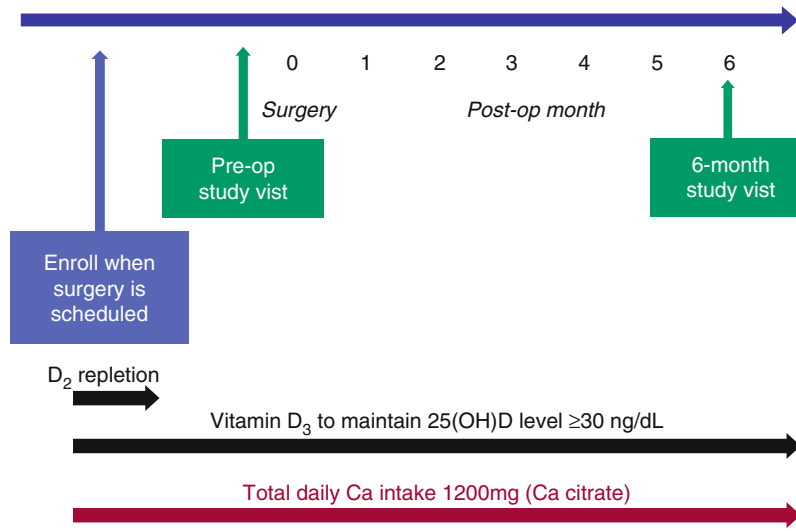

Fig. 25.1 Schema for the study of 33 obese men and women undergoing Roux-en-Y gastric bypass surgery (Schafer et al. [9])

Table 25.1 Key anthropometric, laboratory, and skeletal parameters before and after Roux-en-Y gastric bypass surgery

Parameter	Pre-op	6-months post-op	p-value
Weight (kg)	125 ± 18	93 ± 14	<0.001
BMI (kg/m^2)	45 ± 7	33 ± 6	<0.001
Fractional Ca absorption (%)	33 ± 14	7 ± 4	<0.001
25(OH)D (ng/mL)	41 (33–49)	37 (29–40)	<0.01
24-h urinary calcium (mg)	191 (93–247)	109 (60–159)	<0.001
PTH (pg/mL)	41 (32–53)	48 (39–59)	0.02
1.25(OH)$_2$D (pg/mL)	37 (34–46)	51 (41–63)	<0.001
CTX (ng/mL)	0.293 (0.167–0.347)	0.975 (0.802–1.278)	<0.001
Femoral neck areal BMD (g/cm^2)	0.973 ± 0.132	0.925 ± 0.110	<0.001
Spinal volumetric BMD (g/cm^3)	0.162 ± 0.040	0.152 ± 0.039	<0.001

Values are means ± SDs or medians (IQR)

absorption of calcium in the RYGB patient (*i.e.,* colonic as well as small intestinal, passive as well as active) requires a longer period of time. However, it has been demonstrated that RYGB patients have orocecal transit times similar to obese controls [33]. In the study responsible for that observation, median (interquartile range) time for ingested material to reach the beginning of the large intestine was 180 (173–210) min in those who had undergone RYGB and 180 (150–240) min in obese controls, indicating that calcium spends the majority of a 24-h post-ingestion period in the colon and should have ample time for passive absorption.

The fractional calcium absorption decline we observed presumably resulted from the marked alteration to the gastrointestinal tract with RYGB, with the duodenum and proximal jejunum no longer in contact with ingested food and supplements, but we also found that participants with greater percentage weight loss had greater declines in calcium absorption, in bivariate analysis ($p = 0.05$) and after adjustment for age, sex, and race ($p = 0.02$). This finding is consistent with the observation discussed above that non-surgical weight loss results in a decline in fractional calcium absorption compared to weight maintenance. Alternatively, there may have been confounding by surgical approach: Those with more intestine bypassed (longer biliopancreatic limbs) may have experienced, as a result, more weight loss and also a larger impact on calcium absorption (Fig. 25.2).

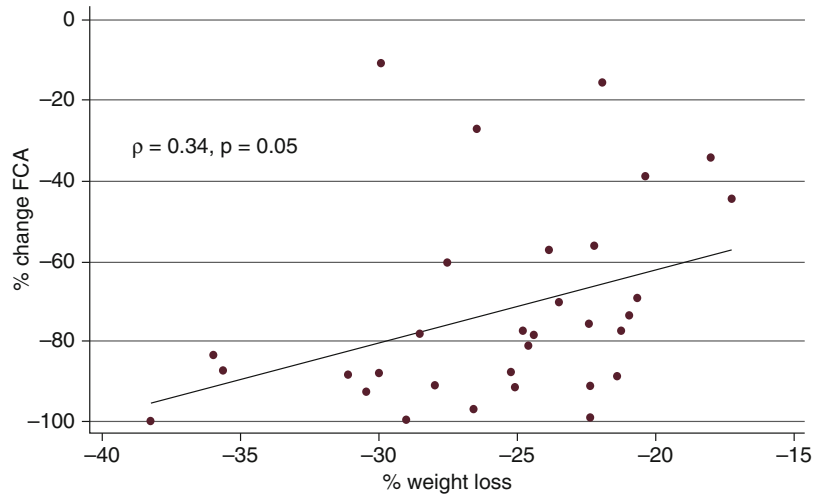

Fig. 25.2 Correlation between percentage changes in fractional calcium absorption and weight loss after Roux-en-Y gastric bypass surgery

We observed correlations with other parameters that reflect weight loss (adiponectin and IGF binding protein-3, a protein that decreased with weight loss and may reflect nutritional status). Otherwise, our search for predictors of calcium absorption decline was largely unrevealing, despite an *a priori* hypothesis that the extent of changes in particular dietary intakes (like dietary protein, fat, and fiber) and hormone levels (like estradiol and IGF-1) might be important. We suspect that the surgical alteration to the gastrointestinal tract was so profound that it overshadowed subtle effects of other potential determinants.

A decline in calcium absorption capacity after RYGB, especially one as dramatic as we observed, raises concern for bone loss and, ultimately, increased fracture risk. Indeed, we found that bone turnover markers increased after RYGB. In particular, serum CTX, a marker of bone resorption, increased by a median 276 % over the 6-month period. Areal BMD decreased at the proximal femur, with a 6-month decline of 4.6 % at the femoral neck and a similar decline at the total hip, and spinal volumetric BMD by QCT decreased by 6.5 %. Participants with greater percentage declines in fractional calcium absorption or lower post-op fractional calcium absorption had greater increases in CTX ($\rho = -0.43$, $p = 0.01$). Despite the substantial declines in fractional calcium absorption and BMD, there was not a statistically significant correlation between the extent of those declines. This likely reflects the influence of other important factors on BMD, and it does not preclude a contribution of the calcium absorption decline to the BMD decline. In fact, those with greater increases in PTH did have greater declines in femoral neck BMD. Also, a period longer than 6 months may be required to see an effect of impaired calcium absorption capacity on BMD.

What are the implications for the clinical care of RYGB patients and for future investigation? RYGB patients may need high calcium intakes—higher than the 1200 mg daily our participants had—to prevent perturbations in calcium homeostasis. The decline in intestinal calcium absorption capacity may be a contributor to the decline in BMD after RYGB, so future research should include efforts to establish the approach to calcium supplementation and to mitigate long-term skeletal fragility.

Future Direction: Intestinal Calcium Absorption After Other Bariatric Surgical Procedures

As trends change in bariatric surgery, it will be critical to understand whether surgical weight loss procedures other than RYGB also have potential negative skeletal effects and how such effects can be abated.

Sleeve gastrectomy is a newer bariatric procedure in which the 80 % of the stomach is removed but the intestinal tract remains unaltered. Its use has risen dramatically, accounting for <1 % of bariatric procedures at US academic medical centers in 2008 then 36 % of procedures in 2012 [34], and it is poised to overtake RYGB. Its rise is largely due to evidence that its lower invasiveness results in fewer complications than RYGB [35–37].

Despite the skyrocketing use of sleeve gastrectomy, its effects on calcium metabolism and bone health have not been defined. The intact intestine in the sleeve gastrectomy patient would suggest unimpeded calcium and vitamin D absorption, but post-operative deficiencies nevertheless occur [35, 38]. Further, other factors thought to contribute to the negative skeletal effects of RYGB also occur with sleeve gastrectomy, including decreased skeletal loading, loss of muscle mass, and changes in fat-secreted hormones and estrogen. As a consequence of our poor understanding of sleeve gastrectomy's effects, professional organizations' recommendations about skeletal health for sleeve gastrectomy patients are extremely limited at this time [16–18]. For example, a recent position statement by the American Society for Metabolic and Bariatric Surgery addressing bone metabolism admits frankly to the "current lack of procedure specific data" for the newer operation [18].

With respect to calcium homeostasis, it is unknown whether sleeve gastrectomy—or any other bariatric surgical procedure—impairs calcium absorption capacity, because direct assessment (*i.e.,* determination of fractional calcium absorption using a dual stable isotope technique) has never been done in sleeve gastrectomy patients. Further, studies published to date have inadequately addressed the vitamin D deficiency that is so common in severe obesity and with dietary restriction. Even though sleeve gastrectomy does not involve resection of intestine, as in RYGB, the procedure changes the nature of food and supplement delivery to the intestine, and it diminishes gastric acidity, which may impede calcium absorption. As discussed earlier in this chapter, it has been shown that nonsurgical weight loss results in a decline in intestinal calcium absorption capacity [24, 25]. Together, these phenomena could be sufficient to impair post-operative calcium absorption, but this hypothesis has not yet been tested (Fig. 25.3).

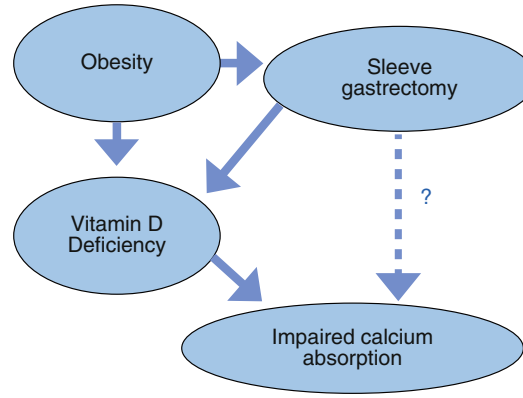

Fig. 25.3 Mechanisms for potential impairment of intestinal calcium absorption capacity following sleeve gastrectomy

Understanding whether intestinal calcium absorption is impaired after sleeve gastrectomy and other bariatric surgical procedures—even with vitamin D optimization—will be critical for evidence-based guidelines about calcium supplementation after the procedures. More broadly, understanding the relative calcium and skeletal effects of bariatric surgical procedures will contribute to the fully informed decision-making of patients and providers, something which may be particularly important for certain groups such as older bariatric patients, postmenopausal women, or those with low bone mass pre-operatively. The ultimate goal is to decrease potential long-term skeletal complications of these otherwise beneficial procedures.

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Nutrition, Adolescent Pregnancy and Bone

26

Kimberly O. O'Brien and Cora M. Best

Abstract

Pregnancy during adolescence often occurs before peak bone mass has been fully consolidated. This may have short or long-term implications for maternal, fetal and neonatal skeletal health. Optimal calcium and vitamin D intake are essential for bone health, but data regarding metabolism of these nutrients in relation to skeletal health among pregnant teens remains limited. This review summarizes calcium and vitamin D homeostasis in relation to bone outcomes in the pregnant teen and her neonate, and adult pregnancy is used as a reference for comparison throughout. The conclusion highlights key gaps in knowledge and challenges for future work in this area.

Keywords

Vitamin D • Parathyroid Hormone • Calcium Absorption • Pregnancy • Adolescence • Bone • Secondary Hyperparathyroidism • Fetal Bone

Adolescent pregnancy continues to be a significant public health problem in the United States. Statistics from the Centers for Disease Control in 2011 indicated that nearly 1,100 adolescents gave birth each day in the United States, 1 in 10 new mothers was an adolescent and teen childbearing cost U.S. taxpayers more than 9 billion dollars annually [1]. In addition to the social, economic and emotional concerns associated with early childbearing, pregnancy during adolescence often occurs before adolescents have consolidated their peak bone mass or stopped growing in linear height. This may have short and/or long-term implications for skeletal health and subsequent risk of low bone mass, but data on this topic are limited given the challenges associated with research in this population. To complicate matters, many pregnant adolescents give birth more than once during their teenage years as fully 1 in 5 births to teenage mothers' ages 15–19 years is a repeat birth [2]. This review compares and contrasts calcium (Ca) and vitamin D homeostasis during pregnancy in adolescents to data obtained

K.O. O'Brien, PhD (✉) • C.M. Best, MHS
Division of Nutritional Sciences, Cornell University, Rm 230 Savage, Ithaca, NY 14853, USA
e-mail: Koo4@cornell.edu

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in adults and discusses possible effects of early childbearing on maternal and fetal/neonatal bone outcomes in the pregnant teen.

Females accrue approximately 40 % of their skeletal mass between Tanner Stages 2 and 5 [3], such that by the age of 16 years, females have a bone mass that is nearly equal to that of their premenopausal mothers [4]. When adolescent skeletal accretion is interrupted by a pregnancy, the adolescent must divert nearly 30 g of Ca to consolidate the fetal skeleton. If this loss is expressed on a daily basis, during late gestation approximately 300 mg of Ca per day are accrued by the fetus [5]. This amount is comparable to the peak daily skeletal calcium deposition observed in adolescent females (284 mg/day at age 11.8 years) [6].

Racial differences in bone mass are known to occur with African-Americans having the lowest risk of osteoporosis at maturity and significantly higher rates of skeletal Ca deposition and Ca absorption, along with lower urinary Ca excretion and alterations in calcitropic hormones (lower 25-hydroxyvitamin D and higher parathyroid hormone and calcitriol) [7, 8]. This point is important to mention as teen pregnancy disproportionately impacts minorities; Hispanic and Black teens have more than double the birth rate (per 1,000 girls ages 15–19 years) when compared to White adolescents [9].

At this time the calcium and vitamin D intake requirements for pregnant adolescents (<19 years of age) are no different than those for their age-matched non-pregnant peers, but the need for more data on the metabolism and role of Ca and vitamin D in this population has been highlighted by the Institute of Medicine [10]. Even without any increase in Ca or vitamin D requirements it is unlikely that pregnant teens will achieve the Estimated Average Requirements (EAR) for these nutrients. Nationally representative data indicate that 87 % of US females age 14–18 years consume less than the EAR for vitamin D and 77 % consume less than the EAR for Ca when considering the combined intake from both food and supplements [11]. Many believe the intake recommendations for vitamin D are too low and this issue remains controversial. The Endocrine Society published guidelines for use by clinicians treating or preventing vitamin D deficiency. These guidelines suggest that the RDA of 600 international units (IU) of vitamin D per day is insufficient to prevent vitamin D deficiency during pregnancy and instead advocate daily vitamin D intakes between 1,500 and 2,000 IU in order to achieve target 25-hydroxyvitamin D (25(OH)D) concentrations of 30 ng/mL [12].

Calcium and Vitamin D Physiology Across Gestation in Adults

Calcium and vitamin D physiology are closely inter-related to maintain circulating Ca and P concentrations at the supersaturated concentrations needed to support skeletal homeostasis and overall health. This is accomplished with the assistance of several calcitropic hormones including parathyroid hormone (PTH), calcitonin, 1,25-dihydroxyvitamin D (calcitriol) and the phosphatonin FGF23. These hormones interact to regulate renal Ca and phosphorus (P) reabsorption, intestinal Ca and P absorption and bone turnover of Ca and phosphorus.

Many studies have characterized changes in Ca dynamics across gestation in adults. Early in gestation the efficiency of Ca absorption increases, well before the period of rapid fetal bone growth [13]. By the end of pregnancy, Ca absorption averages ~50 % in US women consuming ~1,000 mg of Ca per day. This represents an approximate doubling of the pre-pregnancy absorption efficiency [13, 14]. Urinary Ca excretion also increases markedly across gestation and offsets some of the gains in Ca retention that occur due to the increased absorption of this mineral. Pregnancy associated increases in urinary Ca excretion have been reported even among adult Brazilian women ingesting habitual intakes of only ~500 mg of Ca per day [15]. In contrast data from Gambian women ingesting ~350 mg Ca/day found a substantially lower urinary Ca excretion at 20 weeks (~60 mg/day) compared to other published pregnancy data in other geographical locations and this loss did not

significantly differ in Gambian women receiving Ca supplementation across pregnancy (1,500 mg Ca/day) [16].

Marked changes in calcitropic hormones occur to support the Ca demands of pregnancy. Concentrations of 25(OH)D have typically been found to remain static or decrease across gestation in those receiving standard prenatal supplements containing 400 IU of vitamin D [17, 18]. Increased attention has been placed on not only the total concentration of this prohormone but also on the biological activity of 25(OH)D. Bioavailable (free) 25(OH)D is impacted by concurrent concentrations of vitamin D binding protein (DBP) and this binding protein is also known to increase significantly across gestation [19]. Data on bioavailable 25(OH)D are constrained by challenges associated with the techniques utilized to quantify free 25(OH)D. A new assay that is purported to have more sensitivity in quantifying free 25(OH)D found that while DBP concentrations increased across gestation, free 25(OH)D concentrations remained unchanged from the 2nd to the 3rd trimester perhaps as a consequence of alterations in the affinity of DBP for 25(OH)D [20].

Alterations in bioavailable 25(OH)D across pregnancy would impact the cellular use of this prohormone for intracrine functions. Genetic polymorphisms in DBP may also influence bioavailable vitamin D concentrations. The prevalence of individual genetic polymorphisms in DBP has been found to significantly differ between African Americans and Caucasians, with African-Americans having an increased prevalence of a polymorphism that is associated with lower concentrations of DBP [21]. Additional studies are needed to identify how these polymorphisms impact the amount of free versus bound 25(OH)D, to ensure that assays utilized for DBP equally recognize all DBP polymorphisms and to evaluate some of the assumptions that are utilized in the equations used to quantify bioavailable vitamin D [22].

Calcitriol, the hormonal form of vitamin D, significantly increases within the first trimester of pregnancy [14, 23]. In women consuming typical diets and standard prenatal supplements, final concentrations of calcitriol in late gestation are increased ~2–2.5 fold compared to pre-pregnancy values [18, 23]. Vitamin D supplementation with 2,000 or 4,000 IU of D_3/day leads to even greater increases in calcitriol as well as gains in 25(OH)D across gestation [17, 24]. Other longitudinal studies have reported slight decreases in calcitriol from late gestation until term in pregnant teens who did not receive high dose vitamin D supplementation [25].

Data on pregnancy associated changes in intact PTH (iPTH) remain controversial. Some reviews report that iPTH is low to undetectable across pregnancy in most women except for those from Asia or the Gambia [26], but recent studies contradict this finding. Pregnancy associated elevations in PTH have now been reported in several US populations including women ingesting average Ca intakes of ~1,000 mg/day [17], low income and predominantly minority women ingesting Ca intakes of 1,085 mg/day [27], and pregnant adolescents (predominantly minority) consuming Ca intakes averaging 913 mg/day [25].

Evaluation of serum analytes in pregnant women is challenging as these must be evaluated in relation to the marked hemodilution that is known to occur across pregnancy. Decreases in concentrations of serum biomarkers may be due to plasma volume expansion while constant concentrations may actually reflect increased synthesis or failure to appropriately expand the plasma volume. Total Ca concentrations decrease across pregnancy a finding that may be driven by hemodilution given the increase in plasma volume and concurrent decreases in serum albumin. Ionized Ca concentrations, in contrast, do not decrease between early and late pregnancy [18].

The net impact of pregnancy-associated alterations in Ca retention and calcitropic hormones on bone turnover has been examined using stable Ca isotopes. These studies have reported significant increases in bone deposition and resorption rates during pregnancy compared to values evident in the non-pregnant state [28]. Some of these studies have also found that higher Ca intake across gestation is associated with an improvement in Ca balance [28].

Calcium and Vitamin D Physiology Across Gestation in Adolescents

The growing literature on gestational changes in Ca physiology in pregnant adolescent populations indicates that the physiological adaptations to pregnancy in adolescents largely mirror those observed among adults. Adolescents experience a similar magnitude of increase in Ca absorption, similar elevations in urinary Ca losses, and comparable hormonal changes across gestation [29]. Because adolescent pregnancy disproportionately impacts minorities, and darker skin tones are a risk factor for lower 25(OH)D, it is perhaps not surprising that vitamin D insufficiency is prevalent in this population. We found in a recent study of 168 pregnant teens ≤18 years of age (65 % were African-American and 25 % were Hispanic) residing in Rochester, NY (latitude of 43° N), that at delivery 47 %, 31 %, and 18 % of teens had 25(OH)D under 20 ng/mL, 16 ng/mL and 12 ng/mL respectively [25]. In these adolescents fully 25 % of teens exhibited elevated PTH at delivery (>60 pg/mL) while ingesting mean Ca intakes of 900 mg/day [25]. A depiction of the typical temporal changes in Ca and vitamin D parameters across gestation in adults and adolescents is presented in Figure 26.1.

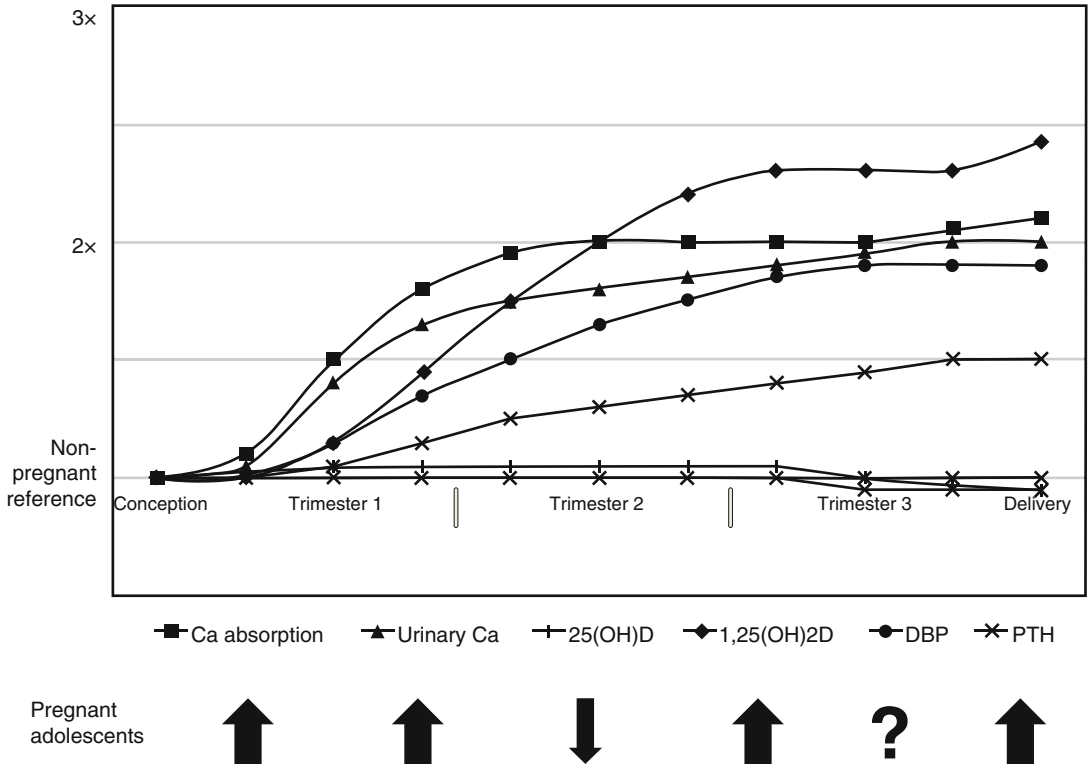

Fig. 26.1 Typical temporal changes in markers of calcium and vitamin D metabolism are presented across trimesters as fold changes relative to pre-pregnancy values. The patterns of change depicted are based on published data from observational and experimental studies conducted in U.S. pregnant adults. The *y*-axis reflects a 2× (double the non-pregnant value, i.e. a 100 % increase) or 3× increase (triple the non-pregnant value, i.e. a 200 % increase). *Arrows* presented below the *x*-axis depict the direction and magnitude of changes in these same parameters based on data obtained in pregnant adolescent study populations. No data on vitamin D binding protein have been published in pregnant adolescents to date. Data on PTH concentrations across gestation are mixed with some studies finding no change, a decrease, or an increase in this hormone by late gestation. Several recent U.S. studies in individuals consuming average Ca intake of ~1000 mg/day have found that from 11 to 25 % of women studied exhibit elevated PTH (>60 pg/mL) during pregnancy

Secondary Hyperparathyroidism During Pregnancy

The prevalence of secondary hyperparathyroidism in our study of pregnant adolescents at mid-gestation was 6.6 % (intact PTH (iPTH) >60 pg/mL) at 26.3 ± 3.6 weeks of gestation and 25 % at delivery [25]. This can be compared to data from another study of 1,116 low-income and minority pregnant subjects, in which 11 % of women studied exhibited iPTH >62 pg/mL at 13.8 weeks of gestation [27]. Furthermore, in a vitamin D supplementation trial by Hollis et al., women in the lowest supplementation dose group (400 IU) had iPTH concentrations of 18.1 pg/mL at baseline; this increased by ~17 % to 21.0 pg/mL by 1-month prior to delivery [17].

Factors associated with those who exhibit secondary hyperparathyroidism across pregnancy have not been fully elucidated. In the vitamin D supplementation study by Hollis et al., PTH elevations were blunted in African Americans receiving higher dose vitamin D supplementation (2,000 and 4,000 IU D_3/day), and higher 25(OH)D concentrations also resulted in increased calcitriol [17]. A similar vitamin D supplementation study by Wagner et al., which compared 2,000 IU vs 4,000 IU of vitamin D_3, found that women in the 2,000 IU group had an increase in PTH across gestation while those in the 4,000 IU group experienced a net 1.0 pg/mL decrease, a difference between treatment groups that was significant (P = 0.03) [24]. In our adolescent study, one-quarter of teens studied had elevated PTH at delivery. PTH was inversely associated with maternal 25(OH)D concentrations in the cohort as a whole and in teens with 25(OH)D < 20 ng/mL. Of note this relationship did not remain significant when examined only in the sub-group of teens with 25(OH)D concentrations over 20 ng/mL. Mid-gestation 25(OH)D was also inversely associated with calcitriol at delivery, irrespective of Ca intake [25].

The secondary hyperparathyroidism observed in some women during pregnancy suggests that a subset of adult and adolescent women experience greater Ca stress across gestation. Additional research is needed to identify those at risk for secondary hyperparathyroidism and to determine if this response is associated with adverse maternal, fetal or neonatal outcomes. Towards this goal, Scholl et al. recently measured serum iPTH, 25(OH)D and Ca intake in relation to birth outcomes in a group of 1,116 low-income pregnant women and adolescents from Camden, NJ (23 % of the population was <19 years of age). In these women, 11 % exhibited elevated PTH (>62 pg/mL) at entry into prenatal care (13.8 weeks of gestation), the only time point that serum was obtained [27]. The authors categorized women as having calcium metabolic stress if they exhibited elevated PTH in combination with either a low dietary Ca intake (<60 % of the EAR) or vitamin D insufficiency (defined as 25(OH)D < 20 ng/mL). Concentrations of PTH in early gestation were independently associated with both 25(OH)D and Ca intake. However, elevated PTH was common in those with insufficient 25(OH)D, even in the face of Ca intakes that met or exceeded the recommended dietary allowance (RDA). Women with elevated PTH and either or both low Ca intake and low 25(OH)D status had a 2–3 fold increased risk of small for gestation age birth (SGA) and gave birth to infants with significantly lower birth weight, birth length and head circumference [27]. Alterations in serum PTH were highly predictive of adverse fetal outcomes; women with insufficient 25(OH)D or low Ca intake who did not have elevated PTH did not experience reductions in fetal growth. Stable Ca isotope studies have also found that rates of bone calcium deposition and resorption in late pregnancy are significantly positively associated with PTH [28]. If Ca economy is strained, then there may be insufficient substrate to fully support maternal and fetal skeletal health and both maternal and fetal mineralization may be adversely impacted. Interactions between PTH, 25(OH)D and calcitriol in a pregnant adolescent population may well differ from those identified to date among pregnant adults due to the rapid bone deposition that typically occurs during adolescence and the increased competition for calcium between the pregnant adolescent and her developing fetus.

Teen Pregnancy and Bone Mass

A 2012 literature review of all total body and site specific bone mineral data across gestation found net bone changes ranged from losses of −2.0 % to non-significant gains of 0.5 % [30]. These authors noted that a 2 % loss in total body bone mineral in adult women reflects a net loss of ~25 g of Ca, an amount approaching the 30 g of Ca that are thought to be present in the neonate at birth [30]. This same 30 g loss would reflect nearly 4 % of an adolescents' total body Ca content given their lower bone mass [29].

Stable Ca isotope absorption studies in pregnant adolescents (ages 13.5–18.3 years) have reported average percent Ca absorption values of ~53 % in teens ingesting Ca intakes averaging 1,200 mg/day. Using concurrent measures of urinary Ca excretion and estimates of endogenous fecal Ca secretion, this translated to an estimated Ca balance of 240 mg/day during the third trimester. This amount is comparable to reported peak rates of fetal Ca accretion but would not be sufficient to achieve the combined peak rates of fetal and adolescent bone Ca accretion [29].

Data on the degree to which Ca or vitamin D status influences maternal bone outcomes across gestation are mixed. Many studies have utilized heel ultrasound measures to track changes across pregnancy since this method does not involve radiation exposure. These studies report significant declines in calcaneal measures across gestation in both adults and adolescents [31–33]. In one study that recruited both pregnant teens (n = 45) and pregnant adults (n = 199), teens experienced significantly greater deficits in the quantitative ultrasound index outcome (−5.5 % vs. −1.9 %) across the interval from 16 ± 7 weeks gestation to 6 ± 1 week postpartum [31].

We recently assessed possible associations between adolescent heel ultrasound measures across gestation (n = 156 adolescents ≤18 years at entry into the study) in relation to maternal dietary Ca and vitamin D intake and maternal vitamin D status. In pregnant adolescents, maternal bone loss across pregnancy was not significantly associated with maternal Ca or vitamin D intake, nor was it impacted by 25(OH)D status [32]. Loss of calcaneal bone among UK pregnant women was also not found to be correlated with milk intake or Ca supplement use, except that women ingesting less than a pint of milk per day before pregnancy were found to experience a greater loss in calcaneal bone (measured by speed of sound) across pregnancy [33].

Of interest, while Ca intake and vitamin D status did not impact maternal bone loss across pregnancy, adolescent pre-pregnancy body mass index (ppBMI) was a significant determinant of calcaneal bone outcomes across gestation. Teens entering pregnancy with a lower BMI experienced significantly greater decreases in all bone quality outcomes across gestation [32]. Body composition was also found to be a key determinant of gestational bone losses in a group of 307 pregnant adults; reductions in heel ultrasound measures across gestation were attenuated in women with greater fat stores (based on maternal mid upper arm circumference in late pregnancy) [33]. Similarly, pre-gravid weight and weight gain across pregnancy were predictive of reductions in heel ultrasound measures in pregnant adults and adolescents [31].

Site specific alterations in calcaneal bone quality across pregnancy may or may not translate to net changes in total body or site specific bone mineral content or density. This question is challenging to evaluate in pregnant adolescents as the peak bone mass that would have been achieved had the pregnancy not occurred is unknown, it is difficult to obtain pre-pregnancy data from teens that subsequently become pregnant and this group is challenging to follow in the post-partum period to evaluate postpartum gains in bone mass once menses resume.

Given the well-known effects of estrogen on closure of the epiphyseal growth plate [34], it is possible that the elevated estrogen concentrations that occur during pregnancy may accelerate epiphyseal senescence and fusion of the growth plate [35]. Studies in adolescents have found lower cortical BMD and whole body BMC in the early postpartum period compared to age and ethnicity matched controls [29, 36], but these studies are generally small and must rely on estimates of

expected peak bone mass. Supplementation studies in Brazilian adolescents (14–19 years) found that Ca (600 mg) and D supplementation (200 IU) over the last 14 weeks of pregnancy resulted in significantly higher lumbar spine bone mineral content and bone area at 5 weeks post-partum when compared to the placebo group [37].

Using other approaches, epidemiological studies have evaluated determinants of bone mass in women with and without histories of early childbearing. Studies have found an adverse effect of early childbearing on postmenopausal BMD at several sites [35, 38]. These findings remain controversial and the long-term impact of any acute bone losses on peak bone mass and subsequent risk of low bone mass and osteoporosis at maturity has not been sufficiently characterized.

Teen Pregnancy and Fetal Skeletal Growth

Nutrients necessary for bone mineralization may be disproportionately partitioned between the growing adolescent and her fetus. Adolescent sheep models have frequently been utilized to study the perturbed nutrient partitioning to the fetus that is often evident in animals that are still growing at the time of conception [39]. In humans, many studies have found average birth weights of neonates born to pregnant teens are lower than birth weights observed among adult populations even when adjusting for race to account for the smaller birth weights evident among African-Americans compared to Caucasians [40].

In our adolescent studies, while maternal Ca and D intake were not associated with changes in maternal bone outcomes, increased maternal dairy intake was found to have a significant positive impact on fetal femur length [41]. A limitation of this whole food based approach is that the observed associations with dairy intake cannot be attributed to any one individual nutrient. In a subsequent study, the impact of adolescent Ca and vitamin D intake and 25(OH)D concentrations across gestation was evaluated in relation to longitudinal measures of fetal femur and humerus length [42]. Although maternal 25(OH)D and intakes of Ca and D were not significantly associated with changes in maternal calcaneal outcomes [32], longer fetal femur and humerus length were present in the adolescents consuming Ca intakes ≥1,050 mg/day. Significant positive associations were also evident between fetal femur and humerus z-scores in teens with 25(OH)D >20 ng/mL [42]. In this same study, a significant association was observed between dietary Ca intake and fetal femur z-scores and birth length, but only in teens with vitamin D insufficiency (25(OH)D < 20 ng/mL). These findings highlight the interactions that are evident between adequate Ca and vitamin D status and the negative impact that insufficiency of either of these nutrients can have on fetal bone growth (Fig. 26.2) [42].

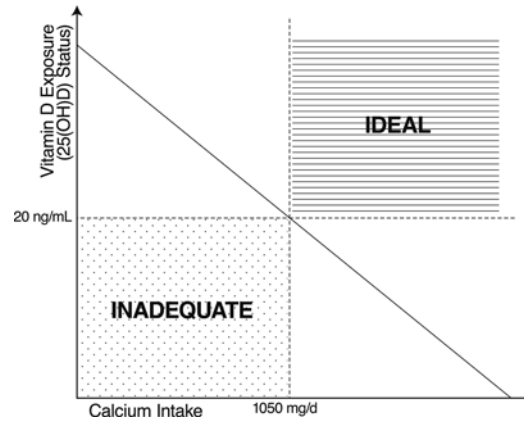

Fig. 26.2 Optimal maternal calcium (Ca) intake and adequate 25-hydroxyvitamin D (25(OH)D) status are integral to optimal fetal bone growth. In a group of 169 pregnant adolescents, maternal dietary calcium intake ≥1050 mg/d was associated with a significantly more positive fetal femur and humerus length z score. Similarly, pregnant teens with 25(OH)D concentrations ≥20 ng/mL exhibited significantly higher fetal femur and humerus length z scores. Interactions between maternal Ca intake and 25(OH)D status were evident. Maternal Ca intakes and vitamin D status (as determined by 25(OH)D) were only significantly associated with fetal femur and humerus length z scores when intake/status of the other nutrient was inadequate. In a pregnant adolescent population, consuming either an adequate Ca intake or achieving sufficient 25(OH)D status was found to attenuate deficits in fetal long-bone growth that occurred in those with suboptimal status/intake of both nutrients

In contrast, other recent data in pregnant adolescents habitually ingesting ~600 mg Ca/day found that additional Ca supplementation (600 mg/day) over the last trimester of pregnancy did not significantly impact fetal bone measures or infant BMC, BMD or BA at 5 weeks postpartum [43]. The authors concluded that early infant bone mass in those born to this age group is largely met by maternal bone mobilization when Ca intake is low [37, 43].

Data on the effects of maternal Ca intake and fetal bone outcomes remains controversial. Calcium supplementation (1,500 mg/day Ca) across the second half of pregnancy to women habitually ingesting only ~350 mg of Ca per day was associated with lower maternal bone mineral at the hip and a significant increase in maternal bone loss over the following 12 months of lactation compared to the placebo group [44]. In these Ca supplemented women no changes in fetal growth (based on birth weight and crown-heel length within 5 days of birth) or neonatal whole body BMC at 2 weeks of age were noted. The rates of total body bone mineral content and bone area accumulation at 52 weeks of age were slower in babies born to Ca supplemented women compared to the placebo group [45]. Given that the majority of bone mass is genetically determined, more studies are needed in different population groups of women with variable combinations of Ca, P and vitamin D status to more fully identify environmental and genetic determinants of maternal, fetal and neonatal bone health.

Gaps in Knowledge

Until recently it was assumed that phosphorus homeostasis was largely maintained by serum PTH but it is now known that the phosphatonin, fibroblast growth factor 23 (FGF23), is integral to systemic phosphorus regulation [46]. At this time little is known about FGF23 homeostasis across human pregnancy and how FGF23 interacts with the dynamic gestational changes in calcitriol and PTH that may also occur at this time. Similarly, there are few data on vitamin D kinetics during pregnancy and more data on basic aspects of production and catabolism of calcidiol and calcitriol during pregnancy are needed in order to better inform nutritional requirements and clinical practice. Concurrent data on DBP concentrations are needed when measures of calcidiol and calcitriol are obtained in order to estimate how net concentrations are related to free concentrations of vitamin D metabolites.

Because there are no easily applied methods to quantify plasma volume it is assumed that plasma volume expansion is comparable between individuals when interpreting biochemical indicators at a given stage of gestation. This has not been sufficiently evaluated in adolescent or adult pregnancies. For iron it is known that there is a U-shaped distribution with respect to hemoglobin concentrations and adverse birth outcomes in adolescents [47] and adults [48]. The increased risks evident in women with high hemoglobin concentrations may be associated with a failure to appropriately expand plasma volume across gestation. Similar perturbations in plasma volume expansion would also influence the interpretation of any calcitropic serum biomarker; greater attention to this issue is warranted when evaluating outcomes and nutrient requirements across gestation.

Optimal maternal nutrition during pregnancy must support health in both the adolescent and her developing fetus. Studies evaluating Ca and vitamin D requirements in pregnant women often do not concurrently consider fetal and neonatal outcomes. The nutrient intakes required to prevent maternal deficiency may not be identical to those necessary to optimize maternal health or the fetal environment as it relates to developmental programming. Additional studies on this point are needed.

Finally, when evaluating the possible benefits of vitamin D, non-bone health outcomes should be considered given the recently identified role of vitamin D in immune system function and the numerous associations linking vitamin D status to increased risk of adverse birth outcomes [49].

Conclusion

Adolescence is a time of rapid bone acquisition that culminates in the fusion of the epiphyseal growth plate and cessation of linear

growth. When adolescence is interrupted by pregnancy, females that should be consolidating their own skeletal mass must divert substantial amounts of Ca to their developing fetus. The physiological adaptations that occur to support this process may not be sufficient to fully support maximum maternal and fetal skeletal growth and consolidation. Further data are needed in order to evaluate acute and long-term implications of early childbearing on maternal and offspring bone health. Optimal Ca and vitamin D requirements in this age group are challenging to identify given that the peak bone mass that would have been achieved is unknown. Additional research is needed to fully characterize changes in calcitropic hormones across gestation in pregnant adolescents and adults in order to quantify bioactive concentrations of vitamin D metabolites and their interaction with total and ionized Ca and phosphorus while including measures of serum albumin to account for possible differences in plasma volume.

Accumulating data indicates that a subset of pregnant women exhibit elevated parathyroid hormone combined with low 25(OH)D status and/or low Ca intake, and this may increase the risk of adverse birth outcomes. While genetic factors are known to be responsible for the majority of bone mass, many of these factors have yet to be identified, and additional work is needed to identify genotypes that predispose pregnant women and their developing fetuses to mineral insufficiency. Finally, greater focus on the placental interface will help identify factors that influence placental trafficking of Ca and vitamin D in support of fetal demands.

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Part VIII

Recommendations

27 Lifestyle Factors That Affect Peak Bone Mass Accrual: Summary of a Recent Scientific Statement and Systematic Review by the National Osteoporosis Foundation

Connie M. Weaver, Catherine M. Gordon, Kathleen F. Janz, Heidi J. Kalkwarf, Joan M. Lappe, Richard Lewis, Megan O'Karma, Taylor C. Wallace, and Babette S. Zemel

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C.M. Weaver, PhD
Department of Nutritional Sciences, Women's Global Health Institute, Purdue University, 700 W. State Street, West Lafayette, IN 47907, USA

C.M. Gordon, MD, MSc
Division of Adolescent and Transition Medicine, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC 4000, Cincinnati, OH 45229, USA

Department of Pediatrics, University of Cincinnati College of Medicine, 3230 Eden Avenue, Cincinnati, OH 45267, USA

K.F. Janz, EdD
Departments of Health and Human Physiology and Epidemiology, University of Iowa, 130 E FH, Iowa City, IA 52242, USA

H.J. Kalkwarf, PhD
Department of Pediatrics, University of Cincinnati College of Medicine, 3230 Eden Avenue, Cincinnati, OH 45267, USA

Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC 2010, Cincinnati, OH 45229, USA

J.M. Lappe, PhD, RN
Schools of Nursing and Medicine, Creighton University, 601 N. 30th Street, Omaha, NE 68131, USA

R. Lewis, PhD, RD
Department of Foods and Nutrition, University of Georgia, Dawson Hall, Athens, GA 30602, USA

M. O'Karma, MS
Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia Research Institute, 3535 Market Street, Room 1560, Philadelphia, PA 19104, USA

T.C. Wallace, PhD, CFS (✉)
Department of Nutrition and Food Studies, George Mason University, MS 1F8, 10340 Democracy Lane, Fairfax, VA 22030, USA

National Osteoporosis Foundation, 1150 17th Street NW, Suite 850, Washington, DC 20036, USA
e-mail: taylor.wallace@me.com

B.S. Zemel, PhD
University of Pennsylvania Perelman School of Medicine, 3535 Market Street, Room 1560, Philadelphia, PA 19104, USA

Division of Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, 3535 Market Street, Room 1560, Philadelphia, PA 19104, USA

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Abstract

Optimization of factors that influence peak bone mass is one strategy to reduce the risk of osteoporosis later in life. The National Osteoporosis Foundation recently issued a scientific statement providing evidence-based guidance for the purpose of helping individuals achieve optimal peak bone mass early in life. This chapter summarizes the role of individual nutrients, food patterns, lifestyle issues (e.g., smoking), and physical activity on bone mass and strength development in children and adolescents. The best evidence is for positive effects of calcium intake and physical activity on bone mass and density. Good evidence was also available for positive effects of vitamin D and dairy consumption.

Keywords

Peak bone mass • Osteoporosis • Low bone mass • Calcium • Vitamin D • Nutrients • Food patterns • Dairy • Physical activity • Exercise

Introduction

The National Osteoporosis Foundation (NOF) recently issued a Scientific Statement providing evidence-based guidance for the purpose of helping individuals achieve optimal peak bone mass early in life [199]. This chapter summarizes the findings of this scientific statement and examines the role of individual nutrients, food patterns, special issues, and physical activity on bone mass and strength development in children and adolescents. The Scientific Statement was endorsed by the following scientific societies: American Bone Health, American College of Sports Medicine, Endocrine Society, and Society for Women's Health Research.

Bone Accretion

During growth and development, skeletal growth occurs primarily as a consequence of bone modeling whereby bone deposition and resorption are delicately coordinated to allow bones to expand through periosteal apposition of cortical bone and lengthen through endochondral ossification [1, 2]. This process occurs from fetal growth through to fusion of the growth plates at the cessation of linear growth. Bone mineral accretion is particularly rapid during adolescence, reaching a peak shortly after peak height gain (Fig. 27.1). It has been estimated that 39 % of total body bone mineral is gained within ±2 years of this peak [3].

The structure and composition of bone, such as the amount of epiphyseal cartilage, cortical and trabecular tissue, change as children progress through puberty. Trabecular bone density of the spine and long bones increases in mid puberty [4–6], but cortical bone volumetric density increases more rapidly as epiphyseal fusion occurs, and continues into the third decade of life [7]. Increasing structural strength of long bones is attained through changes in their outer dimensions and cortical thickness. This process involving accumulation of bone mineral and changes in density and structural strength proceeds into the third decade of life, depending on the skeletal site.

Peak bone mass is most commonly defined as the amount of bone gained by adulthood when the skeleton has reached a stable state. For an individual, "peak bone mass" sometimes refers to the optimal or genetic potential for bone mass (or bone strength). At the population level, peak bone mass is considered to be the average or median value for a bone outcome at the age when age-related changes are no longer positive and have attained a plateau [8].

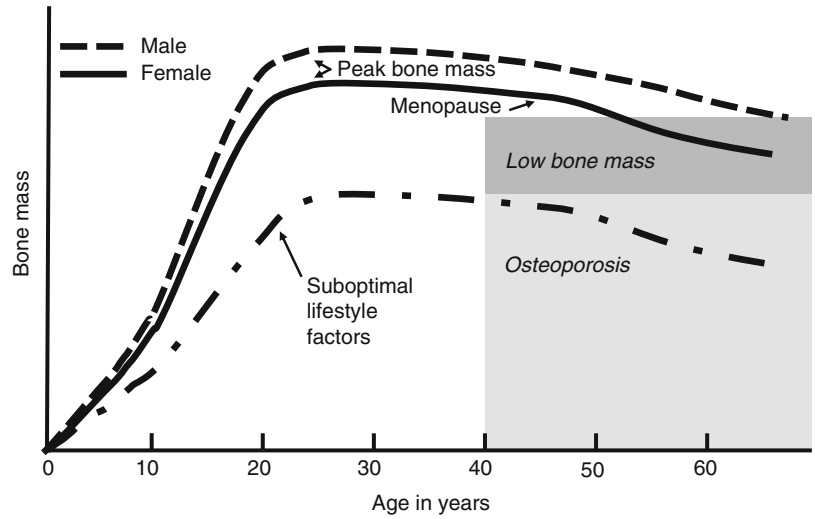

Fig. 27.1 The influence of optimal and suboptimal lifestyle choices on bone health across the lifespan

The timing of peak bone mass varies depending on the skeletal site and bone compartment. For example, the longitudinal Canadian Multicentre Osteoporosis Study used dual energy x-ray absorptiometry (DXA) to identify the ages of peak bone mass: for women, areal bone mineral density (aBMD) peaked between the ages of 33 and 40 years for the spine and between 16 and 19 years for the total hip [8]. Another study showed that women aged 20–29 years ($n=15$) were losing trabecular bone at a rate of 1–1.75 % per year at the distal radius and lumbar spine, but also demonstrated gains in cortical bone at a rate of 0.25 % per year in the tibia [7].

Promoting optimal bone accretion during growth and attainment of peak bone mass may have life long benefits by preventing current or future fractures. In both children and adults, bone mass, density, and strength are associated with fracture risk [9–11]. Fractures are common in children; approximately 50 % of boys and 30 % of girls will experience at least one fracture by age 18 years, and 20 % will have two or more fractures [14, 15]. There is an 89 % increase in fracture risk for each SD decrease below the norm in bone mass [12] or density [13] among children. Most childhood fractures (30–50 %) occur in the forearm [14, 16–19]. The skeleton is particularly vulnerable to fracture during the rapid growth period of late childhood and early adolescence, when the rate of growth in length precedes mineral accrual (Fig. 27.2) [20].

Bone mass and strength tend to "track," or remain stable, from childhood to peak bone mass, with tracking correlations ranging from 0.5 to 0.9 depending on the skeletal site, trait, and duration of follow-up [13, 21–25]. The fact that tracking correlations are far from unity suggests that lifestyle factors can alter bone status in both positive and negative directions. It is important to establish and retain behaviors that contribute to skeletal health, including region-specific changes (e.g., hip, spine). Moreover, until the lifelong importance of peak bone mass is fully understood [26], it is prudent to assume that these behaviors are needed to sustain skeletal health through the life cycle.

Methods for Measuring Peak Bone Mass

Studies of childhood bone accretion and development of peak bone mass primarily used DXA and quantitative computed tomography (QCT) to quantify different characteristics of bone strength. DXA is a rapid, safe, widely available, and precise technique involving low radiation exposure (effective dose ranges from 0.03 to 15.2 μSV) [27] that measures bone mass (or bone mineral content [BMC]) and areal BMD (aBMD). QCT and peripheral quantitative computed tomography (pQCT) are less widely used,

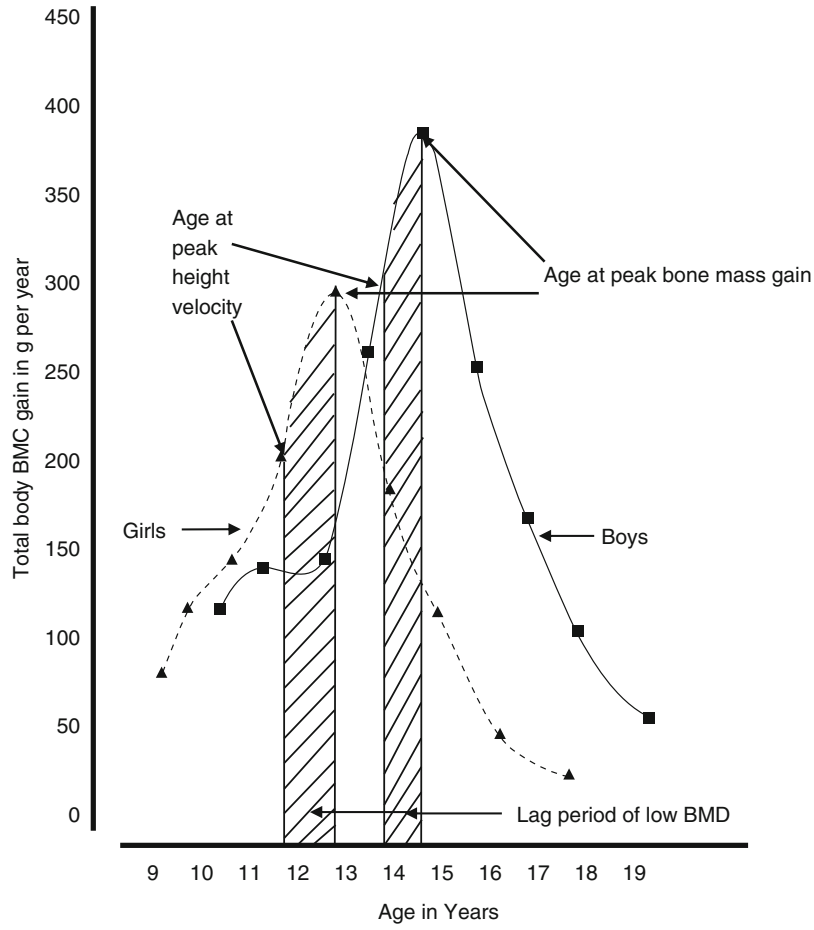

Fig. 27.2 Peak BMC gain and peak height velocity in boys and girls from longitudinal DXA analysis (Adapted from Bailey et al. [34])

but have the advantage of being able to separately measure the cortical and trabecular bone compartments for measures of volumetric bone mineral density (vBMD), bone structure, and, in some cases, microarchitecture. Cortical bone forms the external shell that protects trabecular bone and bone marrow. Trabecular or cancellous bone forms a sponge-like structure within the cortical shell, adding to the structural strength of bone. The patterns of cortical and trabecular bone accretion differ during growth, as does their responsiveness to modifiable factors such as impact-loading physical activity, hormonal changes, disease effects, and medications. There is still much to be learned about the roles of cortical and trabecular bone in optimizing peak bone mass and minimizing both childhood and adult fracture risk.

Mechanical Loading

Physical activity comprises any body movement produced by muscle contraction resulting in energy expenditure above a resting level [28]. Exercise is a more restrictive concept and is defined by planned, organized, and repetitive physical activity aimed at maintaining or enhancing one or more components of physical fitness or a specific health outcome, such as bone strength [29]. The randomized controlled trials (RCTs) reviewed in this chap-

ter and in the recent scientific statement by the NOF [199] used targeted exercise as an intervention to improve bone strength, whereas most of the longitudinal studies measured physical activity including active transportation and activities of everyday life [30]. Physical activity has long been regarded as a behavior likely to influence bone health [31, 32]. Epidemiological and clinical trial research dating back >2 decades confirm the positive impact of regular physical activity on bone [32–37]. However, researchers are only beginning to quantify the specific dimensions, dose, and timing of physical activity needed for optimal bone strength. What is known, primarily from animal studies, is that increased mechanical loads placed on bone through both impact and muscle forces cause deformation (strains) of whole bone [38, 39]. These strains activate mechanosensitive cells (i.e., osteocytes), embedded within the cortex, which signal molecules to activate osteoblasts and osteoclasts. The signaling begins the process of bone adaptation in response to changes in physical activity, as well as other mechanical loads (e.g., an increase in body weight). To initiate an osteogenic response, bone must be subjected to a strain magnitude that surpasses a given threshold determined by the habitual strain range in the predominant loading direction. The threshold varies between individuals (and also bone sites) according to physical activity habits and other factors (e.g., maturity status). Thus, children and adolescents may respond differently to similar mechanical loading conditions. Some children (who are mainly inactive) may respond to low-impact loading and improve bone mass or structure, whereas others will need a higher mechanical load to promote a skeletal response [40].

The skeleton needs to be strong for load bearing and light for mobility. A manner of minimizing the amount of bone mass needed in a cross-section without decreasing strength is to modify the distribution of bone mass (bone structure). Throughout life, but mainly during growth, periosteal apposition increases the diameter of long bones and endocortical resorption enlarges the marrow cavity (Fig. 27.3). Cortical thickness is determined by the net changes occurring at the periosteal and endosteal surface of bone. However, even without an increase in cortical thickness, the displacement of the cortex (periosteal apposition) increases bending strength because resistance to bending is proportional to the fourth power of the distance from the neutral axis. In addition to the independent effect of physical activity on mass

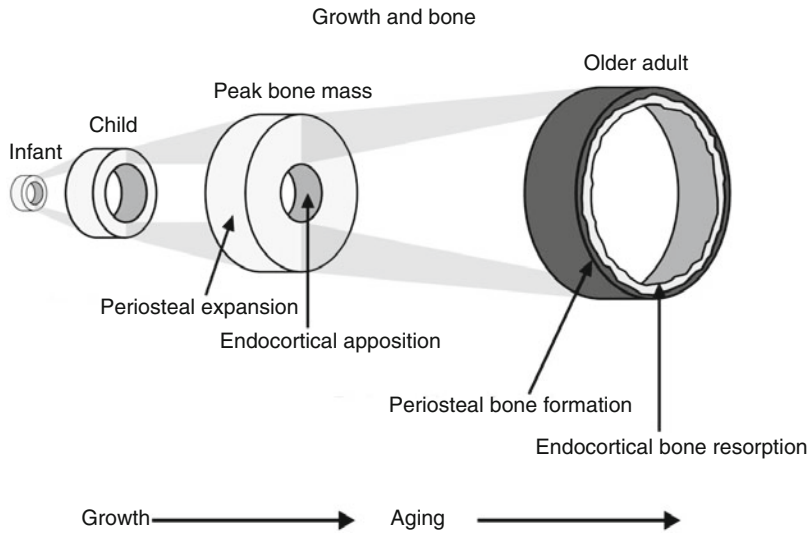

Fig. 27.3 Changes in structural composition of bone throughout the lifespan

and density, increased mechanical loading via physical activity may influence structural changes in bone to increase strength in response to the new loading condition [41–43].

Bone is most responsive to physical activities that are dynamic, moderate to high in load magnitude, short in load duration, odd or non-repetitive in load direction, and applied quickly [40]. The load magnitude is produced by impact with the ground (tumbling, jumping), impact with an object (racquet sports), or muscle power moves such as the lift phase in jumping and vaulting. On the other hand, due to desensitization of the osteocytes, static loads and repetitive low-magnitude loads are not osteogenic [44–46]. Although physical activity is a modifiable factor that contributes to peak bone mass and strength, the understanding of how to quantify osteogenic movement dimensions (including frequency, intensity, time, and type) in observational and clinical trial studies is incomplete.

Nonmodifiable Factors

Genetics

Heritability may explain up to 60–80 % of the variability in peak bone mass and osteoporosis risk. The remainder is influenced by environmental factors, sex hormone levels and growth factors during puberty [47]. Bone density is lower among both men and women with first-degree relatives who have osteoporosis [48, 49]. Genome-wide association studies have been conducted in adults [50, 51] and children [2, 52–55] to identify genes associated with bone density and osteoporotic fractures. Ethnic differences in bone density in pediatric cohorts also support the genetic basis for bone density [4, 56, 57]; African Americans having greater aBMD than other ethnic groups. Individuals of European ancestry have been shown to exhibit greater aBMD values as compared to Asians and Hispanics. These findings are supported by QCT studies showing greater cortical bone dimensions and trabecular vBMD among African Americans compared to others [4, 58–60].

Sex

At most ages during childhood and adolescence, males have greater BMC and aBMD by DXA than females. These differences increase with the onset and progression through puberty [56, 57, 61–63]. This sex difference in DXA measures is due, in large part, to larger cortical bone dimensions; pQCT measures of the tibia show lower values for most cortical bone outcomes (BMC, bone area, periosteal circumference, and section modulus) in females. However, as puberty advances, cortical vBMD was greater in females compared to males [60, 64–66].

Maturation

The hormonal changes of puberty result in rapid bone accretion as measured by pronounced increases in BMC and aBMD, as well as cortical and trabecular vBMD. Maturational timing is also important. Earlier maturation in girls is associated with greater DXA BMC and aBMD at skeletal maturity [67, 68]. Later puberty in boys was associated with lower radius aBMD (−4.2 %, by DXA), and lower cortical (−0.7 %) and trabecular vBMD (−4.8 %, by pQCT) [69]. Further studies are needed to determine whether the effects of pubertal timing on peak bone mass have life long consequences.

Modifiable Factors

Diet and physical activity are the primary modifiable factors associated with bone accrual, although other lifestyle and environmental factors may contribute to optimizing peak bone mass. The bony mineral tissue is composed of hydroxyapatite, a calcium-phosphate compound, with magnesium and trace amounts of other minerals. The connective tissue is composed primarily of the protein, collagen. The role of many

micronutrients in bone is to assist in connective tissue synthesis and maturation. Iron, zinc, magnesium, copper, manganese, and vitamin K are cofactors in enzymes responsible for bone metabolism, collagen synthesis, and cross-linking. Vitamin D is metabolized to a steroid-like hormone that increases calcium absorption through a saturable, facilitated diffusion pathway. Mechanical loading from physical activity is essential to stimulate bone modeling to provide the stimulus necessary to develop a strong skeleton to support growth and development. In children and adolescents, the important focus is on bone accrual, with careful monitoring of growth parameters. Unfortunately, in contrast with adults, children and adolescents have not been the focus of research in many studies relating lifestyle factors to bone structure and strength.

Both physical activity and calcium had the strongest and most abundant evidence available, meriting priority action for public health efforts. Importantly, engagement in physical activity and adequate calcium intake are not achieved to recommended levels by many young people. A large difference in the nature of the evidence between physical activity and calcium is apparent. The evidence for physical activity and bone mass is a global approach, whereas the evidence for calcium and bone mass is a reductionist approach. The research available for physical activity does not try to individually examine the effects of various types of exercise, and few studies examine the dose loading effects of any one type of exercise. Even though physical activity is important for growing bone, the characteristics of physical activity that impact bone or other characteristics such as mode, frequency, intensity, and duration are not yet fully understood. What is known from RCTs will be presented later in this chapter as a best practices exercise prescription. On the other hand, evidence for diet is usually looking at a single nutrient effect with much less evidence for diet quality as a whole. There is opportunity for researchers in both fields to consider the approaches of the other field.

Macronutrients

Fat

The majority of studies examining fat intake and bone have been conducted in adults and the findings are equivocal with respect to improvements in bone mass [70]. Prospective studies and RCTs in children and adolescents are lacking, and it is premature at this time to draw conclusions regarding the influences of dietary fat on bone during growth.

Protein

It has been hypothesized that higher intakes of protein greater than the recommended dietary allowance (RDA) have a positive impact on bone growth and development. Furthermore, higher-protein diets and specific dietary proteins with higher sulfur-containing amino acids may lead to lower bone quality associated with potential renal acid load (PRAL). The majority of prospective [71–74] and cross-sectional [75–78] studies support a positive relationship between protein intake and bone. The Dortmund Nutritional and Anthropometric Longitudinally Designed Study [71, 72] demonstrated that protein intake was positively, and PRAL was negatively, associated with the geometrical properties of the forearm in a stepwise multiple regression model. Further supporting a positive effect of dietary protein on bone growth, data from the University of Saskatchewan Pediatric Bone Mineral Accrual Study showed that protein intake over approximately 8 years predicted total body BMC net gain [74]. The prospective studies collectively lend support for a positive effect of protein on bone in growing children; however, one study reported negative relationships between dietary protein intakes and bone in children that may have been linked with low calcium intakes [79]. Consistent with this notion, Vatanparast et al. [74] reported that the positive effect of dietary protein on bone mass is most evident in those consuming adequate calcium (>1000 mg/day). Higher dietary protein, accompanied by low calcium intakes (i.e., lower calcium/protein ratio), could lead to increased urinary calcium excretion [80] and

lower bone mass; however, RCTs are needed to prove this assumption.

Micronutrients

Calcium

The requirements for dietary calcium during the first two decades of life are driven primarily by skeletal growth. Although severe calcium deficiency in children and adolescents can cause rickets [81, 82] even moderate deficiency can lead to harmful effects on the skeleton. Balance studies have shown that calcium is a threshold nutrient (i.e., calcium retention increases with increases in intake until a plateau is reached). Race and sex differences are seen in the calcium intake plateau and the peak maximal retention in adolescents. In girls, maximal retention is highest in black girls, followed by whites and then Chinese-Americans [83–85]. The plateau intake is lower in Chinese-American girls than in white and black girls (1300 mg/day versus 970 mg/day). White boys have higher peak calcium retention rates than white girls, but similar intake at the plateau occurs [86]. Both maximal calcium retention rates and intakes for maximal retention are higher in Chinese-American boys than Chinese-American girls [85, 86]. Mexican-American boys and girls have similar rates of calcium retention [85]; however, Mexican-American boys and girls do not have different rates of calcium retention compared with non-Hispanic white boys, but their rates are higher than those for non-Hispanic white girls [87]. The adolescent RDA for calcium intake is based on the maximal retention for white adolescents [88].

Although many studies have been conducted to determine the effects of calcium on growing bone in humans, study designs are inconsistent regarding baseline calcium intake, calcium dose, skeletal outcome variables, and adjustment for size [89]. However, review of the current evidence finds that calcium supplementation consistently increases gain in skeletal mass and density measures in growing youth, usually between 1.0 and 5.0 % [90].

Vitamin D

Four of the nine vitamin D RCTs were conducted in females, primarily aged 10–17 years, and provided moderate evidence to support vitamin D supplementation effects on childhood and adolescent bone mineral accrual [91–95]. The RCT that presented the most compelling evidence was a Lebanese trial showing that supplementation with 2000 IU improved hip BMC [91] and geometrical properties of the femoral neck [92] in females, but not in males. The vitamin D effect was more pronounced in pre-pubertal or early pubertal versus post-pubertal girls, as well as in those with lower versus higher baseline 25(OH)D. Two RCTs demonstrated improvements in BMC after supplementation when using a compliance-based analysis [93, 94]; the beneficial effects of vitamin D supplementation on total body BMC were also shown, but only when vitamin D was combined with calcium [94, 95].

Several issues complicate the ability to compare the findings from these RCTs. For example, the doses used in the RCTs ranged from approximately 130–3200 IU/day, with no consistent dose response in the positive studies. In two RCTs, vitamin D was not the only test nutrient (i.e., calcium was also added). The assays used to measure serum 25(OH)D differed among RCTs and not all study laboratories participated in the Vitamin D External Quality Assessment Scheme. Diverse statistical approaches were used with analyses being presented as intent to treat or compliance based, and potential confounding factors varied among studies.

It is important to note that mean baseline serum 25(OH)D concentrations for all RCTs included in the review as well as in a recent NOF meta-analysis [199] ranged between 11 and 48 nmol/L, which is lower than the 50 nmol/L Institute of Medicine (IOM) cutoff used to define vitamin D sufficiency [96]. Even with deficient-to-low baseline serum 25(OH)D in these RCTs, vitamin D supplementation did not consistently promote BMC gains.

The osteogenic effects of calcitriol are attributed to its role in serum calcium homeostasis, partially through regulation of intestinal calcium

absorption [97]. Calcium absorption was not assessed in the RCTs included in this review, although one dose–response RCT in children entering the early stages of puberty with a mean baseline serum 25(OH)D of 70 nmol/L showed no effects of 12-weeks supplementation (400 IU to 4000 IU) on fractional calcium absorption [98]. These results are compatible with comments in a systematic review by Winzenberg et al. [99] that the beneficial effects of vitamin D on bone are less likely to occur in children with sufficient serum 25(OH)D. An increase in calcium absorption with vitamin D supplementation most likely occurs in children with low serum 25(OH)D concentrations, although the threshold or cutoff value is unknown.

Three critical gaps deserve further investigation. First, only one study was conducted in males, and it is therefore premature to make conclusions regarding sexual dimorphism with respect to vitamin D supplementation and bone. Second, subgroup analyses were conducted in several studies in an attempt to identify critical maturational periods during which supplementation may be most effective on bone. The results reported for premenarche, the early stages of puberty, or the postmenarche years were equivocal. Third, the majority of studies were conducted in white females. It is unknown whether the bone response to vitamin D supplementation differs by population ancestry.

Other Micronutrients

Magnesium, phosphorus, vitamin C, zinc, vitamin K, and other micronutrients play important structure-function roles for bone; however clinical trial evidence is limited to one RCT assessing the effects of magnesium. Supplementation with magnesium was found to increase hip BMC gains in a small group of young white females and only at one level of supplementation. Cross-sectional studies reported positive associations between vitamin C intakes and tibia and femur bone strength in girls [100] and vitamin C and femoral neck BMC in boys, but not girls [101]. The benefits of vitamin C in boys and not girls in the study by Prynne et al. [101] are most likely because the girls were more sexually mature. Benefits to younger girls were apparent in the study by Laudermilk et al. [100]. Moreover, zinc intakes were positively associated with femur and tibia cortical vBMD in young girls [100]. Phosphorous intakes may be a marker for intakes of either cola or protein, while dietary sodium intakes may negatively impact bone through their direct influence on urinary calcium excretion [102].

Food Patterns

The strongest dietary pattern associated with benefits to bone identified from this systematic review was for dairy products. Fruit and vegetable intakes were also positively associated with higher bone mass. The explanation for the benefit of fruit and vegetable intake to bone is not clear. The active constituents may be essential nutrients, such as potassium, magnesium, and/or vitamin C [101, 103]; bioactive ingredients from specific fruits and vegetables, such as flavonoids [104]; or their alkaline ash-forming properties.

A negative effect of carbonated beverage and cola consumption was found for BMC, aBMD, and bone strength. We observed a higher fracture rate in several cross-sectional analyses, especially in girls [196–198]. Whether this is due to milk displacement, a direct physiological effect, or both is not entirely clear [176, 178].

Novel fibers that influence the gut microbiome in ways that enhance calcium absorption and bone accrual are of recent interest [180–183]. In a 1-year trial in boys and girls, a combination of short- and long-chained fructo-oligosaccharides showed a significant benefit to BMC accrual [105].

Infant Nutrition

Breastfeeding during the first year of life has long been suggested to be optimal for infant nutrition; however, the available literature is conflicting in terms of bone accretion and fracture outcomes. Formula feeding may increase short-term BMC and bone mineral density (BMD) outcomes

[106, 107], possibly due to higher amounts of nutrients such as calcium in most infant formulas compared with breast milk (to note, infant formula contents likely vary between and within observational studies). An older landmark study [108] supports this hypothesis and suggests that during the first 6 months of life, bone accretion is less in infants fed human or low-mineral formula but is greater in the second 6 months of life. Data from observational studies are also inconsistent and give little insight into any potential effects of breastfeeding versus infant formula. Additional studies on the impact of the duration of breastfeeding on peak bone mass development are also needed since current observational data have shown inconsistent findings [109–112]. It is important to note possible confounding bias because mothers who breastfeed have been shown to adopt other healthy behaviors for their children. Data from the RCT assessing the effects of infant formula enriched with palm olein suggests positive effects in relation to total body BMC at 3 and 6 months [113]. However, observational data have demonstrated that children aged 4.5 and 10 years who consumed infant formula with either added palm olein or *sn*-2 palmitate during their infancy showed no difference in total body BMC [114, 115].

Body Composition

Lean body mass is among the strongest correlates of bone mineral accretion during childhood [116–119]. The concept of the functional muscle-bone unit speaks to the mechanism that underlies the strong association between lean mass and bone accretion [1, 120]. According to Wolff's law, bone adapts to the mechanical stresses to which it is exposed. These forces are largely generated through the exertion of muscle forces on bone. However, the relationship between BMC and lean mass changes as a function of puberty [121], and lean mass does not fully account for sex and race differences in cortical dimensions of the tibia (by pQCT) [60]. Both lean mass and bone mass have a strong heritable component, and the positive association of lean mass with measures of bone mass, density, and strength may be attributable, in part, to the fact that they covary due to genetic determinants.

The effect of fat mass on bone mineral accretion and attainment of peak bone mass are complex and controversial. The impact of weight-bearing activity on bone is greater as body weight increases, and overweight and obese children have greater amounts of both lean and fat mass. Fat mass is often a positive independent predictor of total body BMC [116, 122], but it may have negative effects on trabecular and cortical vBMD of the tibia by pQCT after adjustment for lean mass [123]. The source of adipose tissue (visceral versus subcutaneous) is also important; visceral adipose tissue may be deleterious to bone because of its metabolic effects. Gilsanz et al. [124] found that subcutaneous fat was positively associated with femur size and strength, and visceral fat had a similar but negative association. Other studies using DXA have similar findings [125, 126]. In the context of obesity, adipose infiltrations of muscle and bone marrow adversely affect bone [127]. The overall effects of childhood obesity on bone density and strength and fracture rates are inconsistent [128–133]. Furthermore, the effects of obesity may be site specific such that overweight or obese children have greater bone strength (as assessed by pQCT) in the tibia, but not the radius, compared to healthy weight children [133–135].

Special Issues

Alcohol

Despite the association between excessive alcohol intake and poor bone health, there is little evidence to suggest that alcohol intake among adolescents affects attainment of peak bone mass. However, the reported amount of alcohol consumed by adolescents studied was relatively low; thus, the impact of moderate to high alcohol intakes or binge drinking on bone health among adolescents is unknown.

Smoking

Studies that have examined the association between smoking and bone health in adolescence have been hampered by methodological challenges in quantifying smoke exposure and confounding due to other lifestyle factors associated with smoking. Despite methodological challenges, results of the studies reviewed in a recent NOF scientific statement [199] support the contention that smoking in adolescents may reduce peak bone mass. The strongest evidence comes from large studies of young adult military recruits, demonstrating that a history of smoking is associated with reduced bone density. Adolescents who smoke are more likely to smoke in adulthood. Thus, the deleterious impact of smoking during adolescence on bone health is likely to compound over time, increasing risk of osteoporosis and fracture later in life.

Contraception

Data are inconsistent regarding the effect of combined oral contraceptives (OCs) on bone density among adolescent females. OC pill use by healthy, white, teenage females did not show an affect on acquisition of peak bone mass in one study [136]. However, other studies examining the effect of low-dose estrogen OCs suggest otherwise. Some data suggest that long-term treatment of an ultra low-dose oral monophasic contraceptive (ethinylestradiol 20 μg + desogestrel 0.150 mg) may lead to suboptimal peak bone mass acquisition [137]. The skeletal effects of combined OCs are of larger concern in adolescents compared to adult females who have already attained peak bone mass. OCs suppress endogenous estradiol production by inhibiting activity of the hypothalamic-pituitary-ovarian axis. However, some studies have had inherent limitations such as lack of control for smoking status and other confounders, small sample size, and so forth [140].

Injectable depot medroxyprogesterone acetate (DMPA) is associated with hip and spinal skeletal deficits when initiated before peak bone mass acquisition. DMPA results in an estrogen-deficient state [141]. DMPA injections may also have selective glucocorticoid properties. However, weight gain while on DMPA may mitigate bone loss among adolescent users [142]. Skeletal losses in female adolescents receiving DMPA for contraception appears to be partly or fully reversible following discontinuation of DMPA, with quicker recovery rates at the spine than hip [143]. DMPA is still used commonly in adolescents, but with caution, given the potential skeletal implications.

Pregnancy and Lactation

Pregnancy and lactation are times of notable transfer of calcium from the mother to fetus and into breast milk. Because adolescents need to accrue bone for their own skeleton, there has been concern that pregnancy and lactation may potentially compromise peak bone mass attainment. Similar to that of adult women, physiologic alterations in calcium homeostasis occur in adolescents during pregnancy and lactation. Most notable are the increased intestinal calcium absorption during pregnancy [144] and decreased urinary calcium excretion during lactation [145]. Pregnant adolescents consuming a low calcium intake (≈500 mg/day) show even further reductions in urinary calcium excretion [145], illustrating the importance of physiologic compensations in the overall calcium economy.

Few data are available on the skeletal implications of pregnancy during adolescence. The most robust data come from the Third National Health and Nutrition Examination Survey, which found that by age 20–25 years, hip bone density did not differ in women with a history of adolescent pregnancy compared to nulliparous women [146].

Adolescents experience a 2–9 % decrease in aBMD during lactation, which is recovered after weaning [144, 145, 147–149]. This recovery occurs even in adolescents with calcium intakes <500 mg/day [145, 149].

Anorexia Nervosa

Adolescents and young adults with anorexia nervosa exhibit numerous hormonal alterations that affect bone and have a decreased fat mass

[150, 151], additionally compromising bone [152]. Adolescent girls with anorexia nervosa have reduced aBMD [153], suppressed bone formation and resorption markers [154], and reduced bone accrual [155]. aBMD was 1.0 SD lower in at least one skeletal site in 92 % and by at least 2.5 SD in 38 % of adult women with anorexia nervosa [156]. Adolescent boys with anorexia nervosa also have lower aBMD throughout the skeleton and weight recovery is not associated with consistent skeletal gains [157, 158]. One recent study showed that after 1 year of weight recovery in 79 adolescents with anorexia nervosa, DXA-acquired bone parameters had not been re-established to original levels [158].

Physical Activity, Exercise, and Peak Bone Mass

The evidence of a positive effect of physical activity on bone mass and density is strong. The evidence is less clear in support of a positive effect of physical activity on bone structure. Similar RCTs designed to assess bone structure have resulted in positive effects [37, 159–162], no effects [41, 163–170], and different effects based on gender or maturity status [42]. However, despite the inconsistencies in RCT results, the evidence provided by well-designed RCTs [37] and prospective cohort studies [171] supports a positive effect on structure, including those using objective measures of physical activity [172]. Unlike RCTs with bone mass and density outcomes, the multitude of structural measures, sites for measurement (distal, proximal), and inconsistencies in adjustment for bone size present a unique challenge in evaluating the quality of studies examining and interpreting exercise effects on structure. The recent NOF scientific statement reviewed RCTs on mass, density, and structure [199]. The authors noted that the RCTs shared a design limitation, which included an inability to adequately assess the physical activity levels of controls, the degree of effort in the exercisers, and the activity levels of the exercisers during periods of nonintervention. In short, issues of compliance were common threats to internal validity. In addition, physical activity interventions, in general, are susceptible to compensation effects (i.e., the intervention group does less physical activity outside of the intervention session to maintain a "normal" activity routine) [173].

There is a need to more precisely deliver the exercise dose and to understand the levels of physical activity in control and intervention groups. Laboratory-based work indicates an osteogenic effect at or above mechanical loads of 4.2 g-force [174], whereas RCTs suggest an osteogenic effect at or above 3.5 g-force [175, 176]. RCTs also suggest 3 days/week with 100 loads per session, and approximately 7 months of intervention are needed to detect change [175–177]. Due to the overlap in time and frequency in interventions that show change and those that do not, specific recommendations for these exercise dimensions (time and frequency) are equivocal [41, 42, 161, 168, 169]. At present, based on successful RCTs [42, 175, 176] a best practices exercise prescription for bone health in youth should be seen as at least 3.5 g-force, 100 loads per session, and 3 days/week.

Almost all RCTs used jumping as the primary exercise type. This is a sound decision because jumping is the gross motor skill that mechanically and non-repetitively loads the clinically important site of the hip via muscle loading during takeoff and via impact loading during landing. Animal and human studies have shown that jumping imposes a greater anabolic stimulus on bone than running or walking [176, 178], and the latter are activities commonly prescribed for metabolic health and obesity prevention. The known differences in types of physical activities for different targeted health outcomes suggest a need to promote physical activities that incorporate multiple motor skills (e.g., soccer, tumbling, tennis) or promote diverse physical activity patterns.

In addition to the above-described RCTs and prospective longitudinal studies, other types of research support physical activity as a causal factor for healthy bone mass, density, and structure [179]. Many of the mechanisms and pathways have been elucidated in laboratory studies [38, 178, 180] and the theoretical underpinning of why physical

activity is expected to influence mass, density, and structure is clearly described in Frost's mechanostat model [31, 32], which is well respected in the greater scientific community [181–183].

Research Gaps

Many questions have been identified that will drive a future research agenda. The following areas merit further investigation: differing effects of interventions depending on the life stage of growth, gene–environment interactions and how they may impact the development of peak bone mass, the need to identify and utilize biomarkers of exposure and effects, and the interaction of bone with other tissues throughout the body. In considering knowledge, it is important to recognize that a young skeleton with its open epiphyses differs from that of a fully grown adult skeleton after peak bone mass has been attained. Additional interventions and longitudinal studies are needed to understand the relationship between growth and measures of bone fragility and fractures and to identify lifestyle interventions that may prevent fractures during this period of susceptibility.

Implementation

Dietary intakes and physical activity levels of most Americans during the development of peak bone mass do not support maximal bone mass accretion for one's genetic potential. This increases risk for fracture both during childhood and later in life. Adherence to the US Department of Agriculture (USDA)/US Department of Health and Human Services (HHS) Dietary Guidelines for Americans and HHS Physical Activity Guidelines for Americans is an important and positive step toward ensuring healthy bone growth and/or maintenance throughout the lifecycle.

Diet

Shortfall food groups include dairy, fruits, and vegetables. Consequently, intakes of nutrients provided by these food groups often do not meet national recommendations. Dairy products provide most of the calcium and vitamin D in the diet as well as high-quality protein and significant amounts of other essential nutrients such as magnesium and potassium. In the US, 66 % of boys and 83 % of girls do not meet the recommended intakes of milk during the time of peak height velocity [184]. Low intakes of fruits and vegetables can lead to insufficient intakes of vitamins A, C, E, and K and potassium. Potassium has only recently been associated with bone health [103]. Additional research is needed to confirm why higher fruit and vegetable intakes seem to contribute to pediatric bone health. At present, continuing to advocate for children and adolescents to obtain recommended intakes of fruits and vegetables as described by the Dietary Guidelines for Americans has no downside, and it may offer a potential benefit toward attainment of peak bone mass.

Recommended intakes of vitamin D are particularly difficult to achieve without fortified foods or supplements. Enriched and fortified foods provide almost 60 % of dietary vitamin D and 30 % of vitamin A as well as substantial amounts of B vitamins and iron [185]. Fortified foods provide the greatest amount of the vitamin D in the US diet [88, 186]. Most US milk is voluntarily fortified with 100 IU of vitamin D per 8 ounce serving [88]. Breakfast cereals are often fortified with vitamin D, as are many brands of orange juice, yogurt, margarine, and soy beverages. Mandatory fortification of infant formula to contain a minimum of 40 IU and a maximum of 100 IU of vitamin D per 100 kcal (21 CFR 107.100) is present in the US. Low-income, overweight/obese, and minority populations of children and adolescents in the United States have been shown to have lower dietary intakes of both vitamin D and calcium [187].

Very few foods naturally contain vitamin D. Fatty fish (e.g., salmon, tuna, and mackerel) and fish liver oils are the best sources, whereas beef liver, cheese, and egg yolks contribute small amounts [88]. UV-treated mushrooms have the potential to provide vitamin D [188–190], but are scarce on the market.

There is recent growing interest in the possibility that intake of 25(OH)D, the metabolized form of vitamin D that is also present in animal foods such as meat, poultry, and eggs, may contribute to vitamin D status in humans [191]. Amounts of 25(OH)D in foods currently are not included in the USDA databases. Studies that have reported discrepancies between estimated vitamin D intakes and serum levels of 25(OH)D are driving the interest in determination of 25(OH)D in a plethora of food products.

Physical Activity

Regular physical activity in youth promotes healthier bones throughout childhood and adolescence. As part of the federally recommended ≥60 min of daily physical activity, children and adolescents should include bone-strengthening physical activity at least 3 days of the week. As mentioned above, bone-strengthening activities are those that are dynamic, moderate to high in load magnitude, short in load duration, odd or non-repetitive in load direction, and applied quickly [40, 192].

Although complete data are lacking, the IOM estimates that only about one-half of youth meet the current HHS Physical Activity Guidelines for Americans' recommendation for ≥60 min of daily moderate-to-vigorous intensity physical activity. The number of youth meeting this recommendation decreases with age [193] and precipitously declines in early adolescence, a time when bone appears most responsive to physical activity. Throughout childhood and adolescence, girls are less active than boys and are clearly missing opportunities to optimize bone health. The US Centers for Disease Control and Prevention (CDC) reports that as many as one-third of youth report no physical activity in the preceding 5 days [194]. Regrettably, participation in bone-strengthening physical activities is not measured in the CDC Youth Risk Behavior Surveillance System. Daily opportunities for incidental physical activity have declined for both children and adolescents as a result of factors such as increased reliance on motorized transportation, automation of activities for daily living, and greater opportunities for sedentary behavior. Disparities in opportunities for physical activity exist across racial, ethnic, and socioeconomic profiles [193].

Taking Action

A multilayered approach must be applied to achieving the recommendations of the Dietary and Physical Activity Guidelines for Americans.

Families

Adults must model healthy behaviors and engage with family members during mealtime and emphasize exercise. Government resources such as MyPlate and the Youth Physical Activity Guidelines Toolkit are informative resources for consumers of all ages.

Schools

Schools must continue to implement optimal nutrition standards through programs such as the National School Lunch Program and the National School Breakfast Program. Strategies for creating enhanced physical education, extracurricular physical activities, and active classrooms have been recommended by the 2012 *Physical Activity Guidelines for American's Midcourse Report*: *Strategies to Increase Physical Activity Among Youth* [195] and the recent IOM report *Educating the Student Body*: *Taking Physical Activity and Physical Education to School* [193]. Early knowledge of nutrition and physical activity through consumer sciences and physical education courses should be mandatory in school. Recess should be mandatory for every K–5 school.

Healthcare System

All allied healthcare providers should be required to be proficient in counseling for both nutrition and physical activity through their required curriculum and continuing education. Scientific groups such as the NOF, the Academy of Nutrition and Dietetics, the American Society for Nutrition, the American College of Sports Medicine, and the Society for Health and Physical

Educators should provide allied healthcare providers with tool kits and educational materials with consistent messaging on nutrition and physical activity for bone health.

Federal, State, and Local Policy

Government support for healthy growth and development should reach beyond obesity to antecedents of chronic disease, including osteoporosis. Subsidizing foods for bone health through programs such as Head Start, the National School Breakfast Program, the National School Lunch Program, the Supplemental Nutrition Assistance Program, and the Special Supplemental Nutrition Program for Women, Infants, and Children helps to ensure that all children and adolescents meet their requirements. Examples of how physical activity requirements are supported include the Federal Safe Routes to Schools Program and the Let's Move program, as well as public–private sector collaborations such as the NFL Play 60 Challenge and the Partnership for a Healthier America. Through zoning, incentives, and innovative cooperative agreements with businesses, local governments can help to ensure access to fresh foods as well as parks and youth recreation opportunities. Expanding successful government nutrition and physical activity programs as well as facilitating innovative collaboration between the public and private sectors is critical to transforming to a society in which bone health matters.

Conclusions

There is a vital need for more research focusing on children, adolescents, and the transition to young adulthood. Future research should consider sex, population ancestry, and maturation. The best evidence is available for positive effects of calcium intake and physical activity, especially during the late childhood and adolescence—a critical period for bone acquisition. Good evidence was also available for a role of vitamin D and dairy consumption, as well as a detriment of DMPA injections. However, more work is needed on physical activity dose response and the potential interaction between physical activity and diet quality. Weaker but physiologically plausible evidence is available and emerging for the effects of macronutrients and other micronutrients on bone among youth. It is important to address the factors most strongly linked to developing peak bone mass and strength from the current evidence through multilayered public health strategies. It is equally important to develop a research agenda to better understand other lifestyle factors that are less clearly understood for the purpose of building strong and healthy bones. Adherence to federal guidelines for intakes of nutrients and physical activity while cautioning against harmful behaviors is a priority strategy.

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28 Fracture Prevention Recommendations for Long Term Care

Hope A. Weiler

Abstract

Population projections estimate that almost 10 % of the population will be over the age of 80 years by 2063. Thus optimizing care for the frailest of the elderly will require effective novel treatments for common age related chronic diseases such as osteoporosis. Achieving healthy vitamin D status is emphasized in the treatment of osteoporosis. This chapter will address the growing need for new strategies in long term care in the prevention and management of osteoporosis using vitamin D intake as an example. The unique needs of the elderly including dysphagia will be highlighted along with strategies to reduce polypharmacy.

Keywords

Nutrition • Calcium • Vitamin D • Fractures • Osteoporosis

Introduction

In 2014, 15.7 % of Canadians were ≥65 years of age [1]. Population projections estimate that by year 2041 the life expectancy at birth for Canadian men approximates 81 years and for women 86 years [2]. Furthermore, as many as 23 % of the population will be over the age of 65 years by 2063 with almost 10 % represented by those over 80 years of age [3]. These estimates are similar to other high-income countries [4]. Thus optimizing care in the primary setting, which is often in a long term care facility for the frailest of the elderly, will require effective novel treatments for common age related chronic diseases such as osteoporosis. Vitamin D intake as a means to improve vitamin D status is highly regarded in the treatment of osteoporosis [5]. The Institute of Medicine (IOM) has updated the Dietary Reference Intakes for Calcium and Vitamin D [6]; modifying the Adequate Intake (AI) values to Estimated Average Requirements (EAR) and Recommended Dietary Allowances (RDA). For adults of over 70 years, the daily AI of 600 IU (15 μg) was replaced with a daily EAR of 400 IU

H.A. Weiler, RD, PhD
School of Dietetics and Human Nutrition,
McGill University, 21111 Lakeshore Rd,
Ste-Anne-de-Bellevue, QC H9X 3V9, USA
e-mail: hope.weiler@mcgill.ca

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(10 μg) and RDA of 800 IU (20 μg). The Upper Tolerable Intake Level (UL) was doubled to 4000 IU (100 μg) per day. Fracture risk was stated as the most important indicator of status due to associated mortality and morbidity risks. This chapter will address the growing need for new strategies in long term care in the prevention and management of osteoporosis using vitamin D intake as an example. The unique needs of the elderly including dysphagia will be highlighted along with strategies to reduce polypharmacy based on research into the preferences of residents in long term care.

Prevalence of Falls and Fractures and Entrance into Long Term Care

Recent NHANES 2005–2008 data suggest that the prevalence of osteoporosis in women over 50 years of age is lowest in non-Hispanic Blacks and highest in Mexican Americans [7]. Prevalence rates for osteoporosis in adults are estimated to be 9 % of the population and for osteopenia or low bone mass as high as 49 %. In Canada earlier estimates of osteoporosis suggest the prevalence of low bone mass is higher than in other countries located predominantly south of 40°N [8]. More recently through the Canadian Community Health Survey, spanning 2008 and 2009, a diagnosis of osteoporosis in adults 65 years of age over approximates 30 % for women and just below 10 % for men (Fig. 28.1). This coincides with a similarly high proportion of men and women experiencing falls (Fig. 28.1) that when combined with osteoporosis places them at very high risk for fracture.

Osteoporotic fractures most commonly arise in the vertebrae, Colles' fracture of the distal forearm or various regions of the hip. Even though all fractures will affect quality of life, hip fractures are associated with the highest morbidity and mortality as well as institutionalization [9, 10]. Over the period of 2007–2008, 57,413 osteoporosis related fractures were reported in Canada. The mean hospital stay was 14.5 days with hip fractures accounting for half of the hospitalization needs and just over half (53 %) of the total health care costs related to fracture [9]. Thus fractures represent a heavy cost to the health care system amounting to $1.2 billion yearly with 112,740 emergency visits during the same time. While hip fractures carry the heaviest mortality risk, wrist fractures are more common accounting for up to 85 % of emergency visits and a high proportion of same day surgeries (30 % vs 23 % for hip fracture). Following fracture and the initial hospitalization period, continuing care can be significant requiring almost 3 months of care through various health care systems. Upon fracture, approximately 65 % of adults were living in the community, whereas after fracture this number is reduced to 38 %. Life after fracture and discharge from acute care often means entrance into long term care, home care, chronic care or rehabilitation programs resulting in significant changes to autonomy [9]. While the majority of fracture cases come from the community, as many as 12 % or more emanate from long term care.

In view of the statistics above, it is therefore important in the primary prevention of osteoporosis and public health programs that suitable screening programs exist and that these are updated according to population needs, changing demographics (i.e., aging population) and research progress. In the USA, the Healthy People 2020 goals related to osteoporosis are to "prevent illness and disability related to arthritis and other rheumatic conditions, osteoporosis, and chronic back conditions" [11]. In Canada similar goals are not as specific but Health Canada and the Public Health Agency of Canada outline ways to minimize risk including eating well, being active, and falls prevention [12]. Few guidelines exist for those already in long term care to both prevent fractures or re-occurrences of falls and fractures. The need for best clinical practice guidelines both for the general population and those in long term care is thus high in view of the aging population and the high prevalence of falls, osteoporosis and fractures.

Clinical Practice Guidelines

Clinical practice guidelines for the primary prevention and management of osteoporosis include

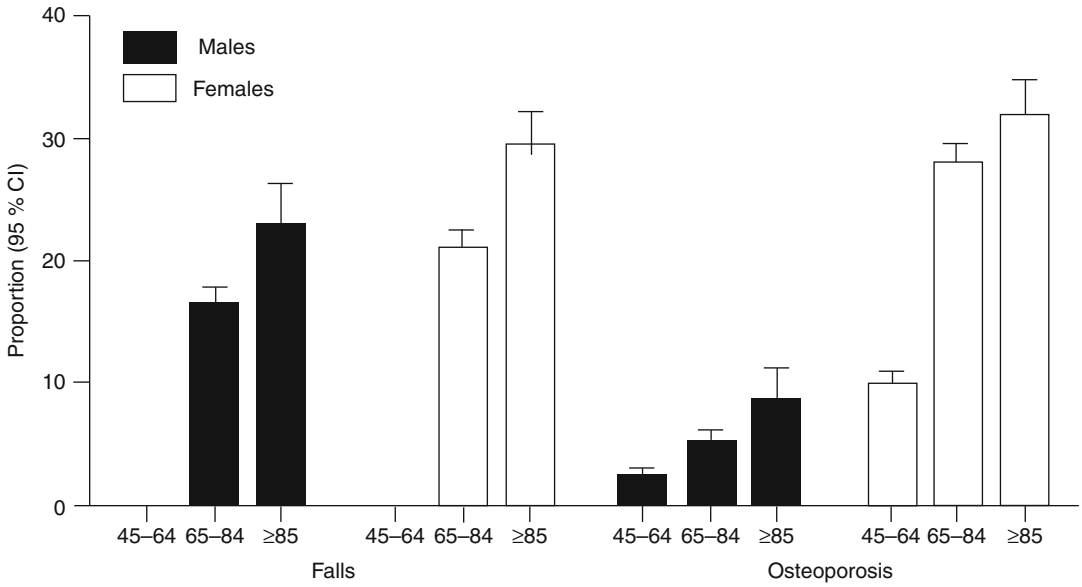

Fig. 28.1 Prevalence of falls and osteoporosis in men and women based on the 2008–2009 Canadian Community Health Survey data (Data source: Statistics Canada. Table 105-1200 – Health aging indicators, by age group and sex, household population ages 45 and over, Canada and provinces, occasional, CANSIM (Database), accessed June 2, 2015)

weight-bearing activity, calcium, and vitamin D and screening criteria [13]. Often clinical practice guidelines suggest higher intakes of calcium and vitamin D for high risk groups of the population whereas the EAR recommendation is recommended for the general population. The Canadian guidelines suggest for adults over the age of 50 years at moderate risk of deficiency, an total intake of 1200 mg of calcium including dietary sources is necessary along with 800–1000 IU of supplemental vitamin D and that dosages of 1000–2000 IU may be required and are considered safe [13]. When vitamin D is used as pharmacotherapy, assessment of vitamin D status 3–4 months later is advised. However, this may no longer be covered by the health care system if a diagnosis has not yet been made [14].

In both Canada and the U.S., screening for osteoporosis is part of the public health system. The U.S. Preventive services task force recommends that women of all races over 65 years of age, or those 50–64 years who's 10-year fracture risk is believed to be as high as a 65 year old with no other risk factors, should be screened for osteoporosis using dual-energy x-ray absorptiometry scans of the lumbar spine or hip [15]. This recommendation is grade B level, meaning that "there is high certainty that the net benefit is moderate or there is moderate certainty that the net benefit is moderate to substantial". At the time, the task force proclaims that there is insufficient evidence for such screening in men. According to the 2008 National Osteoporosis Foundation, men and women over the age of 50 years should be considered for treatment for osteoporosis [16]. The Canadian guidelines are similar, however these state that women and men over 50 years of age should be screened for risk factors for osteoporosis and fracture and that a bone density assessment be obtained in select individuals including those over 65 years of age as well as those with other clinical risk factors [13]. Whether these guidelines, that are designed primarily based on research of community dwelling older adults, are applicable to long term care has been questioned. In a review of studies supporting the Canadian guidelines, only 6 trials specifically included older adults living in long term care [17]. The other 96 % of studies were based on community-dwelling adults with median ages of 52–84 years;

thus excluding the oldest and frailest adults including those in long term care. Fracture risk in long term care is challenged by lack of clear guidelines and correspondingly many physician-perceived barriers to assessing fracture risk [18]. Barriers include lack of information and resources, difficulty obtaining patient information for fracture risk assessment and inconsistent prescribing of vitamin D and calcium at the time of admission [19].

Calcium and Vitamin D in Long Term Care

The most well studied age group with respect to vitamin D nutrition is adults, with emphasis on those over 65 years of age, although not necessarily those in long term care. Vitamin D supplementation trials have shown that higher intakes of vitamin D associate with higher vitamin D status, which is associated with improved bone density, tooth retention, fewer falls and some improvements in physical ability [20]. This research area is well reviewed in the National Institutes of Health systematic review [20] and thus not detailed herein. From that review however, the majority of studies have used 800 IU of vitamin D as the upper end of the dose–response [20]. The response of 25-hydroxyvitamin D (25(OH)D), the best known biomarker of vitamin D status, to vitamin D fortification of foods seems to be better than from supplements [20]. However, the benefits of food *versus* supplemental vitamin D on bone health have not been as rigorously tested in aging adults. Nonetheless, the importance of achieving total vitamin D intake at or above the EAR is viewed as being beneficial to preventing hip fractures in the long term when combined with sufficient calcium intakes [21]. The level of evidence is of a high grade indicating that further research is not likely to change confidence in the recommendations. In addition, another systematic review specifically in long term care indicates the rate of falls is reduced [rate ratio 0.63, 95 % CI 0.46, 0.86] when vitamin D is prescribed along with calcium or as a multivitamin [22]. It is thus not surprising that 71 % of physicians surveyed in one study in Ontario, Canada, felt there were no barriers to prescribing a vitamin D supplement; yet barriers to calcium supplementation were split 1:1:1 among no barriers, side effects or poor compliance [23].

Despite clinical guidelines and evidence to recommend calcium and vitamin D in aging, institutionalized Canadians often have low serum values of 25(OH)D (means of 40–45 nmol/L) and insufficient calcium and vitamin D intake despite health care supervision [24, 25]. Only two studies [26, 27] reported in Canadian long term care demonstrate intakes of calcium and vitamin D that approximate the recommendations (Fig. 28.2). The majority of vitamin D was from supplemental sources since food sources are limited; natural sources include fatty fish such as salmon, mackerel, tuna, plus small amounts in beef, eggs and irradiated mushrooms. Canadian legislation mandates fortification of milk and margarine with vitamin D [28]. Other foods with added vitamin D such as meal supplements, apple or orange juice and yogurts are available and provide small amounts of vitamin D per serving. It is thus not surprising that in other Canadian studies, where supplementation was not as common, calcium and vitamin D intakes were well below the EAR [24, 29–31]. Supplementation is important since exposure to ultraviolet beta radiation from the sun is limited in long term care. This is demonstrated in recent work [27] where institutionalized elderly participants (aged 84.9 ± 3.6 years; n = 30) were served 440 ± 200 IU/day of vitamin D with little variation over the seasons; the only evidence of endogenous synthesis was in August and it was very modest. In addition, this study emphasized that intakes are not in accordance with food provided. Actual consumption was 66 % of that served. Only one participant met the then daily AI of 600 IU once in 9 days. In fall/winter, ~40 % of participants had 25(OH)D concentration <50 nmol/L indicating that dietary or supplemental interventions are required. Surprisingly, more met the intakes for calcium, highlighting the need for vitamin D specific interventions. In other long term care studies, only 35 % received a vitamin D supplement and 20 % a multivitamin [32]. One approach could be

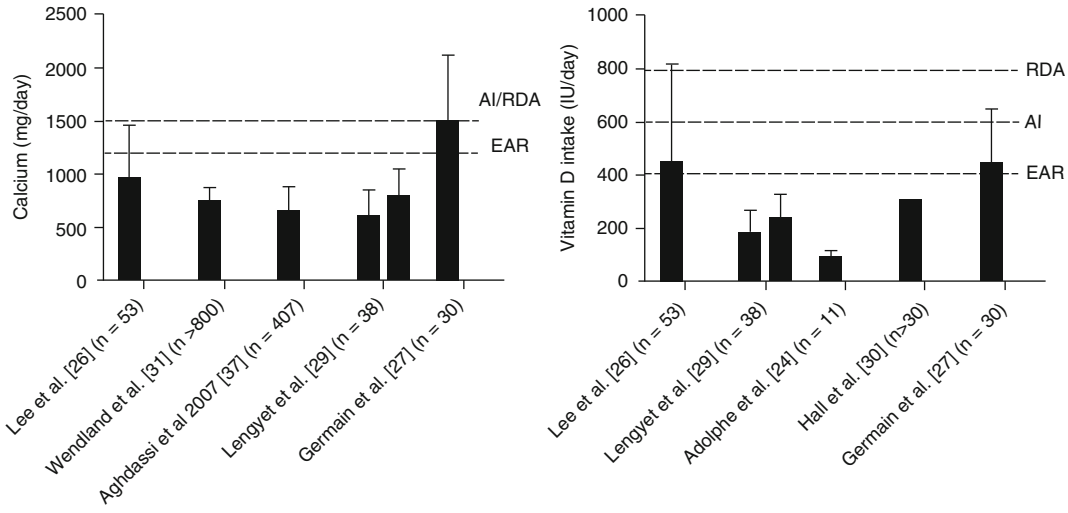

Fig. 28.2 Estimates of calcium and vitamin D intakes in residents of long term care in Canada. *Dashed lines* represent dietary recommendations set by the Institute of Medicine

to prescribe vitamin D to all current and in coming residents. However, vitamin D supplements contribute to polypharmacy which can exceed 11 prescriptions daily [27]. Other barriers to supplementation include oro-pharyngeal dysphagia that can be common among 7–40 % of residents in long term care [33]. While pills can be crushed and mixed with small volumes of food or a liquid formulation chosen, this still does not address the issue of polypharmacy.

To meet the RDA 800 IU of vitamin D per day, specially fortified foods for the elderly in long-term care are needed. Meta-analyses demonstrate vitamin D fortified foods and supplements improve vitamin D status [6, 20, 34]. One recent study suggests that specially fortified foods can help to increase vitamin D intakes in long term care [24]. Whether this results in improved vitamin D status and musculoskeletal outcomes is unclear. To date one small trial published in abstract form suggests that uniquely fortified foods are indeed advantageous and preferred in long term care both in terms of biological response using serum 25(OH)D and resident preferences [35]. In this study, control foods were compared to identical foods with 500 or 1000 IU of added vitamin D over 6 months in men (n = 60) on average 89 years of age. Study foods were designed to be bite-sized and no vitamin D supplementation from other sources was permitted during this time. The study spanned January through July including measurement of serum 25(OH)D using a chemiluminescence immune assay (Liaison, Diasorin Inc.). After 6 months of consuming the foods, 81 % of the participants indicated they preferred the food-based delivery over previous supplements in pill form. In addition, vitamin D status was maintained over the study period in the 500 and 1000 IU groups (74 ± 16.1–78 ± 17.3 nmol/L 25(OH)D, respectively) compared to control where a significant decline to 56 ± 10.3 nmol/L 25(OH)D was observed [36]. The 500 and 1000 IU groups not only prevented declines in 25(OH)D concentration, but demonstrated an upward significant trend from baseline values (500 IU: 65.2 ± 13.8; 1000 IU 69.6 ± 12.8 mmol/L 25(OH)D). This was enough to result in a positive dose-response in change in distal forearm bone mineral density over the 6 months as measured using a peripheral instantaneous x-ray imager (PIXI, Lunar GE) and measurement of the non-dominant arm (Fig. 28.3). Overall, this preliminary study provides support for the design of novel approaches to meeting vitamin D intake recommendations in the prevention and management of osteoporosis. Whether such intakes would also limit falls and fractures requires further study.

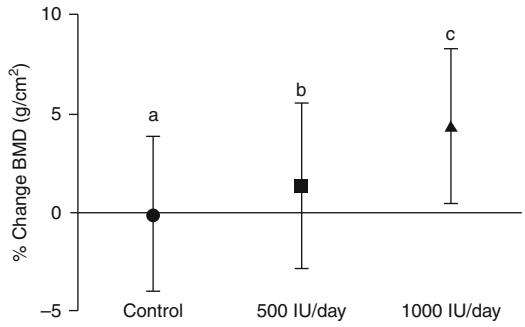

Fig. 28.3 Percent change in distal forearm bone mineral density (*BMD*) in men receiving control foods or the same foods with 500 IU and 1000 IU of vitamin D added. Different *superscript letters* indicate significant differences, $P<0.001$. Data are mean ± SD, n = 17 control, n = 19 500 IU and n = 17 1000 IU vitamin D groups, data are from 36

Conclusion

In summary, longevity of the population coincides with an increased prevalence of falls and osteoporosis in both men and women [1]. Falls and fractures increase reliance on long term care [9] where vitamin D intakes are typically too low to support bone health [24, 29–31]. Most health professionals agree that vitamin D intake should reach the recommended intake values in the prevention and management of osteoporosis [13, 23]. However, recommendations specific for long term care are lacking. New strategies to achieve vitamin D intakes in long term care should aim to address the issues of dysphagia [31] and polypharmacy [27] while considering preferences of food based supplementation.

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Promotion of Bone-Friendly Nutrition

29

Peter Burckhardt

The Problem

Although the scientific knowledge about the positive effects of certain nutriments and certain diets on bone has tremendously increased in the last two decades, its application to nutritional habits is difficult to achieve. Therefore it is of interest to discuss the possibilities and means for promoting this knowledge to the population. What is the most promising intervention and life stage for optimal bone health? Which is the best investment for public health? The target is the whole population, and certainly not only osteoporotic patients. Obviously, subsets of the population are at higher risk for developing or worsening osteoporosis than others, such as undernourished pregnant women or frail elderly persons, and need nutritional advice. But healthy children, adolescents, adults and older persons also should optimize their bone health by appropriate nutrition. However, the means applied to promote bone-friendly nutrition must be specific for each targeted subpopulation.

How can we as specialists and scientists, who are not necessarily trained in marketing, reach the general population, or at least the subgroup for which our specific knowledge is relevant? We have to use any available form of promotion, although we can hardly measure its impact, only its uptake. In addition we know that the impact of nutritional advices is weak when it is not enforced by the fear of a given disease such as diabetes or recurrent myocardial infarction. Even diseased obese patients hardly follow the nutritional advices they get. Despite these difficulties, promotion of bone-friendly nutrition has to follow closely the pleasing development of the awareness, that osteoporosis matters.

The Means

Publications

As scientists we are used to transfer our knowledge by publications. For an optimal uptake by the targeted subpopulation we have to choose the appropriate form of publication. Scientific papers and review articles reach mostly a medical reader only, but the medical specialist can use this knowledge for teaching, advising and treating patients.

Review articles in the lay press certainly reach the general population, but usually represent a one-day action, probably with a short influence, although the effort of writing such an article is considerable.

Articles in magazines probably have more impact, because magazines target a specific group of readers: house-wives, adolescents,

P. Burckhardt, MD
Internal Medicine, Clinique Bois Cerf,
Avenue D'Ouchy 31, Lausanne 1006, Switzerland
e-mail: p_burckhardt@bluewin.ch

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business-men, health-professionals etc. They offer the possibility to focus nutritional messages and advices to subsets of the population which might have a specific interest for picking them up.

Flyers are concentrated messages and, when well conceived and attractively illustrated, can be persuasive and of practical value. They have to be written for and distributed to a specific target population, such as children at school, young adults in their professional environment (work place, high school, university), adults at home, patients in the waiting room of their physician, general public or health professionals at their meetings etc.

Publications and flyers addressed to adults often contain lists and tables which are not very useful. For being applicable by the reader, they must contain the content of the given nutriment in the given substance, such as Calcium, Vitamin D, proteins, Potassium, acid load, etc., then size of a serving, and finally the content of a serving.

Courses and Conferences

We can easily give conferences on the results of nutritional research in the bone field to clinical professionals of all specialties, as long as we are invited to. This means, that we have to suggest nutrition as an appealing topic whenever we are involved in programming a medical meeting. By this we can develop an interest in nutrition in internists, gynecologists, endocrinologists, general practitioners and family doctors. Directly effective are the conferences we can and should give to lay audiences. In the frame of our own environment it might even be possible, to create such an opportunity.

Internet

The spectrum of targeted age-groups is probably wider by promoting nutritional messages on the internet. It reaches children as well as adults, and is widely used. For "Nutrition and Bone Health" an impressive number of websites are available, containing not only explanatory texts, but also lists, tables, recipes, and slideshows with photographs of appropriate food. The interest for a given website can be evaluated by the number of hits it gets.

Video clips promoting bone-friendly nutrition can not only be placed on internet, but also included in conferences and slide shows. They probably capture more easily the attention of children than texts or slides.

Practical Advices

Another form of teaching is the support of practical activities, not only by writing recipes, but also by organizing courses of cooking or common meals, for example after a course of gymnastic. The advice which products and nutriments to by at the supermarket, can be given by flyers or by articles in magazines. For diabetic and obese patients there are even dieticians in some supermarkets, who teach elderly and diseased customers how to read the instructions on the packages for making the appropriate choices. These dieticians could also be trained in bone-friendly nutrition.

In addition, frail persons and osteoporotic patients have also to be taught how to carry home the purchased goods or to by a caddy.

The Costs

The choice of the means applied obviously depends largely from the available financial support. TV clips are very expensive, video clips too, internet messages are more affordable, while publications in magazines are cost-free. The costs of flyers vary from cheap to expensive depending on the quality of the graphic support and the extent of the distribution. Teaching by conferences and lessons probably is the less expensive way of promoting knowledge, but is restricted to the audience gathered for this occasion.

Public Health Authorities

Public health authorities have to become aware, that the nutritional behavior of the population not only should respect the advices given for the

prevention of cardiovascular diseases, but that the decrease of the risk of osteoporosis by encouraging bone-friendly nutrition, is also cost-effective measure. But how can the scientific community reach the public health authorities? Connie Weaver stated at the ISNAO 2009, that "public health policies still favor for their policies the use of evidence based reviews which prioritize high quality randomized controlled trials" (Weaver CM, Hill KM. Estimating calcium requirements. In: Burckhardt P, Dawson-Hughes B, Weaver C, editors. *Nutritional influences on bone health*. London: Springer; 2010. p. 41–9). This means that the publication of high quality research is an efficient, indirect way of influencing health policies. But this way could certainly be enforced by publishing scientific data in journals which are read by health authorities. There are many, such as Public Health Nutrition, Journal of Public Health – an international one and many national ones, Evidence-based Healthcare & Public Health etc.

On the other hand it has to be reminded that messages which should reach Public Health Authorities need strong and suggestive terms.

Conclusion

Most members of the community engaged in research on the influence of nutrition on bone health are active in promotional activities. But eventually they are not aware of all the above mentioned forms of promotion, which all can be very efficient. They not only vary in their targeted population, but also in their affinity to the talents and knowledge of each author.

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