Peter Burckhardt
Bess Dawson-Hughes
Connie M. Weaver *Editors*

Nutritional Influences on Bone Health

8th International Symposium

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Preface

For the eighth time, specialists interested in the role of nutrition in bone development and preservation met in Lausanne, Switzerland, to hear and discuss recent research findings. Participants ranged from graduate students to senior research scientists. As in the first symposium, held in 1991, and each succeeding symposium, calcium and vitamin D dominated the program. Since the last symposium 3 years ago, controversies have arisen about the safety of calcium supplements and about the optimal amount of vitamin D needed for bone health. These and other aspects of calcium and vitamin D nutrition were addressed. New topics included the connections between fat, inflammation, and bone and the interaction of exercise and nutrition in relation to bone. Other sessions included the role of protein, with and without calcium, flavonoids, and the acid–base balance of the diet on bone and muscle. Presenters have generously provided proceedings of their presentations, which have been compiled herein. We hope that this book will be a useful compendium of the science related to areas of current interest in the field of nutrition and bone health.

Peter Burckhardt
Bess Dawson-Hughes
Connie M. Weaver

Acknowledgments

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Nutrition, Aging, and Chronic Low-Grade Systemic Inflammation in Relation to Osteoporosis and Sarcopenia

1

Robin M. Daly

Abstract

Aging is accompanied by a chronic low-grade systemic inflammation state, characterized by an increase in circulating levels in inflammatory mediators, that has been strongly implicated in the pathophysiology of common chronic diseases, including osteoporosis and fractures, sarcopenia, and disability. While a range of genetic, hormonal, environmental, and lifestyle factors have been reported to contribute to increased levels of inflammation, various dietary patterns, foods, and nutrients have also been reported to have anti-inflammatory effects, particularly in people with chronic diseases characterized by increased inflammation such as cardiovascular disease, type 2 diabetes, and cancer. With regard to musculoskeletal and functional outcomes, the findings from cross-sectional and prospective studies and randomized controlled trials on the effects of dietary/supplemental calcium, vitamin D, protein, vitamin K, omega-3 fatty acids, or their combination or food products such as dairy on markers of inflammation are mixed. Currently there is little or no evidence that these nutrients or foods attenuate circulating inflammatory cytokines in healthy middle-aged and older adults. In contrast, in people with chronic disease and/or increased inflammation, including those with osteoporosis and sarcopenia, a limited number of human intervention trials, mostly conducted over 12–16 weeks, have reported that calcium-vitamin D supplementation, high-dairy diets, and increased dietary protein, vitamin K, or omega-3 fatty acids alone or in combination with resistance training can produce modest reductions in inflammation. Whether these short-term reductions in inflammatory markers are clinically important and translate into positive effects on muscle and bone health and function or reduced disability remains unknown. Further randomized controlled trials in older

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adults and the elderly with or at increased risk of chronic disease are needed to evaluate the long-term efficacy of different nutrients or combination of nutrients and dietary interventions on markers of inflammation and their relation to musculoskeletal health outcomes.

Keywords
Inflammation • Osteoporosis • Fracture • Sarcopenia • Calcium • Vitamin D • Protein • Vitamin K • Omega-3 fatty acids • Aging • Cytokines

Introduction

It is well established that inflammation is part of the normal immune response to injury or infection. This rapid and acute process typically lasts 2–3 days and is tightly regulated to promote healing and restore homeostasis at damaged or infected sites. However, it is now recognized that aging per se is associated with changes in, and a dysregulation of, the immune system (termed *immunosenscence*), including its inflammatory components [1, 2]. Indeed, there is considerable evidence that aging is associated with a persistent increase in proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 (IL-6), IL-one-beta (IL-1β), and acute phase reactants [C-reactive protein (CRP)], and a reduction in anti-inflammatory cytokines, particularly IL-10 [3–6]. This age-related systemic, chronic, but low-grade inflammation has been termed *inflammaging* [7] and is typically characterized by a two- to fourfold increase in circulating inflammatory mediators (cytokines and acute phase proteins) in older people [8]. Although this inflammatory response is just one aspect of the multiple of changes occurring in the immune system with aging, there is consistent evidence that this chronic low-grade systemic inflammation contributes to the pathophysiology of many common chronic diseases, including cardiovascular disease, diabetes, arthritis, cancer, dementia, Alzheimer's disease, metabolic syndrome, as well as osteoporosis, sarcopenia, frailty, disability, and mortality [2, 9]. While many factors have been reported to contribute to *inflammaging*, including genetic, hormonal, lifestyle, and environmental factors, as well as the natural aging process itself, there has been considerable interest in identifying both pharmacological and non-pharmacological strategies to combat inflammation given its link to many common chronic diseases.

Regular physical activity and exercise is recognized as one modifiable safe and effective non-pharmacological approach to reduce systemic inflammation in older people [10, 11]. There is also a growing body of evidence supporting an anti-inflammatory effect of various diets, nutrient, and micronutrients, including calcium, vitamin D, protein, vitamin K, and omega-3 fatty acids, all of which can play an important role in optimizing bone and/or muscle health. The aim of this chapter is to provide an overview of the current evidence on the role of nutritional strategies for targeting inflammation and their putative effects on slowing bone loss or preventing osteoporosis and related fractures and age-related changes in muscle mass and function in older people.

Origins of Chronic, Low-Grade Systemic Inflammation

At present the underlying mechanism(s) contributing to the age-related "proinflammatory" state remains to be determined, but it is generally accepted that it has multiple origins. It has been hypothesized that it may be due to an impairment of the mechanisms that induce the inflammatory response [4] or a failure of anti-inflammatory mechanisms to neutralize inflammatory responses that are continuously triggered throughout life [1, 12]. Others have reported that a range of lifestyle and hormonal factors contribute to the age-related

increase in inflammatory markers, including smoking, obesity, inactivity, excessive alcohol intake, malnutrition, infection, stress, anxiety and depression, and decreased sex steroids [1, 2, 4, 13–15]. Oxidative stress, which represents an imbalance in oxidant and antioxidant levels, has also been reported to induce an increase in inflammatory markers [16, 17]. While it is beyond the scope of this chapter to review the role of oxidative stress on inflammation and disease, it is important to note that increases in inflammation and oxidative stress are closely linked, with the latter strongly implicated in the etiology of a range of musculoskeletal and age-related diseases [9, 17]. This chapter will focus predominantly on the effects of nutrition on common inflammatory mediators, including CRP, IL-6, TNF-α, IL-1β, and IL-10, and their link to bone and muscle health.

Since some inflammatory mediators are used as markers of disease risk, it has been suggested that the proinflammatory state in the elderly may be largely due to the presence of comorbidities [1, 4]. Currently there is still ongoing debate about whether chronic low-grade, systemic inflammation is a cause or an effect of age-related diseases and/or the aging process per se. In a cross-sectional study of 1,327 community-dwelling adults aged 20–85+ years, Ferrucci et al. [4] reported that a battery of inflammatory markers (serum IL-6, sIL-6, IL-1ra, IL-18, CRP, and fibrinogen) increased significantly with age in both men and women (Fig. 1.1, panels a and b). However, adjusting for cardiovascular risk factors and morbidity substantially reduced the effects of age of many of the markers (Fig. 1.1, panels c and d), indicating that part of the proinflammatory state in older persons is likely to be related to the presence of risk factors for disease. Despite these findings, there are reports that increased circulating inflammatory mediators are associated with the development of various diseases, including osteoporosis, sarcopenia, and reduced muscle function, independent of comorbidities [13, 15]. The following sections will provide a brief overview of the effects of low-grade systemic inflammation on age-related bone and muscle loss, osteoporosis, sarcopenia, falls, and fractures.

Chronic Low-Grade Systemic Inflammation, Sarcopenia, and Falls Risk

Sarcopenia is a term used to describe the age-related loss in muscle mass, strength, and function, all of which have been associated with an increased risk of falls, fractures, and frailty which can lead to a loss of independence and quality of life [18, 19]. While the precise mechanism(s) underlying the age-associated changes in muscle remains to be fully determined, it has been reported that chronic inflammation can exert a catabolic effect on muscle tissue. Indeed, numerous cross-sectional studies have found that elevated levels of various circulating inflammatory cytokines, including IL-6, TNF-α, and CRP, are associated with low muscle mass, size, strength, and power [20–22], reduced physical performance [21] and increased disability in older adults [23]. Several prospective studies conducted over 2–5 years have also reported that higher levels of inflammatory markers and their soluble receptors, which may be more representative of prolonged and severe inflammation, are associated with accelerated losses in muscle strength and lean mass [24–27] and a greater decline in function as measured by walking speed [28]. However, these findings are not consistent, and there is evidence that the association between higher levels of serum IL-6, CRP, and TNF-α with muscle loss is attenuated after adjusting for changes in weight [26]. This suggests that weight-associated changes (most likely fat mass) may play an important role in mediating age-related muscle loss.

Adipose tissue, particularly visceral fat, acts as an active endocrine organ that can secrete a large number of proinflammatory cytokines (termed *adipokines*) as well as attenuate the release of anti-inflammatory markers, such as adiponectin [29]. Thus, it has been hypothesized that the loss of muscle mass with age may be related to concurrent increases in visceral and intermuscular fat, mediated by increases in circulating levels of inflammatory markers and/or insulin resistance (Fig. 1.2) [30, 31]. That is, increased fat mass may result in a systemic "spillover" of inflammatory mediators to other organs/tissues and/or local

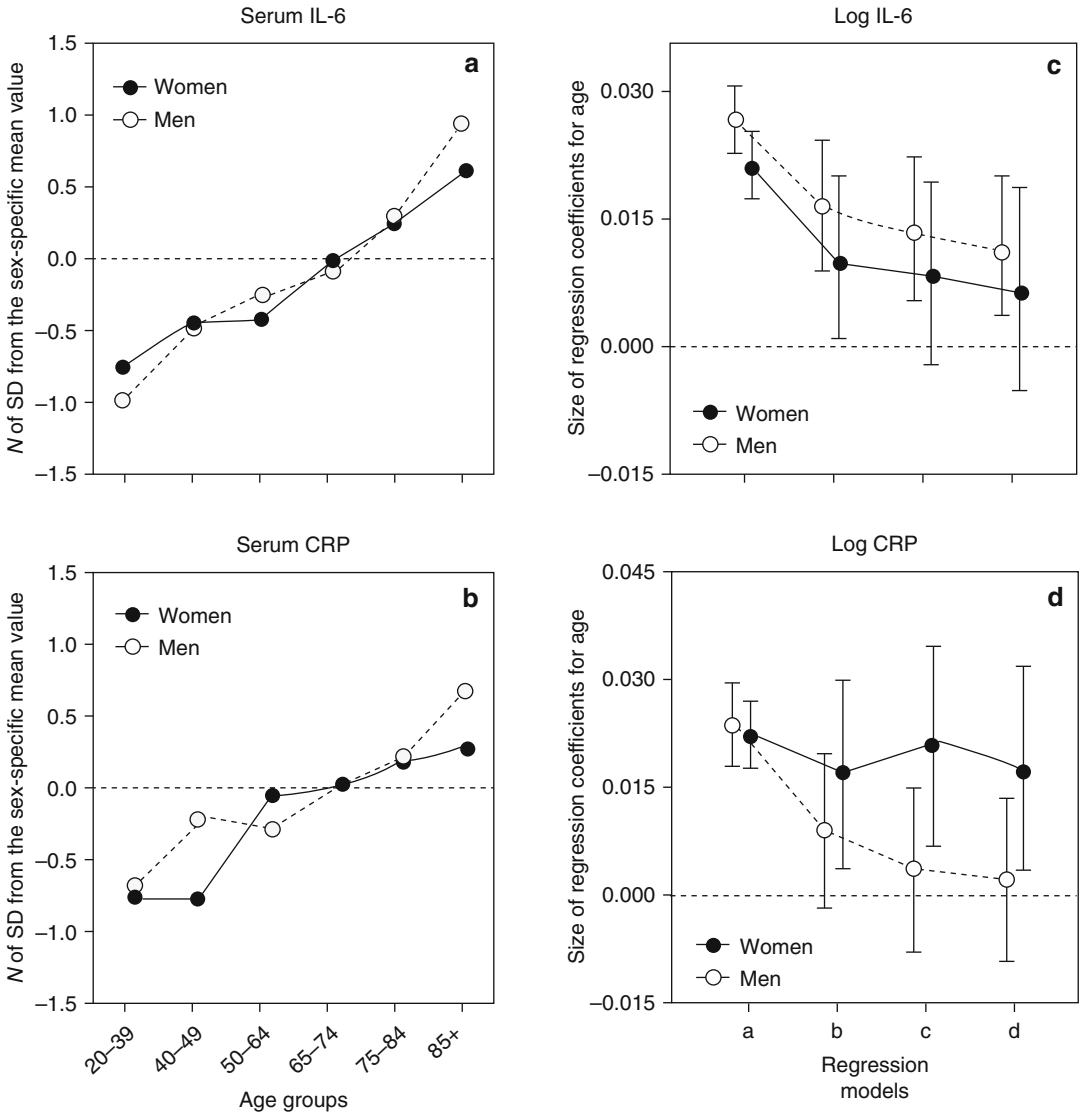

Fig. 1.1 Panels (**a**) and (**b**) show the mean serum IL-6 and CRP levels by sex and age groups expressed as number of standard deviations from the population mean. Panels (**c**) and (**d**) show the age regression coefficients and their 95 % confidence intervals estimated from linear regression models predicting level of IL-6 and CRP. Model "a" estimates the crude effect of age, model "b" is adjusted for cardiovascular risk factors, model "c" also adjusts for subclinical cardiovascular diseases, and model "d" is adjusted for major chronic diseases (CHD, CHF, stroke, PAD, COPD, diabetes, hypertension, osteoporosis, CRF, cancer, dementia, and depression) (Based on data from Ref. [4])

inflammatory reactions within the muscle itself that could have a catabolic effect by impairing muscle protein synthesis [9, 31, 32]. In addition, excess fat can also reduce the anabolic effects of insulin in stimulating muscle protein synthesis [31, 33]. Findings from the Health, Aging, and Body Composition study (Health ABC) involving 2,307 men and women aged 70–79 years showed that greater fat mass at baseline was associated with significantly greater loss in leg lean mass in both men and women over 7 years of follow-up [34]. However, the inclusion of various cytokines

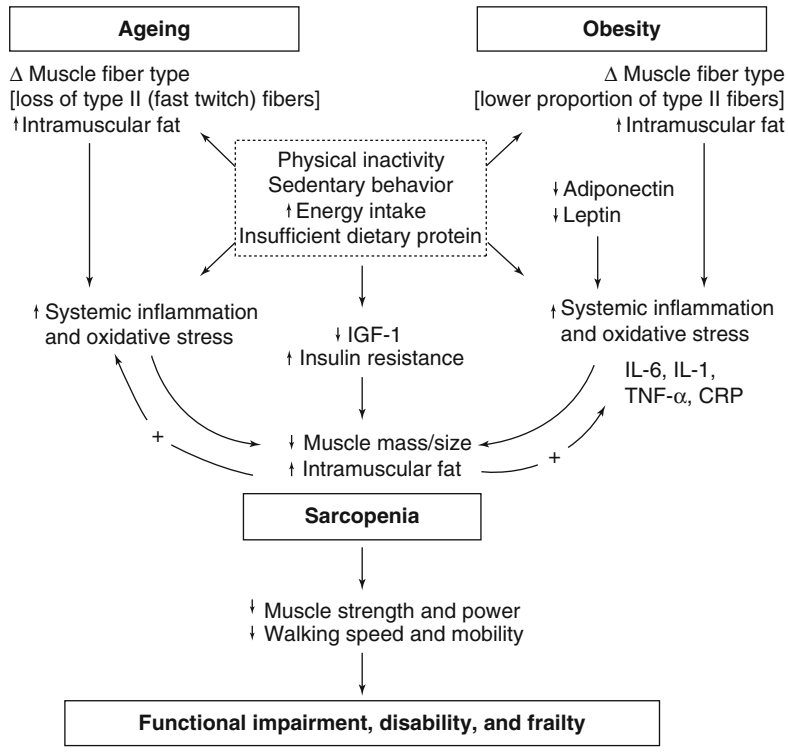

Fig. 1.2 A proposed model illustrating the key factors contributing to sarcopenia in obese people and older adults (*IGF-1* insulin-like growth factor-1, *IL-6* interleukin-6, *IL-1* interleukin-1, *TNF-α* tumor necrosis factor-α, *CRP* C-reactive protein). The symbol + denotes a positive effect (Adapted from Vincent et al. [31]. With permission from Elsevier)

or insulin resistance in the model did not change the results. This suggests that the accelerated loss of leg lean mass with greater fat mass was not related to higher levels of adipokines or insulin resistance, but further research is still needed to disentangle this interrelationship between muscle, fat, and inflammation.

It is widely recognized that multiple lifestyle, nutritional, and hormonal factors can contribute to the development of sarcopenia and its consequences, including decreased physical activity, inadequate energy intake and dietary protein, neurological changes, as well as age-related reductions in serum concentrations of sex steroids, growth factors [IGF-1], and circulating 25-hydroxyvitamin D [25(OH)D] levels [18, 19]. While it is difficult to ascertain the relative influence of each of these factors on age-related changes in muscle since they are often interrelated, a recent study examined the influence of both catabolic (serum IL-6, IL-1RA, TNF-α, CRP) and anabolic [serum insulin-like growth factor -1 (IGF-1), bioavailable testosterone, dehydroepiandrosterone sulfate (DHEA-S)] biomarkers on muscle loss after 6 years of follow-up in 716 men and women aged ≥65 years [35]. The main finding from this study was that having a greater number of elevated inflammatory (catabolic) markers was associated with a greater rate of decline in muscle strength than just having an increase in one inflammatory marker. In contrast, simultaneous reductions in several anabolic hormones were not associated with a greater strength decline than deficiency in just one marker alone [35]. Based on these findings, the authors concluded that a catabolic dysregulation is a major factor underlying age-related loss in muscle strength. However, as will be discussed in more detail below, there may be a synergy between certain anabolic and catabolic markers [20, 36]. For instance, women with low circulating IGF-1 levels and high IL-6 concentrations have been shown to be at higher risk of developing walking limitations and mobility disability than those with high IGF-1 and low IL-6 levels [36].

Chronic Low-Grade Systemic Inflammation, Osteoporosis, and Fracture Risk

Elevated circulating and local levels of proinflammatory cytokines have also been implicated in the etiology of age- and menopause-related bone loss, osteoporosis, and fractures. In vitro and experimental studies in animals have shown that various hormones and proinflammatory cytokines, including IL-6, TNF-α, IL-1, and IL-11, can interact to alter the bone remodeling balance [37–39]. More detailed information about the role of various proinflammatory and inhibitory cytokines on regulating osteoblast and osteoclast differentiation and activity is provided in several excellent reviews [37–39]. Briefly, the accelerated loss of bone associated with menopause and subsequent estrogen withdrawal has been related to an upregulation of proinflammatory cytokines by bone marrow and bone cells which can exert a catabolic effect on bone by stimulating osteoclastogenesis while simultaneously inhibiting osteoblast function through the regulation of the RANKL/RANK/OPG pathway [37–39]. It is important to highlight that these findings reflect the influence of cytokines at the local bone microenvironment, whereas most human studies measure circulating cytokine concentrations and relate these to BMD or fracture risk.

To date, the vast majority of human studies investigating the link between inflammation and bone have been cross-sectional, conducted in postmenopausal and older women and focused predominantly on the circulating cytokines CRP, IL-6, and TNF-α. While several of these studies reported that higher levels of high-sensitivity (hs)-CRP and IL-6 were associated with lower BMD in middle-aged and older adults [40, 41], others have failed to detect any significant association [42, 43] or differences in various cytokines between osteoporotic women and age-matched controls [44]. However, several recent prospective studies have reported that higher levels of circulating inflammatory cytokines were associated with increased bone loss [45–47]. In a study involving 168 healthy community-dwelling men and women aged 50–79 years who were followed for a mean of 2.9 years, Ding et al. [45] found that various inflammatory markers at baseline and their changes over time, particularly IL-6, were significantly associated with total body, lumbar spine, and hip bone loss. Others have reported that polymorphisms in cytokine genes [IL-1 receptor antagonist (IL-1ra), 174 GG polymorphism in IL-6], which alter the expression of a given cytokines, were associated with lower BMD, increased bone turnover, and fracture risk [48–50]. In addition, studies using antibodies against specific cytokines and/or their receptors or animals that do not express IL-6 provide further evidence implicating inflammation in bone loss [51–53].

Chronic, low-grade systemic inflammation has also been associated with an increased risk of osteoporotic fracture. In the Health ABC study involving 2,985 well-functioning white and black women and men aged 70–79 years, increased serum levels of inflammatory markers, particularly high receptor levels of proinflammatory cytokines, predicted a higher incident of fractures over 5.8 years of follow-up, independent of known risk factors including BMD. The finding that cytokine soluble receptors strongly predicted fracture risk is important because they may be more representative of a prolonged and severe underlying inflammatory state compared to the markers themselves which have a short half-life and often change transiently [54]. Another important finding from this study was that the risk of fracture was even greater in participants with two or more inflammatory markers in the highest quartile. This suggests that measuring several inflammatory markers may improve risk assessment compared to a single marker alone. While this study was limited to an assessment of all nontraumatic fractures, similar findings were observed in a recent nested case-control study with hip fracture as the outcome [55]. In this study, women aged 50–79 years with elevated levels (highest quartile) of inflammatory markers for all three cytokine soluble receptors (IL-6 SR, TNF SR1, and TNF SR2) had a 2.4- to 2.8-fold increased risk of incident hip fracture over a median of 7.1 years of follow-up compared to women with zero or one high inflammatory marker

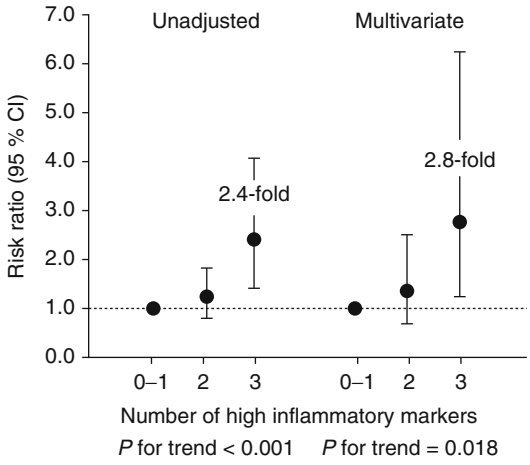

Fig. 1.3 Risk ratios with 95 % confidence intervals (CI) of hip fracture, according to the number of high inflammatory markers in the top quartile based on the distribution of cytokine soluble receptor concentrations among the controls (Based on data from Ref. [55])

(Fig. 1.3). Together, these findings highlight that identifying strategies to reduce inflammation may represent an important approach to reduce bone loss and fracture risk in the elderly.

Nutritional Strategies to Combat Inflammation and Musculoskeletal Disease

Exercise, weight loss, and caloric restriction are recognized to play an important role in reducing chronic systemic inflammation, but maintaining a healthy diet has also been associated with lower circulating concentrations of inflammatory markers. As discussed in a comprehensive review on the influence of dietary factors on low-grade inflammation [1], there is sound evidence to support an anti-inflammatory role of healthy eating patterns, such as the Mediterranean diet and vegetarian diets, and specific dietary factors, including whole grains, fruit and vegetables, fish and omega-3 fatty acids, and certain vitamins. From a disease-specific perspective, the vast majority of research into the role of diet on inflammation has been conducted in people with cardiovascular disease, type 2 diabetes, and cancer, all of which are characterized by increased levels of inflammation. This chapter will provide an overview of the evidence on the influence of nutrients specific to musculoskeletal health, such as calcium and dairy foods, vitamin D, protein, vitamin K, omega-3 fatty acids, or a combination of these factors, on inflammatory markers and their association to bone and muscle health.

Calcium and Dairy Foods

It has been reported that an increased intake of dietary calcium or a high-dairy diet may reduce inflammation (and oxidative stress), particularly in overweight and obese people, by promoting lipid metabolism and a loss of body fat [56–58]. Laboratory studies in rodents have shown that dietary calcium can inhibit lipogenesis and induce lipolysis by reducing 1,25-dihydroxyvitamin D-induced calcium signaling in adipocytes [59, 60]. While others contend that the effects of dietary calcium on lipolysis and/or lipogenesis are equivocal, there is consistent evidence that dietary calcium can promote modest weight loss through increased fecal-fat excretion [61]. Since obesity and related disorders are associated with increased inflammation, it has been proposed that calcium may play a key role in modulating adipose tissue cytokine production [56, 62]. This is supported by research in mice which has shown that high-calcium diets can decrease the expression of proinflammatory factors, such as TNF-α and IL-6, in visceral fat, adipocytes, and plasma and stimulate the expression of the anti-inflammatory marker IL-15 as well as adiponectin [56, 62]. In the same rodent model, it was also reported that a high-dairy diet (nonfat dry milk) may be more effective for reducing inflammation and oxidative stress than supplements (calcium carbonate) [62]. Together, these findings provide evidence that increased calcium or a high-dairy diet can modulate adipocyte cytokine production in a mouse model of obesity, but less is known about the influence of dietary and supplemental calcium or a high-dairy diet on inflammation in humans.

It is well known that the beneficial effects of calcium supplementation on maintaining or slowing bone loss and preventing osteoporotic fractures

are associated with suppression of parathyroid hormone (PTH). Since PTH can regulate circulating levels of IL-6 and TNF-α, which stimulate production of CRP [63], it has been suggested that the benefits of calcium supplements on bone could be mediated in part by a reduction in inflammation. To our knowledge, only one study has investigated the anti-inflammatory effects of calcium supplementation alone without other therapies. In a 12-month randomized controlled trial in healthy postmenopausal women that were part of a larger study of calcium supplementation on fracture incidence, Grey et al. [64] reported that supplementation with 1,000 mg/day of calcium citrate had no effect on CRP levels. Several intervention trials which have evaluated the effects of calcium plus vitamin D supplementation on inflammation in relatively healthy older adults also observed no improvements in inflammatory markers. For example, Gannage-Yared et al. [65] observed no effect of calcium (1,000 mg/day) plus vitamin D (800 IU/day) supplementation on circulating IL-6, TNF-α, or CRP concentrations in 47 healthy postmenopausal women in a 12-week randomized controlled trial. Similarly, secondary analysis of a 3-year randomized, double-blinded, placebo-controlled trial in adults aged ≥65 years showed that calcium (500 mg/day) plus vitamin D (700 IU/day) supplementation had no effect on either circulating CRP or IL-6 levels, despite beneficial effects on BMD [66]. In contrast, the findings from a recent pilot, randomized, double-blind, placebo-controlled, 2×2 factorial clinical trial in a high-risk patient group [colorectal adenoma patients (n=92)] showed that supplementation with calcium (2,000 mg/day), vitamin D (800 IU/day), or the combination for 6 months led to a 8–50 % nonsignificant reduction in various proinflammatory markers compared to placebo controls [67]. While the lack of a statistically significant effect is likely due to the small sample size, when they examined the effects of calcium and/or vitamin D on a combined inflammatory marker z-score that included all six measured cytokines, they found that the z-score decreased by 77 % in the vitamin D-only group (P=0.003); the change in the calcium (48 %, P=0.18) or combined group (33 %, P=0.40) was not significant. These preliminary findings provide some evidence that calcium and/or vitamin D supplementation may help to ameliorate inflammation in "high-risk" individuals with diseases known to have an inflammatory pathogenesis, but further intervention trials with adequate sample sizes are needed to test this hypothesis.

Since dairy foods contain additional factors that may have anti-inflammatory properties, such as angiotensin-converting enzyme inhibitors, vitamin D, protein, and related bioactive peptides, a number of studies have investigated the acute and long-term effects of a high-dairy diet on inflammation (and oxidative stress). In a retrospective analysis of archival samples from two clinical trials, Zemel and Sun [62] reported that high-dairy (~1,100–1,200 mg/day of calcium) compared to low-dairy (~400–500 mg/day of calcium) diets were effective in suppressing CRP levels and increasing adiponectin during weight loss and maintenance in obese adults. However, these findings must be interpreted with caution because of the concomitant reductions in adiposity. In a subsequent acute (28-day) blinded, randomized, crossover trial comparing a high-dairy (three daily serves, calcium 1,200–1,400 mg/day) versus soy-based placebo diet (calcium 500–600 mg/day) in overweight and mildly obese adults, circulating TNF-α and IL-6 levels decreased (as well as markers of oxidative stress) and adiponectin increased in the high-dairy group; the opposite effect was observed in the soy-protein group [58]. Given the crossover design and the lack of any changes in body composition in this study, these findings provide some confirmation that an increase in dairy food intake, even over a short period, may represent an effective strategy to reduce inflammation (and oxidative stress) in overweight and obese adults. Consistent with these findings, the results from a 12-week randomized controlled trial comparing an adequate-dairy (~3.5 daily serves) versus low-dairy (<0.5 daily servings) weight maintenance diet showed that the adequate-dairy diet significantly attenuated markers of inflammatory and oxidative stress in adults with metabolic syndrome [68]. Similarly, 12-weeks of supplementation with a vitamin D or calcium-vitamin D-enriched yoghurt drink reduced various

inflammatory markers (IL-1β, IL-6, fibrinogen, hs-CRP) and increased adiponectin in adults aged 30–60 years with type 2 diabetes [69]. In contrast, a 6-month trial in middle-aged and older adults with metabolic syndrome reported no effect of increased dairy intake (3–5 portions of dairy products daily) on markers of inflammation, adiponectin, or oxidative stress [70]. However, in this study the baseline calcium intake in the dairy group was 815 mg/day, which may have been too high to observe any effects from the additional dairy products. Taken together, these findings support previous work indicating that high-calcium or high-dairy diets may be most effective for attenuating inflammation in individuals with an inflammatory-related chronic disease(s).

To date, few studies have examined the anti-inflammatory effects of a high-dairy or calcium-vitamin D-enriched diet in relatively healthy older adults. In an 18-month, factorial 2 × 2 design randomized controlled trial in healthy middle-aged and elderly men which was designed to examine the independent and combined effects of exercise and calcium-vitamin D-fortified milk on bone and muscle health, we previously reported that serum IL-6 concentrations tended to increase in men who received low-fat, calcium (1,000 mg/day)-vitamin D_3 (800 IU/day)-fortified milk and decrease in those assigned to the exercise training [71]. However, these between-group differences did not persist after adjusting for changes in fat mass; there was no effect of the fortified milk or exercise on serum TNF-α or CRP. While the lack of an effect in this study may be explained in part by the high baseline calcium (~900 mg) and serum 25(OH)D levels (~85 nmol/L) in the men, the finding that changes in circulating IL-6 concentrations were largely dependent on changes in fat mass provides further evidence that calcium and/or vitamin D may be most effective for reducing inflammation in overweight or obese individuals or those with other chronic diseases.

Vitamin D

Vitamin D is recognized to have immunomodulatory effects with 1,25-dihydroxyvitamin D_3, the biological active form of vitamin D, shown to influence the differentiation and function of both innate and adaptive immune cell types and to modulate cytokine production (for a recent review refer to Hewison [72]). While the precise mechanism(s) by which vitamin D might attenuate inflammation is not clear, in vitro data suggests that the anti-inflammatory effects of vitamin D may be mediated by 1,25-dihydroxyvitamin D_3 coupling to the vitamin D receptor, which is present in many immune cells, to downregulate or transrepress inflammatory cytokines; various immune cells also have the capacity to regulate the activity of 1-α-hydroxylase, which converts 25(OH)D to 1,25-dihydroxyvitamin D_3 [69, 73]. Data from human epidemiological studies also supports a relationship between vitamin D status and various autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes [72]. However, the findings from observational and epidemiological studies examining the association between serum 25(OH)D concentrations and various inflammatory cytokines in asymptomatic adults or those with common chronic disease(s) are mixed [74–78].

In relatively healthy adults, it has been proposed that any association between vitamin D and inflammation may only exist at low serum 25(OH)D levels. To test this hypothesis, Amer and Qayyum [74] examined the relationship between circulating 25(OH)D and CRP concentrations in 15,167 asymptomatic adults aged ≥18 years involved in the NHANES survey from 2001 to 2006. Using linear spline regression analysis, with a single knot at the median serum 25(OH)D concentration of 21 ng/mL (52.5 nmol/L), they found that there was an inverse relation between serum 25(OH)D at levels <52.5 nmol/L and CRP in both the univariate and multivariate analyses adjusting for traditional cardiovascular risk factors. However, in the multivariate analysis, they also observed a positive relationship between CRP and 25(OH)D at levels above 52.5 nmol/L, suggesting that higher concentrations of 25(OH)D may be proinflammatory. While it is difficult to explain this latter finding, a limitation of this study is that only a single inflammatory marker was assessed and there was no adjustment for

geographic location or time of year. Furthermore, since this was a cross-sectional study, causality cannot be inferred, and thus randomized controlled trials are needed to determine the effects of vitamin D on inflammation.

To date, randomized controlled trials examining the effects of vitamin D supplementation on markers of low-grade inflammation in both healthy young and older adults and those with various chronic diseases have produced inconsistent findings. Most of the studies which have reported a positive effect of vitamin D supplementation on pro- and/or anti-inflammatory markers have been conducted in people with chronic disease, including chronic heart failure [79], chronic kidney disease [80], type 2 diabetes [81], multiple sclerosis [82], those with prolonged critical illness [83], and osteoporosis [84]. In the study in postmenopausal women ($n = 70$) with osteoporosis, 6 months of supplementation with 0.5 μg/day of calcitriol and 1,000 mg/day of calcium significantly increased lumbar spine, trochanteric, and intertrochanteric BMD and reduced serum IL-1 and TNF-α levels; there were no changes in the calcium-alone group [84]. It is difficult to compare these findings with other trials since the active vitamin D metabolite (calcitriol) was used. However, in the study by Pittas et al. [66] reported earlier, combined calcium (500 mg/day) plus cholecalciferol (700 IU/day) supplementation for 3 years had no effect on circulating CRP or IL-6 levels in subjects with normal or impaired fasting glucose, despite improvements in BMD. Based on these limited findings, it is difficult to make any conclusion as to whether the beneficial effects of vitamin D (or calcium plus vitamin D) on bone health and fractures are mediated, at least in part, by their effects on inflammation.

In recent years a number of intervention trials have investigated the effects of different doses of vitamin D on inflammatory markers in healthy adults and those who are overweight or obese. In a 6-month weight loss trial in healthy overweight adults with low 25(OH)D concentrations (mean 30 nmol/L), supplementation with 83 μg/day (3,332 IU/day) of cholecalciferol resulted in a more pronounced reduction in serum TNF-α, but not IL-6 or CRP, compared to weight loss alone [85]. In contrast, supplementation with 4,000 IU/day for 12 weeks in healthy overweight and obese adults with serum 25(OH)D levels around 50 nmol/L participating in a resistance training program had no effect on circulating inflammatory markers [86]. Similarly, incremental doses of 5, 10, and 15 μg/day of vitamin D (200, 400, and 600 IU/day) for 22 weeks throughout winter did not alter cytokine concentrations in either healthy young (aged 20–40 years) or free-living older adults (aged ≥64years) [87]. There are several likely explanations for this finding: (1) the baseline median 25(OH)D concentrations were 71 and 55 nmol/L in the young and older adults, respectively, which many consider to be sufficient; (2) the vitamin D dosing regimen did not improve serum 25(OH)D levels in the young adults, and the 400–600 IU doses only increased levels to ~70 nmol/L in the older adults; and (3) the baseline cytokine levels were already very low prior to the intervention, providing little scope for improvement.

It has been suggested that serum 25(OH)D concentrations as high as 80–100 nmol/L may be required for optimal immune function [88]. Thus, a recent study investigated the effects of high-dose vitamin D supplementation (40,000 or 20,000 IU of vitamin D_3 per week or a placebo) on markers of inflammation in 437 healthy overweight adults aged 21–70 years with a mean serum 25(OH)D concentration of 56 nmol/L [76]. Despite marked increases in serum 25(OH)D levels in the supplemented groups [median 25(OH)D levels at follow-up were 141 and 98 nmol/L for the 40,000 and 20,000 IU dose groups, respectively], there were no between-group differences for the change in a panel of 11 inflammatory cytokines after 12 months. These findings are consistent with previous research suggesting that the immunomodulatory effects of vitamin D (and other nutrients) may only be seen when the immune system is stimulated, as is evident in people with a chronic disease(s), and/or when circulating 25(OH)D levels are insufficient. Since many questions still remain as to the optimal level of serum 25(OH)D needed for health benefits, further long-term clinical trials should

also investigate if there is a concentration of 25(OH)D and dose of vitamin D that might be effective for improving immune function.

Protein

Adequate dietary protein is considered to be essential for the maintenance of both bone and muscle health, which has been largely attributed to the positive effect of protein on IGF-1 levels [89]. However, it has also been suggested that increased dietary protein may have an indirect effect on muscle and bone via a reduction in inflammation mediated by an increase in IGF-1; high levels of IL-6 decrease circulating levels of IGF-1, and low levels of IGF-1 stimulate IL-6, indicating that IL-6 may oppose the effect of IGF-1 on muscle and bone [20, 90]. There is also evidence that TNF-α can interfere with IGF-1 signaling and inhibit the signaling pathways downstream of the IGF-1 receptor and thus decrease muscle protein synthesis [91].

The loss of muscle with advancing age results from an imbalance between muscle protein synthesis and muscle protein breakdown. In older adults and the elderly, it has been reported that the stimulatory effect of an anabolic stimuli such as protein (amino acids) on muscle protein synthesis is blunted, an effect that has been coined *anabolic resistance* [92, 93]. To stimulate muscle protein synthesis and promote a positive net protein balance to optimize muscle health, it has been suggested that older adults require a higher daily dietary protein intake, particularly when undertaking progressive resistance training [92, 93]. A comprehensive review on the type, dose, and timing of protein needed to enhance muscle protein synthesis and increase or maintain muscle mass in the elderly is provided in several recent reviews [92, 93]. While the precise cause(s) of anabolic resistance in aging muscle remains unknown, studies in both rodents and humans have reported that an increase in proinflammatory markers is associated with a decrease in muscle protein synthesis [94, 95]. By blocking low-grade inflammation using a nonsteroidal anti-inflammatory drug (ibuprofen), Rieu et al. [96] found that the anabolic effects of food intake on muscle protein metabolism were maintained in older rats resulting in a decrease in muscle mass loss. Together, these findings support the results from several human prospective studies discussed earlier which showed that higher levels of inflammation were associated with accelerated losses in muscle mass and strength in older adults [24–28].

To our knowledge, few human studies have investigated the interactive effects of IGF-1 and inflammation on muscle or bone health. Data from a population-based study involving 526 adults aged 20–102 years found that there was a reciprocal relationship between IGF-1 and IL-6 on muscle strength and power [20]. In the analysis stratified according to tertiles of IL-6, serum IGF-1 was positively related to both muscle strength and power only in those in the lowest IL-6 tertile. Cappola et al. [36] also reported that a combination of low IGF-1 and high IL-6 levels was associated with an increased risk for incident walking limitation, mobility disability, disability in activities of daily living, and death in older women. To examine whether a synergistic relationship exists between dietary protein, inflammation, and changes in muscle strength in the elderly, Bartali et al. [97] followed 598 men and women aged 65 years and over for 3 years. Interestingly, dietary protein intake at baseline was not associated with changes in muscle strength, but there was a significant interaction between protein intake and serum CRP, IL-6, and TNF-α on changes in muscle strength. That is, in those with high levels of inflammatory markers, a lower protein intake was associated with a greater decline in muscle strength, independent of the presence of chronic conditions. These findings indicate that chronic inflammation can alter protein metabolism and may reduce the efficiency of protein on muscle, but this needs to be confirmed in a clinical trial.

There are few intervention studies which have examined whether a high-protein diet or protein supplementation can reduce inflammation, either directly or indirectly via an increase in IGF-1, and whether any subsequent changes in cytokine concentrations are related to changes in muscle mass,

strength, and/or function. A recent systematic review and meta-analysis on the effects of higher- versus lower-protein diets on a range of health outcomes reported no significant effect of higher-protein diets on circulating CRP levels [98]. However, the results from an 18-month randomized, open-label, crossover study in 41 elderly outpatients with sarcopenia found that nutritional supplementation with amino acids (8 g of essential amino acids twice daily) resulted in significant gains in lean mass with a parallel increase in IGF-1 and a reduction in TNF-α compared to those assigned to the placebo control group [99]. In a recent, as yet unpublished study, we found that daily consumption of lean red meat for 4 months, equivalent to a protein intake of ~1.3 g/kg/day, with progressive resistance training (PRT) led to a greater reduction in the proinflammatory marker IL-6 and significantly greater increases in serum IGF-1, lean mass, and muscle strength compared to elderly women assigned to PRT alone (Daly R unpublished observation). Similarly, in overweight and obese premenopausal women involved in a 16-week trial, consumption of diets higher in protein with an emphasis on dairy foods during a diet- and exercise-induced weight loss program improved markers of bone turnover as well as adipokine levels (adiponectin and leptin), serum osteoprotegerin (OPG), and RANKL, important regulators of bone formation/resorption that are influenced of cytokines, compared to those assigned to a low-dairy diet [100]. While there is currently inconclusive evidence with regard to the effects of dietary protein on bone health and fracture risk [101], the above findings provide some evidence that diets higher in protein, dairy foods, and dietary calcium, when combined with exercise, represent an effective approach to reduce inflammation via an increase in IGF-1 levels and thereby enhance both muscle and bone health.

Others have suggested that soy protein and other dairy proteins and peptides may also have anti-inflammatory properties [1]. However, a review on the effects of soy foods and soy isoflavones on inflammation found that there was no consistent evidence for an effect on the cytokines IL-6 and TNF-α [102]. Similarly, the findings from several human trials have shown that specific milk/dairy proteins (whey or casein protein or milk peptides) have no effect on inflammatory markers in overweight adults [103], mildly hypertensive people [104], or postmenopausal women [105]. Whether other specific branched chained amino acids, particularly leucine, which has a strong stimulatory effect on muscle protein synthesis, have anti-inflammatory properties and can modulate skeletal muscle health remains to be determined [106].

Vitamin K

Vitamin K has been strongly implicated in bone health due to its function as a cofactor in the post-translational γ-carboxylation of several vitamin K-dependent proteins, including osteocalcin, which plays an important role in bone mineralization [107]. While it is beyond the scope of this chapter to provide a comprehensive review on the effects of vitamin K supplementation on BMD and fracture risk, the findings from several recent reviews and meta-analyses of clinical trials indicate that supplementation with either form of vitamin K (phylloquinone, vitamin K_1; menaquinone, vitamin K_2), particularly at higher doses (phylloquinone >1,000 μg/day or menaquinone >45 mg/day), in combination with calcium and vitamin D, can improve indices of hip bone strength and protect against fractures [108, 109]. Interestingly however, the effects of vitamin K treatment on BMD are equivocal [107, 110], which has been attributed to the fact that there is considerable between study heterogeneity and publication bias in the studies that have been conducted [110].

If vitamin K does play a role in reducing fracture risk, many questions still remain as to the underlying mechanism(s) of action. One theory is that vitamin K may reduce circulating concentrations of proinflammatory cytokines. This is supported by data from in vitro studies which have shown that treatment with vitamin K was associated with a decrease in proinflammatory markers [111, 112]. Similarly, the findings from several human cross-sectional studies in middle-aged and older adults have shown that plasma phylloquinone

concentrations, which represent a marker of vitamin K status, and/or dietary phylloquinone intake were inversely associated with various inflammatory markers, including CRP and IL-6 [78, 113]. Unfortunately, a follow-up intervention study examining the effects of 3 years of supplementation with vitamin K (500 μg phylloquinone), together with additional calcium and vitamin D, failed to detect any significant effect on circulating levels of IL-6 or CRP [113] or BMD [114] in healthy older men and women. It is possible that the lack of a cytokine response in this study might be related to the supplemental dose (500 μg) of phylloquinone used [113]. In a previous trial, 1,000 μg/day of phylloquinone was found to be effective for slowing bone loss in postmenopausal women [115]. Nevertheless, the above findings are consistent with the results from calcium and/or vitamin D supplementation trials which generally observed no effects on inflammation in healthy adults.

Omega-3 Fatty Acids

It has been suggested that diets high in omega-3 ($n-3$) fatty acids or with a lower omega-6 ($n-6$) to $n-3$ ratio may have beneficial effects on both bone and muscle health, which might be mediated, at least in part, by a decrease in proinflammatory cytokines [116–119]. There is considerable evidence to support an anti-inflammatory effect of a high dietary intake of omega-3 fatty acids, particularly in those with chronic disease (for a review refer to Calder et al. [1]) However, the findings from human studies on the effects of omega-3 fatty acids or the $n-6$ to $n-3$ ratio ($n-6$ tends to increase inflammation) on BMD and fracture risk are mixed, with some studies reporting a protective effect of omega-3 fatty acids against bone loss [116, 120] and fractures [121], while others have observed no significant effect or even an increased fracture risk [117, 122, 123]. This is supported by the findings from a systematic review which found that only one of four randomized controlled trials with BMD as the primary outcomes showed significant improvements or maintenance of BMD following 18 months of omega-3 supplementation [primrose oil (high in linoleic acid) and fish oil] with calcium versus a placebo control of coconut oil and calcium [117]. Interestingly, this study was conducted in elderly women with osteopenia or osteoporosis, a group which may have chronic systemic inflammation, whereas the others trials which observed no effect involved healthy pre- and/or postmenopausal women or healthy older men. In a recent systemic review, Rangel-Huerta et al. [118] reported that omega-3 fatty acid supplementation was associated with lower inflammation in patients with acute and chronic diseases, but not in healthy subjects. While there is currently no consensus on the type or dose required to exert an anti-inflammatory effect and/or improve bone health [118], it is possible that any beneficial effect of omega-3 fatty acids on bone might be limited to those with osteoporosis, inflammatory disease(s), or chronic low-grade systemic inflammation. However, this needs to be confirmed in future intervention trials.

In recent years there has been some interest in the role of omega-3 fatty acids and fish oil supplementation alone or in combination with progressive resistance training as a strategy to treat and prevent sarcopenia. Data from an epidemiological study in adults aged 59–73 years revealed that fatty fish consumption was a strong positive predictor of grip strength [124]. In elderly women, combining fish oil supplementation with progressive resistance training led to greater increases in muscle strength and function than resistance training alone [125]. While no studies appear to have examined the association between omega-3 fatty acids and muscle mass, the findings from an 8-week trial in older adults revealed that omega-3 supplementation enhanced the rate of muscle protein synthesis in response to amino acid feeding; there was no effect on basal muscle protein synthesis rate [126]. This suggests that omega-3 fatty acids may attenuate the "anabolic resistance" commonly seen in the elderly, that is, enhance the sensitivity of muscle to anabolic stimuli such as amino acids. Importantly, they also found that increased omega-3 fatty acid intake was not associated with a decrease in inflammatory cytokines but did result in an increase in muscle anabolic signaling activity. While this suggests that omega-3 fatty acids may

have a positive effect on muscle via a mechanism independent of their anti-inflammatory effects, it is important to note that the participants in this study were healthy older adults with low circulating inflammatory markers. Again, further long-term trials are warranted in the elderly and at risk groups which should include measures of lower limb muscle mass or size.

Conclusion

Aging is accompanied by a chronic low-grade systemic inflammation state (termed *inflammaging*), characterized by higher circulating levels in inflammatory mediators, that has been strongly implicated in the pathophysiology of many chronic diseases, including osteoporosis and fractures, sarcopenia, and disability. While a wide range of factors have been reported to contribute to *inflammaging*, including genetic, hormonal, environmental, and lifestyle factors, various dietary patterns, foods, and nutrients have been reported to have anti-inflammatory effects, particularly in people with chronic diseases characterized by increased inflammation such as cardiovascular disease, type 2 diabetes, and cancer. With regard to musculoskeletal health and function, there are conflicting findings from cross-sectional and prospective studies and randomized controlled trials on the effects of nutrients such as dietary calcium, vitamin D, protein, vitamin K, omega-3 fatty acids, or their combination or food products such as dairy, on markers of inflammation. In healthy middle-aged and older adults, there is little or no evidence that these nutrients or foods attenuate circulatory inflammatory cytokines. In contrast, there is evidence from a limited number of human intervention trials, mostly conducted over 12–16 weeks, indicating that calcium-vitamin D supplementation, high-dairy diets, and increased dietary protein, vitamin K, or omega-3 fatty acids alone or in combination with exercise (resistance training) can produce modest reductions in inflammation in high-risk groups with chronic disease and/or increased inflammation, including people with osteoporosis and sarcopenia. Whether these short-term reductions in inflammatory markers are clinically important and translate into positive effects on muscle and bone health, improved muscle function, or reduced disability remains unknown. Further randomized controlled trials in older adults and the elderly with or at increased risk of chronic disease are needed to evaluate the long-term efficacy of different nutrients or combination of nutrients and dietary interventions on markers of inflammation and their relation to musculoskeletal health outcomes.

References

1. Calder PC, Ahluwalia N, Brouns F, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106 Suppl 3:S5–78.
2. Singh T, Newman AB. Inflammatory markers in population studies of aging. Ageing Res Rev. 2011;10:319–29.
3. Ballou SP, Lozanski FB, Hodder S, et al. Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. Age Ageing. 1996;25:224–30.
4. Ferrucci L, Corsi A, Lauretani F, et al. The origins of age-related proinflammatory state. Blood. 2005;105:2294–9.
5. Saurwein-Teissl M, Blasko I, Zisterer K, Neuman B, Lang B, Grubeck-Loebenstein B. An imbalance between pro- and anti-inflammatory cytokines, a characteristic feature of old age. Cytokine. 2000;12:1160–1.
6. Wei J, Xu H, Davies JL, Hemmings GP. Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci. 1992;51:1953–6.
7. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244–54.
8. Vasto S, Candore G, Balistreri CR, et al. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev. 2007;128:83–91.
9. Chung HY, Cesari M, Anton S, et al. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev. 2009;8:18–30.
10. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol. 2005;98:1154–62.
11. Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. Clin Chim Acta. 2010;411:785–93.
12. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev. 2007;128:92–105.
13. Bruunsgaard H, Pedersen BK. Age-related inflammatory cytokines and disease. Immunol Allergy Clin North Am. 2003;23:15–39.

14. Hunt KJ, Walsh BM, Voegeli D, Roberts HC. Inflammation in aging part 1: physiology and immunological mechanisms. Biol Res Nurs. 2010;11:245–52.
15. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. Exp Gerontol. 2004;39: 687–99.
16. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol. 2007;292:R18–36.
17. Meng SJ, Yu LJ. Oxidative stress, molecular inflammation and sarcopenia. Int J Mol Sci. 2010;11: 1509–26.
18. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39: 412–23.
19. Fielding RA, Vellas B, Evans WJ, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. J Am Med Dir Assoc. 2011;12:249–56.
20. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. Am J Physiol Endocrinol Metab. 2003;284:E481–7.
21. Cesari M, Penninx BW, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. J Gerontol A Biol Sci Med Sci. 2004;59:242–8.
22. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci. 2002;57:M326–32.
23. Ferrucci L, Penninx BW, Volpato S, et al. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc. 2002;50:1947–54.
24. Aleman H, Esparza J, Ramirez FA, Astiazaran H, Payette H. Longitudinal evidence on the association between interleukin-6 and C-reactive protein with the loss of total appendicular skeletal muscle in free-living older men and women. Age Ageing. 2011;40:469–75.
25. Payette H, Roubenoff R, Jacques PF, et al. Insulin-like growth factor-1 and interleukin 6 predict sarcopenia in very old community-living men and women: the Framingham Heart Study. J Am Geriatr Soc. 2003;51:1237–43.
26. Schaap LA, Pluijm SM, Deeg DJ, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci. 2009;64:1183–9.
27. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med. 2006;119:526 e9–17.
28. Verghese J, Holtzer R, Oh-Park M, Derby CA, Lipton RB, Wang C. Inflammatory markers and gait speed decline in older adults. J Gerontol A Biol Sci Med Sci. 2011;66:1083–9.
29. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89:2548–56.
30. Roubenoff R. Sarcopenic obesity: does muscle loss cause fat gain? Lessons from rheumatoid arthritis and osteoarthritis. Ann N Y Acad Sci. 2000;904:553–7.
31. Vincent HK, Raiser SN, Vincent KR. The aging musculoskeletal system and obesity-related considerations with exercise. Ageing Res Rev. 2012;11:361–73.
32. Peake J, Della Gatta P, Cameron-Smith D. Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. Am J Physiol Regul Integr Comp Physiol. 2010;298:R1485–95.
33. Chevalier S, Gougeon R, Choong N, Lamarche M, Morais JA. Influence of adiposity in the blunted whole-body protein anabolic response to insulin with aging. J Gerontol A Biol Sci Med Sci. 2006;61:156–64.
34. Koster A, Ding J, Stenholm S, et al. Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? J Gerontol A Biol Sci Med Sci. 2011;66:888–95.
35. Stenholm S, Maggio M, Lauretani F, et al. Anabolic and catabolic biomarkers as predictors of muscle strength decline: the InCHIANTI study. Rejuvenation Res. 2010;13:3–11.
36. Cappola AR, Xue QL, Ferrucci L, Guralnik JM, Volpato S, Fried LP. Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. J Clin Endocrinol Metab. 2003;88:2019–25.
37. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. Immunol Rev. 2005;208:207–27.
38. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. New Engl J Med. 1995;332:305–11.
39. Troen BR. Molecular mechanisms underlying osteoclast formation and activation. Exp Gerontol. 2003;38: 605–14.
40. Koh JM, Khang YH, Jung CH, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporos Int. 2005;16:1263–71.
41. Papadopoulos NG, Georganas K, Skoutellas V, Konstantellos E, Lyritis GP. Correlation of interleukin-6 serum levels with bone density in postmenopausal women. Clin Rheumatol. 1997;16:162–5.
42. Kania DM, Binkley N, Checovich M, Havighurst T, Schilling M, Ershler WB. Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. J Am Geriatr Soc. 1995;43:236–9.
43. McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL. Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. J Bone Miner Res. 1994;9:1313–8.
44. Khosla S, Peterson JM, Egan K, Jones JD, Riggs BL. Circulating cytokine levels in osteoporotic and normal women. J Clin Endocrinol Metab. 1994;79: 707–11.

45. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. J Clin Endocrinol Metab. 2008;93:1952–8.
46. Gertz ER, Silverman NE, Wise KS, et al. Contribution of serum inflammatory markers to changes in bone mineral content and density in postmenopausal women: a 1-year investigation. J Clin Densitom. 2010;13:277–82.
47. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, et al. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab. 2001;86:2032–42.
48. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab. 2003;88:255–9.
49. Kim JG, Lim KS, Ku SY, Kim SH, Choi YM, Moon SY. Relations between interleukin-1, its receptor antagonist gene polymorphism, and bone mineral density in postmenopausal Korean women. J Bone Miner Metab. 2006;24:53–7.
50. Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF. Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1 – receptor antagonist gene but not with polymorphisms in the interleukin-1beta gene. J Bone Miner Res. 2000;15:402–14.
51. Jilka RL, Hangoc G, Girasole G, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 1992;257:88–91.
52. Lorenzo JA, Naprta A, Rao Y, et al. Mice lacking the type I interleukin-1 receptor do not lose bone mass after ovariectomy. Endocrinology. 1998;139:3022–5.
53. Poli V, Balena R, Fattori E, et al. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J. 1994;13:1189–96.
54. Cauley JA, Danielson ME, Boudreau RM, et al. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. J Bone Miner Res. 2007;22:1088–95.
55. Barbour KE, Boudreau R, Danielson ME, et al. Inflammatory markers and the risk of hip fracture: the Women's Health Initiative. J Bone Miner Res. 2012;27:1167–76.
56. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D3 regulation of adipokine expression. Obesity (Silver Spring). 2007;15:340–8.
57. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res. 2005;13:1218–25.
58. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. Am J Clin Nutr. 2010;91:16–22.
59. Shi H, Dirienzo D, Zemel MB. Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. FASEB J. 2001;15:291–3.
60. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. FASEB J. 2000;14:1132–8.
61. Soares MJ, Murhadi LL, Kurpad AV, Chan She Ping-Delfos WL, Piers LS. Mechanistic roles for calcium and vitamin D in the regulation of body weight. Obes Rev. 2012;13:592–605.
62. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr. 2008;138:1047–52.
63. Grey A, Mitnick MA, Shapses S, Ellison A, Gundberg C, Insogna K. Circulating levels of interleukin-6 and tumor necrosis factor-alpha are elevated in primary hyperparathyroidism and correlate with markers of bone resorption – a clinical research center study. J Clin Endocrinol Metab. 1996;81:3450–4.
64. Grey A, Gamble G, Ames R, Horne A, Mason B, Reid IR. Calcium supplementation does not affect CRP levels in postmenopausal women – a randomized controlled trial. Osteoporos Int. 2006;17:1141–5.
65. Gannage-Yared MH, Azoury M, Mansour I, Baddoura R, Halaby G, Naaman R. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. J Endocrinol Invest. 2003;26:748–53.
66. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care. 2007;30:980–6.
67. Hopkins MH, Owen J, Ahearn T, et al. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. Cancer Prev Res (Phila). 2011;4:1645–54.
68. Stancliffe RA, Thorpe T, Zemel MB. Dairy attenuates oxidative and inflammatory stress in metabolic syndrome. Am J Clin Nutr. 2011;94:422–30.
69. Neyestani TR, Nikooyeh B, Alavi-Majd H, et al. Improvement of vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. J Clin Endocrinol Metab. 2012;97:2005–11.
70. Wennersberg MH, Smedman A, Turpeinen AM, et al. Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. Am J Clin Nutr. 2009;90:960–8.
71. Peake JM, Kukuljan S, Nowson CA, Sanders K, Daly RM. Inflammatory cytokine responses to progressive resistance training and supplementation with fortified milk in men aged 50+ years: an 18-month randomized controlled trial. Eur J Appl Physiol. 2011;111:3079–88.
72. Hewison M. Vitamin D, and immune function: an overview. Proc Nutr Soc. 2012;71:50–61.
73. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev. 2005;26:662–87.

74. Amer M, Qayyum R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol. 2012;109:226–30.
75. Hypponen E, Berry D, Cortina-Borja M, Power C. 25-Hydroxyvitamin D and pre-clinical alterations in inflammatory and hemostatic markers: a cross sectional analysis in the 1958 British Birth Cohort. PLoS One. 2010;5:e10801.
76. Jorde R, Sneve M, Torjesen PA, Figenschau Y, Goransson LG, Omdal R. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine. 2010;50:175–80.
77. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond). 2008;5:10.
78. Shea MK, Booth SL, Massaro JM, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol. 2008;167:313–20.
79. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006;83: 754–9.
80. Matias PJ, Jorge C, Ferreira C, et al. Cholecalciferol supplementation in hemodialysis patients: effects on mineral metabolism, inflammation, and cardiac dimension parameters. Clin J Am Soc Nephrol. 2010; 5:905–11.
81. Shab-Bidar S, Neyestani TR, Djazayery A, et al. Improvement of vitamin D status resulted in amelioration of biomarkers of systemic inflammation in the subjects with type 2 diabetes. Diabetes Metab Res Rev. 2012;28:424–30.
82. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol. 2003;134:128–32.
83. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003;88:4623–32.
84. Inanir A, Ozoran K, Tutkak H, Mermerci B. The effects of calcitriol therapy on serum interleukin-1, interleukin-6 and tumour necrosis factor-alpha concentrations in post-menopausal patients with osteoporosis. J Int Med Res. 2004;32:570–82.
85. Zittermann A, Frisch S, Berthold HK, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr. 2009;89:1321–7.
86. Carrillo AE, Flynn MG, Pinkston C, et al. Vitamin D supplementation during exercise training does not alter inflammatory biomarkers in overweight and obese subjects. Eur J Appl Physiol. 2012;112:3045–52.
87. Barnes MS, Horigan G, Cashman KD, et al. Maintenance of wintertime vitamin D status with cholecalciferol supplementation is not associated with alterations in serum cytokine concentrations among apparently healthy younger or older adults. J Nutr. 2011;141:476–81.
88. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005;135:317–22.
89. Genaro Pde S, Martini LA. Effect of protein intake on bone and muscle mass in the elderly. Nutr Rev. 2010;68:616–23.
90. De Benedetti F, Alonzi T, Moretta A, et al. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest. 1997;99:643–50.
91. Grounds MD, Radley HG, Gebski BL, Bogoyevitch MA, Shavlakadze T. Implications of cross-talk between tumour necrosis factor and insulin-like growth factor-1 signalling in skeletal muscle. Clin Exp Pharmacol Physiol. 2008;35:846–51.
92. Breen L, Phillips SM. Skeletal muscle protein metabolism in the elderly: interventions to counteract the 'anabolic resistance' of ageing. Nutr Metab (Lond). 2011;8:68.
93. Hulmi JJ, Lockwood CM, Stout JR. Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: a case for whey protein. Nutr Metab (Lond). 2010;7:51.
94. Balage M, Averous J, Remond D, et al. Presence of low-grade inflammation impaired postprandial stimulation of muscle protein synthesis in old rats. J Nutr Biochem. 2010;21:325–31.
95. Toth MJ, Matthews DE, Tracy RP, Previs MJ. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. Am J Physiol Endocrinol Metab. 2005;288:E883–91.
96. Rieu I, Magne H, Savary-Auzeloux I, et al. Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats. J Physiol. 2009;587:5483–92.
97. Bartali B, Frongillo EA, Stipanuk MH, et al. Protein intake and muscle strength in older persons: does inflammation matter? J Am Geriatr Soc. 2012;60:480–4.
98. Santesso N, Akl EA, Bianchi M, et al. Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. Eur J Clin Nutr. 2012;66:780–8.
99. Solerte SB, Gazzaruso C, Bonacasa R, et al. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. Am J Cardiol. 2008;101:69E–77.
100. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets higher in dairy foods and dietary protein support bone health during diet- and exercise-induced weight loss in overweight and obese premenopausal women. J Clin Endocrinol Metab. 2012;97:251–60.

101. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 2009;90:1674–92.
102. Beavers KM, Jonnalagadda SS, Messina MJ. Soy consumption, adhesion molecules, and pro-inflammatory cytokines: a brief review of the literature. Nutr Rev. 2009;67: 213–21.
103. Pal S, Ellis V. The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. Obesity (Silver Spring). 2010;18:1354–9.
104. Lee YM, Skurk T, Hennig M, Hauner H. Effect of a milk drink supplemented with whey peptides on blood pressure in patients with mild hypertension. Eur J Nutr. 2007;46:21–7.
105. Beavers KM, Serra MC, Beavers DP, Cooke MB, Willoughby DS. Soy and the exercise-induced inflammatory response in postmenopausal women. Appl Physiol Nutr Metab. 2010;35:261–9.
106. Nicastro H, da Luz CR, Chaves DF, et al. Does branched-chain amino acids supplementation modulate skeletal muscle remodeling through inflammation modulation? Possible mechanisms of action. J Nutr Metab. 2012;2012:136937.
107. Gundberg CM, Lian JB, Booth SL. Vitamin K-dependent carboxylation of osteocalcin: friend or foe? Adv Nutr. 2012;3:149–57.
108. Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. Arch Intern Med. 2006;166:1256–61.
109. Iwamoto J, Sato Y, Takeda T, Matsumoto H. High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: a review of the literature. Nutr Res. 2009;29:221–8.
110. Fang Y, Hu C, Tao X, Wan Y, Tao F. Effect of vitamin K on bone mineral density: a meta-analysis of randomized controlled trials. J Bone Miner Metab. 2012;30:60–8.
111. Ohsaki Y, Shirakawa H, Hiwatashi K, Furukawa Y, Mizutani T, Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci Biotechnol Biochem. 2006;70:926–32.
112. Reddi K, Henderson B, Meghji S, et al. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine. 1995;7:287–90.
113. Shea MK, Dallal GE, Dawson-Hughes B, et al. Vitamin K, circulating cytokines, and bone mineral density in older men and women. Am J Clin Nutr. 2008;88:356–63.
114. Booth SL, Dallal G, Shea MK, Gundberg C, Peterson JW, Dawson-Hughes B. Effect of vitamin K supplementation on bone loss in elderly men and women. J Clin Endocrinol Metab. 2008;93:1217–23.
115. Braam LA, Knapen MH, Geusens P, et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. Calcif Tissue Int. 2003;73:21–6.
116. Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr. 2011;93:1142–51.
117. Orchard TS, Pan X, Cheek F, Ing SW, Jackson RD. A systematic review of omega-3 fatty acids and osteoporosis. Br J Nutr. 2012;107 Suppl 2: S253–60.
118. Rangel-Huerta OD, Aguilera CM, Mesa MD, Gil A. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. Br J Nutr. 2012;107 Suppl 2:S159–70.
119. Ubeda N, Achon M, Varela-Moreiras G. Omega 3 fatty acids in the elderly. Br J Nutr. 2012;107 Suppl 2:S137–51.
120. Weiss LA, Barrett-Connor E, von Muhlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. Am J Clin Nutr. 2005;81:934–8.
121. Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults. J Nutr. 2011;141:1146–53.
122. Virtanen JK, Mozaffarian D, Cauley JA, Mukamal KJ, Robbins J, Siscovick DS. Fish consumption, bone mineral density, and risk of hip fracture among older adults: the cardiovascular health study. J Bone Miner Res. 2010;25:1972–9.
123. Virtanen JK, Mozaffarian D, Willett WC, Feskanich D. Dietary intake of polyunsaturated fatty acids and risk of hip fracture in men and women. Osteoporosis Int. 2012;23:2615–24.
124. Robinson SM, Jameson KA, Batelaan SF, et al. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. J Am Geriatr Soc. 2008;56:84–90.
125. Rodacki CL, Rodacki AL, Pereira G, et al. Fish-oil supplementation enhances the effects of strength training in elderly women. Am J Clin Nutr. 2012;95: 428–36.
126. Smith GI, Atherton P, Reeds DN, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr. 2011;93:402–12.

Interactions of Dietary Patterns, Systemic Inflammation, and Bone Health

2

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Abstract

Examination of combinations of foods, as described by dietary patterns in relation to health indices, may be an important approach to further our understanding of chronic disease prevention. Bone loss is a common factor in many chronic inflammatory conditions, although it is unclear whether low-grade systemic inflammation may have similar long-term effects. In this chapter we summarize current evidence relating dietary patterns and chronic low-grade systemic inflammation to bone health. Consideration is then given to potential mechanisms whereby dietary eating patterns may affect inflammatory status. Dietary patterns rich in fruits and vegetables consistently appear to have a protective effect on bone mineral density, likely due to their abundance of micronutrients, minerals, and bioactive compounds. Current evidence relating low-grade systemic inflammation to indices of bone health is limited and contradictory, although modification of dietary eating habits (increasing intakes of plant-based foods and reducing the omega-6 to omega-3 fatty acid ratio) may be important in the management of chronic inflammatory status. Longitudinal studies assessing dietary patterns in relation to bone mineral density/fracture incidence and biomarkers of inflammation could further our understanding of these complex interactions.

Keywords

Dietary patterns • Systemic inflammation • Bone mineral density • Fracture • Chronic disease prevention

Introduction

Nutritional research in relation to chronic disease prevention has historically focused on the effects of single nutrients, foods, or food groups on incident disease events or surrogate markers of risk. Poor nutrition may play a role in the pathogenesis

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of osteoporosis. Research in relation to bone health has tended to focus on vitamin D and calcium, with adequate intake of these nutrients required for the prevention and cure of rickets in children [1]. Convincing evidence for dietary supplementation of calcium alone or in combination with vitamin D to reduce fracture incidence in older adults remains somewhat equivocal [2]. In studies of other nutrients and food groups such as fruits and vegetables [3–5], potassium [5], vitamin K [6], caffeine [7, 8], and protein [9] in relation to bone heath, clear relationships have not yet been elucidated.

The majority of foods and nutrients are typically consumed in combinations, of which, many are likely to be interactive or have synergistic effects [10]. It is possible that discrepancies from single nutrient studies may relate in part to inherent imprecision associated with food composition databases or that the extent of effect of a single nutrient or food on disease risk/outcome may be too small to overcome potential confounding factors [11]. We would suggest it may therefore be appropriate to examine combinations of foods as described by dietary patterns [10, 12]. Such combinations, which reflect dietary preferences of the individual, are influenced by a mixture of socioeconomic, cultural, environmental, and lifestyle factors [13].

Dietary patterns can be generated using *a priori* knowledge (under which circumstances the dietary patterns are generated by the investigator), or empirically. In *a priori* analyses, the investigator may utilize national dietary guidelines from which to base a dietary pattern and score foods according to how much they represent a particular "healthy eating" pattern. Empirical analyses employ data reduction techniques such as cluster analysis and factor analysis, using commonly available statistical software packages. Details of the different methodologies employed in dietary pattern analysis, covering the advantages and disadvantages of the general approach, have been reviewed previously [14]. Such methods appear to consistently derive similar dietary patterns reflective of differences in diets which are nutrient poor and nutrient rich [15].

Chronic inflammatory diseases are frequently associated with bone loss [16]. A comprehensive explanation of the mechanisms behind these associations has yet to be established although interactions of inflammatory cells, cytokines, and bone cells affecting the bone remodeling cycle may be important. While associations between chronic inflammatory diseases and bone loss are well recognized, it is less clear whether low-grade systemic inflammation has similar effects. In this chapter we summarize current evidence relating dietary patterns to bone health. We discuss chronic low-grade systemic inflammation as it relates to bone physiology and indices of bone health, with particular reference to bone mineral density. Consideration is given to potential mechanisms whereby dietary eating patterns may affect inflammatory status. Finally we present evidence from a recent cross-sectional study assessing associations of dietary patterns with chronic low-grade systemic inflammation.

Dietary Patterns and Bone Health

There have been relatively few studies to date investigating the impact of dietary patterns on BMD, bone mineral content (BMC), or fracture incidence [17–25]. The results of these studies, which vary markedly in terms of size, participant population, and analysis methodology, are summarized in Table 2.1. Food types included in dietary patterns which appear to be associated with greater BMD at various sites are fruits and vegetables [17–19, 21], oily fish [18, 19], and meat [25]. It has been suggested that fruits and vegetables may be beneficial because of the alkaline salts they provide by balancing excessive dietary acidity [26], although we have previously reported no effect of supplementary potassium citrate (high dose, 55.6 mmol/day ($n=56$); low dose, 18.5 mmol/day ($n=54$); placebo ($n=55$)) on BMD or markers of bone turnover in a 2-year parallel group RCT of postmenopausal women [5]. Potentially beneficial effects of this food group on bone health are more likely to be related to their micronutrient (vitamin C, K, and B vitamins), phytochemical (including flavonoids and phytoestrogens), and dietary fiber

Table 2.1 Studies assessing association of dietary patterns with BMD, BMC, or fracture incidence

Study, year [reference]	Cohort (country)	Women (%)	Participants, *n*; mean age, years (SD)	Main dietary patterns (ascertainment method)	Association with BMD/ fracture risk	Covariates[a]
Tucker et al., 2002 [14]	Framingham Osteoporosis Study (USA)	62	Elderly women, 345; 75.1 (4.9) Elderly men, 562; 75.3 (4.8)	1. Fruit, veg, cereal 2. Candy (CA)	1. Greater BMD at RF in men ($P<.05$) 2. Lower BMD at radius in women ($P<.01$) and RF in men ($P<.05$)	1–8
Okubo et al., 2006 [15]	JMETS Study (Japan)	100	Premenopausal women, 291; 46.4 (3.7)	1. Healthy – fruit, veg, fish 2. Western – fats/oils, processed meat (FA)	1. Positive association with FA BMD ($P<.05$)	1, 3, 6, 7, 9 11
Kontogianni et al., 2009 [16]	(Greece)	100	Premenopausal women, 100; 38.0 (8.7) Peri-/postmenopausal women, 96; 56.7 (6.4)	1. Mediterranean type – fish and olive oil, low red meat (PCA)	1. Positive association with LS BMD ($P=.017$) and total body BMC ($P=.05$)	1, 3–6, 12
Langsetmo et al., 2010 [17]	Canadian Multicentre Osteoporosis Study (Canada)	71	Women, 4,611; 61.2 (12.2) Men, 1,928; 58.8 (13.5)	1. Nutrient dense – fruit, veg, whole grains 2. Energy dense (FA)	1. No association with primary outcome (FN BMD)	
Hardcastle et al., 2011 [18]	APOSS (United Kingdom)	100	Women, 3,236; 55.1 (2.2)	1. Fruit, veg, rice/pasta 2. Processed food 3. Snack food (PCA)	1. Negative association with FN BMD ($P<.001$) 2. Positive association with FN BMD ($P<.001$)	2, 3, 5, 6, 12, 14, 17
Langsetmo et al., 2011 [19]	Canadian Multicentre Osteoporosis Study (Canada)	68	Women, 3,539; 67.6 (8.6) Men, 1,649; 64.6 (10.0)	1. Nutrient dense – fruit, veg, whole grains 2. Energy dense (FA)	1. Lower risk of fracture per 1SD in women (HR: 0.86; 95 % CI: 0.76, 0.98). Similar trend in men (HR: 0.83; 95 % CI: 0.64, 1.08)	1, 6, 7, 10, 15, 16
McNaughton et al., 2011 [20]	Twin and Sister Bone Research Program (Australia)	100	Women, 527; 39.4 (10.2)	1. Legumes, seafood, seeds, wine, rice, veg 2. Processed meat/ cereals, fats/oils (FA)	1. Positive association with BMC (TB $P=.016$) and BMD (total hip $P=.042$; LS $P<.0001$) 2. Negative association with BMC (TB $P=.01$)	2–7, 14

(continued)

Table 2.1 (continued)

Study, year [reference]	Cohort (country)	Women (%)	Participants, *n*; mean age, years (SD)	Main dietary patterns (ascertainment method)	Association with BMD/ fracture risk	Covariates[a]
Karamati et al., 2012 [21]	(Iran)	100	Postmenopausal women, 154; 60.0 (8.4)	1. High-fat dairy, organ/ red/processed meat 2. French fries, oils, mayo, sweets/desserts (PCA)	Those in high category for pattern 1 and 2 had greater probability of below median BMD at LS (OR 2.29; 95 % CI: 1.05–4.96) and FN (OR 2.83; 95 % CI: 1.31–6.09)	1,3–7, 10, 11, 13, 14
Whittle et al., 2012 [22]	Young Hearts Project (Northern Ireland)	49	Women, 238; 22.8 (1.7) Men, 251; 22.4 (1.6)	1. Nuts and meat – nuts, chocolate, meat dishes 2. Refined – desserts, snack food, soft drinks (PCA)	1. Greater FN BMD for women in top vs. bottom quintile ($P = .05$) 2. Lower FN BMC for men in top vs. bottom quintile ($P = .05$)	1, 3–6, 14

BMD bone mineral density, *BMC* bone mineral content, *TB* total body, *RF* right femur, *FA* forearm, *LS* lumbar spine, *FN* femoral neck, *CA* cluster analysis, *FA* factor analysis, *PCA* principal components analysis

[a] *1* BMI, *2* height, *3* age, *4* energy intake, *5* physical activity level, *6* smoking status, *7* medication and supplement use, *8* season, *9* grasping power, *10* falls/fracture history, *11* age at menarche, *12* menopausal status, *13* parity, *14* social deprivation category/education, *15* BMD, *16* milk consumption, *17* weight

content [27]. Beneficial effects of oily fish and meat in nutrient-dense dietary patterns may be related to vitamin D (particularly at northerly latitudes) and protein (to adequately support bone remodeling), respectively, although the relationship between dietary protein and bone health is controversial with high dietary protein traditionally thought to act negatively on bone via an increased acid load [26]. In a relatively recent review of the literature in relation to dietary protein and bone health interactions, the authors conclude that this macronutrient has a modest beneficial effect on bone density, although recommendations about its use should be reserved for groups at higher risk of bone loss (such as the elderly) and that consideration of the interaction between dietary protein and other components in a mixed diet, such as calcium and fruits and vegetables, may be important [28].

Inflammatory Disease and Bone Loss

Conditions which include rheumatoid arthritis [29, 30], inflammatory bowel disease [31, 32], systemic lupus erythematosus [33], ankylosing spondylitis [34], and chronic obstructive pulmonary disease [35] share common mechanisms by which bone can be lost. For example, in osteoblasts and bone marrow stromal cells, a wide variety of cytokines have been found to impact on the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL) (involved in signaling of osteoblasts to osteoclasts) system to affect osteoclastogenesis and bone resorption. Cytokines with stimulatory effects on osteoclastogenesis include tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-11, and IL-17. Cytokines with predominantly inhibitory effects include interferon (IFN)-γ, IL-4, and transforming growth factor (TGF)-β [36]. A variety of other important signaling mechanisms (beyond the scope of this chapter) may be involved in bone loss during inflammatory disease [16] with an uncoupling of bone formation from resorption in favor of excess bone resorption most commonly attributable to the pathogenic damage to bone.

C-reactive protein (CRP) is an acute-phase reactant produced mainly by the liver that increases in response to inflammatory stimuli [37], with biochemical testing widely used to detect immediate-phase responses to tissue injury, and in infectious and autoimmune diseases. Developments in assay methodologies towards the end of the 1990s allowed for more accurate and precise measurement of this protein at the lower end of its distribution. Serum concentrations of high-sensitivity C-reactive protein (hsCRP) markedly below those associated with an acute-phase response (indicative of chronic low-grade systemic inflammation) have been shown to be associated with prediction of the risk of developing major chronic conditions such as cardiovascular disease [38]. Many other surrogate markers of systemic inflammation such as IL-6, TNF-α, homocysteine, fibrinogen, E-selectin, and serum amyloid A (SAA) have been positively associated with cardiovascular disease risk and events in observational studies [39], although the role for these systemic biomarkers in risk assessment and appropriate prevention interventions is not yet well defined [40].

Synthesis of CRP is induced by IL-6, IL-1, and TNF-α. Concentrations of hsCRP in serum may therefore be an appropriate surrogate marker of the broad extent of chronic low-grade systemic inflammation. The results of recent observational studies assessing the association of serum hsCRP concentration with BMD are summarized in Table 2.2 [41–48]. These data are somewhat conflicting with some studies demonstrating an inverse association between hsCRP concentration and BMD at various skeletal sites [41, 46, 47] and others showing mixed results or no association [42–45, 48]. Two of these studies showed positive associations of hsCRP concentration with fracture risk [43, 44], while another study observed no such association [48]. Substantial variation with regard to participant populations, confounding effects, and BMD measurement methodology may partly explain divergent results.

It has been suggested that longitudinal studies may be warranted to confirm the association of chronic low-grade systemic inflammation (assessed by hsCRP) with BMD. Strategies to modify systemic inflammation could then be tested to

Table 2.2 Studies assessing association of serum hsCRP concentration with BMD or fracture incidence

Study, year [reference]	Cohort (country)	Women (%)	Participants, *n*; mean age, years (SD)	hsCRP association with BMD/ incident fracture	Covariates[a]
Koh et al., 2005 [41]	(Korea)	100	Premenopausal women, 3,662; 42.6 (5.1) Postmenopausal women, 1,031; 57.6 (5.3)	Lower FN BMD in highest vs. lowest quintile of hsCRP ($P=.003$) Lower FN BMD in highest vs. lowest quintile of hsCRP ($P<.001$)	1–6
Ganesan et al., 2005 [42]	NHANES Survey (USA)	100	Postmenopausal women, 2,807; >65years	No association with total hip BMD	
Pasco et al., 2006 [43]	Geelong Osteoporosis Study (Australia)	100	Elderly women, 444; 77.0 (71.2–82.3) (median (IQR))	24–32 % increase in fracture risk for each SD increase in hsCRP (data collected between 1994 and 2002)	1, 2, 7–11
Schett et al., 2006 [44]	Bruneck Study (Italy)	50.9	Men and women, 906; 40–79 years	Incidence of nontraumatic fractures varied from 1.3 to 13.9 per 1,000 person-years in the lowest vs. highest tertile of hsCRP (data collected every 5 years between 1990 and 2005)	1, 2, 5, 6, 10, 12–15
Bhupathiraju et al., 2007 [45]	SIRBL (USA)	100	Postmenopausal women, 184; 54.2 (3.1)	No association with trabecular BMD	
Ding et al., 2008 [46]	Tasmanian Older Adult Cohort Study (Tasmania)	48.2	Men, 100; 63.3 (7.2) Women, 93; 61.9 (6.9)	Baseline hsCRP and hsCRP change negatively associated with Total body BMD change (over 2.9 years; $P<.05$)	1, 4, 7, 10, 12, 16, 17
de Pablo et al., 2012 [47]	NHANES Survey (USA)	49.8	Men, 5,261; 51 [18] Women, 5,214; 51 [19]	BMD (total, subtotal, extremities, ribs, trunk subregions) negatively associated with hsCRP quintiles (total BMD *P* for trend: <.001 for men and women)	1, 2, 4–6, 9, 10, 12, 14, 18–23

Cauley et al., 2007 [48]	Health Ageing and Body Composition Study (USA)	51.5	Men and women, 2,985; 70–79 years	No association of hsCRP with fracture incidence (data collected over 5.8 ± 1.6 years) although in a composite measure of inflammation, ≥3 elevated systemic inflammatory biomarkers were associated with RR (95 % CI) of fracture; 2.65 (1.44–4.89) compared with no elevation ($P < .001$)	1, 4–6, 9, 10, 12, 16, 18, 24

BMD bone mineral density, *FN* femoral neck, *hsCRP* high-sensitivity C-reactive protein

[a] *1* age, *2* BMI, *3* years since menopause, *4* smoking status, *5* alcohol intake, *6* physical activity level, *7* BMD, *8* prevalent fracture, *9* medication and supplement use, *10* disease status, *11* lifestyle, *12* sex, *13* income, *14* serum creatinine, *15* bone turnover markers, *16* weight, *17* height, *18* race/ethnicity, *19* education, *20* socioeconomic status, *21* blood lipids, *22* serum 25(OH)D, *23* blood pressure, *24* falls history

determine their effects on the risk of bone loss over the longer term [47].

Dietary Patterns and Inflammation

One potential strategy to protect against inflammation and chronic disease development is via modification of dietary eating patterns [49]. A Western-type diet, common in industrialized nations, which is characterized by high intakes of refined grains, red meat, sweetened beverages, added fats (including trans fats generated from the processing of polyunsaturated fatty acids in food production), and low intakes of fresh and dried fruits, nuts, vegetables, whole grains, insoluble fiber, and foods rich in omega-3 fatty acids [50], has been identified as a major contributing factor to the promotion of chronic inflammation. High dietary intakes of trans fats may promote inflammation via direct effects on cell surface receptors to trigger proinflammatory signals (elevated CRP, IL-6, E-selectin, and soluble intracellular adhesion molecules (sICAM-1 and sVCAM-1)) [51]. Dietary patterns with a high glycemic index or glycemic load are also associated with inflammation. Excessive glucose intake may induce oxidative stress and upregulate inflammatory processes [52].

Nutrient-dense dietary patterns which tend to contrast with those of the Western-type are associated with a reduced risk for the development of many chronic conditions and diseases [53] and may act to reduce inflammation via a variety of mechanisms. Plant-based foods contain a vast array of secondary metabolites (phytochemicals) [54] ranging from structurally simple alkaloids to more complex polyphenols and steroids, many of which have been shown to have potent anti-inflammatory effects. For example, polyphenols may act to modulate inflammatory processes via inhibition of proinflammatory enzyme activation [55, 56], modulation of the production of proinflammatory cytokines [56, 57], inhibition of proinflammatory cell adhesion molecules [58, 59], and scavenging effects towards reactive oxygen species [60, 61]. Omega-3 fatty acids from fish or plant sources may also be particularly important, acting via inhibitory effects on the arachidonic acid content of cell membranes, alteration of eicosanoid production, and modulation of nuclear receptor activation [62]. Contrastingly, omega-6 fatty acids are found predominantly in grain crops and vegetable oils, and a diet disproportionately high in omega-6 compared to omega-3 fatty acids has been associated with a shift towards proinflammatory processes [63, 64]. Finally, high intakes of dietary fiber from plant sources have consistently been shown to be associated with a reduced inflammatory status [65]. Mechanisms to explain these anti-inflammatory effects are not yet clear, although they may be associated with effects on glycemia [66].

Creating a healthy eating pattern which emphasizes a balanced intake of energy and nutrients tending towards substantial intakes of plant-based foods and reduced ratio of omega-6 to omega-3 fatty acids may be important in the management of chronic systemic inflammation.

Cross-Sectional Analysis of Dietary Patterns and Chronic Low-Grade Systemic Inflammation

Against this background, we explored the relationship between dietary patterns and systemic inflammation, assessed by serum concentrations of hsCRP and BMD. Data collected from the Aberdeen Prospective Osteoporosis Screening Study [67] cohort were used for this investigation. Diet was examined by validated Food Frequency Questionnaire [68] (FFQ) (n=3,238) during study visits conducted between 1997 and 2000, when the mean (SD) age of participants was 55 [2] years. Dietary patterns were generated by principal components analysis. Concentrations of hsCRP from stored serum collected during 1997–2000 study visits were recently measured (n=2,013) using standardized automated procedures (ADIVA 1800 Chemistry System). Inter/intra-assay coefficients of variation were <4 % across the range of concentrations tested. Potential confounding factors (weight, national deprivation category, smoking status, physical activity level, and menopausal status) were measured as described previously [21].

Table 2.3 Characteristics of our study cohort from 1997 to 2000 study visit who completed both dietary questionnaires and provided serum for hsCRP analysis

	N	Mean (SD)
Height (cm)	2,010	160.5 (5.9)
Weight (kg)	2,010	68.5 (12.5)
Age (years)	2,012	54.8 (2.2)
BMI (kg/m^2)	2,010	26.6 (4.6)
PAL (MET.h/week)	2,011	1.83 (0.32)
		Percent
Current smoker	369	18.4
Nonsmoker	1,634	81.6
HRT use and menopausal status		
Postmenopausal	588	29.4
Perimenopausal	126	6.3
Premenopausal	69	3.4
Past HRT user	445	22.2
Present HRT user	775	38.7
National deprivation category[a]		
I	520	26.0
II	873	43.7
III	151	7.6
IV	281	14.1
V–VI	173	8.6

BMI body mass index (calculated as weight in kilograms divided by height in meters squared), *PAL* physical activity level, *HRT* hormone replacement therapy
[a]Based on postcode classification, where I represents the most affluent and VI represents the most deprived

Table 2.4 Concentrations of CLSI biomarkers across quintiles of dietary score for dietary patterns generated from principal components analysis[a]

Diet descriptor							
"Healthy"	*Q1* (*n 396*)	*Q2* (*n 400*)	*Q3* (*n 384*)	*Q4* (*n 414*)	*Q5* (*n 419*)	*P*[b]	*P*[c]
hsCRP mg/L	1.9 (3.5)	1.2 (2.4)	1.1 (2.6)	1.1 (2.3)	1.2 (2.3)	.001	.01
"Bread and butter, low red meat and alcohol"	*Q1* (*n 390*)	*Q2* (*n 411*)	*Q3* (*n 404*)	*Q4* (*n 413*)	*Q5* (*n 395*)	*P*[b]	*P*[c]
hsCRP mg/L	1.6 (3.3)	1.3 (2.4)	1.3 (2.4)	1.2 (2.4)	1.2 (2.4)	.01	.12
"High fat and white fish"	*Q1* (*n 422*)	*Q2* (*n 403*)	*Q3* (*n 380*)	*Q4* (*n 393*)	*Q5* (*n 415*)	*P*[b]	*P*[c]
hsCRP mg/L	1.0 (1.9)	1.3 (2.8)	1.3 (2.8)	1.4 (2.9)	1.6 (2.9)	.009	.45

[a]Data presented as median (IQR) for each quintile of dietary pattern score
[b]Based on ANOVA with inflammatory marker as the independent variable (unadjusted)
[c]Based on ANCOVA with inflammatory marker as the independent variable, dietary pattern quintiles as the fixed factor, and adjustment for the following potential confounding covariates: weight, national deprivation category, smoking status, and physical activity level

ANOVA was used to test the relationship between dietary pattern scores and hsCRP measurements with ANCOVA to control for lifestyle covariates (weight, national deprivation category, smoking status, physical activity level, and menopausal status).

Characteristics of our study cohort who completed both dietary questionnaire and provided serum for hsCRP analysis are shown in Table 2.3. Five dietary patterns (accounting for 26 % of the variance in the diet) were identified [21], three of which were associated with serum hsCRP concentrations (Table 2.4). Women in the highest quintile of the "healthy" dietary pattern (rich in fruits and vegetables, lean meat, and with negative

loadings for high-sugar foods) had lower median serum hsCRP concentration compared with those in the lowest quintile (Table 2.4). This relationship remained significant after confounding adjustment. Concentrations of hsCRP decreased with increasing quintiles of the dietary pattern with positive factor loadings for bread and butter and negative factor loadings for red meat and alcohol (Table 2.4). Finally, hsCRP concentration increased with increasing quintiles of the high-fat/whitefish dietary pattern (Table 2.4). However, these relationships were no longer significant after adjustment for confounding covariates.

Our data confirm that healthy dietary patterns rich in fruits, vegetables, and lean protein appear to suppress chronic low-grade systemic inflammation assessed by the biomarker hsCRP independently of weight and physical activity level.

Conclusions

Dietary components may influence bone health and chronic inflammatory status via both positive and negative effects on inflammatory pathways. A dietary pattern approach may help to further our understanding the role of nutrition on disease processes. Future studies assessing diet in relation to indices of bone health and both traditional and novel biomarkers of inflammation longitudinally may be particularly informative.

References

1. Wharton B, Bishop N. Rickets. Lancet. 2002;362:1389–400.
2. Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367:40–9.
3. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr. 2000;71:142–51.
4. Macdonald HM, New SA, Golden MH, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr. 2004;79:155–65.
5. Macdonald HM, Black AJ, Aucott L, Duthie G, Duthie S, et al. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. Am J Clin Nutr. 2008;88:465–74.
6. Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, et al. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. Arch Intern Med. 2006;166:1256–61.
7. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. Am J Clin Nutr. 2000;71:1003–7.
8. Tucker KL, Morita K, Qiao N, Hannan MT, Cupples LA, et al. Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: the Framingham Osteoporosis Study. Am J Clin Nutr. 2006;84:936–42.
9. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, et al. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000;15:2504–12.
10. Jacobs Jr DR, Steffen LM. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. Am J Clin Nutr. 2003;78:508S–13.
11. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol. 2002;13:3–9.
12. Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? Am J Clin Nutr. 2001;73:1–2.
13. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc. 2004;104:615–35.
14. Macdonald HM, Hardcastle AC. Dietary patterns and bone health. In: Burckhardt P, Dawsonhughes B, Weaver C, editors. Nutritional influences on bone health. London: Springer; 2002.
15. Newby PK, Tucker KL. Empirically derived eating patterns using factor or cluster analysis: a review. Nutr Rev. 2004;62:177–203.
16. Hardy R, Cooper MS. Bone loss in inflammatory disorders. J Endocrinol. 2009;201:309–20.
17. Tucker KL, Chen H, Hannan MT, Cupples LA, Wilson PW, et al. Bone mineral density and dietary patterns in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr. 2002;76:245–52.
18. Okubo H, Sasaki S, Horiguchi H, Oguma E, Miyamoto K, et al. Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. Am J Clin Nutr. 2006;83:1185–92.
19. Kontogianni MD, Melistas L, Yannakoulia M, Malagaris I, Panagiotakos DB, et al. Association between dietary patterns and indices of bone mass in a sample of Mediterranean women. Nutrition. 2009;25:165–71.
20. Langsetmo L, Poliquin S, Hanley DA, Prior JC, Barr S, et al. Dietary patterns in Canadian men and women ages 25 and older: relationship to demographics, body mass index, and bone mineral density. BMC Musculoskelet Disord. 2010;11:20.

21. Hardcastle AC, Aucott L, Fraser WD, Reid DM, Macdonald HM. Dietary patterns, bone resorption and bone mineral density in early post-menopausal Scottish women. Eur J Clin Nutr. 2011;65:378–85.
22. Langsetmo L, Hanley DA, Prior JC, Barr SI, Anastassiades T, et al. Dietary patterns and incident low-trauma fractures in postmenopausal women and men aged >/= 50 y: a population-based cohort study. Am J Clin Nutr. 2011;93:192–9.
23. McNaughton SA, Wattanapenpaiboon N, Wark JD, Nowson CA. An energy-dense, nutrient-poor dietary pattern is inversely associated with bone health in women. J Nutr. 2011;141:1516–23.
24. Karamati M, Jessri M, Shariati-Bafghi SE, Rashidkhani B. Dietary patterns in relation to bone mineral density among menopausal Iranian women. Calcif Tissue Int. 2012;91:40–9.
25. Whittle CR, Woodside JV, Cardwell CR, McCourt HJ, Young IS, et al. Dietary patterns and bone mineral status in young adults: the Northern Ireland Young Hearts Project. Br J Nutr. 2012;108:1494–504.
26. Wachman A, Bernstein DS. Diet and osteoporosis. Lancet. 1968;1:958–9.
27. Macdonald H. Influence of organic salts of potassium on bone health: possible mechanisms of action for the role of fruit and vegetables. In: Burckhardt P, Weaver C, Dawsonhughes B, editors. Nutritional aspects of osteoporosis. London: Elsevier/Academic Press; 2007. p. 268–81.
28. Jesudason D, Clifton P. The interaction between dietary protein and bone health. J Bone Miner Metab. 2011;29:1–14.
29. Gough AK, Lilley J, Eyre S, Holder RL, Emery P. Generalised bone loss in patients with early rheumatoid arthritis. Lancet. 1994;344:23–7.
30. Spector TD, Hall GM, McCloskey EV, Kanis JA. Risk of vertebral fracture in women with rheumatoid arthritis. BMJ. 1993;306:558.
31. Bernstein CN, Blanchard JF, Leslie W, Wajda A, Yu BN. The incidence of fracture among patients with inflammatory bowel disease. A population-based cohort study. Ann Intern Med. 2000;133:795–9.
32. Loftus Jr EV, Crowson CS, Sandborn WJ, Tremaine WJ, O'Fallon WM, et al. Long-term fracture risk in patients with Crohn's disease: a population-based study in Olmsted County, Minnesota. Gastroenterology. 2002;123:468–75.
33. Lane NE. Therapy insight: osteoporosis and osteonecrosis in systemic lupus erythematosus. Nat Clin Pract Rheumatol. 2006;2:562–9.
34. Geusens P, Vosse D, van der Linden S. Osteoporosis and vertebral fractures in ankylosing spondylitis. Curr Opin Rheumatol. 2007;19:335–9.
35. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165–85.
36. Lorenzo J, Horowitz M, Choi Y. Osteoimmunology: interactions of the bone and immune system. Endocr Rev. 2008;29:403–40.
37. Du Clos TW. Function of C-reactive protein. Ann Med. 2000;32:274–8.
38. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836–43.
39. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004;351:2599–610.
40. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women – 2011 update: a guideline from the American Heart Association. Circulation. 2011;123:1243–62.
41. Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporos Int. 2005;16:1263–71.
42. Ganesan K, Teklehaimanot S, Tran TH, Asuncion M, Norris K. Relationship of C-reactive protein and bone mineral density in community-dwelling elderly females. J Natl Med Assoc. 2005;97:329–33.
43. Pasco JA, Kotowicz MA, Henry MJ, Nicholson GC, Spilsbury HJ, et al. High-sensitivity C-reactive protein and fracture risk in elderly women. JAMA. 2006;296:1353–5.
44. Schett G, Kiechl S, Weger S, Pederiva A, Mayr A, et al. High-sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. Arch Intern Med. 2006;166:2495–501.
45. Bhupathiraju SN, Alekel DL, Stewart JW, Hanson LN, Shedd KM, et al. Relationship of circulating total homocysteine and C-reactive protein to trabecular bone in postmenopausal women. J Clin Densitom. 2007;10:395–403.
46. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. J Clin Endocrinol Metab. 2008;93:1952–8.
47. de Pablo P, Cooper MS, Buckley CD. Association between bone mineral density and C-reactive protein in a large population-based sample. Arthritis Rheum. 2012;64(8):2624–31.
48. Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, et al. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. J Bone Miner Res. 2007;22:1088–95.
49. Haddad PS, Azar GA, Groom S, Boivin M. Natural health products, modulation of immune function and prevention of chronic diseases. Evid Based Complement Alternat Med. 2005;2:513–20.
50. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, et al. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr. 2005;81:341–54.

51. Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr. 2005;135:562–6.
52. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem. 1994;269: 9889–97.
53. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003;78:517S–20.
54. Acamovic T, Brooker JD. Biochemistry of plant secondary metabolites and their effects in animals. Proc Nutr Soc. 2005;64:403–12.
55. Guo W, Kong E, Meydani M. Dietary polyphenols, inflammation, and cancer. Nutr Cancer. 2009;61:807–10.
56. Garcia-Lafuente A, Guillamon E, Villares A, Rostagno MA, Martinez JA. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res. 2009;58:537–52.
57. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci. 2004;96:229–45.
58. Lee YH, Choi SJ, Ji JD, Song GG. Associations between ERAP1 polymorphisms and ankylosing spondylitis susceptibility: a meta-analysis. Inflamm Res. 2011;60:999–1003.
59. Park HJ, Jeong SK, Kim SR, Bae SK, Kim WS, et al. Resveratrol inhibits *Porphyromonas gingivalis* lipopolysaccharide-induced endothelial adhesion molecule expression by suppressing NF-kappaB activation. Arch Pharm Res. 2009;32:583–91.
60. Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol. 2006;72:1439–52.
61. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol. 2006;72:1493–505.
62. Visioli F, Poli A, Richard D, Paoletti R. Modulation of inflammation by nutritional interventions. Curr Atheroscler Rep. 2008;10:451–3.
63. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, et al. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation. 2003;108:155–60.
64. Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol. 2009; 77:937–46.
65. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. Eur J Clin Nutr. 2009;63:921–33.
66. Galland L. Diet and inflammation. Nutr Clin Pract. 2010;25:634–40.
67. Barr R, Macdonald H, Stewart A, McGuigan F, Rogers A, et al. Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). Osteoporos Int. 2010;21:457–66.
68. Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. Public Health Nutr. 2003;6:313–21.

Weight Loss and Physical Activity in Obese Older Adults: Impact on Skeletal Muscle and Bone

3

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Abstract

The prevalence of overweight and obesity continues to be a major public health concern worldwide. Obesity is a major risk factor for the development of type II diabetes, cardiovascular disease, and increased mortality. Concerns about obesity and overweight among older adults have been far more controversial. The association of overweight and obesity with increased disease burden persists in older adults, and overweight and obesity are also strongly associated with the development of physical disability in this population. However, the efficacy for treatment of obesity in older adults remains an open debate. For obese older adults, who may require weight loss to reduce the risk of cardiometabolic syndrome, weight loss may not be recommended as the associated loss of bone and muscle could leave these individuals at higher risk for frailty and fracture. Thus, optimal strategies for reducing fat mass while preserving bone and muscle mass need to be further evaluated. This chapter will first review the usual age-related changes in skeletal muscle and bone mass with advancing age, the controversy surrounding intentional weight loss in older adults, and discuss the role of diet and physical activity interventions for the successful loss of body fat with specific reference to their effects on bone and skeletal muscle.

Keywords

Obesity • Weight loss • Sarcopenia • Physical activity • Energy restriction

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Abbreviations

aLM	Appendicular lean mass
BMD	Bone mineral density
FFM	Fat-free mass
FM	Fat mass
LM	Lean mass

Introduction

As the prevalence of overweight and obesity plateaus in the United States and continues to increase worldwide, widespread concern about the associated increase in type II diabetes, cardiovascular disease, and mortality continues to remain at the forefront of public policy debate (overweight >25 kg/m^2; obesity >30 kg/m^2) [1]. What has been less recognized is that obesity and overweight in older adults are also associated with similar increases in cardiometabolic risk, as in young individuals, and are additionally associated with increased risk of declines in physical functioning and disability [2, 3].

The loss of muscle mass (sarcopenia) and bone mineral density parallels each other with advancing age. Weight loss through dietary energy restriction can further exacerbate the age-related decreases in muscle and bone mineral density in adults [4, 5]. For sarcopenic obese older adults, who may need to lose weight to decrease their risk of cardiometabolic syndrome, weight loss may not be recommended as the loss of muscle and bone mass may increase their risk of frailty and fracture. Fractures (especially those of the hip/lower extremity) can be devastating, and many individuals never recover to their pre-fracture levels of functioning [6–8] and remain at a higher risk of hospitalization and institutionalization following recovery [9, 10].

Traditionally, voluntary weight loss through dietary energy restriction has been discouraged for older adults largely due to the concomitant loss of skeletal muscle and bone mass that occur in conjunction with the loss in fat mass. However, the increased risk of cardiovascular disease, metabolic syndrome, and disability in overweight and obese individuals persists and in the case of disability actually increases with advancing age. Thus, optimal strategies that effectively result in loss of fat mass while preserving bone and muscle mass need to be further evaluated in older individuals. This chapter will review the health problems associated with obesity in older adults and the controversy surrounding intentional weight loss in older adults and discuss the role of diet and physical activity interventions for the successful loss of body fat with specific reference to their effects on bone and skeletal muscle. Finally, we will present practical recommendations for safe weight loss in obese older adults, with the goal of preserving muscle and bone mass and optimizing physical functioning.

Skeletal Muscle Loss with Aging: Sarcopenia

Skeletal muscle is required for locomotion, oxygen consumption, whole body energy metabolism, and substrate turnover and storage. Robust skeletal muscle mass is essential for maintaining homeostasis and whole body health [11]. Aging is associated with the loss of skeletal muscle and can lead to declines in physical functioning in older adults [12, 13]. The underlying causes of sarcopenia are multifactorial and include decreased physical activity, increased cytokine activity, increased irregularity of muscle unit firing, and a decrease in anabolic hormones [14, 15]. With the loss of muscle mass and strength, and the concurrent increase in joint dysfunction and arthritis that occurs with aging, a decrease in physical function and an increased risk of disability tends to be the result. Despite the high prevalence and major health implications, sarcopenia still has no broadly accepted clinical definition or diagnostic criteria. The most current definition of sarcopenia includes gait speed <1.0 m/s combined with a low ratio of appendicular lean mass (aLM) to height squared (≤7.23 kg/m^2 in males, ≤5.67 kg/m^2 in women) [15]. Thus, energy restriction that induces concomitant reductions in fat and lean mass may be contraindicated in older adults.

Bone Mineral Density and Aging

There is a linear relationship between age and the loss of BMD [16–18]. Though fractures are more common in women, one out of three fractures occurs in men [19]. Currently, bone mineral density (BMD) is the strongest predictor of osteoporotic fracture [20]. BMD is also strongly associated with body weight at multiple skeletal sites [21–24].

Fractures continue to be a major health concern in older populations. Decline in function has been reported following hip fracture, with many patients never returning to their level of ambulation prior to the fracture, even those who were higher functioning [8, 25, 26]. Patients commonly require walking aids following fracture and are at a higher risk for future fracture [27]. Patients who had deficits of skeletal muscle mass prior to the fracture are at a higher risk of poor recovery after the fracture [28]. This may leave patients at higher risk for decreased physical function and increased risk of institutionalization.

Health-Related Risks of Obesity in Older Adults

The increase of obese older adults is becoming a major public health concern, due to the higher prevalence of medical complications associated with obesity. Obesity has been shown to be associated with and causally linked to several morbidities, including diabetes, hypertension, cardiovascular disease, and arthritis. In addition to the risks of chronic illness, obese individuals have a higher prevalence of impaired quality of life, more specifically on domains involving physical function [29].

Cardiovascular Disease

Cardiovascular disease (CVD) and coronary heart disease (CHD) are major causes of mortality in older populations. Obesity has been shown to increase risk of these conditions. Other conditions that predict cardiac events in older adults include hypertension, diabetes, and hyperlipidemia, all of which are also associated with increased body weight [30, 31]. The Nurse's Health Study showed that among obese older adults, rates of CVD were four times higher compared to the leanest group. The relative risk of CHD also trended significantly with increased body weight ($p<0.001$). Additionally, obese older adults have a higher prevalence of risk factors for cardiovascular disease compared to normal weight older adults. Wannamethee et al. reported data from a cross-sectional analysis, comparing adults between the ages of 60 and 79, and looked at the odds ratio (OR) of diagnosis of many risk factors of CVD. The outcomes included systolic and diastolic blood pressure, hypertension, total cholesterol, HDL cholesterol, and triglycerides. Out of these measures, obese males (BMI ≥30) had a significantly higher OR in all outcomes (except total cholesterol) compared to both normal and overweight groups [3].

When other cardiovascular outcomes, such as heart attack, angina, stroke, major cardiovascular disease, and physician-diagnosed hypertension, have been examined in older adults, there tends to be an increased risk for obese persons compared to normal weight. When separated across four separate BMI categories, normal (BMI 18.5–24.9), overweight (25–27.4 and 27.5–29.9), and obese (≥30), the prevalence of all outcomes was significantly higher in obesity compared to each weight category, demonstrating that older obese men are at higher risk of cardiovascular outcomes compared to overweight and normal weight men at the same age [3]. There is also a strong correlation of increasing BMI and both increased systolic and diastolic blood pressure in older men and women [31–33]. Masaki et al. demonstrated in the Honolulu Heart Project that for each unit increase of BMI, there was an associated increase in systolic blood pressure 1.15 mmHg and diastolic blood pressure of 0.70 mmHg.

Metabolic Syndrome

Diabetes is a growing health concern in many developed countries, and the prevalence is expected to continue to grow, particularly in older populations [34]. Currently approximately 20 % of diabetics are >60 years, and it is estimated that

approximately two thirds of diabetes will be >60 years in 2025 [35]. It is well known that diabetes is associated with advancing age [34], as well as obesity in adults [36]. Not only is obesity associated with diabetes and metabolic syndrome but high amounts of visceral and intermuscular fat are also independent predictors of metabolic syndrome in middle and older adults, even those with normal weight [37]. In a large cross-sectional study, researchers examined the odds ratio of Homeostasis Model Assessment (HOMA) scores in older men, aged 60–79, and compared the results between weight groups: normal weight, overweight, and obese. They showed that the prevalence for a high HOMA score for elderly obese patients is about three times higher than that of overweight and ten times higher than normal weight men at the same age [3]. Other cross-sectional trials support these findings, showing a higher prevalence of diabetes in obese older adults compared to normal and overweight older adults [38].

Arthritis

Osteoarthritis is a major cause of disability in the elderly especially in the lower extremity (knees, hips, ankles). This can inhibit ambulation and make daily activities very difficult. This lack of mobility can lead to muscle atrophy, eventually causing disability. As no cure currently exists, the need for prevention and intervention treatment to reduce pain is necessary.

Several longitudinal studies have demonstrated the relationship between obesity and the development of osteoarthritis, particularly in load-bearing joints such as the knee and hip [39–43]. In NHANES I, a direct increase in osteoarthritis and joint pain was observed with increasing body weight. The prevalence of arthritis tends to increase as body weight increases, as Blaum et al. reported that 60.2 % of the obese women reported osteoarthritis of the knees, compared to 40.7 % of the overweight and 29.2 % of the normal weight groups. The Framingham Heart Study reported that obesity is a major risk factor for arthritis in the elderly [44]. The increase of arthritis in obese older adults is thought to be caused primarily by the increased mechanical stress that is put on load-bearing joints, such as the hip and knee [45]. However, though there is evidence that weight loss and exercise in an obese, elderly population can improve function and decrease joint pain, the effects of weight loss on body composition still show a loss of lean mass, potentially increasing risk of future disability [46, 47].

Mortality Risk in Obese Older Adults and the Controversy Surrounding Weight Loss

Obesity is a risk factor for mortality independent of smoking and history of disease for older adults. The Framingham Heart Study showed that men who were obese at age 40 live 5.8 years less than their normal weight counterparts and women who were obese at age 40 live 7.1 years less [48]. Mortality rate was the lowest among women with BMI between 19.0 and 26.9 kg/m^2.

In the Cancer Prevention Study II, the positive correlation between increased BMI and risk of death was supported. The data suggested that obesity is most strongly linked to all-cause mortality in those who never smoked and had no history of disease further demonstrating that obesity is a significant independent risk factor of mortality. Epidemiological studies clearly show a relationship between obesity and health outcomes in older adults. However, the relationship between mortality and body weight has been described as U shaped, illustrating that those with very low and very high BMI are at higher risk of mortality than those individuals with BMI's in the nadir. Some studies have suggested that older obese adults over 75 years are at a lower relative risk of death compared to those with lower body weight [34, 49]. Although the observational evidence is very clear that obese older adults are at risk for adverse health outcomes, there is a lack of randomized trial data examining disease regression and mortality of this population. In addition, well-documented evidence suggests that underweight older adults have high risk for mortality. However, caution should be used in extrapolating the existing data to all obese older adults particularly for those in the oldest age ranges (>80 years). Because of these limitations, controversy remains surrounding weight loss for obese older adults.

Functional Mobility/Disability and Obesity

There are strong associations between physical function and mobility and obesity in older populations. Elevated BMI is a strong predictor of long-term risk for mobility disability [2, 50]. A strong relationship between body weight and the decline in physical function has also been reported in older adults [51]. The mechanisms by which obesity affect physical function with age may be related to the increased mechanical stress resulting from a greater body mass, the secondary effects of chronic low-grade inflammation, and the deconditioning associated with the sedentary behavior commonly associated with obesity, thus making it difficult to perform daily activities such as walking [2].

Wannamethee et al. examined the difference in self-reported disability with 4,232 men age 60–79 between different BMI categories: normal (18.5–24.9), overweight (25–27.4 and 27.5 and 29.9), and obese (≥30). Subjects were asked to report any illness or disability, resulting in difficulty going up- and downstairs, bending down/straightening up, falling or difficulty with balance, or walking for a quarter of a mile on the level. They were also asked to report any difficulty performing usual activities, such as work, housework, family activities, washing, and dressing. Obese men had a significant prevalence and OR of all self-reported disability outcomes after adjusting for age, smoking, social class, alcohol intake, and physical activity compared to normal weight and overweight men of the same age [3].

Diet and Physical Activity and the Successful Loss of Body Fat

Energy Restriction

The goal of energy restriction is to decrease excess fat mass in order to reduce risk of cardiometabolic disease, arthritis, and other health problems. Though many types of weight loss programs are in existence, they all revolve largely around the concept of decreasing total caloric intake to reduce fat mass. However, when energy intake is restricted, muscle and bone mass are also lost. For most young- and middle-aged overweight and obese adults, weight loss through energy restriction is typically recommended [52], as the muscle and bone loss should not negatively impact other health outcomes. In obese older adults, the loss of skeletal muscle mass and bone mass during energy restriction can have a significantly greater impact on physical function.

Influence of Energy Restriction on Bone

The rate of bone loss increases with age in both men and women [53, 54]. Weight loss can increase the rate of bone loss in older adults [55]. Men and women who lose weight show a greater than average rate of bone mineral density decrease. Additionally, obese older adults tend to lose more bone mass compared to younger adults [54, 56]. Older women who lose weight, both intentional and unintentional, are reported to have twice the risk of fracture compared to women who remain weight stable due to greater loss of bone during energy restriction [53, 57]. Additionally, increases in bone turnover have been seen during energy restriction as well [55, 58–60]. These data highlight that BMD is not the only outcome affected by weight loss in older adults but other risk factors of osteoporosis are affected as well.

The decrease in BMD with weight loss may be due to the reduced mechanical stress that lower body weight puts on the skeleton. This is shown by decreasing bone mineral density in weight-bearing areas, such as the hip and spine. However, reduction of bone mineral density in non-weight-bearing sites is also observed during weight loss, which implies there is an independent factor that protects bone mass in obese individuals.

Protein and calcium supplementation may help to maintain bone mineral density during energy restriction in older adults [61]. Sukumar et al. reported older adults who consumed more protein per day during energy restriction attenuated total bone loss compared to the low-protein group [62]. Calcium supplementation may also be an important factor in the preservation of bone mass during energy restriction. Shapses et al. examined older women and demonstrated that those with adequate calcium supplementation can also preserve bone during weight loss [63].

Influence on Energy Restriction on Muscle

In order to reduce the cardiometabolic complications that are associated with obesity, the primary intervention for most young- and middle-aged patients is diet-induced weight loss. Young and older obese subjects lose approximately 25 % fat-free mass (FFM) and 75 % fat mass (FM) during energy restriction [64, 65]. Although losing a significant amount of FM through energy restriction may be beneficial in preventing cardiovascular, metabolic, and orthopedic disorders, the concomitant loss of approximately 25 % of FFM may significantly impair muscle strength and physical functioning. This is a major reason weight loss therapy is not widely recommended for older adults.

Dietary protein intake may have an effect on muscle preservation during weight loss. In a 3-year follow-up study of older adults, those who consumed more dietary protein lost approximately 40 % less lean mass (LM) and aLM compared to those who consumed less protein [66]. This implies that the restricted amount of protein during energy restriction may be causing the increased loss of muscle.

Understanding the mechanism behind the decrease in FFM during energy restriction may lead to effective therapies to minimize the loss of FFM while maximizing the reduction of FM. Using several models of obesity in rodents, both muscle protein synthesis and degradation have been shown to be suppressed during energy restriction, with muscle protein breakdown being suppressed less, resulting in the decrease in muscle mass [67–69]. In humans multiple studies reported that obese older adults unlike obese rodents who are on a calorie-restricted diet have increased rates of muscle protein breakdown, without a change in muscle protein synthesis during energy restriction [70, 71].

Physical Activity

Physical activity is a major regulator of body weight in adults and also generally declines with age, which may lead to more muscle loss and fat gain [72]. Exercise is usually prescribed to older adults, and walking is the primary mode. Weight-bearing exercises are also generally recommended, but the implementation of these is more difficult for the majority of the population.

Influence of Physical Activity on Bone

There are now many studies that have examined the effect of exercise on BMD in both men and women [73–78]. Aerobic and resistance training exercise interventions have both been investigated, independently and combined, to examine the effects on BMD in middle to older populations. In general, aerobic exercise interventions have been shown to result in a maintenance of BMD in weight-bearing areas, while when resistance training is added, BMD can be increased [55, 79]. However, the mechanism behind this preservation of BMD has yet to be clearly defined.

Influence of Physical Activity on Muscle

Physical activity increases muscle mass in older adults [80–85]. Exercise programs have included both aerobic and resistance training of varying intensity and duration. Older adults who complete these exercise programs have shown significant gains and muscle strength and size. Increasing muscle strength has also been shown to be associated with increased physical function in older populations [86].

Energy Restriction with Physical Activity

Many research trials have shown the isolated effects of weight loss and exercise in older populations. Far fewer have investigated the combined effects of weight loss and exercise, compared to exercise and weight loss alone. Because exercise can preserve and/or increase FFM, it may have the potential to counteract the deteriorating effect of energy restriction on FFM.

Influence of Energy Restriction with Physical Activity on Bone

In a 12-month randomized clinical trial, 107 frail, obese older adults aged 65 years and older were

randomly assigned to a diet group, exercise group, diet and exercise group, and control [55]. The hypothesis of this trial was to determine whether those randomly assigned to the diet and exercise group would attenuate the loss of BMD during energy restriction compared to the group randomized to diet alone. Subjects in the diet or diet and exercise group were instructed to lose 10 % of their total body weight in the first 6 months and then maintain that reduced weight for the following 6 months. Those randomized to the exercise or diet and exercises group completed exercise sessions three times per week. Each session included aerobic exercises, resistance training, and balance and flexibility training. Resistance exercises were progressing and involved all major muscle groups. Intensity gradually increased until subjects performed 2–3 sets at approximately 80 % of the one-repetition maximum, with 6–8 repetitions of each. Aerobic exercises included walking on a track or treadmill, stationary cycling, or stair climbing. Subjects were progressed so that they exercised at 70–85 % of their maximal heart rate. After 12 months, the group exposed to both diet and exercise interventions lost approximately 10 % of body weight but were able to minimize loss in bone mineral density, which decreased approximately 1.1 %. The diet only group lost 2.6 % of BMD, and the exercise group actually increased 1.5 %. This effectively shows that exercise during energy restriction may minimize loss of BMD in obese older adults [55].

Influence on Muscle

Physical function in obese older adults can be maintained during weight loss with exercise. Subjects who completed an exercise program with aerobic and resistance training during weight loss were able to lose weight and still show an increase in physical function compared to a control group [87]. Additionally, when older subjects who were randomly assigned to a 12-month diet and exercise treatments were compared with those assigned to diet or exercise independently, a significant increase in physical function was observed [88].

Body composition can be maintained in obese older adults during energy restriction if exercise is added. Subjects completing a 12-month diet alone, exercise, or diet and exercise were assessed for body composition [55]. The subjects in the diet and exercise group only lost an average of 1.8 kg of FFM after 1 year, approximately 21 % of the total weight lost. The diet group lost an average of 3.2 kg of FFM, approximately 33 % of the total weight lost. The subjects in the exercise group gained an average of 1.3 kg of FFM. The subjects in the diet group also lost about 81 cm^3 of thigh muscle volume compared to a loss of 28 cm^3 in a weight loss and exercise group. There was no difference in fat loss between those two groups. These results demonstrate that FFM can be maintained during energy restriction with regular exercise.

Conclusions

Sarcopenic obese adults have the worst of two worlds. They have relatively low muscle mass and increased weight to carry. This can put an increased amount of stress on joints, making ambulation and the completion of daily activities difficult. The increased fat mass also puts older adults at increased risk for cardiovascular disease, diabetes, and mortality. Though weight loss may be necessary to decrease the risk of these serious health outcomes, it is generally not recommended due to the loss of skeletal muscle and bone mass which is concurrent with weight loss. There is a need to define a more efficient way to lose fat mass while preserving fat-free mass.

For obese older adults, weight loss should be achieved while incorporating methods to maintain both skeletal muscle and bone mass. Table 3.1 shows the recommendations for weight loss in older adults. Calories should be restricted by about 500–700 kcal/day, which translates into approximately 1–2 lb/week. Approximately 1 g of protein per kilogram of body weight should be consumed as well [48].

Table 3.1 Weight loss recommendations for obese older adults

Energy restriction[a]	500–750 kcal/day
Protein intake[a]	1 g/kg body weight

[a]Obesity in older adults: technical review and position statement for nutrition and NAASO, the Obesity Society

Table 3.2 Exercise and recommendations for obese older adults during weight loss

Exercise	Mode	Frequency	Duration	Intensity
Aerobic[a]	Walking	>3 days/week	Work up to at least 30 min/day	Work up to 60–80 % of maximal heart rate
Resistance[a]	Progressive strength training exercises using all major muscle groups	2–3 days/week	1–3 sets of each exercise	65–80 % of maximum
Balance[a]		Daily		
Flexibility[a]		Daily		

[a]Recommendations from bone health and osteoporosis: a report of the surgeon general

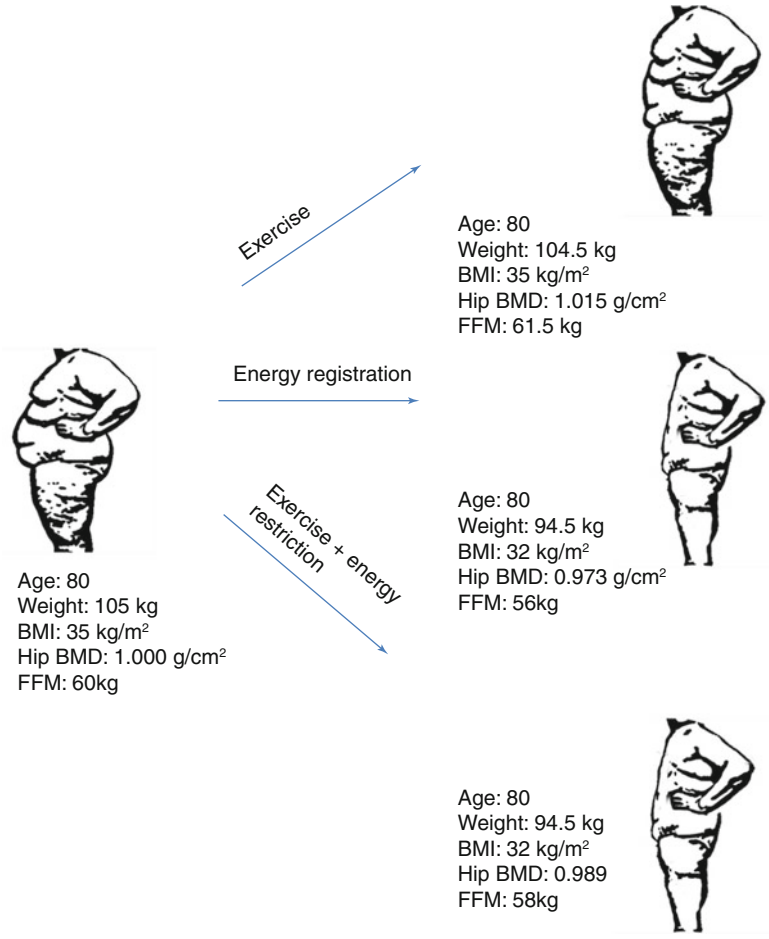

Fig. 3.1 Expected weight, BMI, hip BMD, and FFM of an 80-year-old obese individual following three separate weight loss programs. Exercise only shows an increase in FFM and hip BMD, while weight and BMI remain unchanged. Energy restriction alone shows a decrease in weight and BMI, hip BMD, and FFM. Exercise combined with energy restriction shows a decrease in weight and BMI, but hip BMD and FFM were relatively maintained. Changes in weight, BMI, hip BMD, and FFM were estimated based on the results of previous clinical research (Based on data from Ref. [88])

Adequate calcium and dairy intake are also recommended to reduce bone loss during energy restriction.

In addition to energy restriction, aerobic and resistance exercise should be added in order to maintain skeletal muscle and bone loss. Table 3.2 shows the recommendations for exercises that can prevent muscle and bone loss while building strength. It is important that the program is progressive to avoid a plateau in strength. Balance and flexibility exercise should be added daily to prevent falls.

Figure 3.1 illustrates the effects of energy restriction, exercise, and energy restriction

combined with exercise [88]. The figure shows that energy restriction combined with exercise is able to maintain more FFM and hip BMD than the diet intervention alone.

Future Research

Future studies should focus on the influence of dietary energy restriction on long-term health outcomes in older adults. In addition, future studies should evaluate the impact of obesity in the oldest of the old.

References

1. Flegal KM, et al. Prevalence and trends in obesity among US adults, 1999–2008. JAMA. 2010;303(3):235–41.
2. Launer LJ, et al. Body mass index, weight change, and risk of mobility disability in middle-aged and older women. The epidemiologic follow-up study of NHANES I. JAMA. 1994;271(14):1093–8.
3. Goya Wannamethee S, et al. Overweight and obesity and the burden of disease and disability in elderly men. Int J Obes Relat Metab Disord. 2004;28(11):1374–82.
4. Jensen LB, Quaade F, Sorensen OH. Bone loss accompanying voluntary weight loss in obese humans. J Bone Miner Res. 1994;9(4):459–63.
5. Ballor DL, et al. Resistance weight training during caloric restriction enhances lean body weight maintenance. Am J Clin Nutr. 1988;47(1):19–25.
6. Mossey JM, et al. Determinants of recovery 12 months after hip fracture: the importance of psychosocial factors. Am J Public Health. 1989;79(3):279–86.
7. Koval KJ, et al. Ambulatory ability after hip fracture. A prospective study in geriatric patients. Clin Orthop Relat Res. 1995;310:150–9.
8. Magaziner J, et al. Recovery from hip fracture in eight areas of function. J Gerontol A Biol Sci Med Sci. 2000;55(9):M498–507.
9. Bonar SK, et al. Factors associated with short- versus long-term skilled nursing facility placement among community-living hip fracture patients. J Am Geriatr Soc. 1990;38(10):1139–44.
10. Wolinsky FD, Fitzgerald JF, Stump TE. The effect of hip fracture on mortality, hospitalization, and functional status: a prospective study. Am J Public Health. 1997;87(3):398–403.
11. Haran PH, Rivas DA, Fielding RA. Role and potential mechanisms of anabolic resistance in sarcopenia. J Cachexia Sarcopenia Muscle. 2012;3(3):157–162.
12. Davidson LE, et al. An epidemiologic study of risk factors in two teenage suicide clusters. JAMA. 1989;262(19):2687–92.
13. Roubenoff R. Origins and clinical relevance of sarcopenia. Can J Appl Physiol. 2001;26(1):78–89.
14. Morley JE, et al. Sarcopenia. J Lab Clin Med. 2001;137(4):231–43.
15. Fielding, R.A., et al., Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. J Am Med Dir Assoc, 2011. 12(4): p. 249–56.
16. Ensrud KE, et al. Hip and calcaneal bone loss increase with advancing age: longitudinal results from the study of osteoporotic fractures. J Bone Miner Res. 1995;10(11):1778–87.
17. Greenspan SL, et al. Fall severity and bone mineral density as risk factors for hip fracture in ambulatory elderly. JAMA. 1994;271(2):128–33.
18. Steiger P, et al. Age-related decrements in bone mineral density in women over 65. J Bone Miner Res. 1992;7(6):625–32.
19. Cawthon PM, et al. Loss of hip BMD in older men: the osteoporotic fractures in men (MrOS) study. J Bone Miner Res. 2009;24(10):1728–35.
20. Cummings SR, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet. 1993;341(8837):72–5.
21. Edelstein SL, Barrett-Connor E. Relation between body size and bone mineral density in elderly men and women. Am J Epidemiol. 1993;138(3):160–9.
22. Hannan MT, Felson DT, Anderson JJ. Bone mineral density in elderly men and women: results from the Framingham osteoporosis study. J Bone Miner Res. 1992;7(5):547–53.
23. Salamone LM, et al. Body composition and bone mineral density in premenopausal and early perimenopausal women. J Bone Miner Res. 1995;10(11):1762–8.
24. Glauber HS, et al. Body weight versus body fat distribution, adiposity, and frame size as predictors of bone density. J Clin Endocrinol Metab. 1995;80(4):1118–23.
25. Marottoli RA, Berkman LF, Cooney Jr LM. Decline in physical function following hip fracture. J Am Geriatr Soc. 1992;40(9):861–6.
26. Koval KJ, et al. Dependency after hip fracture in geriatric patients: a study of predictive factors. J Orthop Trauma. 1996;10(8):531–5.
27. Binder EF, et al. Effects of extended outpatient rehabilitation after hip fracture: a randomized controlled trial. JAMA. 2004;292(7):837–46.
28. Visser M, et al. Change in muscle mass and muscle strength after a hip fracture: relationship to mobility recovery. J Gerontol A Biol Sci Med Sci. 2000;55(8):M434–40.
29. Fontaine KR, Barofsky I. Obesity and health-related quality of life. Obes Rev. 2001;2(3):173–82.
30. Harris TB, et al. Carrying the burden of cardiovascular risk in old age: associations of weight and weight change with prevalent cardiovascular disease, risk factors, and health status in the Cardiovascular Health Study. Am J Clin Nutr. 1997;66(4):837–44.

31. Javed F, et al. Association of BMI and cardiovascular risk stratification in the elderly African-American females. Obesity (Silver Spring). 2011;19(6):1182–6.
32. Masaki KH, et al. Association of body mass index with blood pressure in elderly Japanese American men. The Honolulu Heart Program. Hypertension. 1997;29(2):673–7.
33. Mihel S, Milanovic SM. Association of elevated body mass index and hypertension with mortality: the CroHort study. Coll Antropol. 2012;36 Suppl 1:183–8.
34. Harris MI, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988–1994. Diabetes Care. 1998;21(4):518–24.
35. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998;21(9): 1414–31.
36. Mokdad AH, et al. The continuing epidemics of obesity and diabetes in the United States. JAMA. 2001;286(10):1195–200.
37. Goodpaster BH, et al. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med. 2005;165(7):777–83.
38. Blaum CS, et al. The association between obesity and the frailty syndrome in older women: the Women's Health and Aging Studies. J Am Geriatr Soc. 2005;53(6): 927–34.
39. Davis MA, Ettinger WH, Neuhaus JM. The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. J Rheumatol. 1988;15(12):1827–32.
40. Felson DT, et al. Obesity and knee osteoarthritis. The Framingham Study. Ann Intern Med. 1988;109(1):18–24.
41. Bagge E, et al. Factors associated with radiographic osteoarthritis: results from the population study 70-year-old people in Goteborg. J Rheumatol. 1991; 18(8):1218–22.
42. Spector TD, Hart DJ, Doyle DV. Incidence and progression of osteoarthritis in women with unilateral knee disease in the general population: the effect of obesity. Ann Rheum Dis. 1994;53(9):565–8.
43. Cicuttini FM, Spector T, Baker J. Risk factors for osteoarthritis in the tibiofemoral and patellofemoral joints of the knee. J Rheumatol. 1997;24(6):1164–7.
44. Felson DT, et al. Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. Arthritis Rheum. 1997;40(4):728–33.
45. Gunther KP, et al. Prevalence of generalised osteoarthritis in patients with advanced hip and knee osteoarthritis: the Ulm Osteoarthritis Study. Ann Rheum Dis. 1998;57(12):717–23.
46. Miller GD, et al. Intensive weight loss program improves physical function in older obese adults with knee osteoarthritis. Obesity (Silver Spring). 2006;14(7):1219–30.
47. Messier SP, et al. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum. 2004;50(5):1501–10.
48. Peeters A, et al. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. Ann Intern Med. 2003;138(1):24–32.
49. Cornoni-Huntley JC, et al. An overview of body weight of older persons, including the impact on mortality. The National Health and Nutrition Examination Survey I – Epidemiologic Follow-up Study. J Clin Epidemiol. 1991;44(8):743–53.
50. Jensen GL, Friedmann JM. Obesity is associated with functional decline in community-dwelling rural older persons. J Am Geriatr Soc. 2002;50(5):918–23.
51. Villareal DT, et al. Physical frailty and body composition in obese elderly men and women. Obes Res. 2004;12(6):913–20.
52. Stern JS, Thomas PR. A commentary on weighing the options: criteria for evaluating weight-management programs. Obes Res. 1995;3(6):589–90.
53. Ensrud KE, et al. Intentional and unintentional weight loss increase bone loss and hip fracture risk in older women. J Am Geriatr Soc. 2003;51(12):1740–7.
54. Nguyen TV, Sambrook PN, Eisman JA. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology Study. J Bone Miner Res. 1998;13(9):1458–67.
55. Shah K, et al. Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. J Bone Miner Res. 2011;26(12):2851–9.
56. Ensrud KE, et al. Low fractional calcium absorption increases the risk for hip fracture in women with low calcium intake. Study of Osteoporotic Fractures Research Group. Ann Intern Med. 2000;132(5):345–53.
57. Shapses SA, Sukumar D. Bone metabolism in obesity and weight loss. Annu Rev Nutr. 2012;32: 287–309.
58. Chao D, et al. Effect of voluntary weight loss on bone mineral density in older overweight women. J Am Geriatr Soc. 2000;48(7):753–9.
59. Riedt CS, et al. Overweight postmenopausal women lose bone with moderate weight reduction and 1 g/day calcium intake. J Bone Miner Res. 2005;20(3):455–63.
60. Ricci TA, et al. Moderate energy restriction increases bone resorption in obese postmenopausal women. Am J Clin Nutr. 2001;73(2):347–52.
61. Thorpe MP, et al. A diet high in protein, dairy, and calcium attenuates bone loss over twelve months of weight loss and maintenance relative to a conventional high-carbohydrate diet in adults. J Nutr. 2008;138(6):1096–100.
62. Sukumar D, et al. Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: a randomized, controlled trial. J Bone Miner Res. 2011;26(6):1339–48.
63. Shapses SA, et al. Bone turnover and density in obese premenopausal women during moderate weight loss and calcium supplementation. J Bone Miner Res. 2001;16(7):1329–36.
64. Dengel DR, et al. Effects of weight loss by diet alone or combined with aerobic exercise on body composition in older obese men. Metabolism. 1994;43(7):867–71.

65. Gallagher D, et al. Weight loss in postmenopausal obesity: no adverse alterations in body composition and protein metabolism. Am J Physiol Endocrinol Metab. 2000;279(1):E124–31.
66. Houston DK, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008;87(1):150–5.
67. Dunn MA, Houtz SK, Hartsook EW. Effects of fasting on muscle protein turnover, the composition of weight loss, and energy balance of obese and nonobese Zucker rats. J Nutr. 1982;112(10):1862–75.
68. Goodman MN, McElaney MA, Ruderman NB. Adaptation to prolonged starvation in the rat: curtailment of skeletal muscle proteolysis. Am J Physiol. 1981;241(4):E321–7.
69. Clark AS, Mitch WE. Comparison of protein synthesis and degradation in incubated and perfused muscle. Biochem J. 1983;212(3):649–53.
70. Villareal DT, et al. Effect of weight loss on the rate of muscle protein synthesis during fasted and fed conditions in obese older adults. Obesity (Silver Spring). 2012; 20:1780–6.
71. Campbell WW, et al. Resistance training preserves fat-free mass without impacting changes in protein metabolism after weight loss in older women. Obesity (Silver Spring). 2009;17(7):1332–9.
72. Hughes VA, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. J Gerontol A Biol Sci Med Sci. 2001;56(5):B209–17.
73. Chan K, et al. A randomized, prospective study of the effects of Tai Chi Chun exercise on bone mineral density in postmenopausal women. Arch Phys Med Rehabil. 2004;85(5):717–22.
74. Walker M, et al. Longitudinal evaluation of supervised versus unsupervised exercise programs for the treatment of osteoporosis. Eur J Appl Physiol. 2000;83(4–5): 349–55.
75. White MK, et al. The effects of exercise on the bones of postmenopausal women. Int Orthop. 1984;7(4):209–14.
76. Kemmler W, et al. Exercise effects on fitness and bone mineral density in early postmenopausal women: 1-year EFOPS results. Med Sci Sports Exerc. 2002; 34(12):2115–23.
77. Cussler EC, et al. Weight lifted in strength training predicts bone change in postmenopausal women. Med Sci Sports Exerc. 2003;35(1):10–7.
78. Nelson ME, et al. A 1-y walking program and increased dietary calcium in postmenopausal women: effects on bone. Am J Clin Nutr. 1991;53(5):1304–11.
79. Yamazaki S, et al. Effect of walking exercise on bone metabolism in postmenopausal women with osteopenia/ osteoporosis. J Bone Miner Metab. 2004;22(5): 500–8.
80. Taaffe DR, et al. Once-weekly resistance exercise improves muscle strength and neuromuscular performance in older adults. J Am Geriatr Soc. 1999;47(10): 1208–14.
81. Ettinger Jr WH, et al. A randomized trial comparing aerobic exercise and resistance exercise with a health education program in older adults with knee osteoarthritis. The Fitness Arthritis and Seniors Trial (FAST). JAMA. 1997;277(1):25–31.
82. Buchner DM, et al. The effect of strength and endurance training on gait, balance, fall risk, and health services use in community-living older adults. J Gerontol A Biol Sci Med Sci. 1997;52(4):M218–24.
83. Schlicht J, Camaione DN, Owen SV. Effect of intense strength training on standing balance, walking speed, and sit-to-stand performance in older adults. J Gerontol A Biol Sci Med Sci. 2001;56(5):M281–6.
84. Seguin R, Nelson ME. The benefits of strength training for older adults. Am J Prev Med. 2003;25(3 Suppl 2): 141–9.
85. Frontera WR, et al. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. J Appl Physiol. 1988;64(3):1038–44.
86. Brown M, Sinacore DR, Host HH. The relationship of strength to function in the older adult. J Gerontol A Biol Sci Med Sci. 1995; 50 spec no:55–9.
87. Villareal DT, et al. Effect of weight loss and exercise on frailty in obese older adults. Arch Intern Med. 2006;166(8):860–6.
88. Villareal DT, et al. Weight loss, exercise, or both and physical function in obese older adults. N Engl J Med. 2011;364(13):1218–29.

The Hormonal Milieu in Obesity and Influences on the Trabecular, Cortical, and Geometric Properties of Bone

4

Sue A. Shapses and Deeptha Sukumar

Abstract

Obesity is associated with alterations in several endocrine factors, some of which are involved in regulating bone metabolism. The higher serum concentrations of parathyroid hormone (PTH), estradiol, pancreatic hormones, and adipokines such as leptin, resistin, and cytokines and the lower 25-hydroxyvitamin D (25OHD) have specific actions on the skeleton and regulate cortical and trabecular bone differently. Recent evidence suggests that bone quality is altered in obesity with a higher trabecular volumetric bone mineral density (vBMD), while cortical vBMD is lower. Also, the obese are at greater risk of fracture for a given BMD compared to normal weight individuals supporting the evidence that bone quality is altered due to excess adiposity. Higher concentrations of serum PTH have a catabolic effect on cortical bone and may play a role in reducing cortical vBMD in obesity. The lower serum 25OHD, higher leptin and resistin, and lower adiponectin may also independently contribute to the lower cortical vBMD in obesity. There is little evidence to show that higher pancreatic hormones and cytokines influence trabecular and cortical bone in obesity. The altered hormonal milieu in obesity is one important factor that explains bone architectural changes that occur due to excess adiposity. However, other factors such as diet, genetic factors, altered mechanical loading, and/or other environmental factors may also contribute to bone quality and site-specific fracture risk in obesity.

Keywords

Obesity • Hormones • Trabecular • Cortical • Volumetric bone mineral density • Body composition

Introduction

Obesity is a worldwide epidemic and the World Health Organization reports that at least 2.8 million people die each year as a result of excess body weight [1]. Obesity is considered a true epi-

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demic because while rates are disparate across different socioeconomic and racial/ethnic groups, the rise in obesity is similar [2]. It is associated with an increased risk for several comorbidities, including cardiovascular disease, type 2 diabetes, and certain cancers [3]. It has long been established that bone mineral density (BMD) is greater in obesity; however, newer studies suggest that bone quality is altered and show evidence of fractures in this population. The relationship between excess adiposity and bone is likely influenced by the pluripotent stromal cell [4] that differentiates into adipocytes and osteoblasts, as well as chondrocytes. Several endocrine aberrations are seen in obesity, some of which have an important effect on the skeleton. In addition, multiple other factors contribute to BMD and fracture risk in obesity, including mechanical loading of excess body weight on bone [5, 6]. This chapter discusses the unique aspects of bone and fracture risk due to obesity with a focus on the hormonal milieu and its influence on the bone trabecular and cortical compartments of bone and geometry.

Areal and Volumetric Bone Mineral Density: Implications in Obesity

Dual-energy X-ray absorptiometry (DXA) is a valuable two-dimensional bone imaging technique that can assess the relationship between body composition (fat and lean tissue) and bone mineral content (BMC) and areal BMD (aBMD). Although it is the gold standard for BMD measurements, artifacts associated with a two-dimensional measurement of areal bone density (g/cm^2) are considered a limitation at the extremes of BMD (very high or low) or due to excess soft tissue surrounding bone, such as in obesity [7–9]. In addition, the obese have a higher prevalence of vertebral deformities [10] and spinal osteoarthritis [11], which can overestimate BMD and BMC. Careful examination of the lumbar spine measurement for vertebral exclusion is needed in the interpretation of BMD [12] and may require special consideration for the obese.

Information about bone quality can be attained by the inclusion of architectural parameters such as bone size and geometry. This can be assessed with radiography, DXA, peripheral quantitative computed tomography (pQCT), quantitative computed tomography (QCT), or magnetic resonance imaging (MRI). Microarchitectural parameters include cortical and trabecular structural detail which can be evaluated by pQCT or by using high-resolution imaging techniques such as multidetector CT, MRI, and higher-resolution pQCT which will allow for high-precision images and estimation of additional biomechanical properties. In addition, microcomputed tomography techniques are used to examine human bone biopsy samples or excised bone in rodent studies. Bone strength, defined as the force required to cause a material to fail under a given loading condition [13], can be measured directly using biomechanical testing methods in excised bone or can be estimated, in clinical trials, by the amount of mineralized material (BMD) and geometrical properties [14]. These three-dimensional methodologies can assess volumetric BMD (vBMD; mg/cm^3) and bone structural parameters, distinguish between cortical and trabecular bone, and determine the relationship with soft tissue (e.g., muscle and fat cross-sectional area). Measurements of true vBMD that use the density of fat tissue as zero have been found to reduce errors as compared to an areal measurement, such as using DXA technology. Quantitative computed tomography measurements of bone can measure axial sites by QCT and peripheral sites using pQCT. In obesity, potential BMD artifacts may be attenuated or removed by measuring a peripheral (rather than axial) site because less soft tissue surrounds the bone of the arm or leg. In addition, most clinical studies examining vBMD and bone architectural parameters use pQCT due to ease of use and because, compared to QCT, the method produces very low radiation exposure to the patient at only peripheral sites, and therefore is appropriate for both adult and pediatric populations. For example, the total radiation dose is <7 uSv when measuring the tibia and radius using the Stratec-Orthometrix pQCT, and this dose is similar to a DXA measurement at both the hip and spine. In comparison, this dose is less than 1 day of background radiation (~8 uSv/day) or a cross-country flight (~40 uSv).

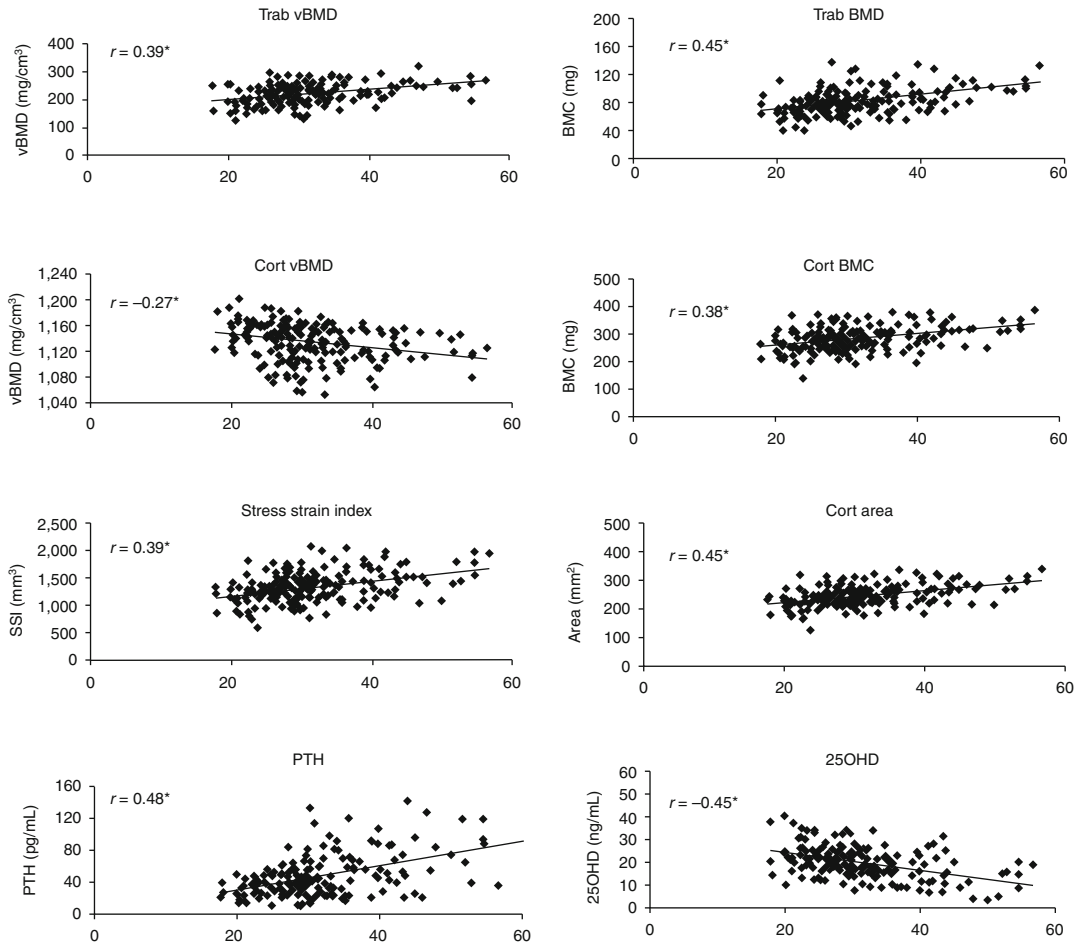

Fig. 4.1 Relationship between body mass index, cortical, and trabecular bone parameters (volumetric bone mineral density (vBMD) and content (BMC), cortical area, and stress-strain index) and serum parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OHD) in 211 women. *$p<0.001$ (Reprinted from Sukumar et al. [19]. With permission from Springer Science + Business Media)

There is an association of peripheral QCT (pQCT) outcomes with fracture, although these studies are limited compared to measurements with DXA.

Volumetric BMD and Bone Quality in Obesity

An altered bone quality may partially explain the greater than expected fracture risk in the overweight and obese for a given BMD. Trabecular and cortical vBMD and geometry differ in the obese compared to normal weight individuals. In children, a higher body weight is associated with a higher trabecular bone, but not cortical vBMD, and may decrease bone strength [15–17]. Lower forearm bone strength found in overweight children has been attributed to the greater fat to muscle ratio in the overweight than normal weight children [18]. In adults, obesity is also associated with higher trabecular and some cortical bone parameters and lower cortical vBMD. In a study with 211 women, we found that the higher trabecular parameters and lower cortical vBMD remain significantly different in the obese even when controlling for confounders (i.e., lean mass and physical activity) (Fig. 4.1) [19]. Also, a higher

Table 4.1 Bone variables obtained by peripheral quantitative computed tomography at the radius and tibia comparing obese to normal weight groups[a,b]

References	Population	Groups	Bone site	Trabecular bone vBMD	Cortical bone vBMD	SSI
				Obese[b] compared to normal weight (%)		
Ducher et al. [18]	Boys and girls	Normal wt. ($n=334$)	Radius	+7.9*	+0.5	+16.2*
	7–10 years	Overweight ($n=93$)	Tibia	+6.9*	0	+21.0*
Pollock et al. [15]	Females	Normal fat ($n=93$)	Radius	−2.9	+0.3	−0.7
	18 years	High fat ($n=22$)	Tibia	−1.5	−0.1	−5.6
Pollock et al. [150]	Black females	Normal fat ($n=33$)	Radius	+5.1	+0.4 %	+5.0
	19 years	High fat ($n=15$)	Tibia	−0.04	−0.5 %	−3.2
Wetzsteon et al. [16]	Boys and girls	Health weight ($n=302$)	Tibia	NA	0	+15.4*
	9–11 years	Overweight ($n=143$)				
Sukumar [19]	Female	Normal ($n=42$)				
	24–75 years	Overweight ($n=119$)	Tibia[c]	+15.5*	−0.3	+6.8*
		Obese ($n=50$)	Tibia[d]	+19.3*	−1.2*	+3.18*
Uusi Rasi et al. [21][e]	Male and female	BMI measured at 12 years of age	Radius	F +5.1	F −0.2	F +14.7
				M −0.8	M −0.9	M +8.6
	36 years	Normal ($n=767$) or overweight ($n=65$)	Tibia	F +7.2	F −0.6	F +17.9
				M −0.9	M −1.1	M +12.9

Abbreviations: *vBMD* volumetric bone mineral density, *SSI* stress-strain index
[a]Studies reported only for those that also included a normal weight control group
[b]"Obese" refers to excess body weight described as either obese, overweight, or high-fat groups based on the terminology used in the study
[c]Overweight compared to normal weight group
[d]Obese compared to normal weight group
[e]Statistical data unavailable for comparison with normal weight
*Significantly different from normal weight

body mass index (BMI) does not confer a positive effect on other cortical parameters such as BMC and area, thickness, and strength indices [19]. Taes et al. [20] showed that greater fat mass is associated with smaller bone size in men at 25–45 years of age. A recent study [21], however, shows that a history of being overweight in childhood is associated with greater total cross-sectional area of long bone sites in men and women (36 years of age) and a 5 % higher trabecular density at the distal radius and tibia in the adult women, but not in men. Hence, the effect of obesity on bone may vary due to gender and a history of obesity. Studies examining the influence of excess body weight on the cortical and trabecular compartments of bone compared to normal weight populations are summarized in Table 4.1.

Fracture Risk in Obesity

In an epidemiological perspective of osteoporosis and fracture risk in overweight and obese individuals, researchers demonstrate that osteoporotic fractures are more problematic in this population than previously believed and that obese men may be particularly susceptible [22, 23]. For example, hip fracture incidence is highest in the underweight, but there is a higher prevalence of fracture in overweight and obese individuals in the USA because they represent the largest portion of the population [24]. Others suggest that a BMI greater than 35 kg/m^2 increases the risk of fracture, when adjusted for BMD [25]. In women presenting with low-trauma fracture, 59 % of obese and 73 % of morbidly obese women had normal BMD, and only 12 and 5 %, respectively,

had evidence of osteoporosis [23]. The normal BMD and higher risk of fracture in obesity are either the result of compromised bone quality or greater forces on the bone during a fall despite the extra body fat padding. It is also possible that excess adiposity overestimates BMD in obese subjects due to measurement artifacts.

The risk of fracture in the obese differs by anatomic site and has been shown in a few epidemiological studies. In a longitudinal study with nearly 11,000 women, high BMI significantly increased the risk of proximal humerus and ankle fractures but was associated with a lower risk at the forearm, spine, and hip [26]. In addition, other researchers have also found a higher humerus fracture risk in obese women [27]. Compston and colleagues report that obese compared to nonobese women have more ankle and upper leg fractures [28]. In 22,444 men, the increased risk of fracture risk with a high BMI only shows a trend for higher fracture risk at the ankle and is lower at other sites, including the proximal humerus [26]. Further analysis is needed to establish a fat-fracture relationship in older men [23, 29] and to distinguish whether it differs from women or if there are racial/ethnic differences. A specific effect of obesity on vertebral fracture compared to normal weight individuals is not clear; however, adiposity is associated with vertebral deformity in obese women and is attributed to excess loading on the thoracic spine [10]. Therefore, obesity is associated with lower hip fracture, but higher risk of proximal humerus fracture and possibly ankle and upper leg fractures in women [23, 26–28]. These findings are consistent with higher forearm fracture risk in children [30]. Also, fractures in both obese pediatric and adult patients increase recovery time and involves more complications [31, 32], so preventing fractures in this population is especially important.

Overall, the strong evidence that bone quality is compromised in obesity may explain fracture risk in this population. In addition, because fractures occur more at certain anatomical sites, the alterations in microarchitecture that is either rich in trabecular or cortical bone may influence the susceptible to fracture. It is also possible that the force upon falling and an altered balance are factors contributing to site-specific fractures in the obese. The different ratios of lean to fat tissue mass or fat depots in obesity may help in understanding the etiology and implications for BMD and fracture risk and is discussed below.

Relationship of a BMD with Soft Tissue

Body composition and its relationship to bone have been examined in numerous studies, and most agree that lean and fat mass are both independent determinants of bone mass. Lean mass and fat mass are strongly influenced by age, gender, dietary intake, and the level of physical activity among other factors which in turn can independently affect bone.

Lean Tissue Mass

When measuring lean tissue mass using DXA, it consists of both skeletal muscle and BMC. For studies that have differentiated these compartments, the term "fat-free soft tissue" is used to indicate skeletal muscle tissue without the inclusion of BMC. The positive effect of a higher fat-free soft tissue on BMD can be attributed to lifestyle factors, steroid hormone sufficiency, genetic influences, or a combination of these factors. Importantly, muscle mass has an independent effect on better balance to prevent frailty and falls associated with osteoporotic fracture risk. The excess weight in obesity consists primarily of excess adipose tissue, yet in general, there is also higher fat-free soft tissue. It has been suggested that the positive effect of a higher body weight on bone and fracture risk reduction occurs only when it is primarily composed of fat-free soft tissue [33, 34]. It is possible that in older individuals, the obese compared to normal weight have a higher incidence of combined sarcopenia and osteopenia due to reduced mobility in this population [35]. In a large study of elderly white and black women and men where hip fracture was validated over a 7-year period, it was found that a decrease of one standard deviation in thigh muscle Hounsfield Unit (an indicator of intramuscular fat) conferred a nearly 40 % increase in fracture risk. Hence, measurement of total fat or

lean mass by DXA may not be able to adequately capture changes in muscle composition in older individuals, suggesting that thigh muscle fat may provide a better estimate of muscle strength and hip fracture risk [29]. Although no defined recommendations are available to consider muscle-related parameters in clinical bone assessments, there is now a greater effort in the field to address these relationships with new trials using 3D bone techniques that are ongoing.

Fat Mass

Because adipose tissue acts as an endocrine organ [36], the hormones and adipokines produced will have a major influence on the bone and this is discussed below. Fat mass, unlike muscle mass, does not always show a direct correlation with bone. It appears to be age and gender specific so while there is a correlation between fat and bone in postmenopausal women [37, 38], this has not been found in children and young adults [39, 40]. Only some of these studies have corrected for muscle mass to determine the independent effect of fat on bone; this may explain some of the different findings in these studies. Also, the influence of soft tissue on bone mass is complicated by variability in the bone site being evaluated [41, 42]. Varying amounts of trabecular or cortical content in different bones, as well as weight bearing of the specific site, may confound the observations. For example, a study in older women showed that total weight influenced BMD at weight-bearing sites, yet only adiposity influenced non-weight-bearing sites, including the radius [43].

Fat Depot

Bone may be influenced by the location and type of white adipose tissue accumulation, including visceral adipose tissue (VAT) compared to subcutaneous tissue. Excess VAT has a greater association with symptoms of metabolic syndrome than the increased total body adipose tissue per se. The metabolic syndrome symptoms (such as dyslipidemia, insulin resistance, and higher inflammatory cytokines) each have independent effects on bone and may explain the inconsistent findings for the influence of excess VAT on bone. For example, the positive influence of VAT on bone reported in postmenopausal women has not been shown in children or men [44–47]. It is also possible that inconsistent findings for an inverse relationship between visceral fat and bone are due to different methodologies and protocols used in each study. Because most of the studies either use waist to hip ratio or measure trunk fat using DXA to estimate VAT, which include both subcutaneous and visceral depots, this limits the interpretation. In addition, studies examining the VAT and bone relationship use different anatomical bone sites. Studies using more precise techniques to measure adipose tissue, such as QCT or MRI, will be important to better understand how the type of fat differentially influences BMD or BMC.

Besides white adipose tissue (subcutaneous and visceral fat), other types of fat (brown fat and bone marrow fat, also referred to as “yellow” fat) may influence BMD. Brown adipose tissue has been reported to maintain bone based on a study in women with anorexia nervosa compared to healthy controls [48], whereas increased bone marrow fat tissue is associated with lower BMD [49]. In addition, a recent study in obese women showed that vertebral bone marrow fat is positively associated with visceral fat and inversely associated with insulin-like growth factor (IGF-1) [50] and BMD. Further studies examining the endocrine function of bone marrow fat in regulating bone and differentiation of mesenchymal stem cells are needed to advance the field.

In summary, the amount and type of soft tissue mass results in differential mechanical support and endocrine regulation of bone that would be expected to influence growth and maintenance. Both fat-free soft tissue and the type and location of adipose tissue are important influences on BMD and may change with age or in different populations. These differences may explain the large body of conflicting data that link body adiposity, bone mass, and fracture risk. Hormonal alterations are influenced by the type and location of fat depots, and the amount of fat-free soft tissue may explain the etiology for the altered bone quality and fracture risk in obesity and is discussed in the next section.

Hormonal Milieu in Obesity That Influence BMD at Cortical and Trabecular Sites and Bone Geometry

Adipose tissue is a metabolically active tissue containing a vast variety of cell types, the more abundant being adipocytes, preadipocytes, immune cells, and endothelial cells [36]. The adipose tissue secretes adipocyte-derived factors that have effects on many organs in the body, including the bone. The altered hormonal milieu and adipokines in obesity have specific actions on BMD, its geometry, and microarchitectural properties that are discussed below.

Sex Steroids

The obese individual has higher levels of serum estrogen and lower sex hormone-binding globulin (SHBG). Serum estrogens in postmenopausal women are largely derived from the metabolism of circulating androstenedione by peripheral tissues, and concentrations are higher in obesity. The higher concentrations of serum estrone in obesity are largely derived from the metabolism of circulating androstenedione by adipose tissue and may also be responsible for higher BMD due to excess body weight [51]. In addition, the adipose-derived enzymes, such as aromatase and hydroxyl steroid dehydrogenase, are elevated in obesity and have known anabolic actions on the osteoblast [52, 53]. Obese men, on the other hand, have low total and free testosterone and low SHBG [54, 55]. The sex steroids, including bioavailable estradiol and testosterone, have been shown to be the major positive hormonal determinants of trabecular microstructure in elderly men and women [56], and the age-related loss of cortical bone is associated with sex steroid deficiency [57]. The GOOD Study [58] in young men shows that free estradiol is an independent negative predictor of cortical parameters such as cross-sectional area, periosteal circumference, and endosteal circumference, whereas it is a positive independent predictor of cortical vBMD at both the tibia and radius. Conversely, free testosterone is an independent positive predictor of cortical cross-sectional area, periosteal circumference, and endosteal circumference, but is not associated with vBMD [58]. SHBG is an independent positive predictor of cortical cross-sectional area and periosteal and endosteal circumference [58]. An obesity-induced association between higher circulating estrogen and lower testosterone concentrations in older adults would be expected to increase trabecular and possibly reduce cortical BMD, but the influence of sex steroids on these bone compartments in obesity has not been specifically addressed.

Serum 25-Hydroxyvitamin D and Parathyroid Hormone

Obesity is associated with higher parathyroid hormone (PTH), lower 25-hydroxyvitamin D (25OHD), and possibly lower 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and all have specific actions on bone. The lower circulating concentrations of 25OHD in obesity are possibly due to greater deposition in the excess adipose tissue or lower sun exposure in obese individuals [59–61]. In addition, there is a rise in serum 25OHD with weight loss and it has been shown to be proportional to loss of body weight [62]. In the InCHIANTI study conducted in Italy, serum 25OHD was positively associated with total cross-sectional area and cortical vBMD, while PTH was negatively associated with cortical vBMD in women, but not in men [63]. Another rodent study showed that vitamin D deficiency in young growing male rats results in a significant reduction in femoral trabecular bone volume, while cortical bone is maintained [64]. On the other hand, in adult patients with primary hyperparathyroidism (PHPT), in those with low serum 25OHD (<20 ng/mL), there is evidence of higher serum PTH concentrations and a greater catabolic effect on cortical bone and anabolic effect on trabecular bone compared to PHPT patients without low 25OHD [65]. Another study also assessed the association of 25OHD with cortical and trabecular bone parameters in men of Caucasian and African ancestry [66]. Among Caucasians, serum 25OHD was positively associated with cortical vBMD, total BMC, cortical thickness, and strength param-

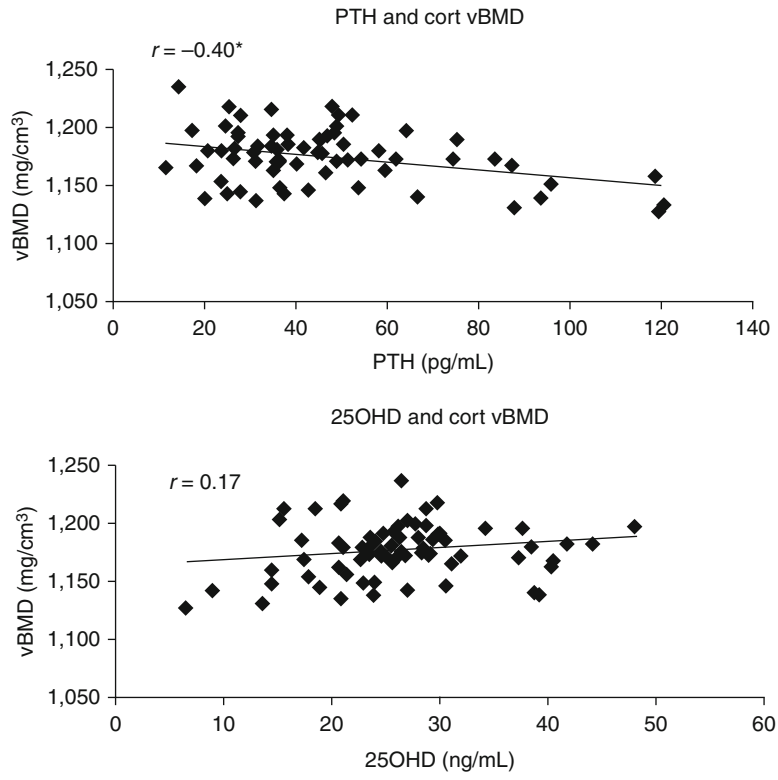

Fig. 4.2 Relationship between cortical volumetric bone mineral density (vBMD) and serum parathyroid hormone (PTH) is positively correlated, but there is no relationship with 25-hydroxyvitamin D (25OHD). ($n = 73$ premenopausal women); *$p < 0.001$ (Modified from Sukumar et al. [19]. With permission from Springer Science + Business Media)

eters at the distal radius. Results also showed that there was an inverse association between serum 25OHD and the cortical cross-sectional area and stress-strain index in men of African ancestry. Whether or not the effects of vitamin D deficiency on bone compartments differ by ethnic/racial difference or are due to a direct effect on bone or due to its parallel increases in PTH is unclear. Currently, there is no data to support a significant association between the lower levels of 25OHD levels in obesity and cortical/trabecular bone [19].

Parathyroid hormone is positively correlated with excess body fat [67, 68]. While short-term increases in PTH are associated with increased calcium absorption and an increase in BMD, chronically elevated PTH will alter calcium metabolism and increase proinflammatory cytokines [69, 70], which would have a detrimental effect on bone. Chronically elevated PTH reduces cortical BMD and inhibits bone collagen synthesis. In contrast, elevated serum PTH preserves or increases trabecular, possibly by increasing osteoblast recruitment [71]. For example, patients with either primary or secondary hyperparathyroidism have increased spine BMD, which is rich in trabecular bone, but decreased cortical bone mass [72, 73]. Patients with osteoporosis who are treated with PTH show higher spine BMD but lower cortical BMD, especially at the distal radius as compared to bisphosphonate treatment [74]. In support of the bone site-specific action of PTH, obese postmenopausal women with high PTH who had a history of gastric bypass surgery compared to obese controls with normal PTH have higher lumbar spine BMD (rich in trabecular bone) and BMC and lower BMC at the femoral neck [75].

The effect of higher PTH levels on bone in 211 women with a wide range of body weights has been examined in a cross-sectional study in our laboratory [19]. The obese women showed a lower cortical vBMD, and in the total population of women with a wide range of body weights, there was a negative association between PTH and cortical vBMD (Fig. 4.2) [19]. It is thus possible that the lower cortical bone in obesity is due to their higher PTH levels. Others have found lower

cortical vBMD in obese children [15] and young adults [20], but circulating hormones were not measured in these studies. Thus, there are currently only limited studies that support the hypothesis that the elevated PTH in obesity is responsible for the lower cortical BMD [19, 75] and none that can establish a cause and effect relationship.

Adipose-Derived Hormones and Peptides, Pancreatic Hormones, and Cytokines

The adipose-derived hormones, adiponectin, leptin, and resistin are altered by obesity and also influence bone. Obesity reduces circulating adiponectin [36], and in vitro observations show it increases osteoblastic activity [76]. Most clinical studies [77–80], but not all [81], show that adiponectin is negatively associated with BMD in adults and children. Adiponectin is also inversely correlated with trabecular and cortical BMD [82]. Consistent with these findings, fracture studies suggest that higher adiponectin is associated with greater fracture risk but may be gender specific [83, 84]. The Health Aging and Body Composition (Health ABC) Study in 3,075 men and women showed that men in the highest tertile of adiponectin had a 94 % higher risk of fracture [hazard ratio (HR) = 1.94; 95 % confidence interval (CI) 1.20–3.16] compared with the lowest tertile, but it was not significant in women [83]. The Osteoporotic Fractures in Men (MrOS) Study also shows that the risk of fracture increases with increasing serum adiponectin with a hazard ratio HR/SD of 1.46 (95 % CI, 1.23–1.72) [85].

Leptin suppresses appetite and increases energy expenditure and there is resistance to leptin associated with the high serum concentrations in obesity. Leptin also has both direct and centrally mediated effects on bone remodeling. The centrally mediated effect on bone occurs through sympathetic tone. It has been shown to inhibit bone formation and enhance bone resorption [86]. In contrast, in vitro studies show a direct effect of leptin on osteoblast differentiation [87, 88]. These different central and peripheral effects of leptin may explain why clinical trials have reported both positive and negative effects of leptin on bone [81, 89–91]. One report suggests that leptin is negatively associated with cortical bone size in adolescents and young men. In obese mice, serum leptin levels negatively correlates with trabecular, but not cortical bone [92]. The two genetic models of obesity, the ob/ob (leptin-deficient) and db/db (leptin null) mouse, have short limbs with thin cortical bone, low trabecular bone volume and BMD, and high marrow adiposity, whereas vertebrae are larger, with elevated BMD and trabecular bone volume, and lower marrow adiposity [93]. Furthermore, there are higher levels of pancreatic hormones such as insulin, amylin, and preptin in the obese, which have anabolic actions on bone [94–96]. In young mice, lower amylin leads to lower trabecular bone volume and thickness [97]; however, its effect on bone compartments in obesity is unclear.

There are also higher circulating concentrations of inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor (TNF-α), monocyte chemoattractant protein-1, and C-reactive protein (CRP) in obesity. Higher inflammatory cytokines have been associated with higher bone turnover [98–101] and have differential effects on cortical and trabecular bone. In mice, IL-6 transgenic mice show severe alterations in cortical and trabecular bone microarchitecture [102]. Serum levels of CRP do not seem to be associated with trabecular [103] or cortical bone [104] in adults. In one study of older women and men (BMI of 27.5 kg/m^2), IL-1 was negatively associated with cortical vBMD, and surprisingly TNF-α was positively associated with total and cortical cross-sectional area [63]. The effect of cytokines on trabecular and cortical bone has not specifically been examined in obese individuals, but may be dependent on the presence of higher serum PTH concentrations [69]. It is possible that the low level of chronic inflammation in obesity is counterbalanced by adipose-derived estrogen, lower adiponectin, and greater weight bearing that act to prevent bone loss in the obese compared to leaner populations.

Conditions of excess adiposity are associated with reduced growth hormone [105, 106] and IGF-1 and insulin-like growth factor binding protein (IGFBP)-1 [107]. However, the implications of low serum IGF-1 concentrations [105] are not

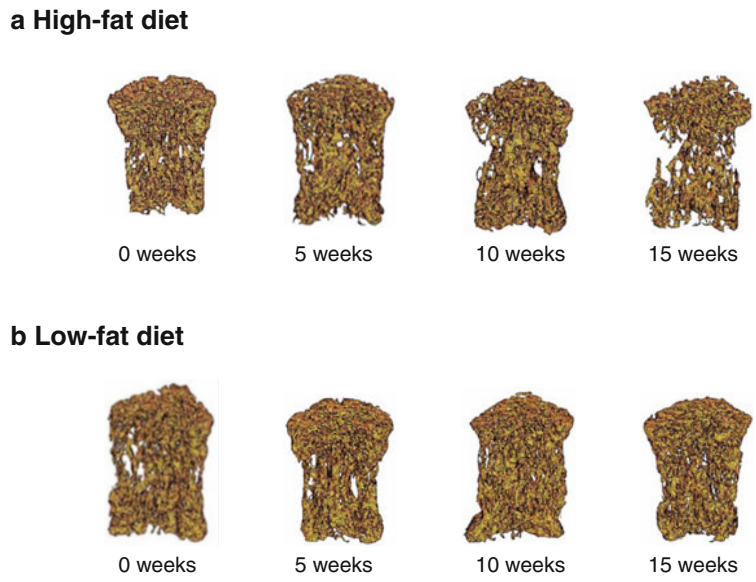

Fig. 4.3 Image shows the typical 3D trabecular microarchitectural changes in the L4 vertebral bodies with micro-CT-based finite element model. In this study, young mice (6 weeks of age) were fed a high-fat diet or a normal low-fat diet for 15 weeks. The HFD (**a**) increases trabecular space, number, and loss of metaphyseal trabeculae compared to control (**b**) (Modified from Woo et al. [114]. With permission from Elsevier)

entirely clear, since it has been reported that *free or bioactive* IGF-1 concentrations are either similar or higher in obese compared to normal weight subjects [106, 107]. In young men, higher serum IGF-I and IGFBP-3 concentrations are associated with conversion of thick trabecular into more numerous and thinner trabeculae from aging to mid-life [56], but this study found that in the older population, sex steroids are the major determinants of trabecular microstructure. In a study of older women, serum IGF-1 was found to be significantly related to cortical, but not to trabecular density [108]. Consistent with these findings, mice with low serum levels of IGF-1 exhibit reduced cortical but normal trabecular bone [109] suggesting a more pronounced role of systemic IGF-1 on cortical than on trabecular bone. It is possible that the lower cortical vBMD in obesity is related to a lower IGF. However, these findings should be confirmed in larger prospective studies.

Obesity also alters the gut peptides, including ghrelin, incretins, CCK, pancreatic polypeptide (PPY), and peptide YY (PYY). These peptides not only regulate satiety but also have reported effects on bone. A meta-analysis [84] shows no convincing data to support an association between visfatin and ghrelin and BMD. In the case of ghrelin, this appetite stimulant is high in obesity, and it increases osteoclastic bone resorption during fasting [110], but also increases bone formation in other studies [111–113]. However, the meta-analysis by Biver et al. [84] did not find an association between ghrelin and BMD. Overall, there are a limited number of studies examining the effect of these peptides on cortical and trabecular bone compartments and the data remain unclear.

Bone and Diet-Induced Obesity Models

Besides hormonal factors, the skeletal consequences of obesity will vary depending on the age at onset, duration, and composition of the diet. Animal models offer an opportunity to determine these effects. Most diet-induced obesity in rodents during growth has shown that it lowers BMD and impairs bone quality [114–116] (Fig. 4.3). Some studies suggest that age influences the BMD response to a high-fat diet (HFD) because the effect may be exaggerated during rapid growth as compared to a more mature skeleton. One study examined the effect of a 16-week HFD in very young (3 weeks of age) and 3-month-old mice [117]. The HFD resulted in greater lean and fat

tissue mass and lower cortical bone biomechanical properties, as compared to the low-fat diet (LFD) [117]. The HFD also increased serum IGF-1 and leptin levels compared to controls, but the rise in IGF-1 was markedly higher in the young compared to adult mice [117]. This may explain the greater bone size in the younger mice vs. smaller bone size in the adult mice compared to their lean counterparts [117]. In mice (9 weeks of age), excessive fat and sucrose intake for 10 weeks impaired bone geometry and mechanical properties of cortical bone in mice [118]. The bone changes are attributed to the upregulation of receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA suggesting higher osteoclast activity with obesity. In addition, it was found that the detrimental effects of a high-fat high-sucrose diet (HF/HS) on bone are exacerbated in the femoral neck and lumbar vertebrae after long-term feeding (2 years), showing that duration of dietary exposure is also important [119]. Other studies where the diet was initiated in adolescent or adult rodents have not found a detrimental effect of a HFD on bone. In a study in our lab, 2-month-old female rats were fed either a HFD or control LFD. At 8 months of age, the obese vs. lean rats showed no difference in femoral aBMD and femoral neck vBMD, or trabecular thickness and number [120]. In addition, in 11-month-old male rats who had been fed a HF/HS diet for 16 weeks, most bone parameters were greater than the low-fat controls, except for a lower cortical porosity [121]. Others have studied the effect of excessive caloric intake on bones in rodents by examining different types of dietary sugars. For example, excessive intake of fructose or glucose has been shown to produce a detrimental effect on BMD, BMC, and/or mechanical strength in rats [122, 123]. Protein source during excessive energy intake may also influence the bone response. Researchers studied the bones of 4-month-old rats that were fed 8 weeks of powdered skim milk, casein, or whey added to a HF/HS diet [124]. The rats given the skim milk showed an attenuated weight gain and increased trabecular bone architecture as compared to casein or whey alone [124]. Whether diet composition is influencing the bone parameters measured by pQCT in clinical obesity studies is not known. Overall, it is likely that diet duration and composition, the level of adiposity, and skeletal age are important factors influencing the detrimental effects reported on bone mass, size, and biomechanical properties.

Effect of Weight Loss on Cortical/ Trabecular Bone

Weight loss is associated with 1–2 % bone loss at the hip and possibly more at highly trabecular sites, such as the trochanter and radius [125–133]. Epidemiological studies show that only 5% weight loss is associated with increased fracture risk in both men and women [126, 134, 135]. A variety of anatomical sites are reported to have higher fracture risk in individuals with a history of weight loss. These fracture sites include hip [126, 136], non-vertebral fractures [137], and distal forearm fractures [138]. Bone loss and increased fracture risk due to moderate weight reduction occur in both older women and men [131], but neither has been demonstrated in younger individuals [139–142] unless there is severe weight reduction.

Few studies have evaluated the effect of weight loss on trabecular and cortical bone parameters. In a 1-year study in older women, 7 % weight reduction decreased aBMD at the radius (distal and 33 % sites) and hip [128]. Weight loss also reduced vBMD and area of the tibia, but there were no significant changes in trabecular vBMD and geometry and only a trend to decrease and increase cortical area and vBMD, respectively [128]. In a 3-month study in premenopausal women, a very low-energy diet resulted in a 10 % loss of body weight and a slight increase in cortical vBMD at the radius [142]. However, because there was also a rise in bone turnover markers, it is possible that bone loss may have occurred at other anatomical sites or would occur in a longer-term study.

In rodent studies, energy restriction is associated with a marked decrease in femoral cortical bone mass, but no change in trabecular bone volume fraction [143]. Both age and initial body weight appear to be important factors influencing the effect of energy restriction on bone. For example, older (14 months) compared to younger (6 months)

mature energy-restricted rats result in a greater reduction in biomechanical properties of bone [144]. Others have studied the effect of energy restriction in very young male mice (3 weeks of age) [145]. After energy restriction, there was greater inhibition of cortical and trabecular bone mass accrual in the limbs than in the spine [145]. In addition, energy restriction decreased appendicular cortical and trabecular bone mass while preserving trabecular bone in the spine [145]. In skeletally mature 8-month-old obese and lean female rats [120], energy restriction in obese rats does not decrease BMD compared to ad-libitum fed controls. However, the lean energy-restricted rats had a lower BMD at the femoral neck and distal femur compared to their lean ad libitum-fed controls [120]. Hence, the age and initial body weight before caloric restriction appear to not only affect whether there will be any bone loss but may also differentially influence the anatomical sites, compartments, and geometry of bone.

Several bone-regulating hormones are altered during caloric restriction and may explain at least some of the bone changes associated with weight loss. For example, a reduction in estrogen levels, rise in cortisol [120, 146], and reduction in IGF-1 and leptin [143] occur during energy restriction and have direct detrimental effects on BMD [133]. The importance and role of exogenous hormones in regulating bone during caloric restriction and preventing BMD loss has also been studied [146–149]. Medications to treat osteoporosis, such as estrogen and raloxifene, during weight reduction will prevent bone loss in postmenopausal women [147]. In rodent studies, treatment with IGF-1 [149] or with low-dose PTH [148] has been shown to maintain normal bone formation during rapid weight loss in a rodent study. Importantly, dietary and exercise interventions will influence the hormonal response to caloric restriction and can also attenuate bone loss due to weight reduction [133].

Conclusions

There is strong evidence that bone quality and fracture risk is altered by obesity in both clinical trials and in rodent studies. In addition, the amount, type, and location of the excess adipose tissue; the ratio with muscle mass; and the altered hormonal milieu are important determinants of bone quality and fracture risk in obesity. The higher circulating estrogens and/or lower testosterone due to excess adiposity may have gender-specific effects on trabecular and cortical bone. Higher serum PTH in obesity appears to play a role in reducing cortical BMD, but the lower serum 25OHD associated with obesity may not be low enough to negatively affect bone. The higher leptin and resistin and lower adiponectin may also contribute to the lower cortical vBMD in obesity. There is currently inadequate information on whether the higher pancreatic hormones in obesity alter trabecular or cortical bone compartments. Cytokines have a catabolic effect on both bone compartments; however, higher circulating concentrations do not explain the higher trabecular and lower cortical vBMD in obesity. Because the altered hormonal milieu in obesity does not completely explain bone architectural changes that occur due to excess adiposity, the influence of other factors such as genetics, altered mechanical loading, diet, physical activity, and/or other environmental factors may have independent effects on bone quality and site-specific fracture risk in obesity.

References

1. World Health Organization. Obesity and overweight. Geneva: World Health Organization; May 2012. http://www.who.int/mediacentre/factsheets/fs311/en/. Last Accessed on 29 Aug 2012.
2. Ljungvall A, Zimmerman FJ. Bigger bodies: long-term trends and disparities in obesity and body-mass index among U.S. adults, 1960–2008. Soc Sci Med. 2012;75:109–19.
3. FastStats (Centers for Disease Control and Prevention). Obesity and overweight. Atlanta: CDC; 2011. http://www.cdc.gov/obesity/adult/causes/index.html. Accessed 9 Sept 2012.
4. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. J Cell Biochem. 2006;98:251–66.
5. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab. 2007;92:1640–6.
6. Frost HM. Obesity, and bone strength and "mass": a tutorial based on insights from a new paradigm. Bone. 1997;21:211–4.

7. Hangartner TN, Johnston CC. Influence of fat on bone measurements with dual-energy absorptiometry. Bone Miner. 1990;9:71–81.
8. Bolotin HH. A new perspective on the causal influence of soft tissue composition on DXA-measured in vivo bone mineral density. J Bone Miner Res. 1998;13:1739–46.
9. Tothill P. Dual-energy x-ray absorptiometry measurements of total-body bone mineral during weight change. J Clin Densitom. 2005;8:31–8.
10. Laslett LL, Just Nee Foley SJ, Quinn SJ, Winzenberg TM, Jones G. Excess body fat is associated with higher risk of vertebral deformities in older women but not in men: a cross-sectional study. Osteoporos Int. 2012;23:67–74.
11. Liu G, Peacock M, Eilam O, Dorulla G, Braunstein E, Johnston CC. Effect of osteoarthritis in the lumbar spine and hip on bone mineral density and diagnosis of osteoporosis in elderly men and women. Osteoporos Int. 1997;7:564–9.
12. Hansen KE, Vallarta-Ast N, Krueger D, Gangnon R, Drezner MK, Binkley N. Use of the lowest vertebral body T-score to diagnose lumbar osteoporosis in men: is "cherry picking" appropriate? J Clin Densitom. 2004;7:376–81.
13. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone. 1993;14:595–608.
14. Rauch F, Tutlewski B, Schonau E. The bone behind a low areal bone mineral density: peripheral quantitative computed tomographic analysis in a woman with osteogenesis imperfecta. J Musculoskelet Neuronal Interact. 2002;2:306–8.
15. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. Am J Clin Nutr. 2007;86:1530–8.
16. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. J Bone Miner Res. 2008;23:1946–53.
17. Cole ZA, Harvey NC, Kim M, Ntani G, Robinson SM, Inskip HM, Godfrey KM, Cooper C, Dennison EM. Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. Bone. 2012;50(2):562–7.
18. Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: implications for bone strength at the distal forearm. Am J Clin Nutr. 2009;90:1104–11.
19. Sukumar D, Schlussel Y, Riedt CS, Gordon C, Stahl T, Shapses SA. Obesity alters cortical and trabecular bone density and geometry in women. Osteoporos Int. 2011;22:635–45.
20. Taes YE, Lapauw B, Vanbillemont G, Bogaert V, De BD, Zmierczak H, Goemaere S, Kaufman JM. Fat mass is negatively associated with cortical bone size in young healthy male siblings. J Clin Endocrinol Metab. 2009;94:2325–31.
21. Uusi-Rasi K, Laaksonen M, Mikkila V, Tolonen S, Raitakari OT, Viikari J, Lehtimaki T, Kahonen M, Sievanen H. Overweight in childhood and bone density and size in adulthood. Osteoporos Int. 2012;23:1453–61.
22. Nielson CM, Marshall LM, Adams AL, Leblanc ES, Cawthon PM, Ensrud K, Stefanick ML, Barrett-Connor E, Orwoll ES. BMI and fracture risk in older men: the osteoporotic fractures in men study (MrOS). J Bone Miner Res. 2011;26:496–502.
23. Premaor MO, Pilbrow L, Tonkin C, Parker RA, Compston J. Obesity and fractures in postmenopausal women. J Bone Miner Res. 2010;25:292–7.
24. Nielson CM, Srikanth P, Orwoll ES. Obesity and fracture in men and women: an epidemiological perspective. J Bone Miner Res. 2012;27:1–10.
25. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton III LJ, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005;16:1330–8.
26. Holmberg AH, Johnell O, Nilsson PM, Nilsson J, Berglund G, Akesson K. Risk factors for fragility fracture in middle age. A prospective population-based study of 33,000 men and women. Osteoporos Int. 2006;17:1065–77.
27. Gnudi S, Sitta E, Lisi L. Relationship of body mass index with main limb fragility fractures in postmenopausal women. J Bone Miner Metab. 2009;27:479–84.
28. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, Pfeilschifter J, Silverman S, Diez-Perez A, Lindsay R, Saag KG, Netelenbos JC, Gehlbach S, Hooven FH, Flahive J, Adachi JD, Rossini M, Lacroix AZ, Roux C, Sambrook PN, Siris ES. Obesity is not protective against fracture in postmenopausal women: GLOW. Am J Med. 2011;124:1043–50.
29. Lang TF, Cauley J, Tylavsky F, Bauer D, Cummings S, Harris T. Computed tomographic measurements of thigh muscle cross-sectional area and attenuation coefficient predict hip fracture: the health, aging and body composition study. J Bone Miner Res. 2010;25(3):513–9.
30. Goulding A, Grant AM, Williams SM. Bone and body composition of children and adolescents with repeated forearm fractures. J Bone Miner Res. 2005;20:2090–6.
31. Di Monaco M, Vallero F, Di Monaco R, Mautino F, Cavanna A. Body mass index and functional recovery after hip fracture: a survey study of 510 women. Aging Clin Exp Res. 2006;18:57–62.
32. Leet AI, Pichard CP, Ain MC. Surgical treatment of femoral fractures in obese children: does excessive body weight increase the rate of complications? J Bone Joint Surg Am. 2005;87:2609–13.
33. Salamone LM, Glynn N, Black D, Epstein RS, Palermo L, Meilahn E, Kuller LH, Cauley JA. Body composition and bone mineral density in premenopausal and early perimenopausal women. J Bone Miner Res. 1995;10:1762–8.

34. Travison TG, Araujo AB, Esche GR, Beck TJ, McKinlay JB. Lean mass and not fat mass is associated with male proximal femur strength. J Bone Miner Res. 2008;23:189–98.
35. Binkley N, Buehring B. Beyond FRAX: it's time to consider "sarco-osteopenia". J Clin Densitom. 2009;12: 413–6.
36. Halberg N, Wernstedt-Asterholm I, Scherer PE. The adipocyte as an endocrine cell. Endocrinol Metab Clin North Am. 2008;37:753, xi.
37. Chen Z, Lohman TG, Stini WA, Ritenbaugh C, Aickin M. Fat or lean tissue mass: which one is the major determinant of bone mineral mass in healthy postmenopausal women? J Bone Miner Res. 1997;12: 144–51.
38. Lindsay R, Cosman F, Herrington BS, Himmelstein S. Bone mass and body composition in normal women. J Bone Miner Res. 1992;7:55–63.
39. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, Gilsanz V. Fat mass is not beneficial to bone in adolescents and young adults. J Clin Endocrinol Metab. 2007;92:143–7.
40. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr. 2001;139: 509–15.
41. Blain H, Vuillemin A, Teissier A, Hanesse B, Guillemin F, Jeandel C. Influence of muscle strength and body weight and composition on regional bone mineral density in healthy women aged 60 years and over. Gerontology. 2001;47:207–12.
42. Hla MM, Davis JW, Ross PD, Wasnich RD, Yates AJ, Ravn P, Hosking DJ, McClung MR. A multicenter study of the influence of fat and lean mass on bone mineral content: evidence for differences in their relative influence at major fracture sites. Early Postmenopausal Intervention Cohort (EPIC) Study Group. Am J Clin Nutr. 1996;64:354–60.
43. Glauber HS, Vollmer WM, Nevitt MC, Ensrud KE, Orwoll ES. Body weight versus body fat distribution, adiposity, and frame size as predictors of bone density. J Clin Endocrinol Metab. 1995;80:1118–23.
44. Warming L, Ravn P, Christiansen C. Visceral fat is more important than peripheral fat for endometrial thickness and bone mass in healthy postmenopausal women. Am J Obstet Gynecol. 2003;188:349–53.
45. Kuwahata A, Kawamura Y, Yonehara Y, Matsuo T, Iwamoto I, Douchi T. Non-weight-bearing effect of trunk and peripheral fat mass on bone mineral density in pre- and post-menopausal women. Maturitas. 2008; 60:244–7.
46. Makovey J, Naganathan V, Sambrook P. Gender differences in relationships between body composition components, their distribution and bone mineral density: a cross-sectional opposite sex twin study. Osteoporos Int. 2005;16:1495–505.
47. Gilsanz V, Chalfant J, Mo AO, Lee DC, Dorey FJ, Mittelman SD. Reciprocal relations of subcutaneous and visceral fat to bone structure and strength. J Clin Endocrinol Metab. 2009;94:3387–93.
48. Bredella MA, Fazeli PK, Freedman LM, Calder G, Lee H, Rosen CJ, Klibanski A. Young women with cold-activated brown adipose tissue have higher bone mineral density and lower pref-1 than women without brown adipose tissue: a study in women with anorexia nervosa, women recovered from anorexia nervosa, and normal-weight women. J Clin Endocrinol Metab. 2012;97(4):E584–90.
49. Shen W, Chen J, Punyanitya M, Shapses S, Heshka S, Heymsfield SB. MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. Osteoporos Int. 2007; 18:641–7.
50. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, Rosen CJ, Klibanski A, Miller KK. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. Obesity (Silver Spring). 2011;19:49–53.
51. Frumar AM, Meldrum DR, Geola F, Shamonki IM, Tataryn IV, Deftos LJ, Judd HL. Relationship of fasting urinary calcium to circulating estrogen and body weight in postmenopausal women. J Clin Endocrinol Metab. 1980;50:70–5.
52. Peng XD, Xie H, Zhao Q, Wu XP, Sun ZQ, Liao EY. Relationships between serum adiponectin, leptin, resistin, visfatin levels and bone mineral density, and bone biochemical markers in Chinese men. Clin Chim Acta. 2008;387:31–5.
53. Pistilli EE, Gordish-Dressman H, Seip RL, Devaney JM, Thompson PD, Price TB, Angelopoulos TJ, Clarkson PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Hoffman EP, Gordon PM. Resistin polymorphisms are associated with muscle, bone, and fat phenotypes in white men and women. Obesity (Silver Spring). 2007;15:392–402.
54. Stanworth RD, Jones TH. Testosterone in obesity, metabolic syndrome and type 2 diabetes. Front Horm Res. 2009;37:74–90.
55. Wang C, Jackson G, Jones TH, Matsumoto AM, Nehra A, Perelman MA, Swerdloff RS, Traish A, Zitzmann M, Cunningham G. Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. Diabetes Care. 2011;34:1669–75.
56. Khosla S, Melton III LJ, Achenbach SJ, Oberg AL, Riggs BL. Hormonal and biochemical determinants of trabecular microstructure at the ultradistal radius in women and men. J Clin Endocrinol Metab. 2006;91: 885–91.
57. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, Amin S, Rouleau PA, Khosla S. A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss in young adult women and men. J Bone Miner Res. 2008;23:205–14.

58. Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. J Bone Miner Res. 2005;20:1334–41.
59. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3.
60. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab. 2003;88:157–61.
61. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. J Clin Endocrinol Metab. 2004;89:1196–9.
62. Mason C, Xiao L, Imayama I, Duggan CR, Bain C, Foster-Schubert KE, Kong A, Campbell KL, Wang CY, Neuhouser ML, Li L, Jeffery W, Robien K, Alfano CM, Blackburn GL, McTiernan A. Effects of weight loss on serum vitamin D in postmenopausal women. Am J Clin Nutr. 2011;94:95–103.
63. Lauretani F, Bandinelli S, Russo CR, Maggio M, Di IA, Cherubini A, Maggio D, Ceda GP, Valenti G, Guralnik JM, Ferrucci L. Correlates of bone quality in older persons. Bone. 2006;39:915–21.
64. Lee AM, Anderson PH, Sawyer RK, Moore AJ, Forwood MR, Steck R, Morris HA, O'Loughlin PD. Discordant effects of vitamin D deficiency in trabecular and cortical bone architecture and strength in growing rodents. J Steroid Biochem Mol Biol. 2010; 121:284–7.
65. Stein EM, Dempster DW, Udesky J, Zhou H, Bilezikian JP, Shane E, Silverberg SJ. Vitamin D deficiency influences histomorphometric features of bone in primary hyperparathyroidism. Bone. 2011; 48:557–61.
66. Barbour KE, Zmuda JM, Horwitz MJ, Strotmeyer ES, Boudreau R, Evans RW, Ensrud KE, Gordon CL, Petit MA, Patrick AL, Cauley JA. The association of serum 25-hydroxyvitamin D with indicators of bone quality in men of Caucasian and African ancestry. Osteoporos Int. 2011;22:2475–85.
67. Bolland MJ, Grey AB, Ames RW, Horne AM, Gamble GD, Reid IR. Fat mass is an important predictor of parathyroid hormone levels in postmenopausal women. Bone. 2006;38:317–21.
68. Pitroda AP, Harris SS, Dawson-Hughes B. The association of adiposity with parathyroid hormone in healthy older adults. Endocrine. 2009;36:218–23.
69. Sukumar D, Partridge NC, Wang X, Shapses SA. The high serum monocyte chemoattractant protein-1 in obesity is influenced by high parathyroid hormone and not adiposity. J Clin Endocrinol Metab. 2011; 96:1852–8.
70. Grey A, Mitnick MA, Shapses S, Ellison A, Gundberg C, Insogna K. Circulating levels of interleukin-6 and tumor necrosis factor-alpha are elevated in primary hyperparathyroidism and correlate with markers of bone resorption – a clinical research center study. J Clin Endocrinol Metab. 1996;81:3450–4.
71. Onishi T, Hruska K. Expression of p27Kip1 in osteoblast-like cells during differentiation with parathyroid hormone. Endocrinology. 1997;138:1995–2004.
72. Duan Y, De Luca V, Seeman E. Parathyroid hormone deficiency and excess: similar effects on trabecular bone but differing effects on cortical bone. J Clin Endocrinol Metab. 1999;84:718–22.
73. Charopoulos I, Tournis S, Trovas G, Raptou P, Kaldrymides P, Skarandavos G, Katsalira K, Lyritis GP. Effect of primary hyperparathyroidism on volumetric bone mineral density and bone geometry assessed by peripheral quantitative computed tomography in postmenopausal women. J Clin Endocrinol Metab. 2006;91:1748–53.
74. Shen L, Xie X, Su Y, Luo C, Zhang C, Zeng B. Parathyroid hormone versus bisphosphonate treatment on bone mineral density in osteoporosis therapy: a meta-analysis of randomized controlled trials. PLoS One. 2011;6:e26267.
75. Goode LR, Brolin RE, Chowdhury HA, Shapses SA. Bone and gastric bypass surgery: effects of dietary calcium and vitamin D. Obes Res. 2004;12:40–7.
76. Oshima K, Nampei A, Matsuda M, Iwaki M, Fukuhara A, Hashimoto J, Yoshikawa H, Shimomura I. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Biochem Biophys Res Commun. 2005;331:520–6.
77. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, Kanaya AM, Harris TB, Cauley JA. The effects of adiponectin and leptin on changes in bone mineral density. Osteoporos Int. 2012;23(6):1699–710.
78. Tubic B, Magnusson P, Swolin-Eide D, Marild S. Relation between bone mineral density, biological markers and anthropometric measures in 4-year-old children: a pilot study within the IDEFICS study. Int J Obes (Lond). 2011;35 Suppl 1:S119–24.
79. Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, Freedman BI, Langefeld CD, Carr JJ, Bowden DW. Adiponectin as a novel determinant of bone mineral density and visceral fat. Bone. 2003;33:646–51.
80. Jurimae J, Jurimae T. Adiponectin is a predictor of bone mineral density in middle-aged premenopausal women. Osteoporos Int. 2007;18:1253–9.
81. Kontogianni MD, Dafni UG, Routsias JG, Skopouli FN. Blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in perimenopausal women. J Bone Miner Res. 2004;19: 546–51.
82. Napoli N, Pedone C, Pozzilli P, Lauretani F, Ferrucci L, Incalzi RA. Adiponectin and bone mass density: the InCHIANTI study. Bone. 2010;47(6):1001–5.
83. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, Kanaya AM, Harris TB, Bauer DC, Cauley JA. Adipokines and the risk of fracture in older adults. J Bone Miner Res. 2011; 26:1568–76.

84. Biver E, Salliot C, Combescure C, Gossec L, Hardouin P, Legroux-Gerot I, Cortet B. Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2011;96(9):2703–13.
85. Johansson H, Oden A, Lerner UH, Jutberger H, Lorentzon M, Barrett-Connor E, Karlsson MK, Ljunggren O, Smith U, McCloskey E, Kanis JA, Ohlsson C, Mellstrom D. High serum adiponectin predicts incident fractures in elderly men: osteoporotic fractures in men (MrOS) Sweden. J Bone Miner Res. 2012;27:1390–6.
86. Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. Cell Metab. 2006;4:341–8.
87. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology. 1999; 140:1630–8.
88. Cornish J, Callon KE, Bava U, Lin C, Naot D, Hill BL, Grey AB, Broom N, Myers DE, Nicholson GC, Reid IR. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. J Endocrinol. 2002;175:405–15.
89. Blum M, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, Dawson-Hughes B. Leptin, body composition and bone mineral density in premenopausal women. Calcif Tissue Int. 2003;73:27–32.
90. Pasco JA, Henry MJ, Kotowicz MA, Collier GR, Ball MJ, Ugoni AM, Nicholson GC. Serum leptin levels are associated with bone mass in nonobese women. J Clin Endocrinol Metab. 2001;86:1884–7.
91. Yamauchi M, Sugimoto T, Yamaguchi T, Nakaoka D, Kanzawa M, Yano S, Ozuru R, Sugishita T, Chihara K. Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. Clin Endocrinol (Oxf). 2001;55:341–7.
92. Fujita Y, Watanabe K, Maki K. Serum leptin levels negatively correlate with trabecular bone mineral density in high-fat diet-induced obesity mice. J Musculoskelet Neuronal Interact. 2012;12:84–94.
93. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. Bone. 2004;34: 376–83.
94. Cornish J, Callon KE, Bava U, Watson M, Xu X, Lin JM, Chan VA, Grey AB, Naot D, Buchanan CM, Cooper GJ, Reid IR. Preptin, another peptide product of the pancreatic beta-cell, is osteogenic in vitro and in vivo. Am J Physiol Endocrinol Metab. 2007;292:E117–22.
95. Bronsky J, Prusa R, Nevoral J. The role of amylin and related peptides in osteoporosis. Clin Chim Acta. 2006;373:9–16.
96. Clowes JA, Khosla S, Eastell R. Potential role of pancreatic and enteric hormones in regulating bone turnover. J Bone Miner Res. 2005;20:1497–506.
97. Davey RA, Moore AJ, Chiu MW, Notini AJ, Morris HA, Zajac JD. Effects of amylin deficiency on trabecular bone in young mice are sex-dependent. Calcif Tissue Int. 2006;78:398–403.
98. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 1992;257: 88–91.
99. Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, Kim GS. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporos Int. 2005;16:1263–71.
100. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature. 1986;319:516–8.
101. Mundy GR. Osteoporosis and inflammation. Nutr Rev. 2007;65:S147–51.
102. De BF, Rucci N, Del FA, Peruzzi B, Paro R, Longo M, Vivarelli M, Muratori F, Berni S, Ballanti P, Ferrari S, Teti A. Impaired skeletal development in interleukin-6-transgenic mice: a model for the impact of chronic inflammation on the growing skeletal system. Arthritis Rheum. 2006;54:3551–63.
103. Bhupathiraju SN, Alekel DL, Stewart JW, Hanson LN, Shedd KM, Reddy MB, Hanson KB, Van Loan MD, Genschel U, Koehler KJ. Relationship of circulating total homocysteine and C-reactive protein to trabecular bone in postmenopausal women. J Clin Densitom. 2007;10:395–403.
104. Rolland T, Boutroy S, Vilayphiou N, Blaizot S, Chapurlat R, Szulc P. Poor trabecular microarchitecture at the distal radius in older men with increased concentration of high-sensitivity C-reactive protein – the STRAMBO study. Calcif Tissue Int. 2012; 90:496–506.
105. Brick DJ, Gerweck AV, Meenaghan E, Lawson EA, Misra M, Fazeli P, Johnson W, Klibanski A, Miller KK. Determinants of IGF1 and GH across the weight spectrum: from anorexia nervosa to obesity. Eur J Endocrinol. 2010;163:185–91.
106. Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, Lee HC, Huh KB. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. Int J Obes Relat Metab Disord. 1997;21:355–9.
107. Frystyk J, Brick DJ, Gerweck AV, Utz AL, Miller KK. Bioactive insulin-like growth factor-I in obesity. J Clin Endocrinol Metab. 2009;94:3093–7.
108. Boonen S, Cheng XG, Nijs J, Nicholson PH, Verbeke G, Lesaffre E, Aerssens J, Dequeker J. Factors associated with cortical and trabecular bone loss as quantified by peripheral computed tomography (pQCT) at the ultradistal radius in aging women. Calcif Tissue Int. 1997;60:164–70.

109. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev. 2008;29:535–59.
110. Costa JL, Naot D, Lin JM, Watson M, Callon KE, Reid IR, Grey AB, Cornish J. Ghrelin is an osteoblast mitogen and increases osteoclastic bone resorption in vitro. Int J Pept. 2011;2011:605193.
111. Fukushima N, Hanada R, Teranishi H, Fukue Y, Tachibana T, Ishikawa H, Takeda S, Takeuchi Y, Fukumoto S, Kangawa K, Nagata K, Kojima M. Ghrelin directly regulates bone formation. J Bone Miner Res. 2005;20:790–8.
112. Maccarinelli G, Sibilia V, Torsello A, Raimondo F, Pitto M, Giustina A, Netti C, Cocchi D. Ghrelin regulates proliferation and differentiation of osteoblastic cells. J Endocrinol. 2005;184:249–56.
113. Kim SW, Her SJ, Park SJ, Kim D, Park KS, Lee HK, Han BH, Kim MS, Shin CS, Kim SY. Ghrelin stimulates proliferation and differentiation and inhibits apoptosis in osteoblastic MC3T3-E1 cells. Bone. 2005;37:359–69.
114. Woo DG, Lee BY, Lim D, Kim HS. Relationship between nutrition factors and osteopenia: effects of experimental diets on immature bone quality. J Biomech. 2009;42:1102–7.
115. Patsch JM, Kiefer FW, Varga P, Pail P, Rauner M, Stupphann D, Resch H, Moser D, Zysset PK, Stulnig TM, Pietschmann P. Increased bone resorption and impaired bone microarchitecture in short-term and extended high-fat diet-induced obesity. Metabolism. 2011;60:243–9.
116. Cao JJ, Sun L, Gao H. Diet-induced obesity alters bone remodeling leading to decreased femoral trabecular bone mass in mice. Ann N Y Acad Sci. 2010;1192:292–7.
117. Ionova-Martin SS, Wade JM, Tang S, Shahnazari M, Ager III JW, Lane NE, Yao W, Alliston T, Vaisse C, Ritchie RO. Changes in cortical bone response to high-fat diet from adolescence to adulthood in mice. Osteoporos Int. 2011;22(8):2283–93.
118. Lorincz C, Reimer RA, Boyd SK, Zernicke RF. High-fat, sucrose diet impairs geometrical and mechanical properties of cortical bone in mice. Br J Nutr. 2010;103:1302–8.
119. Zernicke RF, Salem GJ, Barnard RJ, Schramm E. Long-term, high-fat-sucrose diet alters rat femoral neck and vertebral morphology, bone mineral content, and mechanical properties. Bone. 1995;16: 25–31.
120. Hawkins J, Cifuentes M, Pleshko NL, Ambia-Sobhan H, Shapses SA. Energy restriction is associated with lower bone mineral density of the tibia and femur in lean but not obese female rats. J Nutr. 2010;140:31–7.
121. Gerbaix M, Metz L, Mac-Way F, Lavet C, Guillet C, Walrand S, Masgrau A, Linossier MT, Vico L, Daniel C. Impact of an obesogenic diet program on bone densitometry, micro architecture and metabolism in male rat. Lipids Health Dis. 2012;11:91.
122. Douard V, Suzuki T, Sabbagh Y, Lee J, Shapses S, Lin S, Ferraris RP. Dietary fructose inhibits lactation-induced adaptations in rat 1,25-(OH)(2)D(3) synthesis and calcium transport. FASEB J. 2012; 26:707–21.
123. Tsanzi E, Light HR, Tou JC. The effect of feeding different sugar-sweetened beverages to growing female Sprague-Dawley rats on bone mass and strength. Bone. 2008;42:960–8.
124. Fried A, Manske SL, Eller LK, Lorincz C, Reimer RA, Zernicke RF. Skim milk powder enhances trabecular bone architecture compared with casein or whey in diet-induced obese rats. Nutrition. 2012;28: 331–5.
125. Salamone LM, Cauley JA, Black DM, Simkin-Silverman L, Lang W, Gregg E, Palermo L, Epstein RS, Kuller LH, Wing R. Effect of a lifestyle intervention on bone mineral density in premenopausal women: a randomized trial. Am J Clin Nutr. 1999;70:97–103.
126. Langlois JA, Mussolino ME, Visser M, Looker AC, Harris T, Madans J. Weight loss from maximum body weight among middle-aged and older white women and the risk of hip fracture: the NHANES I epidemiologic follow-up study. Osteoporos Int. 2001;12:763–8.
127. Ensrud KE, Fullman RL, Barrett-Connor E, Cauley JA, Stefanick ML, Fink HA, Lewis CE, Orwoll E. Voluntary weight reduction in older men increases hip bone loss: the osteoporotic fractures in men study. J Clin Endocrinol Metab. 2005;90:1998–2004.
128. Sukumar D, Ambia-Sobhan H, Zurfluh R, Schlussel Y, Stahl TJ, Gordon CL, Shapses SA. Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: a randomized, controlled trial. J Bone Miner Res. 2011;26:1339–48.
129. Riedt CS, Cifuentes M, Stahl T, Chowdhury HA, Schlussel Y, Shapses SA. Overweight postmenopausal women lose bone with moderate weight reduction and 1 g/day calcium intake. J Bone Miner Res. 2005;20:455–63.
130. Villalon KL, Gozansky WS, Van Pelt RE, Wolfe P, Jankowski CM, Schwartz RS, Kohrt WM. A losing battle: weight regain does not restore weight loss-induced bone loss in postmenopausal women. Obesity (Silver Spring). 2011;19(12):2345–50.
131. Bleicher K, Cumming RG, Naganathan V, Travison TG, Sambrook PN, Blyth FM, Handelsman DJ, Le Couteur DG, Waite LM, Creasey HM, Seibel MJ. The role of fat and lean mass in bone loss in older men: findings from the CHAMP study. Bone. 2011;49:1299–305.
132. Nguyen TV, Sambrook PN, Eisman JA. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology Study. J Bone Miner Res. 1998;13:1458–67.
133. Shapses SA, Sukumar D. Bone metabolism in obesity and weight loss. Annu Rev Nutr. 2012;32: 287–309.

134. Langlois JA, Harris T, Looker AC, Madans J. Weight change between age 50 years and old age is associated with risk of hip fracture in white women aged 67 years and older. Arch Intern Med. 1996;156:989–94.
135. Meyer HE, Tverdal A, Selmer R. Weight variability, weight change and the incidence of hip fracture: a prospective study of 39,000 middle-aged Norwegians. Osteoporos Int. 1998;8:373–8.
136. Mussolino ME, Looker AC, Madans JH, Langlois JA, Orwoll ES. Risk factors for hip fracture in white men: the NHANES I Epidemiologic Follow-up Study. J Bone Miner Res. 1998;13:918–24.
137. Wilsgaard T, Jacobsen BK, Ahmed LA, Joakimsen RM, Stormer J, Jorgensen L. BMI change is associated with fracture incidence, but only in non-smokers. The Tromso Study. Osteoporos Int. 2011;22:1237–45.
138. Omsland TK, Schei B, Gronskag AB, Langhammer A, Forsen L, Gjesdal CG, Meyer HE. Weight loss and distal forearm fractures in postmenopausal women: the Nord-Trondelag health study, Norway. Osteoporos Int. 2009;20:2009–16.
139. Shapses SA, Von Thun NL, Heymsfield SB, Ricci TA, Ospina M, Pierson Jr RN, Stahl T. Bone turnover and density in obese premenopausal women during moderate weight loss and calcium supplementation. J Bone Miner Res. 2001;16:1329–36.
140. Riedt CS, Schlussel Y, von Thun N, Ambia-Sobhan H, Stahl T, Field MP, Sherrell RM, Shapses SA. Premenopausal overweight women do not lose bone during moderate weight loss with adequate or higher calcium intake. Am J Clin Nutr. 2007;85:972–80.
141. Redman LM, Rood J, Anton SD, Champagne C, Smith SR, Ravussin E. Calorie restriction and bone health in young, overweight individuals. Arch Intern Med. 2008;168:1859–66.
142. Uusi-Rasi K, Rauhio A, Kannus P, Pasanen M, Kukkonen-Harjula K, Fogelholm M, Sievanen H. Three-month weight reduction does not compromise bone strength in obese premenopausal women. Bone. 2010;46:1286–93.
143. Hamrick MW, Ding KH, Ponnala S, Ferrari SL, Isales CM. Caloric restriction decreases cortical bone mass but spares trabecular bone in the mouse skeleton: implications for the regulation of bone mass by body weight. J Bone Miner Res. 2008;23:870–8.
144. Talbott SM, Cifuentes M, Dunn MG, Shapses SA. Energy restriction reduces bone density and biomechanical properties in aged female rats. J Nutr. 2001;131:2382–7.
145. Devlin MJ, Cloutier AM, Thomas NA, Panus DA, Lotinun S, Pinz I, Baron R, Rosen CJ, Bouxsein ML. Caloric restriction leads to high marrow adiposity and low bone mass in growing mice. J Bone Miner Res. 2010;25:2078–88.
146. Cifuentes M, Advis JP, Shapses SA. Estrogen prevents the reduction in fractional calcium absorption due to energy restriction in mature rats. J Nutr. 2004;134:1929–34.
147. Gozansky WS, Van Pelt RE, Jankowski CM, Schwartz RS, Kohrt WM. Protection of bone mass by estrogens and raloxifene during exercise-induced weight loss. J Clin Endocrinol Metab. 2005;90:52–9.
148. Turner RT, Iwaniec UT. Low dose parathyroid hormone maintains normal bone formation in adult male rats during rapid weight loss. Bone. 2011;48:726–32.
149. Berrigan D, Lavigne JA, Perkins SN, Nagy TR, Barrett JC, Hursting SD. Phenotypic effects of calorie restriction and insulin-like growth factor-1 treatment on body composition and bone mineral density of C57BL/6 mice: implications for cancer prevention. In Vivo. 2005;19:667–74.
150. Pollock NK, Laing EM, Hamrick MW, Baile CA, Hall DB, Lewis RD. Bone and fat relationships in postadolescent black females: a pQCT study. Osteoporos Int. 2011;22:655–65.

5 Emerging Nutritional and Lifestyle Risk Factors for Bone Health in Young Women: A Mixed Longitudinal Twin Study

Jemma J. Christie, Caryl A. Nowson, Suzanne M. Garland, and John D. Wark

Abstract

Late adolescence and early adulthood are times of major behavioral transition in young women as they become more independent and make choices about lifestyle that will affect their long-term health. We prospectively evaluated nutritional and lifestyle factors in 566 15–30-year-old female twins participating in a mixed longitudinal study of diet and lifestyle.

Twins completed 790 visits including questionnaires and measures of anthropometry. Nonparametric tests (chi-square, Mann-Whitney U, and Kruskal-Wallis; SPSS) were used to examine age-related differences in selected variables. Dietary calcium intake by short food frequency questionnaire was relatively low [511 (321,747)] mg/day (median, IQR; 60 % of estimated daily total) and did not vary significantly with age. The number of young women who reported ever consuming alcohol (12+ standard drinks ever) increased from 50 % under 18 years to 93–99 % for the 18+ age groups. Of those who consumed alcohol in the preceding year, monthly intake doubled from under 18 years (5.7, 3.9, 19.0 standard drinks; median, IQR) to 18+ years (12.0, 4.7, 26.0; $P<0.001$) with the highest consumers being 21–23 and 27–29 years. At age 15–17 years, 14 % reported ever smoking and by age 27–29, 51 % had smoked ($P=0.002$). Under the age of 20 years, average cigarette consumption in smokers was six cigarettes per day, increasing to ten above age 20 ($P<0.001$). Participation in sporting activity decreased with age ($P<0.001$): 47.5 % of 15–17-year-olds undertook 4 or more hour/

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week of sport, compared with 23.5 % at age 27–29 years. Conversely, sedentary behavior increased with age: 25.0 % of 15–17-year-olds reported 1 or less hour/week of exercise compared with 50.0 % at age 27–29 years. BMI increased with age ($P=0.011$), from 21.3 (19.5, 23.6; median, IQR) in the youngest to 23.1 (21.5, 25.9) in the oldest.

These highly significant changes in behavior in young women as they transitioned into independent adult living are predicted to impact adversely on bone and other health outcomes in later life. It is crucial to improve understanding of the determinants of these changes and to develop effective interventions to improve long-term health outcomes in young women.

Keywords

Adolescence • Young adult • Lifestyle • Health • Diet • Female • Smoking • Alcohol • Physical activity • BMI

Abbreviations

BMI	Body mass index
IQR	Interquartile range
Fig	Figure
mg/day	Milligrams per day
Cm	Centimeter
Kg	Kilogram
SNS	Social networking sites
Yrs	Years

Introduction

Late adolescence and early adulthood are times of major behavioral transition in young women as they become more independent and make choices about their health-related behaviors and lifestyle. Behaviors and lifestyle choices of young people have far-reaching consequences for future well-being, quality of life, and productivity [1], yet this is a much understudied age group [2]. Women drive health behaviors in our society [3], making it critical to understand the factors shaping health and lifestyle in young women and to implement effective interventions during the crucial ages of 16–25 years when health patterns undergo major transitions (Fig. 5.1) that can shape future health trajectories [4].

Over 30 % of Australia's burden of disease is due to modifiable risk factors (including smoking, alcohol abuse, physical inactivity, high blood pressure and cholesterol, low consumption of fruits and vegetables, overweight/obesity), placing preventive health care at the forefront of Australia's national health strategy [5]. Yet changing health-related behaviors is a major challenge. Smoking causes 7 % of the total burden of disease in Australian women [6] and remains highly prevalent in young women: 18 % of females aged 18–24 were smokers in 2007 [7]. Physical inactivity is second only to smoking as the key risk factor associated with poor health outcomes [6], with poor diet a further contributor. Changes in physical activity and eating patterns during adolescence and early adulthood are associated with rising rates of obesity. The Australian government has called for action to halt this epidemic [8]: obesity rates in children and adults more than doubled over the last two decades [9]. Body mass index (BMI) in youth tracks into adult life [10] and obese or overweight young people are at higher risk of subsequent cardiovascular and metabolic disease [11].

Identifying and addressing risk factors for poor bone and joint health are also of great national importance, since musculoskeletal disorders in later life cause more disability than any other medical condition [12], imposing a large economic burden ($4.7 billion in Australia in 2000–2001 [13]). Musculoskeletal injuries and pain impact upon women's ability to participate in physical and occupational activities and predispose to chronic conditions (e.g., low back pain and knee injuries sustained

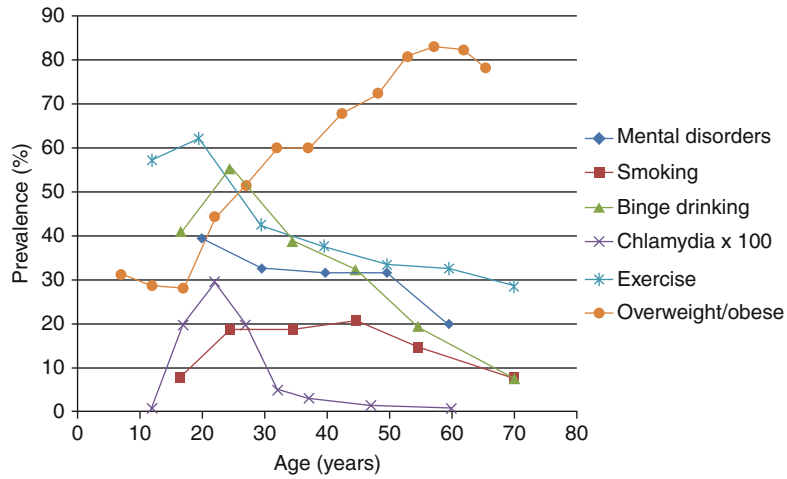

Fig. 5.1 The evolution of health risks in young Australian women (Kindly provided courtesy of Dr. Yeshe Fenner)

during sport, which are common and substantially increase the risk of knee osteoarthritis in later life).

In this study, we investigated the differences of key health and lifestyle factors, dietary calcium, alcohol consumption, smoking, physical activity, and BMI during different stages of adolescence and young adulthood. These factors are related to musculoskeletal development and bone health later in life (among many other health conditions). Having conducted extensive studies of lifestyle, diet, and health in female twins, more recently we have commenced population-based investigations of young women's health determinants using recruitment through social networking sites (SNS) and online data collection methods [14] in a project called the Young Female Health Initiative (YFHI). This research complements the twin studies we report here and offers a powerful approach for ongoing health research in young people. This important line of research will help to determine how health-related behaviors and lifestyle changes influence health in young women and how we can change behaviors to improve their health outcomes.

Methods

We prospectively evaluated nutritional and lifestyle factors in 566 15–30-year-old female twins (both monozygotic and dizygotic). The participants were of various ages at first visit, attended for follow-up at variable intervals, and had variable numbers of visits, depending on opportunities for follow-up. Of the 566 twins, there were a total of 790 visits while they were in the targeted age range. These twins had participated in cross-sectional and longitudinal studies of constitutional, lifestyle, and dietary determinants of bone health in which they completed questionnaires to assess their health and lifestyle [15, 16]. This further analysis looked at the evolution of health risk factors across the crucial late adolescent and young adult years. Current daily calcium intake was measured by a short food frequency questionnaire based on one developed for Australian women [17]. The questionnaire captures approximately 60 % of dietary calcium intake on a typical Australian diet. Physical activity was determined over the previous 12 months by questionnaire [15, 16]. Hours of playing general sport per week and hours of walking per week were each recorded on a categorical scale (0–1, 2–3, 4–7, and >7 h), with the number of hours per week being entered as 0.5, 2.5, 5.5, and 8 h, respectively, for each category. Smoking habits were recorded by participants as ever smoking, current smoking, and smoking amounts. In those who had ever consumed alcohol (more than 12 standard drinks in their lifetime), alcohol consumption was calculated from average monthly consumption over the previous year and expressed as the number of standard drinks (a standard drink contains 10 g of alcohol). BMI was calculated using the standard formula [weight in kilograms/(height in meters × height in meters)]. Height was measured to the nearest 0.1 cm on a wall-mounted stadiometer, and weight was measured on a balance scale to the nearest

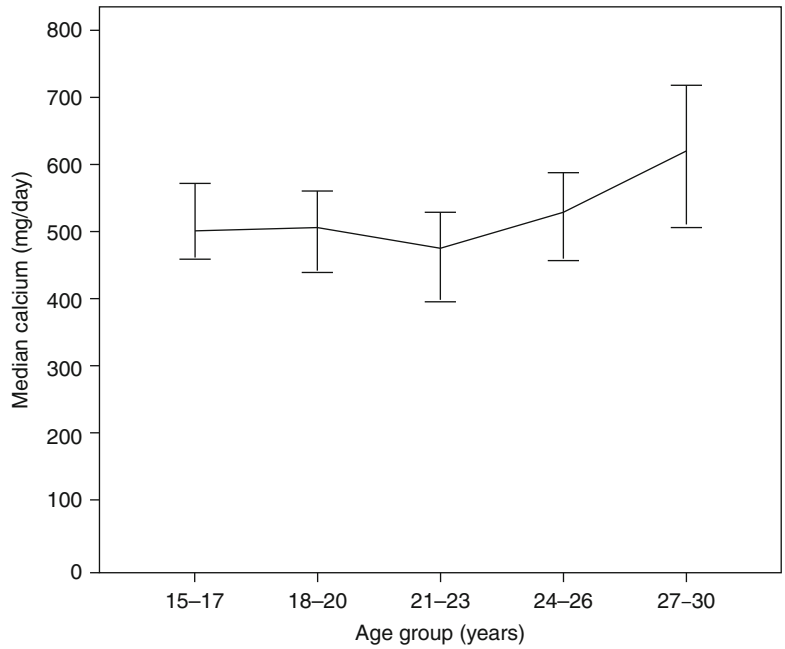

Fig. 5.2 Median (IQR) dietary calcium intake (mg/day) by short food frequency questionnaire representing an estimated 60 % of total calcium intake [17] in different age groups from 15 to 30 years

0.1 kg. All statistical analyses were carried out using SPSS (SPSS® 20.0, Chicago, IL, USA). Unlike previous reports on this cohort [15, 16], twinness was not included in the models since little or no effect on these descriptive data was anticipated. Nonparametric tests (chi-square, Mann-Whitney U, and Kruskal-Wallis) were used to identify significant differences in the selected lifestyle factors between age groups, to examine the age-related differences. One-way ANOVA was used for normal data. *P* values of <0.05 were considered statistically significant.

Results

Dietary calcium intake was relatively low [511 (321,747)] mg/day (median, IQR) and did not vary significantly with age (Fig. 5.2). The questionnaire used to calculate calcium assumes the questionnaire estimates 60 % of total dietary calcium. Taking this into account and that the daily recommended intake for most people is between 800 and 1,500 mg/day [18], almost half of this age group was below the required dietary calcium intake.

The number of young women who reported ever consuming alcohol (defined as12+ standard drinks ever) increased from 50 % in those less than 18 years to 93–99 % for the 18+ age groups ($p<0.001$: Fig. 5.3). Of those who consumed alcohol in the year prior to their visit, monthly intake doubled from under 18 years (5.7, 3.9, 19.0; median, IQR) to 18+ years (12.0, 4.7, 26.0; $P<0.001$) with the highest consumers being 21–23 and 27–29 years (Fig. 5.4).

At age 15–17 years, 14 % reported ever smoking and by age 27–29, 51 % had smoked ($P=0.002$). Under the age of 20 years, average cigarette consumption (Fig. 5.5) in smokers was six cigarettes [4, 10] per day, increasing to ten [2, 15] at 20 years and older ($P<0.001$). When looking at current smoking across the age subgroups, interestingly being a current smoker progressively increased from age 15 to 23 years of age (15 to 17 – 16.8 %, 18 to 20 – 21.4 %, 21 to 23 – 25.0 %), then declined from 24 to 30 years of age (24 to 26 – 21.9 %, 27 to 30 – 14.8 %; $P<0.001$). Therefore, the oldest age group, 27–30 years, had the least current smokers of all ages.

Participation in sporting activity decreased progressively with age ($P<0.001$): 47.5 % of 15–17-year-olds undertook 4 or more hours of sport per week, compared with 23.5 % at age 27–29 years (Table 5.1). Conversely, sedentary behavior increased with age:

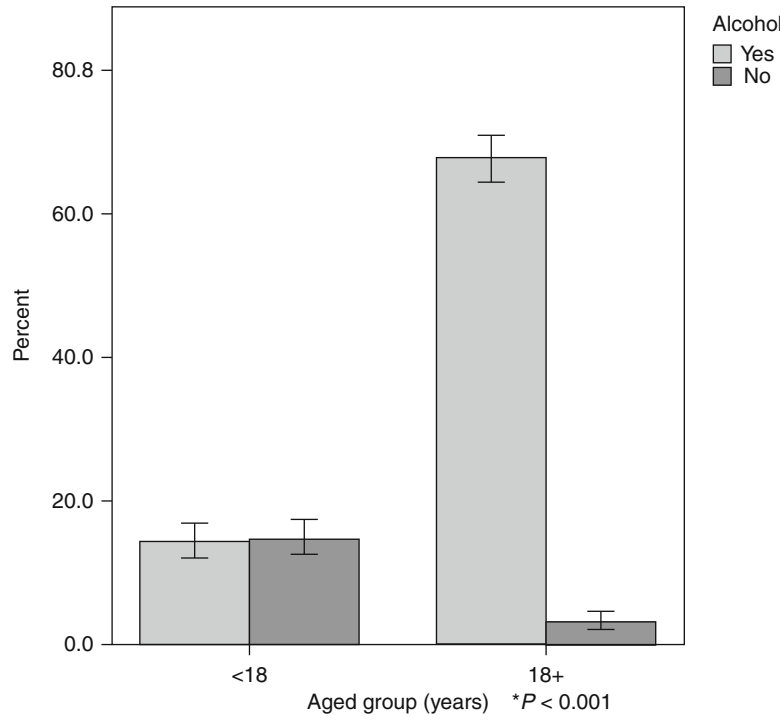

Fig. 5.3 Percentage of young women who have or have not consumed 12+ standard drinks of alcohol ever

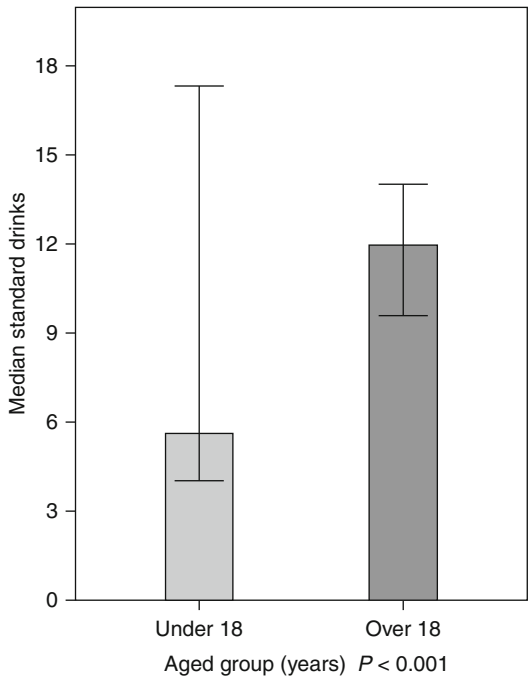

Fig. 5.4 Average monthly alcohol consumption (standard drinks) in the year prior to study visit in young women under 18 years and 18+ years

25.0 % of 15–17-year-olds reported 1 or less hour/week of exercise compared with 50.0 % at age 27–29 years. Changes in walking activity were complex, suggesting an increase with age (Table 5.2).

BMI increased progressively with age ($P=0.011$), the youngest group (15–17 years) having the lowest (21.3, 19.5, 23.6; median, IQR) and the oldest (27–29 years) having the highest (23.1, 21.5, 25.9) BMI (Fig. 5.6). Median BMI in all groups was within a normal range.

There was no difference in height between age groups. Weight (kg) followed the same trend as BMI, progressively increasing with age ($P=0.027$). Again the youngest age group (15–17 years) was the lightest with mean weight 60.0 kg and the oldest age group (27–30 years) was the heaviest at 66.2 kg.

Discussion

These findings demonstrate highly significant and generally adverse changes in health-related behavior in young women as they transition into

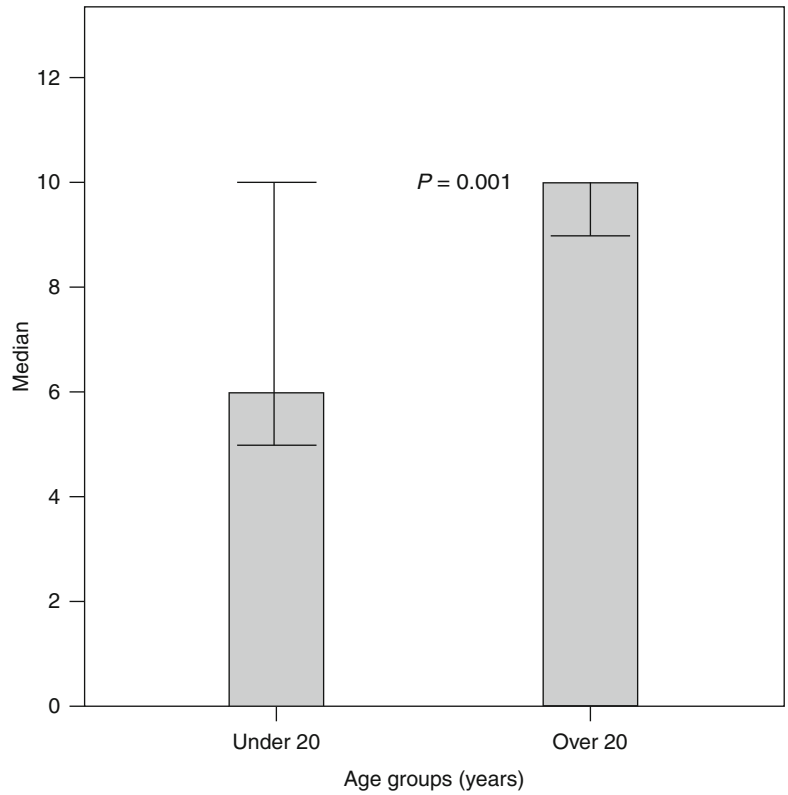

Fig. 5.5 Average daily cigarette consumption (median, IQR) in under 20 years and 20+ years

Table 5.1 Amount of sport (hours) played per week in the year prior to study visit

		Hours of sport per week in the year prior to visit			
Age group (years)		0–1	2–3	4–7	7+
15–17	Count	59	65	57	55
	% Within age group	25.0	27.5	24.2	23.3
	% Within sport	20.7	30.0%	36.5	44.0
18–20	Count	76	40	26	21
	% Within age group	46.6	24.5	16.0	12.9
	% Within sport	26.7	18.4	16.7	16.8
21–23	Count	54	49	40	26
	% Within age group	32.0	29.0	23.7	15.4
	% Within sport	18.9	22.6	25.6	20.8
24–26	Count	62	45	24	16
	% Within age group	42.2	30.6	16.3	10.9
	% Within sport	21.8	20.7	15.4	12.8
27–30	Count	34	18	9	7
	% Within age group	50.0	26.5	13.2	10.3
	% Within sport	11.9	8.3	5.8	5.6

independent adult living. Many of these changes are predicted to impact adversely on bone and other health outcomes in later life. Indeed, studies of adolescent and young adult twins (age range 10–26 years) showed that the contribution of their shared environment to variance in bone mineral density (BMD) diminished dramatically as they started to live more independently in early

Table 5.2 Number of hours spent walking per week in the year prior to study visit

Age group (years)		Hours of walking per week in the year prior to visit			
		0–1	2–3	4–7	7+
15–17	Count	74	91	48	23
	% Within age group	31.4	38.6	20.3	9.7
	% Within walking	34.7	32.4	26.1	21.9
18–20	Count	44	58	43	18
	% Within age group	27.0	35.6	26.4	11.0
	% Within walking	20.7	20.6	23.4	17.1
21–23	Count	38	64	46	21
	% Within age group	22.5	37.9	27.2	12.4
	% Within walking	17.8	22.8	25.0	20.0
24–26	Count	42	48	32	25
	% Within age group	28.6	32.7	21.8	17.0
	% Within walking	19.7	17.1	17.4	23.8
27–30	Count	15	20	15	18
	% Within age group	22.1	29.4	22.1	26.5
	% Within walking	7.0	7.1	8.2	17.1

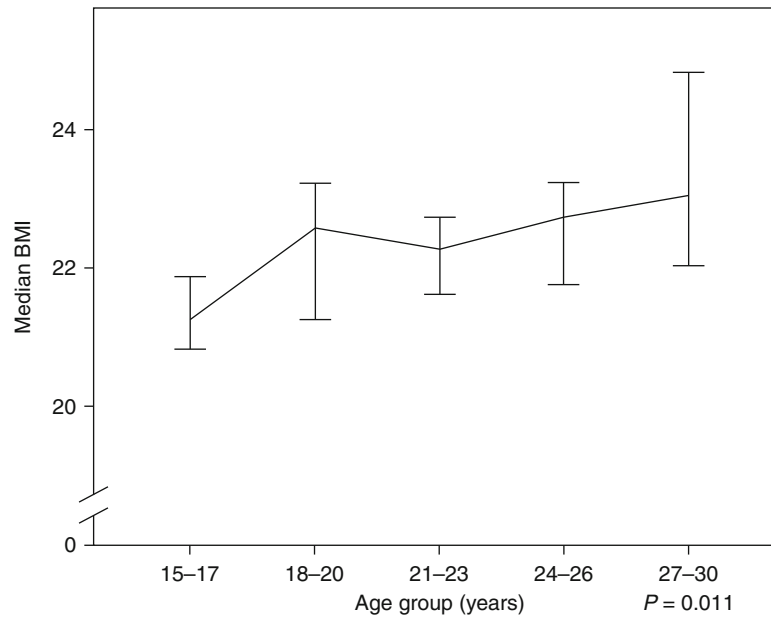

Fig. 5.6 Median (IQR) BMI in different age groups from 15 to 30 years

adult life, when the contribution of environmental factors specific to each individual became evident [19]. These observations are consistent with a change in the environmental sources of variation in BMD across this age range, and most of these environmental changes are likely to relate to the individuals' lifestyle. As we demonstrate here, physical activity, cigarette smoking, and alcohol consumption, all potentially adverse for bone health inter alia, varied significantly in this cohort of young twins across the age range 15–30 years.

There is a pressing need to improve understanding of the determinants of these changes and to develop effective interventions to improve long-term bone health and other outcomes in young

women. The first step towards achieving these goals is to engage effectively with young women. However, young people in particular are underrepresented in medical and population-based studies as they are highly mobile, and recruitment and retention are difficult [2]. Traditional approaches to recruitment and retention of young women in health-related research seem unlikely to be fruitful in future, and it seems much more promising to engage with young women using the mobile and internet-based communication technologies with which they are so familiar and comfortable. Likewise, recruitment via SNS also is very appealing given their wide and almost universal reach in many countries, potential to recruit demographically representative samples where required, and the relatively modest cost of advertising via SNS. Our own group's recent experience [14] in recruiting 16–25-year-old women for health research via *Facebook* was very positive. Over several months, we recruited 278 young women who completed an online health questionnaire, with approximately half choosing to do so at our study center and half remotely. Regional and remotely dwelling participants were well represented. The sample also was representative of the general population in this age range based on socioeconomic level and country of birth. Older subjects and those with a higher educational level were mildly overrepresented. Complete health questionnaire data were provided by >90 % of respondents. Among the results, self-reported weight problems were a major concern, with 24 % of 16–17-year-olds, 33 % of 18–21-year-olds, and 36 % of 22–25-year-olds classified as overweight or obese. Moreover, in a linear regression model, BMI increased by 0.29 kg/m^2 per year of increase in age ($P<0.01$). In contrast, only 5–7 % reported being underweight (BMI < 18.5).

The *Facebook* recruitment method also was highly cost-effective, costing only USD 20 per completing participant. Based on this experience, our group is pursuing *Facebook* recruitment, coupled with online and mobile data collection methods, in a range of observational health studies directed at young women. These methodologies also are readily adaptable for intervention trials, particularly targeting lifestyle and behavioral interventions in areas including nutrition, smoking cessation, physical activity, and sun exposure.

Conclusions

These findings demonstrate highly significant changes in behavior in young women as they transitioned into independent adult living. Many of these changes are predicted to impact adversely on bone and other health outcomes in later life. There is a pressing need to improve understanding of the determinants of these changes and to develop effective interventions to improve long-term bone health and other outcomes in young women. Our recent studies suggest that SNS and other information and communication technologies hold great promise for engaging young women in health research and ultimately for supporting health interventions in this demographic.

References

1. Diet, nutrition, and the prevention of chronic diseases. Report of a WHO Study Group. 1990; Geneva, 6–13 Mar 1989.
2. Faden VB, Day NL, Windle M, Windle R, Grube JW, Molina BSG, et al. Collecting longitudinal data through childhood, adolescence, and young adulthood: methodological challenges. Alcohol Clin Exp Res. 2004;28(2):330–40.
3. Calabretta N. Consumer-driven, patient-centered health care in the age of electronic information. J Med Libr Assoc. 2002;90(1):32–7.
4. UN World Health Organization. Women and health: today's evidence tomorrow's agenda. 2009. Available at: http://www.unhcr.org/refworld/docid/4af82d3d2.html. Accessed 7 Nov 2010.
5. Biggs A, Jolly R. Improving the health of all Australians: the role of preventative health. 2010. Available from: http://www.aph.gov.au/About_Parliament/Parliamentary_Departments/Parliamentary_Library/pubs/BriefingBook43p/preventativehealth. Accessed 29 Aug 2012.
6. Mathers CD, Vos ET, Stevenson CE, Begg SJ. The burden of disease and injury in Australia. Bull World Health Organ. 2001;79(11):1076–84.
7. Scollo MM, Winstandley MH. Tobacco in Australia: facts and issues. 3rd ed. Melbourne: Cancer Council Victoria; 2008.
8. Australian Government. The National obesity taskforce: 2004 overview. 2005. http://www.health.gov.au/internet/healthyactive/Publishing.nsf/Content/activities_2004.pdf/$File/activities_2004.pdf. Accessed 28 Aug 2012.
9. Australian Government Department of Health and Ageing. Promoting healthy weight. 2009. Available from: http://www.health.gov.au/internet/main/publishing.nsf/content/health-pubhlth-strateg-hlthwt-obesity.htm. Accessed 5 Jan 2012.

10. The NS, Suchindran C, North KE, Popkin BM, Gordon-Larsen P. Association of adolescent obesity with risk of severe obesity in adulthood. JAMA. 2010;304(18):2042–7.
11. Morrison J, Friedman L, Wang P, Glueck C. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. J Pediatr. 2008;152:201–6.
12. Australian Bureau of Statistics. National health survey. Canberra: Australian Bureau of Statistics; 2001.
13. Australian Institute of Health and Welfare. Health system expenditure on disease and injury in Australia, 2000–01. Canberra: Australian Institute of Health and Welfare; 2004.
14. Fenner Y, Garland SM, Moore EE, Jayasinghe Y, Fletcher A, Tabrizi SN, et al. Web-based recruiting for health research using a social networking site: an exploratory study. J Med Internet Res. 2012;14(1):e20.
15. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int. 2001;12(6):506–15.
16. Young D, Hopper JL, Nowson CA, Green RM, Sherwin AJ, Kaymakci B, et al. Determinants of bone mass in 10- to 26-year-old females: a twin study. J Bone Miner Res. 1995;10(4):558–67.
17. Angus RM, Eisman JA. Osteoporosis: the role of calcium intake and supplementation. Med J Aust. 1988; 148(12):630–3.
18. Wark JD, Nowson CA. Calcium supplementation: the bare bones. Aust Prescr. 2003;26(8):126–7.
19. Hopper JL, Green RM, Nowson CA, Young D, Sherwin AJ, Kaymakci B, et al. Genetic, common environment, and individual specific components of variance for bone mineral density in 10- to 26-year-old females: a twin study. Am J Epidemiol. 1998;147(1):17–29.

Dietary Fat Composition and Age-Related Muscle Loss

6

Ailsa A. Welch

Abstract

Sarcopenia is the age-related loss of muscle mass and strength, and the consequences include the loss of physical function leading to frailty and disability and to an increased risk of falls and therefore fractures and also mortality. Although age-related muscle loss starts at the age of 30 years, the causes are not yet well established.

Fat could influence muscle mass through its integral association with muscle metabolism and influence on myocellular membrane composition or indirectly through its effects on inflammation and insulin resistance. However, the association between the fat composition of the diet and the skeletal muscle mass has not been previously investigated in a general population. Therefore, we investigated this in 2,689 female twins aged 18–79 years calculated using a Food Frequency Questionnaire (FFQ). Body composition was measured using dual-energy X-ray absorptiometry, and indexes of skeletal muscle mass, fat-free mass (FFM), and fat-free mass index (FFMI, weight/height2) were calculated according to quintile of dietary fat and also adjusted for covariates. Associations per quintile were compared with 10 years of age.

FFM and FFMI were significantly and positively associated with the P:S ratio and inversely associated with total fat, saturated fatty acids, monounsaturated fatty acids, and trans-fatty acids, as a percentage of energy. Comparisons of quintile associations versus those of 10 years of age ranged from 72 % for FFM for the P:S ratio to 95 % for total dietary fat.

Both dietary total fat load and fatty acid composition were associated with skeletal muscle mass. Although the scales of the associations were relatively small, they were significant after multivariate adjustment. These

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novel findings are suggestive that a fat profile that is already associated with CVD protection may also be beneficial for conservation of skeletal muscle mass.

Keywords
Sarcopenia • Age-related muscle loss • Nutrition • Dietary fat composition • Saturated fatty acids • Monounsaturated fatty acids • Trans-fatty acids • P:S ratio • Mechanisms

Introduction

Sarcopenia and Its Consequences

Sarcopenia is the age-related loss of muscle mass and strength associated with aging [1]. The prevalence ranges from 9 to 18 % in those over the age of 65 years [2]. Estimated current costs of sarcopenia in the USA are $18.5 billion ($10.8 billion in men, $7.8 billion in women), about 1.5 % of total health-care expenditure [2, 3]. Although the range for these estimated costs for sarcopenia is between $11.8 and $26.2 billion, the health-care costs amount to $860 for every sarcopenic man and $933 for every sarcopenic woman.

The consequences of the loss of muscle mass are the gradual loss of metabolically active tissue, a decline in energy expenditure, and the loss of physical function leading to frailty and disability [4–7]. Conservation of skeletal muscle mass is important in preventing falls and fractures as it is positively associated with bone density. In addition to its role in maintaining balance, muscle mass may also act as a protective barrier to reduce the impact of falls [8–11]. Sarcopenia, or loss of muscle mass, is also associated with increased rates of mortality in older age [12].

In addition to the direct estimated costs of sarcopenia, sarcopenia contributes to fractures (through the impact on falls) which are a major public health problem costing £2.3 billion/year in health and social care in the UK alone and $17 billion/year in the USA [13, 14].

The age profile of western populations is increasing within the UK with an almost doubling from the current number of over 65-year-olds, which is 10–19 million in 2050. Additionally, of those 19 million, 6 million will be over the age of 80 years [15]. Consequently there will be future increases in the rates of sarcopenia and its associated costs.

The age-related loss of muscle mass is gradual and starts as early as the age of 30, increasing to greater rates of loss of around 1–2 % per year from the age of 50 years; see also Fig. 6.2 for an example [16]. Muscle strength also declines with age (Fig. 6.1) [17]. However, the mechanisms and role of the environment in muscle loss are not well understood. Given the issues with the loss of muscle mass with age, it is important to understand how preventative measures can be made.

Relationship Between Skeletal Muscle Mass and Strength

Muscle loss is a highly important component of sarcopenia. Muscle loss is highly correlated with muscle strength; high correlations between total leg lean mass and muscle strength have been observed (between $r=0.60$ and $P<0.001$ and $r=0.98$ and $P=0.001$) suggesting that lower extremity muscle mass is an important determinant of functionally limited older people (Fig. 6.3) [18, 19]. The remainder of this chapter focuses on the relationship between nutrition and muscle mass.

Association Between Nutrition and Muscle Mass

Rates of sarcopenia vary throughout populations, and given that the environment is likely to influence loss of muscle, nutrition has the potential to influence age-related loss of muscle mass [1, 20, 21]. Nutrients are integral to skeletal muscle metabolism, but although associations between protein and muscle are well established, the association between the fat

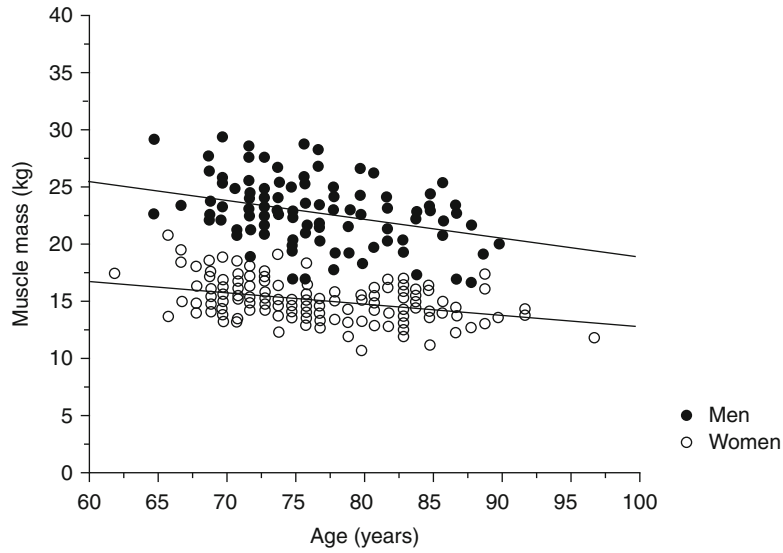

Fig. 6.1 Association between grip strength and age in men and women aged 60–97 years (Reprinted from Baumgartner et al. [16]. With permission from Elsevier)

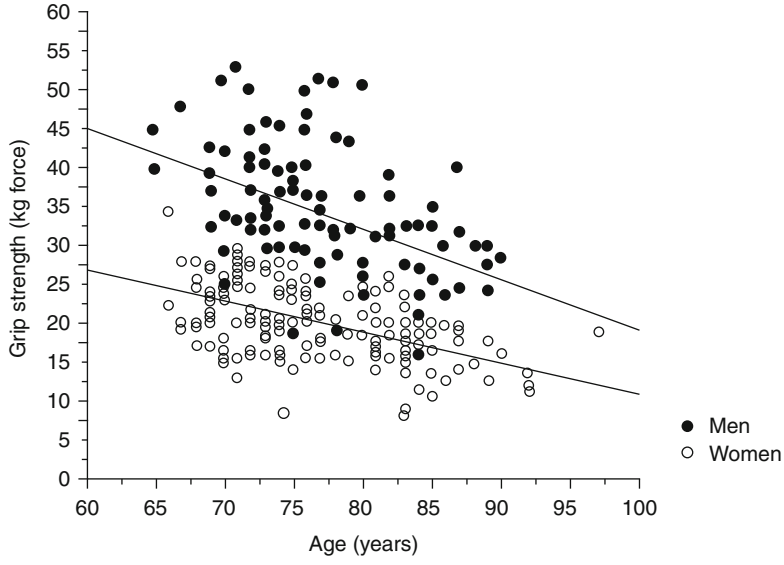

Fig. 6.2 Association between muscle mass and age in men and women aged 60–97 years (Reprinted from Baumgartner et al. [16]. With permission from Elsevier)

composition of the diet and the muscle mass in populations has only previously been studied in a small population, for saturated fatty acids (SFA) [22–29]. The fat composition of the diet varies considerably in quantity, in percentage of energy, and in fatty acid profile and could potentially influence muscle mass, firstly through its integral association with muscle metabolism and influence on myocellular membrane composition and secondly, indirectly, through its effects on inflammation and insulin resistance [22, 30–37].

Fat in the form of fatty acids is the major source of energy for resting and working muscle through mitochondrial beta oxidation [33, 35, 36]. Long-chain fatty acids are an important substrate for ATP production within skeletal muscle, with exercise-induced fatty acid entry into cells principally regulated by the proteins fatty acid translocase (FAT/CD36) and fatty acid-binding protein (FABPpm), although the contribution of fat to oxidative metabolism declines at high intensities of exercise [30, 33–35, 38]. Non-esterified fatty acids (NEFA) are transferred from adipose tissue via plasma lipids, and skeletal muscle is a net consumer of fatty acids [22, 35, 36]. Fatty acids are oxidized differentially with evidence from

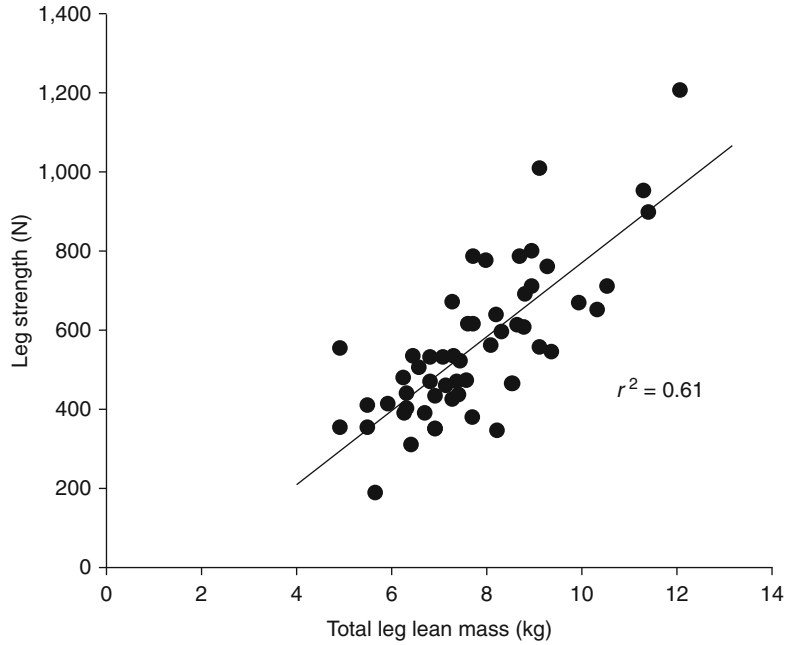

Fig. 6.3 Correlation between leg strength and muscle mass in men and women aged 66–84 years (Reprinted from Baumgartner et al. [17]. With permission from Oxford University Press)

whole-body studies indicating that oleic and unsaturated fatty acids are oxidized preferentially over saturated fatty acids (SFA) [31, 32, 37, 39, 40]. Also in vitro, eicosapentaenoic acid (EPA) increases fatty acid oxidation in myotubules [41, 42].

Fats are also an integral component of cell membranes, and as with other membranes, diet and muscle phospholipid composition have been associated in human and animal studies [31, 37, 43–50]. There is also a significant relationship between the composition of circulating plasma and RBC lipids and that of muscle [51]. The different types of dietary fatty acids could affect membrane fluidity and rigidity and potentially the positioning of proteins and lipid messengers and cellular signaling within the membrane. They could also affect levels of muscle ceramide, diacylglycerol, triacylglycerol, and acylcarnitines [37, 52, 53]. The total amount of fat in the diet may also influence muscle mass through a number of mechanisms including decreased hepatic and skeletal muscle oxidative capacity and, in energy balance situations, by increasing the flux of fatty acids through skeletal muscle for oxidation [54–58].

Inflammation is one established mechanism for muscle loss where increased circulation of inflammatory cytokines such as TNF-α has been associated with loss of muscle mass in elderly people [59]. The fat composition of the diet also has the capacity to influence inflammation through a number of direct (as precursors of eicosanoids) or indirect mechanisms with trans-fatty acids (TFA) and saturated fatty acids (SFA) being considered pro-inflammatory and the polyunsaturated fatty acids (PUFA), particularly the $n-3$ and $n-6$ fatty acids, being considered anti-inflammatory [41, 60–72]. Specific actions of $n-3$ PUFA have been identified either during acute muscle loss (cachexia) or in normal older subjects, suggesting $n-3$ PUFA may attenuate protein degradation and positively influence protein synthesis [73–78].

Insulin resistance may play a role in age-related muscle loss since insulin is key in the regulation of skeletal muscle synthesis and degradation and insulin resistance is also associated with a number of catabolic diseases including cachexia (acute muscle loss) [79, 80].

The remainder of this chapter firstly explores population intakes of dietary fat and describes intakes of fatty acids within the UK and secondly reports on a study investigating the associations between dietary fat composition and fat-free mass in a population of UK women.

Population Intakes of Fat and Cardiovascular Guidelines

Dietary fat and fat composition are established risk factors for cardiovascular disease (CVD) risk. The dietary guidelines recommend reducing total, saturated, and trans fat and replacing saturated fat with polyunsaturated fats [81]. Dietary recommendations within the UK suggest that dietary fat should be below 35 % of energy intake, saturated fat below 11 %, and trans fat 2 % [82]. However, a recent UK wide government survey indicates that while total-fat and trans-fat intake met the recommendations, saturated fat intake did not. Mean intakes for fat were 32.9 % of food energy, were 12.8 % for SFA, and were 0.7–0.9 % for TFA [82]. These figures are largely in agreement with, although a little lower than, a previous government survey in a low-income population with intakes in women aged 50–64 years being fat as percent energy of 35 %, SFA 13.6 %, and TFA 1.2 % [82, 83].

The profile of dietary fat composition is dependent on the fat composition of the types of foods consumed. The contribution of different food types to fat intake in the recent government survey indicates that three food groups contribute to 59 % of fat intake in women (meat and meat products, cereals and products, and milk and milk products), and these same three groups also contribute the most to total saturated fat intake (Figs. 6.4 and 6.5) [82]. However, for PUFA intake, vegetables and vegetable products replace milk and milk products as one of the top three contributors (Fig. 6.6).

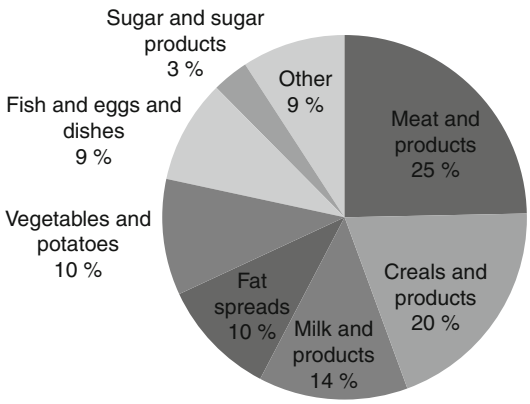

Fig. 6.4 Percentage contribution of foods to total fat intake in UK women aged 19–64 years (Based on data from Ref. [82])

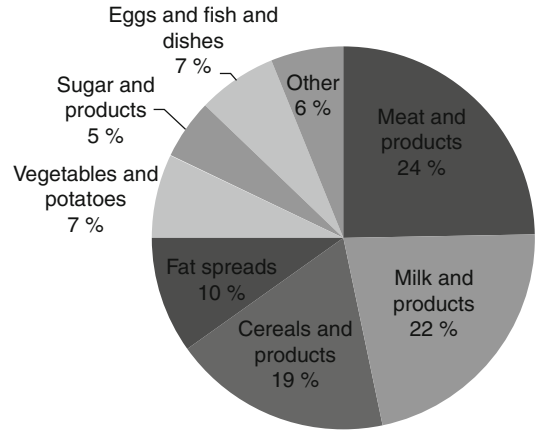

Fig. 6.5 Percentage contribution of foods to total saturated fat intake in UK women aged 19–64 years (Based on data from Ref. [82])

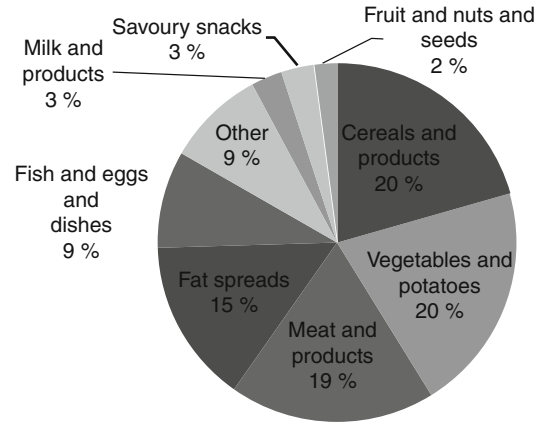

Fig. 6.6 Percentage contribution of foods to polyunsaturated fat intake in UK women aged 19–64 years (Based on data from Ref. [83])

A Population Study of Dietary Fat and Muscle Mass

Since loss of muscle mass is one aspect of the development of sarcopenia, we investigated the association of muscle mass with dietary fat intake. The purpose of this study was to investigate the

fat composition of the diet and indexes of muscle mass in a cohort of healthy free-living women to determine whether the fat composition of the diet could influence total muscle mass. We hypothesized, on the basis of available evidence, that muscle mass would be higher in those who consumed diets higher in PUFA and lower with greater consumption of SFA and TFA. We also investigated the P:S ratio.

Methods and Results

We studied the associations between body compositions (fat-free mass (FFM) and fat-free mass index (FFMI, weight/height^2)) measured using dual-energy X-ray absorptiometry in 2,689 women aged 18–79 years from the Twins UK Study [84]. We calculated the indexes of skeletal muscle mass according to quintile of dietary fat composition (from a Food Frequency Questionnaire) after adjustment for covariates. The Twins UK registry is a study of healthy, mainly female, twins who have undergone extensive clinical assessments for a range of age-related characteristics. A decision was made a priori to analyze the data as a singleton population and this group and has been shown to be representative of adult singleton populations in the UK for both diet and other characteristics [85, 86]. In this study, only those with complete data for body composition, validated health and lifestyle questionnaires, and clinical assessments between 1996 and 2000 were included. Ethical approval was obtained from St Thomas's Hospital Research Ethics Committee with informed consent from all participants.

Dietary intake was calculated using a validated semiquantitative Food Frequency Questionnaire (FFQ) [87, 88]. Fatty acids were derived from a database based on published analytic data for fatty acids with a small amount of additional data from other European national food composition databases [87, 88]. Total fat intake and fatty acids were calculated as a percentage of total energy intake and used in the analyses.

In multivariate statistical analysis, using Stata software, the models were adjusted for age, physical activity, energy intake, energy intake to predicted energy expenditure (to account for potential misreporting), smoking habit and fat mass, and, in a further model, also for percentage protein intake. The robust regression cluster option was used in Stata to control for potential familial aggregation. This method is designed to account for potential bias within the data and to adjust estimates accordingly.

The mean age of the women was 48 years (with 50 % over the age of 50 years), BMI was 24.9 kg/m^2, mean fat-free mass (FFM) was 31.6 kg, and fat-free mass index (FFMI) was 15.0 kg/m^2. The population were predominately physically active (78 %), and 18.4 % were current smokers. Fat intake as a percentage of energy was 31.4 % with SFA comprising the greatest proportion of energy (11.7 %), followed by MUFA (10.3 %), PUFA (6.4 %), TFA (1.1 %), and total $n-3$ PUFA (0.6 %).

FFM and FFMI were significantly and positively associated with the P:S ratio and inversely associated with total fat, SFA, MUFA, and TFA as a percentage of energy. Differences between extreme quintiles of fat intake were 0.6 kg ($P=0.004$) for FFM, 0.28 kg/m^2 ($P<0.001$) for FFMI for fat as a percentage of energy, and 0.7–0.8 kg for FFM (P all <0.01) and 0.26–0.38 kg/m^2 (P all ≤ 0.001) for FFMI, for SFA, MUFA, and TFA intakes. Differences for the P:S ratio were 0.6 kg ($P=0.003$) for FFM and 0.25 kg/m^2 ($P=0.012$) for FFMI.

To further understand the scale of these associations with muscle mass and fat intake, we compared them with that of 10 years of age (an estimate of the population decline in muscle mass over a 10 year period, a decade). We calculated the association with diet to 10 years of age for FFM and FFMI, as a percentage. From this calculation, the association for total fat and FFM was 95 % of the association with 10 years of age and for FFMI was 150 % of the association. For SFA, the corresponding figures were 90 % for FFM and 140 % for FFMI and for the P:S ratio were 72 and 115 %, respectively.

Discussion

We believe this to be the first large-scale cross-sectional study to investigate the associations between detailed dietary fat composition and measures of muscle mass using a precise method

of assessing body composition, DXA. Significant associations between dietary fat composition and FFM and FFMI in women aged 18–79 years were found. A higher P:S ratio was associated with higher FFM and FFMI suggestive of muscle conservation, and conversely, total fat % energy, SFA, MUFA, and TFA were lower suggesting muscle loss. The differences between extreme quintiles of intake were significant after adjustment for the known influences on muscle mass of physical activity and smoking behaviors and also after adjustment for energy intake, potential misreporting, and protein intake. Though these differences between extreme quintiles of fat appear relatively small in relation to the associations per decade, they ranged from 72 % for the P:S ratio and FFM to 95 % for total percentage fat and from 115 % for the P:S ratio to 150 % for total percentage fat for FFMI.

Compared with UK population studies, intakes in the female population in our study were similar. However, fat % energy was lower 31.4 % versus 32.9 %, and SFA intake was also 1.1 % lower in our study and was within the suggested guidelines for CVD of 35 % for total fat and 12 % for SFA, respectively. TFA intake in our study was 1.1 % and higher than in the recent UK government survey, but this likely reflects time trends, since food manufacturers have been gradually reducing the TFA content of foods. The TFA intakes in our study reflect intakes prior to the reductions that have recently occurred.

The strengths of this study include the large sample size, the wide age range of our participants, and the objective assessment of body composition by DXA. The population are also representative of singleton populations for both diet and other characteristics [85]. Apart from one smaller population study that only investigated saturated fat and muscle mass, we are unaware of other studies with detailed fatty acid intakes and muscle mass [24]. The limitations of our study are that as with any cross-sectional study design, no causal associations can be made and we cannot exclude the possibility of residual confounding, despite adjusting for confounders known to be associated with muscle mass. We used a validated FFQ, and although there are limitations with fatty acid databases, due to the temporal changes to the composition of the oils and fats used in food manufacture, our database was derived around the time the FFQs were completed by the study subjects [89].

While further studies are required to confirm these cross-sectional findings, dietary modifications to UK total fat intake that would achieve the current UK CVD dietary guidelines would include reducing consumption of high-fat-containing foods that contribute the most to fat intake, e.g., full fat milk and products, cakes, pies, pastries and biscuits, and meat and meat products, and potentially replacing animal products (milk- and meat-based products) with vegetable-based sources; see Figs. 6.4 and 6.5 [90]. These modifications would also reduce intakes of saturated fats and may also be beneficial for prevention of muscle loss.

Conclusions

In conclusion, we found associations between total percentage dietary fat and individual fatty acid profile and indexes of skeletal muscle mass in women aged 18–79 years. Although the scales of the associations were relatively small, they were significant after accounting for the known influences on muscle mass of age, physical activity, and smoking habit and also for total energy, for protein intake, and for potential dietary misreporting.

The novel findings from this cross-sectional study are suggestive that a fat profile that is already associated with CVD protection may also be beneficial for conservation of skeletal muscle mass. Our data indicate that fat intake is likely to be relevant for muscle mass at all ages. These findings deserve further investigation in prospective studies and intervention trials.

References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412–23.

2. Sayer AA. Sarcopenia. BMJ. 2010;341:c4097 [Editorial].
3. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc. 2004;52(1):80–5 [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].
4. Beenakker KG, Ling CH, Meskers CG, de Craen AJ, Stijnen T, Westendorp RG, et al. Patterns of muscle strength loss with age in the general population and patients with a chronic inflammatory state. Ageing Res Rev. 2010;9(4):431–6.
5. Rolland Y, Czerwinski S, Abellan Van Kan G, Morley JE, Cesari M, Onder G, et al. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. J Nutr Health Aging. 2008;12(7):433–50.
6. Rolland Y, Lauwers-Cances V, Cristini C, Abellan van Kan G, Janssen I, Morley JE, et al. Difficulties with physical function associated with obesity, sarcopenia, and sarcopenic-obesity in community-dwelling elderly women: the EPIDOS (EPIDemiologie de l'OSteoporose) Study. Am J Clin Nutr. 2009;89(6):1895–900.
7. Berger MJ, Doherty TJ. Sarcopenia: prevalence, mechanisms, and functional consequences. Interdiscip Top Gerontol. 2010;37:94–114.
8. Bogl LH, Latvala A, Kaprio J, Sovijarvi O, Rissanen A, Pietilainen KH. An investigation into the relationship between soft tissue body composition and bone mineral density in a young adult twin sample. J Bone Miner Res. 2011;26(1):79–87.
9. MacInnis RJ, Cassar C, Nowson CA, Paton LM, Flicker L, Hopper JL, et al. Determinants of bone density in 30- to 65-year-old women: a co-twin study. J Bone Miner Res. 2003;18(9):1650–6.
10. Salamone LM, Glynn N, Black D, Epstein RS, Palermo L, Meilahn E, et al. Body composition and bone mineral density in premenopausal and early perimenopausal women. J Bone Miner Res. 1995;10(11):1762–8.
11. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. J Bone Miner Res. 1997;12(12):2076–81.
12. Wannamethee SG, Shaper AG, Lennon L, Whincup PH. Decreased muscle mass and increased central adiposity are independently related to mortality in older men. Am J Clin Nutr. 2007;86(5):1339–46.
13. Harvey N, Dennison E, Cooper C. Osteoporosis: impact on health and economics. Nat Rev Rheumatol. 2010;6(2):99–105.
14. Foundation IO. Facts and statistics about osteoporosis and its impact. 2010 [cited 2010 14/01/11]. Available from: http://www.iofbonehealth.org/facts-and-statistics.html. Last Accessed on 2 Oct 2012.
15. Statistics OfN. An executive summary, 2010-based National Population Predictions (NPP) reference volume. 2012 [cited 2012 1/8/2012]. Available from: http://www.ons.gov.uk/ons/dcp171776_253890.pdf. Last Accessed on 2 Oct 2012.
16. Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ. Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev. 1999;107(2):123–36 [Research Support, U.S. Gov't, P.H.S.].
17. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147(8):755–63.
18. Samson MM, Meeuwsen IB, Crowe A, Dessens JA, Duursma SA, Verhaar HJ. Relationships between physical performance measures, age, height and body weight in healthy adults. Age Ageing. 2000;29(3):235–42.
19. Reid KF, Naumova EN, Carabello RJ, Phillips EM, Fielding RA. Lower extremity muscle mass predicts functional performance in mobility-limited elders. J Nutr Health Aging. 2008;12(7):493–8 [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.].
20. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: a review of the evidence and implications for preventive strategies. J Aging Res. 2012;2012:510801.
21. Volkert D. The role of nutrition in the prevention of sarcopenia. Wien Med Wochenschr. 2011;161(17–18):409–15 [Review].
22. Jorgensen SB, Richter EA, Wojtaszewski JF. Role of AMPK in skeletal muscle metabolic regulation and adaptation in relation to exercise. J Physiol. 2006;574(Pt 1):17–31.
23. Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NE, et al. Nutritional recommendations for the management of sarcopenia. J Am Med Dir Assoc. 2010;11(6):391–6.
24. Scott D, Blizzard L, Fell J, Giles G, Jones G. Associations between dietary nutrient intake and muscle mass and strength in community-dwelling older adults: the Tasmanian Older Adult Cohort Study. J Am Geriatr Soc. 2010;58(11):2129–34.
25. Wackerhage H, Rennie MJ. How nutrition and exercise maintain the human musculoskeletal mass. J Anat. 2006;208(4):451–8.
26. Evans WJ. Protein nutrition, exercise and aging. J Am Coll Nutr. 2004;23(6 Suppl):601S–9.
27. Morais JA, Chevalier S, Gougeon R. Protein turnover and requirements in the healthy and frail elderly. J Nutr Health Aging. 2006;10(4):272–83.
28. Paddon-Jones D, Short KR, Campbell WW, Volpi E, Wolfe RR. Role of dietary protein in the sarcopenia of aging. Am J Clin Nutr. 2008;87(5):1562S–6.
29. Meng X, Zhu K, Devine A, Kerr DA, Binns CW, Prince RL. A 5-year cohort study of the effects of high protein intake on lean mass and BMC in elderly postmenopausal women. J Bone Miner Res. 2009;24(11):1827–34.
30. Holloway GP, Bonen A, Spriet LL. Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals. Am J Clin Nutr. 2009;89(1):455S–62.
31. Kien CL. Dietary interventions for metabolic syndrome: role of modifying dietary fats. Curr Diab Rep. 2009;9(1):43–50.

32. Kien CL, Bunn JY, Ugrasbul F. Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. Am J Clin Nutr. 2005;82(2):320–6.
33. Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev. 2006;86(1):205–43.
34. Holloway GP, Luiken JJ, Glatz JF, Spriet LL, Bonen A. Contribution of FAT/CD36 to the regulation of skeletal muscle fatty acid oxidation: an overview. Acta Physiol (Oxf). 2008;194(4):293–309.
35. Frayn KN. Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. Acta Physiol (Oxf). 2010;199(4):509–18.
36. Frayn KN. Non-esterified fatty acid metabolism and postprandial lipaemia. Atherosclerosis. 1998;141 Suppl 1:S41–6.
37. Corcoran MP, Lamon-Fava S, Fielding RA. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. Am J Clin Nutr. 2007;85(3):662–77.
38. Dyck DJ. Adipokines as regulators of muscle metabolism and insulin sensitivity. Appl Physiol Nutr Metab. 2009;34(3):396–402.
39. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr. 2000;72(4):905–11.
40. Jones PJ, Schoeller DA. Polyunsaturated:saturated ratio of diet fat influences energy substrate utilization in the human. Metabolism. 1988;37(2):145–51.
41. Kalupahana NS, Claycombe K, Moustaid-Moussa N. (n-3) fatty acids alleviate adipose tissue inflammation and insulin resistance: mechanistic insights. Adv Nutr. 2011;2:304–16.
42. Wensaas AJ, Rustan AC, Just M, Berge RK, Drevon CA, Gaster M. Fatty acid incubation of myotubes from humans with type 2 diabetes leads to enhanced release of beta-oxidation products because of impaired fatty acid oxidation: effects of tetradecylthioacetic acid and eicosapentaenoic acid. Diabetes. 2009;58(3):527–35.
43. Andersson A, Nalsen C, Tengblad S, Vessby B. Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. Am J Clin Nutr. 2002;76(6):1222–9.
44. Stark KD, Lim SY, Salem N. Docosahexaenoic acid and n-6 docosapentaenoic acid supplementation alter rat skeletal muscle fatty acid composition. Lipids Health Dis. 2007;6:13.
45. Turner N, Lee JS, Bruce CR, Mitchell TW, Else PL, Hulbert AJ, et al. Greater effect of diet than exercise training on the fatty acid profile of rat skeletal muscle. J Appl Physiol. 2004;96(3):974–80.
46. Andersson A, Sjodin A, Hedman A, Olsson R, Vessby B. Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men. Am J Physiol Endocrinol Metab. 2000;279(4):E744–51.
47. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med. 1993; 328(4):238–44.
48. Soriguer FJ, Tinahones FJ, Monzon A, Pareja A, Rojo-Martinez G, Moreno F, et al. Varying incorporation of fatty acids into phospholipids from muscle, adipose and pancreatic exocrine tissues and thymocytes in adult rats fed with diets rich in different fatty acids. Eur J Epidemiol. 2000;16(6):585–94.
49. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. 2008;47(5):348–80.
50. Damsgaard CT, Frokiaer H, Andersen AD, Lauritzen L. Fish oil in combination with high or low intakes of linoleic acid lowers plasma triacylglycerols but does not affect other cardiovascular risk markers in healthy men. J Nutr. 2008;138(6):1061–6.
51. Baur LA, O'Connor J, Pan DA, Wu BJ, O'Connor MJ, Storlien LH. Relationships between the fatty acid composition of muscle and erythrocyte membrane phospholipid in young children and the effect of type of infant feeding. Lipids. 2000;35(1):77–82.
52. Blachnio-Zabielska A, Baranowski M, Zabielski P, Gorski J. Effect of high fat diet enriched with unsaturated and diet rich in saturated fatty acids on sphingolipid metabolism in rat skeletal muscle. J Cell Physiol. 2010;225(3):786–91.
53. Kien CL, Everingham KI, D Stevens R, Fukagawa NK, Muoio DM. Short-term effects of dietary fatty acids on muscle lipid composition and serum acylcarnitine profile in human subjects. Obesity (Silver Spring). 2011;19(2):305–11.
54. Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci USA. 2008;105(22):7815–20.
55. Lossa S, Lionetti L, Mollica MP, Crescenzo R, Botta M, Liverini G. Effect of high-fat feeding on metabolic efficiency and mitochondrial oxidative capacity in adult rats. Br J Nutr. 2003;90:953–60.
56. Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, et al. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes. 2005;54(7):1926–33.
57. Chanseaume E, Malpuech-Brugere C, Patrac V, Bielicki G, Rousset P, Couturier K, et al. Diets high in sugar, fat, and energy induce muscle type-specific adaptations in mitochondrial functions in rats. J Nutr. 2006;136(8):2194–200.
58. Newsom SA, Schenk S, Li M, Everett AC, Horowitz JF. High fatty acid availability after exercise alters the regulation of muscle lipid metabolism. Metabolism. 2011;60(6):852–9.
59. Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci. 2009;64(11):1183–9.
60. Kalogeropoulos N, Panagiotakos DB, Pitsavos C, Chrysohoou C, Rousinou G, Toutouza M, et al.

Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. Clin Chim Acta. 2010;411(7–8):584–91.
61. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. Arterioscler Thromb Vasc Biol. 2006;26(5):995–1001.
62. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr. 2009;101 Suppl 1:S1–45.
63. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, et al. A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. Am J Clin Nutr. 2009;90(6):1656–64.
64. Granados N, Amengual J, Ribot J, Palou A, Bonet ML. Distinct effects of oleic acid and its trans-isomer elaidic acid on the expression of myokines and adipokines in cell models. Br J Nutr. 2011;105(8):1226–34.
65. Dai J, Ziegler TR, Bostick RM, Manatunga AK, Jones DP, Goldberg J, et al. High habitual dietary alpha-linolenic acid intake is associated with decreased plasma soluble interleukin-6 receptor concentrations in male twins. Am J Clin Nutr. 2010;92(1):177–85.
66. Zhang L, Geng Y, Yin M, Mao L, Zhang S, Pan J. Low omega-6/omega-3 polyunsaturated fatty acid ratios reduce hepatic C-reactive protein expression in apolipoprotein E-null mice. Nutrition. 2010;26(7–8):829–34.
67. Poudel-Tandukar K, Nanri A, Matsushita Y, Sasaki S, Ohta M, Sato M, et al. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. Nutr Res. 2009;29(6):363–70.
68. Pot GK, Geelen A, Majsak-Newman G, Harvey LJ, Nagengast FM, Witteman BJ, et al. Increased consumption of fatty and lean fish reduces serum C-reactive protein concentrations but not inflammation markers in feces and in colonic biopsies. J Nutr. 2009;140(2):371–6.
69. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. Ann Nutr Metab. 2009;55(1–3):123–39.
70. Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: the Heart and Soul Study. Atherosclerosis. 2009;205(2):538–43.
71. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary alpha-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr. 2007;85(2):385–91.
72. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. Atherosclerosis. 2003;167(2):237–42.
73. Kumar NB, Kazi A, Smith T, Crocker T, Yu D, Reich RR, et al. Cancer cachexia: traditional therapies and novel molecular mechanism-based approaches to treatment. Curr Treat Options Oncol. 2010;11(3–4):107–17.
74. Magee P, Pearson S, Allen J. The omega-3 fatty acid, eicosapentaenoic acid (EPA), prevents the damaging effects of tumour necrosis factor (TNF)-alpha during murine skeletal muscle cell differentiation. Lipids Health Dis. 2008;7:24.
75. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. Clin Sci (Lond). 2010;121(6):267–78.
76. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr. 2010;93(2):402–12.
77. Khal J, Tisdale MJ. Downregulation of muscle protein degradation in sepsis by eicosapentaenoic acid (EPA). Biochem Biophys Res Commun. 2008;375(2):238–40.
78. Fiaccavento R, Carotenuto F, Vecchini A, Binaglia L, Forte G, Capucci E, et al. An omega-3 fatty acid-enriched diet prevents skeletal muscle lesions in a hamster model of dystrophy. Am J Pathol. 2010;177(5):2176–84.
79. Honors MA, Kinzig KP. The role of insulin resistance in the development of muscle wasting during cancer cachexia. J Cachexia Sarcopenia Muscle. 2012;3(1):5–11.
80. Waterlow JC. Protein turnover. Wallingford/Cambridge: CABI Publishing; 2006.
81. Great Britain. Cardiovascular Review G, Great Britain. Committee on Medical Aspects of Food P. Nutritional aspects of cardiovascular disease: report of the Cardiovascular Review Group, Committee on Medical Aspects of Food Policy. London: H.M.S.O.; 1994.
82. Bates B, Lennox A, Bates C, Swan G. National diet and nutrition survey: headline results from years 1 and 2 (combined) of the rolling programme (2008/2009-2009/10). London: Department of Health; 2011.
83. Nelson M, Great Britain. Food Standards A, National Centre for Social R, King's College. Nutritional Science Research D, University College LMS. Low income diet and nutrition survey. Norwich: TSO; 2007.
84. Welch AA, MacGregor AJ, Skinner J, Spector TD, Cassidy A. Dietary total fat and fatty acid profile are associated with indexes of skeletal muscle mass in women. Am J Clin Nutr. Submitted.
85. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin Res. 2001;4(6):464–77.
86. Teucher B, Skinner J, Skidmore PM, Cassidy A, Fairweather-Tait SJ, Hooper L, et al. Dietary patterns and heritability of food choice in a UK female twin cohort. Twin Res Hum Genet. 2007;10(5):734–48.

87. Welch AA, Luben R, Khaw KT, Bingham SA. The CAFE computer program for nutritional analysis of the EPIC-Norfolk food frequency questionnaire and identification of extreme nutrient values. J Hum Nutr Diet. 2005;18(2):99–116.
88. Agency FS. McCance and Widdowson's the composition of foods sixth summary edition. Cambridge: Royal Society of Chemistry; 2002.
89. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. Am J Clin Nutr. 2010;92(5):1214–22.
90. United States. Department of Health and Human Services., United States. Department of Agriculture, United States. Dietary Guidelines Advisory Committee. Dietary guidelines for Americans, 2010. 7th ed. Washington, D.C.: G.P.O.; 2010.

Relationships Between Body Fat and Bone Mass

7

Ian R. Reid

Abstract

Body weight impacts on both bone turnover and bone density and is therefore an important risk factor for vertebral and hip fractures, ranking in importance alongside that of age. The effect of body weight is probably contributed to by both fat mass and lean mass, though in postmenopausal women, fat mass has been more consistently demonstrated to be important. A number of mechanisms for the fat-bone relationship exist and include the effect of soft tissue mass on skeletal loading and the association of fat mass with the secretion of bone-active hormones from the pancreatic beta cell (including insulin, amylin, and preptin). Insulin circulates in increased concentrations in obesity and exerts anabolic effects on bone. The adipocyte is also an important source of factors that act as circulating regulators of bone metabolism. These include estrogens and the adipokines, leptin, and adiponectin. Leptin acts directly on bone cells, and in some experimental models, these effects are modified by its actions on the central nervous system, which impact on appetite, body weight, and insulin sensitivity. Adipokine levels correlate with bone turnover, suggesting that they dynamically influence bone metabolism. In postmenopausal women they may be among the principal regulators of bone turnover, accounting for their increasing importance as determinants of bone density with age. Of the adipokines, adiponectin appears to have the strongest relationships with bone parameters in postmenopausal women.

This area of research has provided important insights in bone biology. Its greatest importance, however, is to emphasize the critical role that weight maintenance plays in osteoporosis prevention.

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Introduction

Osteoporosis has long been characterized as a problem afflicting small, thin, elderly women. The advent of axial bone densitometry in the 1980s allowed the relationship between body weight and bone density to be quantified, and the expected positive relationship was found. This has now resulted in a substantial literature in which a wide variety of measures of skeletal health have been assessed in relation to a similar diversity of soft tissue assessments. This explosion of the clinical literature has been mirrored in a large number of laboratory studies seeking to understand the pathways which link soft tissue mass to skeletal health. As the diversity of investigations has increased, this has sometimes obscured, rather than clarified, the key observation, which is that thin people have low bone density and more fractures.

Soft Tissue Mass Is Positively Related to Bone Density

Many investigators have consistently shown a positive relationship between bone density throughout the skeleton and either body weight or body mass index (BMI) (Fig. 7.1) [2–5]. With advances in dual-energy X-ray absorptiometry (DXA), it has become straightforward to assess fat mass and lean mass separately, and typical results for such analyses in postmenopausal women are shown in Fig. 7.2. As is demonstrated here, both fat mass and lean mass are positively related to bone density. These cross-sectional relationships are mirrored in the findings of longitudinal studies, in which changes in bone density over a decade in postmenopausal women are found to be impacted on by baseline fat mass and by changes in fat mass [6]. Thus, women with higher fat mass at baseline and who have gained in fat mass over time have slower rates of bone loss.

The relationship between fat mass and bone density tends to be most marked in postmenopausal women [7]. We have found it also to be detectable in premenopausal women [8] but to be further attenuated in premenopausal women who exercise regularly [9]. In men, the effect is less obvious, and once correction has been made for skeletal size (which impacts on DXA measurements of bone mineral density [BMD] and on lean mass), the effect of fat may be lost altogether [8]. These gender differences are not surprising since sex hormones have a profound impact on soft tissue composition as well as on skeletal mass. Thus, if fat mass positively impacts on bone mass in the absence of sex hormones, the introduction of testosterone will tend to reduce this association through its anabolic effects on bone, while its effects on fat mass are quite the opposite. Thus, any underlying relationship will be obscured. The introduction of estrogen will also have positive effects on bone mass without directly reducing fat mass, so a fat/bone relationship is still present in premenopausal women, though attenuated. The introduction of regular exercise is somewhat similar to the effects of androgen, in that it increases bone mass and leads to a reduction in fat mass. These biological considerations account for some of the diversity of findings in the literature.

This diversity is probably also contributed to by the use of different techniques for the assessment of bone and soft tissue. For instance, DXA measures areal bone density, which is inherently positively associated with skeletal size. Measurements of true volumetric BMD by quantitative CT scanning do not have this problem of colinearity. Different DXA softwares may have subtle differences in the separation of lean and fat masses, which may contribute to diversity of outcomes in clinical studies. Other techniques, such as bioimpedance analysis may separate fat and lean tissues quite differently from DXA, and thus result in the finding of different relationships. Some investigators have used cross-sectional measurements of fat area rather than

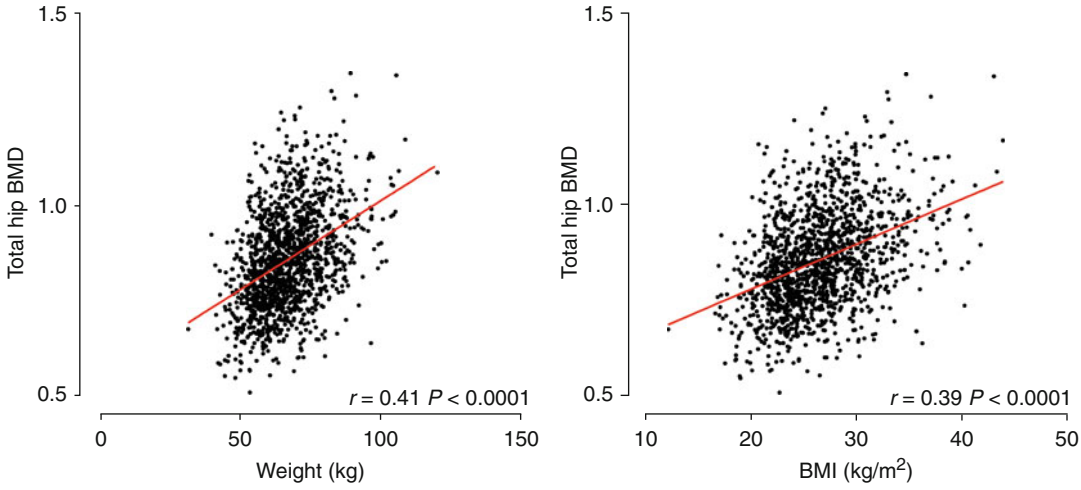

Fig. 7.1 Dependence of total hip bone mineral density (in g/cm^2) on weight and BMI in 1,462 normal postmenopausal women from the Auckland Calcium Study [1]. "*r*" is the Pearson correlation coefficient (Copyright I.R. Reid, used with permission)

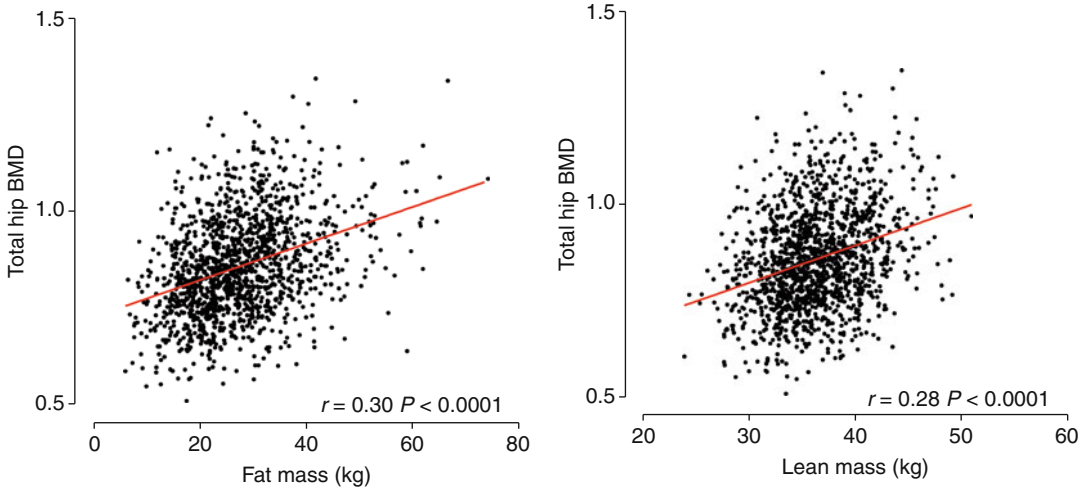

Fig. 7.2 Dependence of total hip bone mineral density (in g/cm^2) on fat mass and lean mass (all determined with dual-energy X-ray absorptiometry, in the same cohort as that in Fig. 7.1. "*r*" is the Pearson correlation coefficient (Copyright I.R. Reid, used with permission)

inferring the actual mass of adipose tissue from DXA scans, and again it would not be surprising if different relationships emerged from these analyses.

Soft Tissue Mass and Fractures

Numerous epidemiological studies have shown low body weight to be a risk factor for fractures. A number of these studies were meta-analyzed by de Laet, who demonstrated that each unit of increase in BMI diminished total fracture risk by 2–3 %, with similar effects being found in men and women [10]. For hip fractures, the dependence on BMI was more dramatic, with fracture risk declining 7 % for each unit increase in BMI. When these analyses were adjusted for individuals' BMD, the protective effect of BMI on total fracture risk was eliminated, implying that BMI prevented fractures by increasing BMD. However, for hip fractures, there was

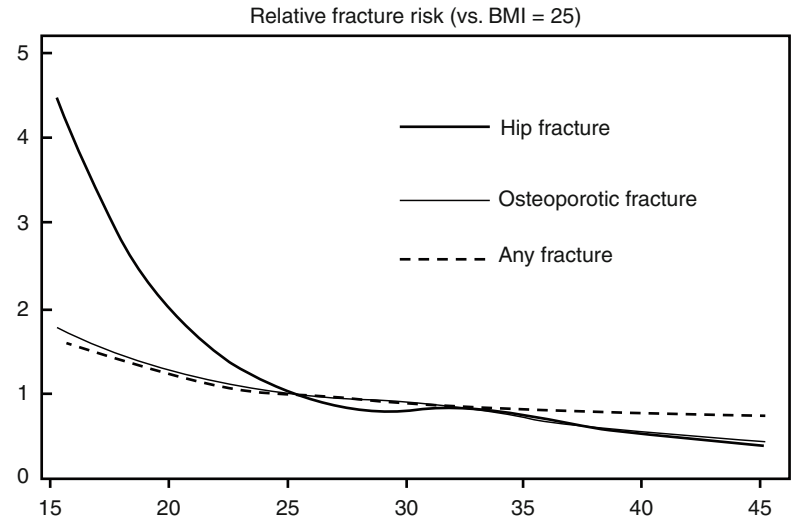

Fig. 7.3 Relative risk of fracture in relation to BMI (kg/m^2) for almost 60,000 men and women from 12 prospective population-based cohort studies. Data are adjusted for age and duration of follow-up (Reprinted from De Laet et al. [10]. With permission from Springer Science + Business Media)

still a residual, though attenuated, protective effect of BMI, implying a non-BMD-mediated mechanism. The direct shock-absorbing capacity of subcutaneous fat overlying the greater trochanter is likely to be at least part of this effect, though the independent effect of height as a risk factor for hip fracture may also be involved in the persistence of a BMI effect after adjustment for BMD. This marked dependence of hip fracture risk on body weight may account for the falling hip fracture rates observed in many Western countries over the last decade. A study of Chinese men has attempted to dissect out the independent effects of fat mass and lean mass on the risk of vertebral fracture and found a more marked protective effect from high fat mass compared with lean mass [11].

This body of data has led to the assumption that fractures are a low health priority in the overweight and elderly. However, with the steadily climbing incidence of obesity combined with similar trends in longevity, there are now substantial numbers of fractures occurring in individuals with high BMI. Thus, Compston et al. [12] have recently demonstrated, in a practice-based study, that a quarter of postmenopausal women with fractures were obese. Their analysis further showed that obesity may impact differently on various types of fractures. In particular, they observed that obesity is a risk factor for ankle fractures, implying that increased propensity to fall or altered fall mechanics in the obese may be contributing to this effect. These findings justify a closer look at the relationships between BMI and fracture risk in the de Laet, population-based analyses. While their findings have typically been interpreted as showing a linear relationship between fracture risk and BMI, in fact, the original publication demonstrates only a weak relationship between fracture risk and BMI in the overweight and obese, in contrast to a dramatic rise in fracture risk when the BMI is less than 25 (Fig. 7.3). Thus, the general conclusion that obesity is protective against fracture should be recast as a statement that "fracture risk rises steeply as BMI decreases below 25."

Methodological Considerations in Separating Fat and Lean Effects

The finding of a positive relationship of bone density with both fat mass and lean mass, together with the knowledge that these two entities are correlated with one another, has led to the use of multiple regression analysis to determine whether fat and lean masses have independent effects on bone density. The correlation between fat mass and lean mass is usually between 0.3 and 0.4, which should not violate the assumption regarding the independence of variables entered into a multiple regression analysis, so we have used this technique in a number of our previous studies. This has demonstrated that, in postmenopausal

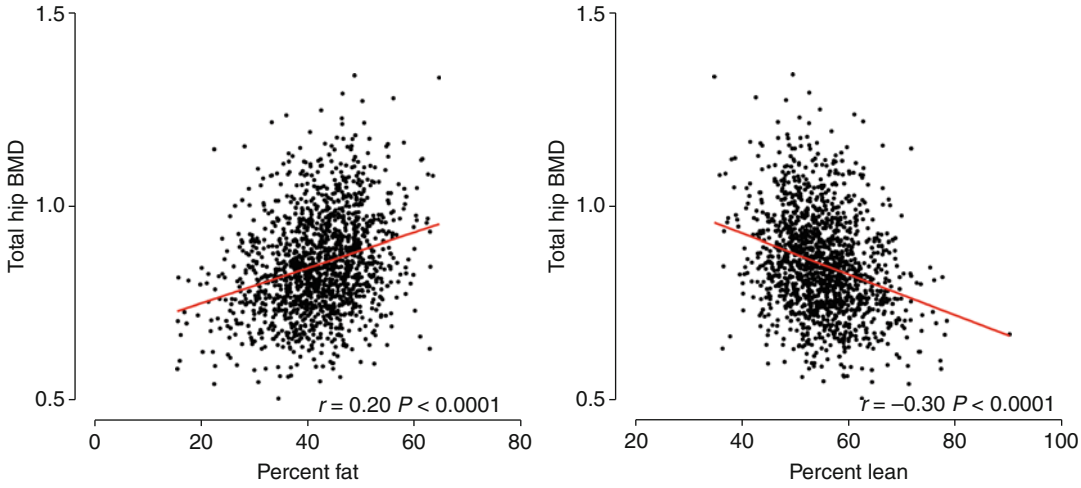

Fig. 7.4 Relationships between total hip bone mineral density (in g/cm^2) and fat mass and lean mass, each expressed as a percentage of body weight, in the cohort of postmenopausal women presented in Fig. 7.1 (Copyright I.R. Reid, used with permission)

women, the relationship between body weight and bone density is substantially driven by fat rather than lean mass, with attenuation of the fat effect in premenopausal women and, to an even greater degree, in men [7, 8]. However, some investigators have chosen to enter both fat mass and body weight or fat mass and BMI into the same multiple regression analysis. The correlations between fat mass and these other variables are greater than 0.9 indicating that there will be a substantial problem of colinearity between the supposedly "independent" variables [13]. This violates the assumptions that underpin multiple regression analysis and will lead to completely misleading findings. This can be demonstrated using the data presented in Figs. 7.1 and 7.2. If we pose the question as to how fat mass and lean mass impact on bone density, independent of weight, and enter all three of these variables into the multiple regression analysis, we find that total hip BMD is directly related to weight, but inversely related to both fat mass and lean mass [13]. Interpreted in a clinical context, this would indicate that we should be encouraging our patients to maximize their weight while minimizing their fat mass and lean mass. This is obviously impossible and the ridiculousness of this finding simply illustrates that if we perform analyses that ignore the mathematical rules on which multiple regression analysis is founded, nonsense will result. However, many investigators have committed this error and have wrongfully concluded that fat mass is inversely related to bone density, because they have adjusted their analyses for either body weight or BMI. All such analyses are flawed and lead to the generation of completely inappropriate advice to patients.

Another way of carrying out these analyses which does not depend on the complicated technique of multiple regression analysis is simply to express either fat or lean mass as a percentage of total body weight. When these analyses are carried out in the same cohort of postmenopausal women, we find that percent fat is *positively* related to hip bone density, whereas percent lean is inversely related to hip bone density (Fig. 7.4) [13]. This is giving essentially the same information as the multiple regression analysis but without the same mathematical assumptions and demonstrates the importance of adequate fat mass to optimal skeletal health in postmenopausal women.

Central Versus Peripheral Fat

In recent years it has become possible to assess both abdominal and visceral fat masses and to contrast their metabolic effects and associations

with either appendicular or subcutaneous fat. A number of investigators have demonstrated that both visceral and subcutaneous fat are positively correlated with bone density [14], though in some studies the relationship between bone mass and visceral fat was weaker, not reaching significance [15]. However, many investigators have adjusted these analyses for either subcutaneous fat mass or for total body fat. The correlation between subcutaneous fat and visceral fat is of the order of 0.7 [15], again raising the major problem of colinearity in these analyses, which invalidates the conclusions drawn. It is a major experimental challenge to dissociate the effects of subcutaneous and visceral fat on bone density, and at the present time, there are no data which satisfactorily do this. At present it can be stated that visceral fat is positively related to bone density, though in some studies more weakly so than the effects seen with total body fat. Yamaguchi has demonstrated that men without vertebral fractures have higher visceral adipose fat mass than those with fractures [14], suggesting that the relationships are similar to what has been observed for total fat mass.

Mechanisms of the Fat-Bone Connection

When the association between fat mass and bone mass was first observed, its mechanism was quite unclear since adipose tissue was widely regarded as an inert depot for energy storage. As a result, fat mass was thought to influence the skeleton simply by increasing skeletal load. While this is no doubt a contributor, the clinical studies reviewed above show similar correlations of weight-bearing and non-weight-bearing bones with indices of adiposity. Therefore, other explanations are required, and endocrine connections between adipose tissue and bone have received the most attention. It is now clear that the adipocyte directly secretes cytokines and hormones and indirectly affects the function of a number of other endocrine glands. Thus, we have moved from a dearth of explanations to a plethora, and the challenge at present is to discern which of the potential mechanisms are the most important.

Adipocyte Hormones

The adipocyte has long been recognized as a site of estrogen production from adrenal androgen precursors. This is probably most important in postmenopausal women and may be one of the reasons why fat and bone are most consistently associated in this group. The adipocyte is also a site of production of interleukin-6, which is known to be bone active, and it produces a number of putative hormones, the function of which is unclear (e.g., resistin). However, the two most investigated hormonal products of the adipocyte are leptin and adiponectin.

The *leptin*-bone literature is effectively divided into two. There are a number of studies which have assessed the action of leptin directly on bone. These studies have established that the leptin receptor is present on osteoblasts [16, 17], that leptin directly stimulates osteoblast proliferation and inhibits osteoclastogenesis [17–20], and that systemic administration of leptin to animals and humans increases bone mass [16, 17, 21–25]. Set against this is a suite of studies, many from the Karsenty laboratory, which have assessed the effects of leptin administered into the third ventricle of mice. Centrally administered leptin results in bone loss, and considerable effort has been invested into delineating the mechanisms that underpin this [26, 27]. However, it is often overlooked that central administration of leptin results in a profound loss in body weight as a result of diminished appetite, and this loss in body weight in turn results in reduced peripheral leptin levels and reduced circulating insulin levels, both of which decrease anabolic effects on bone [28–30]. Indeed, it has been shown that caloric restriction alone results in significant reductions in bone density [31, 32]. Thus, these two sets of experiments (central versus peripheral administration) are not necessarily contradictory. It should be remembered, however, that in normal human physiology, leptin is produced systemically in

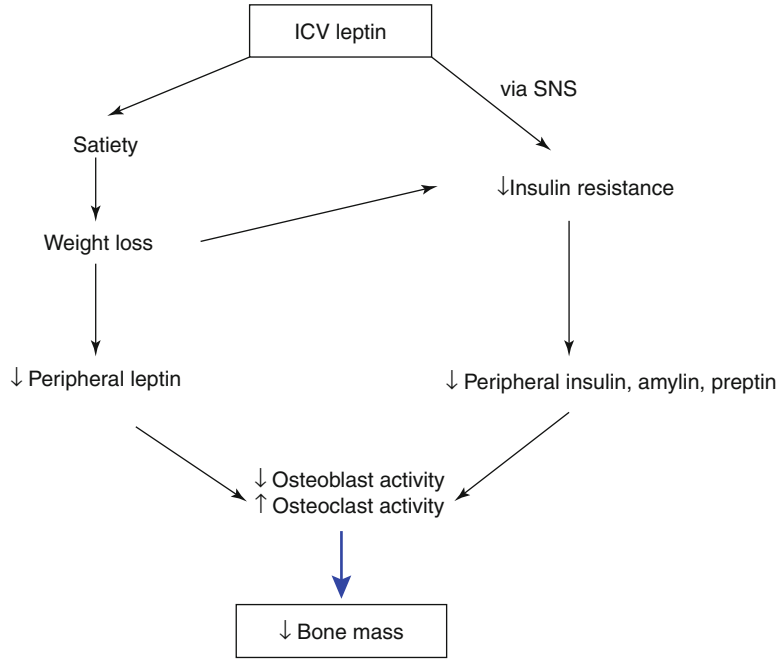

Fig. 7.5 Possible mechanisms for central leptin effects on bone, via reduced peripheral insulin and leptin levels. *SNS* sympathetic nervous system (Copyright I.R. Reid, used with permission)

adipocytes, including the adipocytes in bone marrow, so it will have direct access to bone cells. The fact that most studies of systemic administration result in *increases* in bone mass in both animals and humans indicates that the direct anabolic effects of leptin usually outweigh its negative indirect effects on bone mass, mediated through its central nervous system receptors. These relationships are set out in Fig. 7.5.

Adiponectin is a 28-kDa protein secreted from the adipocyte, whose circulating levels are inversely related to fat mass. Its actions on bone cells have been studied, producing conflicting results (reviewed in Williams et al. [33]). There is evidence that it inhibits osteoclastogenesis. However, it stimulates osteoblast differentiation; binds some growth factors, which might reduce bone formation; and is an insulin sensitizer, so it reduces circulating insulin levels. Further, adiponectin circulates in a number of different molecular forms, which have differing biological properties, so this might contribute to some of the diversity of results that has been found. However, it is now clear that in the adiponectin knockout mouse, bone mass is increased [33], suggesting that the net effect of this hormone is to reduce bone mass. This is consistent with clinical studies which show bone mass to be inversely related to circulating adiponectin levels [34, 35].

Pancreatic β-Cell Hormones

Obesity is associated with hyperinsulinemia, and insulin has been shown to directly stimulate proliferation of osteoblasts in vitro [36] and bone formation in vivo [37]. In vivo, insulin has other actions relevant to bone physiology, through reducing hepatic production of sex hormone-binding globulin (thus increasing free sex hormones) and through directly stimulating ovarian estrogen production in premenopausal women.

The pancreatic β-cell also produces amylin, a peptide related to calcitonin gene-related peptide, which has calcitonin-like effects on bone resorption [38], and also is anabolic to osteoblasts [39]. A further product of the pancreatic β-cell is preptin, a fragment of the IGF2 precursor, which itself is anabolic to osteoblasts [40]. Thus, the pancreatic β-cell produces a trio of bone-active peptides

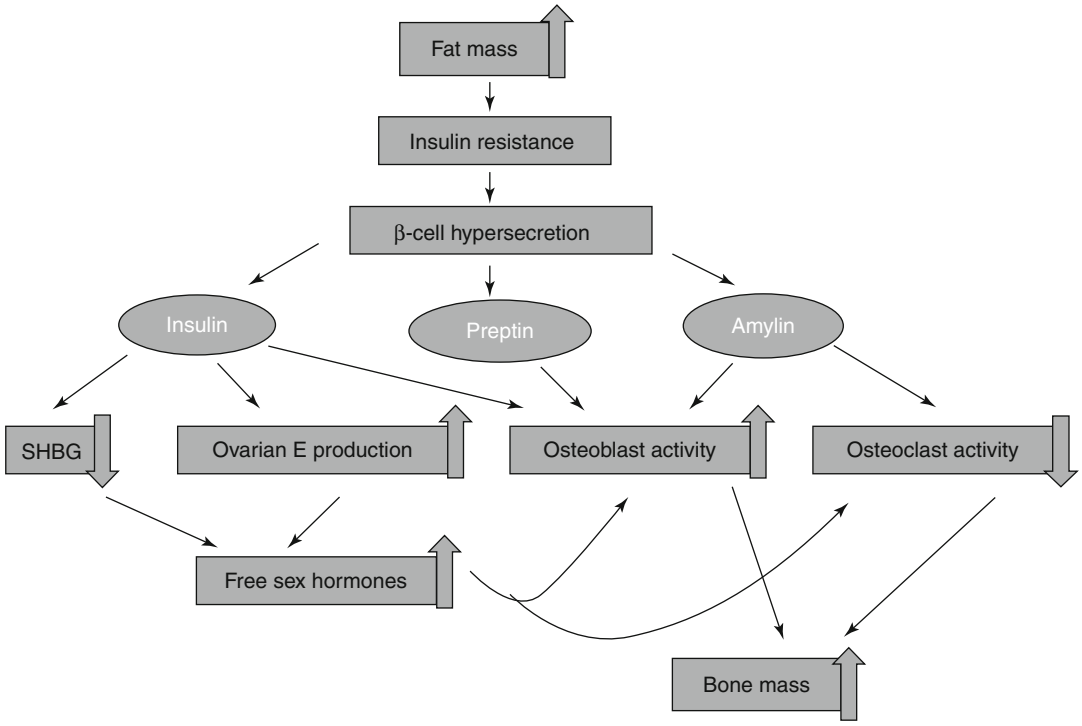

Fig. 7.6 Summary of the principal mechanisms by which the hyperinsulinemia associated with obesity contributes to increased bone mass (Copyright I.R. Reid, used with permission)

which collectively stimulate bone formation and reduce bone resorption, thus tending to increase bone mass (Fig. 7.6).

Integrated Relationships Between Soft Tissue, Adipokines, and Bone

In an attempt to determine which of these factors were the principal drivers of bone density in normal women, we have analyzed hormonal and bone density data from 453 premenopausal women and 215 postmenopausal women [41]. Leptin was positively related to bone density ($r \approx 0.75$) in both groups, weakly positively related to insulin ($r = 0.04$–0.22), and inversely related to adiponectin ($r = -0.13$ to -0.27). In the premenopausal women, multiple regression analyses at the different skeletal sites showed a consistent positive relationship to lean mass and to one or other of the fat-related variables – which one varied from site to site. Bone turnover in the premenopausal women was inversely related to the fat-related variables. In postmenopausal women, however, while the positive relationships with lean mass persisted, adiponectin was inversely related to bone density at all sites, a much more consistent effect than any of the other fat-related variables. Again, turnover was related to fat-related variables. Thus, turnover throughout adult life is influenced by fat mass, which might account for the growing influence of fat mass on BMD in older women. It is not clear why adiponectin is so much more consistently related to BMD in postmenopausal women than are other fat-related variables, since it is less closely linked to fat mass itself than is leptin. This suggests that adiponectin best reflects the metabolic influences that ultimately act to determine bone mass in postmenopausal women. It is clearly important to unravel the mechanisms that underpin this.

Feeding Effects on Bone

With the development of bone turnover markers, it has become clear that feeding results in an acute inhibition of bone resorption, observable

within an hour of meal ingestion [42]. Further, caloric restriction over a period of 5 days has been shown to impact not only on resorption but also on bone formation [43]. Feeding results in increased circulating levels of insulin, amylin, preptin, IGF1, and glucose-dependent insulinotropic polypeptide, all of which tend to promote bone formation. The suppression of bone resorption is probably mediated by increased secretion of calcitonin, amylin, and glucagon-like peptide-2, together with an inhibition of parathyroid hormone (reviewed in Reid [13]). Collectively, these effects cause feeding to have positive effects on bone mass.

Conclusions

As noted above, the relationships between fat and bone have become increasingly complex over the last two decades, but the key conclusions for clinicians are that thin people have an increased fracture risk as a result of low BMD and that both fat and lean masses appear to contribute to this effect. There is an important public health message, particularly targeted at young women, that having a low BMI is likely to increase future risk of osteoporotic fractures, though it is now becoming clear that the converse is not necessarily true – that is, obese individuals are not immune from frailty-related fractures in old age. Continued study of this area is important since it will increase our understanding of bone physiology and may throw up new possibilities for the development of anti-osteoporotic drugs.

Acknowledgement Supported by the Health Research Council of New Zealand

References

1. Reid IR, Mason B, Horne A, Ames R, Reid HE, Bava U, et al. Randomized controlled trial of calcium in healthy older women. Am J Med. 2006;119(9):777–85.
2. Ribot C, Tremollieres F, Pouilles JM, Bonneu M, Germain F, Louvet JP. Obesity and postmenopausal bone loss: the influence of obesity on vertebral density and bone turnover in postmenopausal women. Bone. 1987;8:327–31.
3. Khosla S, Atkinson EJ, Riggs BL, Melton LJ. Relationship between body composition and bone mass in women. J Bone Miner Res. 1996;11(6):857–63.
4. Ravn P, Cizza G, Bjarnason NH, Thompson D, Daley M, Wasnich RD, et al. Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. J Bone Miner Res. 1999;14(9):1622–7.
5. Reid IR. Relationships among body mass, its components, and bone. Bone. 2002;31(5):547–55.
6. Wu F, Ames R, Clearwater J, Evans MC, Gamble G, Reid IR. Prospective 10-year study of the determinants of bone density and bone loss in normal postmenopausal women, including the effect of hormone replacement therapy. Clin Endocrinol. 2002;56(6):703–11.
7. Reid IR, Ames R, Evans MC, Sharpe S, Gamble G, France JT, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women – a key role for fat mass. J Clin Endocrinol Metab. 1992;75(1):45–51.
8. Reid IR, Plank LD, Evans MC. Fat mass is an important determinant of whole body bone density in premenopausal women but not in men. J Clin Endocrinol Metab. 1992;75(3):779–82.
9. Reid IR, Legge M, Stapleton JP, Evans MC, Grey AB. Regular exercise dissociates fat mass and bone density in premenopausal women. J Clin Endocrinol Metab. 1995;80(6):1764–8.
10. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005;16(11):1330–8.
11. Lau EMC, Chan YH, Chan M, Woo J, Griffith J, Chan HHL, et al. Vertebral deformity in Chinese men: prevalence, risk factors, bone mineral density, and body composition measurements. Calcif Tissue Int. 2000;66(1):47–52.
12. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, et al. Obesity is not protective against fracture in postmenopausal women: GLOW. Am J Med. 2011;124(11):1043–50.
13. Reid IR. Fat and bone. Arch Biochem Biophys. 2010;503:20–7.
14. Yamaguchi T, Kanazawa I, Yamamoto M, Kurioka S, Yamauchi M, Yano S, et al. Associations between components of the metabolic syndrome versus bone mineral density and vertebral fractures in patients with type 2 diabetes. Bone. 2009;45(2):174–9.
15. Gilsanz V, Chalfant J, Mo AO, Lee DC, Dorey FJ, Mittelman SD. Reciprocal relations of subcutaneous and visceral fat to bone structure and strength. J Clin Endocrinol Metab. 2009;94(9):3387–93.
16. Steppan CM, Crawford DT, Chidsey-Frink KL, Ke HZ, Swick AG. Leptin is a potent stimulator of bone growth in ob/ob mice. Regul Pept. 2000;92(1–3 Special Issue SI):73–8.
17. Cornish J, Callon KE, Bava U, Lin C, Naot D, Hill BL, et al. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. J Endocrinol. 2002;175(2):405–15.

18. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology. 1999; 140(4):1630–8.
19. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem. 2002;85(4): 825–36.
20. Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, et al. Leptin inhibits osteoclast generation. J Bone Miner Res. 2002;17(2): 200–9.
21. Burguera B, Hofbauer LC, Thomas T, Gori F, Evans GL, Khosla S, et al. Leptin reduces ovariectomy-induced bone loss in rats. Endocrinology. 2001;142(8): 3546–53.
22. Hamrick MW, Della-Fera MA, Choi YH, Pennington C, Baile CA. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. J Bone Miner Res. 2005;20(6):994–1001.
23. Elefteriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, et al. Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci U S A. 2004;101(9): 3258–63.
24. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 1999;341(12): 879–84.
25. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, et al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004;351(10):987–97.
26. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell. 2000;100(2):197–207.
27. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang XL, Liu XY, et al. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature. 2005;434(7032):514–20.
28. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 1995;269:546–9.
29. Iwaniec UT, Boghossian S, Dube MG, Torto R, Arzaga RR, Wronski TJ, et al. Effects of central leptin gene therapy on weight reduction and cancellous bone mass in female rats. J Bone Miner Res. 2005;20 suppl 1:s13–4.
30. Otukonyong EE, Dube MG, Torto R, Kalra PS, Kalra SP. Central leptin differentially modulates ultradian secretory patterns of insulin, leptin and ghrelin independent of effects on food intake and body weight. Peptides. 2005;26(12):2559–66.
31. Brochmann EJ, Duarte ME, Zaidi HA, Murray SS. Effects of dietary restriction on total body, femoral, and vertebral bone in SENCAR, C57BL/6, and DBA/2 Mice. Metabolism. 2003;52:1265–73.
32. Hamrick MW, Ding KH, Ponnala S, Ferrari SL, Isales CM. Caloric restriction decreases cortical bone mass but spares trabecular bone in the mouse skeleton: implications for the regulation of bone mass by body weight. J Bone Miner Res. 2008;23(6):870–8.
33. Williams GA, Wang Y, Callon KE, Watson M, Lin JM, Lam JBB, et al. In vitro and in vivo effects of adiponectin on bone. Endocrinology. 2009;150(8):3603–10.
34. Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, Freedman BI, et al. Adiponectin as a novel determinant of bone mineral density and visceral fat. Bone. 2003;33(4):646–51.
35. Richards JB, Valdes AM, Burling K, Perks UC, Spector TD. Serum adiponectin and bone mineral density in women. J Clin Endocrinol Metab. 2007; 92(4):1517–23.
36. Hickman J, McElduff A. Insulin promotes growth of the cultured rat osteosarcoma cell line UMR-106-01: an osteoblast-like cell. Endocrinology. 1989;124: 701–76.
37. Cornish J, Callon KE, Reid IR. Insulin increases histomorphometric indices of bone formation in vivo. Calcif Tissue Int. 1996;59(6):492–5.
38. Cornish J, Callon KE, Bava U, Kamona SA, Cooper GJS, Reid IR. Effects of calcitonin, amylin and calcitonin gene-related peptide on osteoclast development. Bone. 2001;29:162–8.
39. Cornish J, Callon KE, Cooper GJS, Reid IR. Amylin stimulates osteoblast proliferation and increases mineralized bone volume in adult mice. Biochem Biophys Res Commun. 1995;207(1):133–9.
40. Cornish J, Callon KE, Bava U, Watson M, Xu X, Lin J, et al. Preptin, another peptide product of the pancreatic beta-cell, is osteogenic in vitro and in vivo. Am J Physiol. 2007;292:E117–22.
41. Reid IR, Richards JB. Adipokine effects on bone. Clin Rev Bone Miner Metab. 2009;7:240–8.
42. Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A, Eastell R. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. Bone. 2002;30(6):886–90.
43. Ihle R, Loucks AB. Dose–response relationships between energy availability and bone turnover in young exercising women. J Bone Miner Res. 2004;19(8):1231–40.

Acid–Base Homeostasis and Skeletal Health: Current Thinking and Future Perspectives

8

Helen Lambert, Claire Huggett,
Richard Gannon, and Susan A. Lanham-New

Abstract

We urgently need public health strategies to help with the prevention of poor bone health across the age ranges. It is especially useful to focus attention on factors that are amenable to change, with nutrition and exercise having clear potential. The aim of this chapter is to review the current evidence for a role of acid–base homeostasis in bone. Analysis of existing literature enabled a combination of observational, clinical, and intervention studies to be assessed in relation to dietary alkalinity, dietary acidity and bone health. Mechanisms of action for a dietary alkalinity "component" effect were examined, and the role that fruit and vegetables can play in bone health was addressed. Natural, pathological, and experimental states of acid loading/acidosis have been associated with hypercalciuria, and negative calcium balance and, more recently, the detrimental effects of "acid" from the diet on bone mineral have been demonstrated. At the cellular level, a reduction in extracellular pH has been shown to enhance osteoclastic activity directly, resulting in increased resorption pit formation.

A number of observational, experimental, clinical, and intervention studies have suggested a positive link between fruit and vegetable consumption and the skeleton. Further research is required, particularly with respect to the influence of dietary manipulation using alkali-forming foods on fracture prevention. There remain no long-term Dietary Approaches to Stop Hypertension (DASH) on bone health in younger and older age

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cohorts, and this is urgently required. Should the findings of the DASH/fruit and vegetable studies prove conclusive, a "fruit and vegetable" approach to bone health maintenance may provide a very helpful strategy for bone health development and maintenance throughout the life cycle.

Keywords
Acid–base balance • Dietary acidity • Fruit and vegetables • Bone health • Muscle function • Vegetarianism • Protein • Calcium • Bone metabolism • Vegans

Introduction

General

Public health strategies are urgently required to target, on a population-wide basis, the prevention of poor bone health throughout the life cycle, given the certainty of prediction that hip fractures will rise dramatically over the next decade and beyond [1]. Particular emphasis should be placed on those factors which are amenable to change. Clearly, nutrition (as an exogenous factor) has a crucial role to play in the optimization and maintenance of skeletal integrity, and the development of novel dietary strategies is a top priority. Likewise, it is essential that specific dietary habits, such as the inclusion or exclusion of particular foods/food groups within the overall diet, are carefully monitored to ensure that any such regime does not place the individual/population group at an increased risk of osteoporosis or its associated risk factors [2].

Criticality of Acid–Base Homeostasis to Health

Acid–base homeostasis is critical to health. It is well established that a major challenge to the body's balance is to keep the hydrogen ion concentrations between 0.035 and 0.045 mEq/l, and it is clear that maintaining the H^+ within such narrow limits is essential to survival [3]. Diet and the aging process have been shown to affect systemic acidity; firstly, adult humans on a normal Western diet generate ~1 mEq of acid/kg/day, and the more acid precursors a diet contains, the greater the degree of systemic acidity; secondly, as people age, the overall renal function declines, including the ability to excrete acid [4], and thus with increasing age, humans become slightly but significantly more acidic [5].

Mechanisms of Action for a Skeletal Role in Maintaining Acid–Base Balance

Theoretical considerations of the role alkaline bone mineral may play in the defense against acidosis date back as far as the late nineteenth century [6]. The pioneering work of Lemann and Barzel over three decades ago showed extensively the effects of "acid" from the diet on bone mineral in both man and animal [7, 8]. More recently, Arnett and Dempster have demonstrated a direct enhancement of osteoclastic activity following a reduction in extracellular pH, an effect that has been shown to be independent of parathyroid hormone [9]. Osteoclasts and osteoblasts appear to respond independently to small changes in pH in culture media [10], and there is evidence that a small drop in pH, close to the physiological range, causes a tremendous burst in bone resorption [11, 12]. Furthermore, metabolic acidosis has been shown to stimulate resorption by activating mature osteoclasts already present in calvarial bone as opposed to the inducement of new osteoclast formation [13]. There are also data showing that excess

hydrogen ions directly induce a physiochemical calcium release from bone [14]. However, a number of key questions remain unanswered in this area; specifically, work is urgently required to determine whether metabolically generated acid, which lowers blood pH, exerts an influence on osteoclasts "in vivo."

Potential for a Link Between "Vegetable-Based Foods" and Osteoporosis

Considerations of the potential criticality of acid–base balance to skeletal integrity led to the development in the 1960s of a hypothesis linking the daily diet to the development of osteoporosis. It was noted specifically that "the increased incidence of osteoporosis with age, many represent, at least in part, the results of a lifelong utilization of the buffering capacity of the basic salts of bone for the constant assault against pH homeostasis" [15]. The extent of loss, over a given period of time need not be of astronomical proportions: if 2 mEq/kg/day of calcium is required to buffer approximately 1 mEq/kg/day of fixed acid, over a decade this would account for a 15 % loss of inorganic bone mass, assuming a total body calcium of approximately 1 kg. It is well established that the net production of acid is related to nutrition: a gross quantitative relationship exists between the amount of acid produced (as reflected by urine pH) and the amount of acid ash consumed in the diet [16]. Thus, it was proposed that long-term ingestion of "vegetable-based" diets may have a beneficial effect on bone mineral mass.

Concept of Dietary "Acidity": Potential Renal Acid Load (PRAL)

If the acid–base/skeletal link is to be believed, a possible explanation for there being no difference in indices of bone health between vegetarian vs. omnivorous populations is that vegetable-based proteins generate a large amount of acid in the urine. Work by Remer and Manz on the potential renal acid load (PRAL) of foods has shown that many grain products and some cheeses have a high PRAL level [17]. These types of products are likely to be found in abundance in a lacto-ovo vegetarian diet. The potentially deleterious effect of specific foods on the skeleton has been a topic of recent debate [18, 19].

Protein and Bone Health: Importance of Dietary Calcium and/or Dietary Alkalinity?

Of interest to the protein controversy is the growing recognition that dietary calcium may play a crucial role; that is, dietary protein is not detrimental to bone health provided that dietary calcium is in adequate supply [20]. While there are a number of studies which provide direct support for this [21, 22], there remain plausible mechanisms by which alkali salts may also have a critical role to play [23–25].

Dietary "Acidity" and Bone: Concept of Net Endogenous Acid Production (NEAP)

Determination of the acid–base content of diets consumed by individuals and populations is of help in assessing the impact of diet, and particular the effect of protein, on bone. Since 24-h urine collections (considered as the gold standard for acid–base research) are inappropriate for population-based studies, an alternative is to examine the net acid content of the diet. Frassetto et al. have shown that the protein to potassium ratio predicts net acid excretion, and in turn, net renal acid excretion predicts calcium excretion [26]. They propose a simple algorithm to determine the net rate of endogenous noncarbonic acid production (NEAP) from considerations of the acidifying effect of protein, mainly through sulfate excretion, and the alkalizing effect of potassium, resulting from the dietary intake of potassium as salts of weak organic acids.

Potassium Bicarbonate Administration and Bone: Clinical Applications

The clinical application of the effect of normal endogenous acid production on bone is of considerable interest, with extensive work in this area by Lemann et al. [27] (at the subject level) and Bushinsky [28] (at the cellular level). In 1994, Sebastian and colleagues demonstrated that potassium bicarbonate administration resulted in a decrease in urinary calcium and phosphorus, with overall calcium balance becoming less negative (or more positive) [29]. Changes were also seen in markers of bone metabolism, with a reduction in urinary excretion of hydroxyproline (bone resorption) and an increased excretion of serum osteocalcin (bone formation). Concern has been raised that the level of protein consumed by the women in the study was higher than is typical of American women in this age group, with a call for further studies to be undertaken in which dietary protein is consumed at a more reasonable level [30]. However, the study by Sebastian's group is of significant clinical importance and may have valued implications for the prevention and treatment of postmenopausal osteoporosis [31]. While long-term studies are of course required, administration of alkali may provide women with an alternative therapy for aging bone loss [32, 33].

We have conducted a meta-analysis of the effects of alkali-producing potassium salts on indicators of bone health. Our preliminary results reveal a significant reduction in both urinary calcium excretion and markers of bone turnover with administration of potassium bicarbonate or potassium citrate. The results for bone resorption markers are shown below (Fig. 8.1).

It is not clear to what extent these observations are due to the acid-buffering effect of the anion or to independent skeletal effects of potassium. However, fruit and vegetables are a rich source of both potassium and alkaline anions, and this could therefore indicate a potential mechanism by which a "fruit and vegetable" diet might act positively on bone.

Findings of the DASH I and DASH II Fruit and Vegetable Intervention Trials: Implications for Bone Health

Further support for a positive link between fruit and vegetable intake and bone health can be found in the results of the DASH I and DASH II intervention trials (Dietary Approaches to Stop Hypertension). In DASH I, diets rich in fruit and vegetables were associated with a significant fall in blood pressure compared with baseline measurements. However, of particular interest to the bone field were findings that increasing fruit and vegetable intake from 3.6 to 9.5 daily servings decreased the urinary calcium excretion from 157 to 110 mg/day [40]. The authors suggested this was due to the "high fiber content of the diet possibly impeding calcium absorption." However, a more likely explanation put forward by Barzel

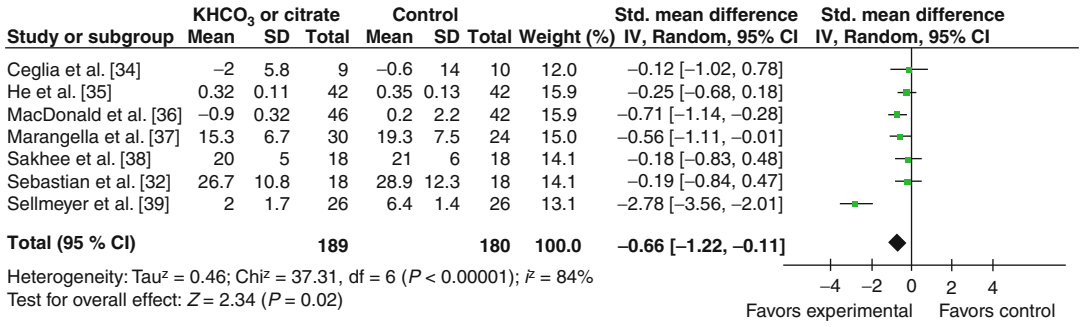

Fig. 8.1 Results of meta-analysis to show the effect of potassium (bicarbonate or citrate) on bone resorption markers. Standardized mean difference –0.66 (95 %CI –1.22, –0.11; $Z = 2.34$; $P = 0.02$), for measures including Ntx, CTx, DPD, and hydroxyproline [29, 34–39]

[41] was a reduction in the "acid load" with the fruit and vegetable diet compared to the control diet. This study is the first population-based fruit and vegetable intervention trial showing a positive effect on calcium economy (albeit a secondary finding).

Lin et al. [42] have reported the findings of the DASH II (DASH-sodium) trial. The impact of two dietary patterns on indices of bone metabolism was examined. The DASH diet emphasizes fruits, vegetables, low-fat dairy products, and reduction in red meats, and in this second DASH II trial, three levels of sodium intake were investigated (50, 100, and 150 nmol/l). Subjects consumed the control diet at the 150 mmol sodium intake/day levels for 2 weeks and were then randomly assigned to eat either the DASH diet or the control diet at all three sodium levels for a further 4 weeks in random order. The DASH diet, compared with the control diet, was found to significantly reduce both bone formation (by measurement of the marker osteocalcin) and bone resorption (by measurement of the marker CTx). Interestingly, sodium intake did not significantly affect the markers of bone metabolism. This is an important intervention study that shows a clear benefit of the high intake of fruit and vegetables on markers of bone metabolism.

Concluding Remarks

There is a clear and urgent need for public health strategies to target prevention of poor bone health on a population-wide basis, and similarly, it is critical that particular dietary habits resulting in the exclusion of specific foods are carefully monitored to ensure that population groups are not placing themselves at an increased risk of osteoporosis or its associated risk factors.

There is growing support from a combination of clinical, observational, and intervention studies for a beneficial effect of fruit and vegetable intake on bone health. The mechanisms behind this "fruit and vegetable" link remain to be fully determined: these foods provide not only a source of dietary alkali (for which there is growing data to suggest a critical role for the skeleton in acid–base homeostasis) but also a wide variety of micronutrients, for which there are potential mechanisms for an effect on bone [43].

The evidence currently available from experimental, clinical, and observational studies suggests a role for skeleton in acid–base homeostasis. There remain no long-term Dietary Approaches to Stop Hypertension (DASH) on bone health in younger and older age cohorts, and this is urgently required. Should the findings of the DASH/fruit and vegetable studies prove conclusive, a "fruit and vegetable" approach to bone health maintenance may provide a very helpful strategy for bone health development and maintenance throughout the life cycle.

References

1. Goulding A. Nutritional strategies for osteoporosis prevention. In: New SA, Bonjour JP, editors. Nutritional aspects of bone health. Cambridge: The Royal Society of Chemistry; 2003. p. 709–32.
2. World Health Organization. Assessment of osteoporotic fracture risk and its role for screening for postmenopausal osteoporosis, WHO technical report series. Geneva: WHO; 1994.
3. Green J, Kleeman R. Role of bone in regulation of systematic acid–base balance (Editorial Review). Kidney Int. 1991;39:9–26.
4. Frassetto LA, Morris Jr RC, Sebastian A. Effect of age on blood acid–base composition in adult humans: role of age-related renal functional decline. Am J Physiol. 1996;271:F1114–22 (Renal Fluid Electrolyte Physiol 40).
5. Frassetto LA, Sebastian A. Age and systemic acid–base equilibrium: analysis of published data. J Gerontol. 1996;51A:B91–9.
6. Goto K. Mineral metabolism in experimental acidosis. J Biol Chem. 1918;36:355–76.
7. Lemann Jr J, Litzow JR, Lennon EJ. The effects of chronic acid load in normal man: further evidence for the participation of bone mineral in the defence against chronic metabolic acidosis. J Clin Invest. 1966;45:1608–14.
8. Barzel US, Jowsey J. The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin Sci. 1969;36:517–24.
9. Arnett TR, Dempster DW. Effect of pH on bone resorption by rat osteoclasts in vitro. Endocrinology. 1986;119:119–24.
10. Kreiger NA, Sessler NE, Bushinsky DA. Acidosis inhibits osteoblastic and stimulates osteoclastic activity in vitro. Am J Physiol. 1992;262:F442–8.

11. Arnett TR, Spowage M. Modulation of the resorptive activity of rat osteoclasts by small changes in extracellular pH near the physiological range. Bone. 1996;18:277–9.
12. Bushinsky DA. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol. 1996;271:F216–22 (Renal Fluid Electrolyte Physiol).
13. Meghji S, Morrison MS, Henderson B, Arnett TR. PH dependence of bone resorption: mouse calvarial osteoclasts are activated by acidosis. Am J Physiol Endocrinol Metab. 2001;280:E112–9.
14. Bushinsky DA, Sessler NE, Glena RE, Featherstone JDB. Proton induced physicochemical calcium release from ceramic apatite disks. J Bone Miner Res. 1994;9:213–20.
15. Wachman A, Bernstein DS. Diet and osteoporosis. Lancet. 1968;1:958–9.
16. Barzel US, Massey LK. Excess dietary protein can adversely affect bone. J Nutr. 1998;128:1051–3.
17. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc. 1995;95:791–7.
18. Fox D. Hard cheese. New Sci. 2001;2329:42–5.
19. New SA, Macdonald HM, Reid DM, Dixon ASJ. Hold the soda. New Sci. 2002;2330:54–5 [Letter].
20. Heaney RP. Excess dietary protein may not adversely affect bone. J Nutr. 1998;128:1054–7.
21. Meyer HE, Pedersen JI, Loken EB, Tverdal A. Dietary factors and the incidence of hip fracture in middle-aged Norwegians. Am J Epidemiol. 1997;145:117–23.
22. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr. 2002;75:773–9.
23. Heaney RP. Protein and calcium: antagonists or synergists? Am J Clin Nutr. 2002;75:609–10.
24. New SA, Millward DJ. Calcium, protein and fruit & vegetables as dietary determinants of bone health. Am J Clin Nutr. 2003;77:1340–1 [Letter].
25. Dawson-Hughes B. Calcium, protein and bone health. Am J Clin Nutr. 2003;77:1341 [Letter].
26. Frassetto L, Todd K, Morris Jr RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from dietary protein and potassium contents. Am J Clin Nutr. 1998;68:576–83.
27. Lemann Jr J, Pleuss JA, Gray RW, Hoffmann RG. Potassium administration increases and potassium deprivation reduces urinary calcium excretion in healthy adults. Kidney Int. 1991;39:973–83.
28. Bushinsky DA. Decreased potassium stimulates bone resorption. Am J Physiol. 1997;272:F774–80 (Renal Fluid Electrolyte Physiology).
29. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris Jr RC. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med. 1994;330:1776–81.
30. Wood RJ. Potassium bicarbonate supplementation and calcium-metabolism in postmenopausal women – are we barking up the wrong tree. Nutr Rev. 1994;52(8):278–89.
31. Morris R, Masud T. Measuring quality of life in osteoporosis. Age Ageing. 2001;30(5):371–3.
32. Sebastian A, Hernandez RE, Portale AA, Colman J, Tatsuno J, Morris RC. Dietary potassium influences kidney maintenance of serum phosphorus concentration. Kidney Int. 1990;37(5):1341–9.
33. Kraut JA, Coburn JW. Bone, acid and osteoporosis. N Engl J Med. 1994;330(235):1821–2.
34. Ceglia L, Harris SS, Abrams SA, Rasmussen HM, Dallal GE, Dawson-Hughes B. Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption. J Clin Endocrinol Metab. 2009;94:645–53.
35. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD, Dalton RN, Kaski JC, MacGregor GA. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension. 2010;55:681–8.
36. MacDonald HM, Black AJ, Aucott L, Duthie G, Duthie S, Sandison R, Hardcastle AC, Lanham-New SA, Fraser WD, Reid DM. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. Am J Clin Nutr. 2008;88:465–74.
37. Marangella M, Di Stefano M, Casalis S, Berutti S, D'Amelio P, Isaia GC. Effects of potassium citrate supplementation on bone metabolism. Calcif Tissue Int. 2004;74:330–5.
38. Sakhaee K, Maalouf NM, Abrams SA, Pak CYC. Effects of potassium alkali and calcium supplementation on bone turnover in postmenopausal women. J Clin Endocrinol Metab. 2005;90:3528–33.
39. Sellmeyer DE, Schloetter M, Sebastian A. Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. J Clin Endocrinol Metab. 2002;87:2008–12.
40. Appel LJ, Moore TJ, Obarzanek E, Vallmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA. A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med. 1997;336:1117–24.
41. Barzel US. Dietary patterns and blood pressure. N Engl J Med. 1997;337:637 [Letter].
42. Lin P, Ginty F, Appel L, Aickin M, Bohannon A, Garnero P, Barclay D, Svetky L. The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults. J Nutr. 2003;133(10):3130–6.
43. New SA. The role of the skeleton in acid–base homeostasis. The 2001 Nutrition Society Medal Lecture. Proc Nutr Soc. 2002;61:151–64.

When Is Low Potential Renal Acid Load (PRAL) Beneficial for Bone?

9

Thomas Remer, Danika Krupp, and Lijie Shi

Abstract

Potential metabolic influences of dietary acid load on bone health have been discussed controversially. Here, we review the available findings in adults and healthy children regarding certain methodological aspects including (i) appropriate use of urinary biomarkers – potential renal acid load (PRAL) and net acid excretion (NAE), (ii) problems in the interpretation of results on calcium balance and bone turnover markers, and (iii) possible influences of selection bias regarding baseline diets of the population groups of randomized controlled trials. Based on the available evidence, it is concluded that calcium balance measurements and bone turnover markers are no adequate and sensitive tools to evaluate the modest but long-term prevailing influence of nutrition on bone status. Findings in children and adults exclusively conducted on the most reliable outcomes, that is, bone densitometric structure analyses, suggest that a low-PRAL diet may be especially relevant in certain population groups, for example, in children with higher dietary protein intakes, in postmenopausal women with impaired bone status, and probably in adults on a habitually acidifying nutrition. The mechanisms mediating detrimental bone effects of higher dietary acid loads under discussion include changes in endocrine–metabolic milieu, for example, impairment of GH/IGF-1 axis and higher glucocorticoid secretion as well as direct bone–cell-related changes by higher acid load. In conclusion, to identify moderate alterations in bone status exerted through nutritional influences, not only appropriate assessments of dietary proton load but also outcome measurements that are closely related to long-term bone structure are required.

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Keywords
Dietary acid load • Biomarker • Potential renal acid load • Net acid excretion • Bone • Calcium balance

Abbreviations

BMD	Bone mineral density
DEXA	Dual-energy X-ray absorptiometry
NAE	Net acid excretion
NEAP	Net endogenous acid production
OA	Organic acids
pQCT	Peripheral quantitative computed tomography (pQCT)
PRAL	Potential renal acid load
RCT	Randomized controlled trial

Introduction

Among the modifiable influences that act on the skeleton, nutrition is – along with physical activity – one of the most relevant factors. Vitamins, especially vitamin D and K; antioxidative polyphenols; calcium; and protein intake as well as acid load with the daily nutrition are under discussion. Particularly, the latter potential metabolic influence of a higher or a lower dietary proton [H^+] delivery to the body has triggered controversies concerning its bone health relevance. Clinical studies in patients with hypercalciuria or with chronic kidney disease have proven the beneficial impact of oral or dietary alkalization for the maintenance of skeletal integrity. However, similar results in healthy subjects or humans with rather moderate medical conditions are rare. Of several available methodological approaches to assess dietary acid loads, one is the potential renal acid load (PRAL) model. PRAL is calculated from the difference of nonbicarbonate anions and mineral cations [1]. This has the advantage that it can be used directly with recorded dietary intake data and in the same way also noninvasively as a clinical–chemical biomarker if appropriate urine collections are available. Although several meaningful diet-related observational and intervention studies on bone health did not directly present PRAL values, it is possible to calculate PRAL data (from the measurement values provided) at least for some of the trials and to assess their implications for bone outcomes. We complemented these assessments of the findings in adults with own study results in healthy children and provided possible metabolic mechanisms that may explain the bone catabolic influence of long-term high-PRAL diets.

Potential Renal Acid Load (PRAL): A Biomarker for the Diet-Related Acid Load

Protein is one of the most anabolic nutrients increasing not only the body's protein synthesis and muscularity [2] but also bone strength, at least moderately [3]. Despite these positive effects, dietary protein additionally represents the major nutritional source of protons metabolized from the sulfur-containing amino acids as well as from phosphorus bound to various protein structures in the form of phosphoproteins.

Accordingly, if renal acid excretion mechanisms are insufficient or immature as in preterm infants and neonates or if the systemic buffer capacity is reduced, mild forms of metabolic acidosis, not necessarily prominent in a clinically sense, can occur. Decreases in systemic pH and circulating bicarbonate levels are usually modest and still lie within the normal range. Despite this, growth retardation in low-birthweight infants [4] or impaired protein synthesis in elderly [5] can be a consequence of these mostly subclinical acidosis forms, which are positively responsive to dietary measures or manipulations [1, 6], but difficult to detect by

blood measurements [4, 6]. Appropriate noninvasive urine measurements are useful to identify states of high, medium, or low nutritionally and metabolically initiated daily proton loads. The classical way to do this is to measure net acid excretion (NAE) which formally represents the sum of urinary PRAL and the total amount of renally excreted organic acids (OA), with urinary PRAL reflecting the major renally excreted mineral anions minus the major urinary mineral cations.

$$\begin{aligned} \text{NAE} &= \text{urinary PRAL} + \text{OA} \\ &= \text{mineral anions} - \text{mineral cations} + \text{OA} \\ &= (\text{SO4}+\text{PO4}+\text{Cl}) - (\text{K}+\text{Mg}+\text{Ca}+\text{Na}) + \text{OA} \end{aligned}$$

Consumption of a rather plant-based fruit- and vegetable-rich diet results in higher intakes of mineral cations, especially of potassium and magnesium which even in absolute terms exceed the phosphorus and sulfur intake originating from dietary protein. In this situation, PRAL is negative or at least varies around zero (provided that sodium and chloride excretion rates, mostly reflecting salt intake, are nearly equimolar).

The PRAL model for the calculation of the diet-dependent daily acid load in humans assumes a theoretical metabolic steady state (for data standardization and practicability) during which the amounts of mineral cations and anions (or anion precursors) intestinally absorbed correspond to the amounts renally excreted. Urinary PRAL represents a biomarker providing the advantage that it reflects clearly defined nutritional influences on net proton loads to the body, whereas the NAE measurement which includes the large fraction of OAs has a strong body size-related component. For normal mixed diets, most of the OAs excreted stem from physiological daily degradation processes related to energy metabolism and body size [7]. Therefore, depending on the research question, quantification of urinary PRAL can be advantageous compared with NAE titration (for more details see [8]).

PRAL Inappropriately Used in Bone Health Studies

In epidemiological studies estimates of endogenous H^+ loads like PRAL or net endogenous acid production (NEAP) [9] commonly applied are diet based. Until now only a few studies have made use of the noninvasive biomarker urinary PRAL that relies on clinical–chemical measurements of mineral anions and cations in urine samples. One of these recent studies reporting urinary PRAL values suggested that neither a low urine pH nor a high acid excretion predicts bone fractures or the loss of bone mineral density. In this prospective cohort study, the authors examined 2-h urine samples that were collected in the morning under fasting conditions in a random sample of Canadian adults 25 years of age and above participating in the Canadian Multicentre Osteoporosis Study [10]. The authors (i) claimed that the use of urine to measure the diet acid load did allow them to avoid random and systematic errors inherent in food intake measurements and (ii) considered it possible that the *fasting-morning-2-h-second-void urine* samples they have analyzed are more reliable measures of dietary acidity than 24-h collections which are more prone to incomplete collection. In this study of Fenton et al. [10], no associations between the above-outlined urine measure of dietary acid load and changes in bone mineral density (BMD) over 5 years (lumbar spine, femoral neck, or total hip) or the occurrence of fragility fractures over 7 years were found. In this context, however, it has to be stated that a 2-h-fasting urine (collected after an 8-h fast) does by no means reflect nutrition: not of that day and even less of the habitual nutrition over years. Such an approach rather yields a snapshot of particular circadian characteristics of renal mineral excretions. In order to properly use urinary PRAL or other noninvasive biomarkers of dietary intake, the collection of 24-h urine samples (preferably, more than one sample for each subject) is necessary. Possible urine collection errors that cannot be fully excluded over a 24-h period bear tolerable risks of inaccuracies, which in any case are inherent in food intake measurements.

Calcium Balance and Bone Turnover Marker: Less Appropriate Outcomes for Examining Long-Term Acid–Base Effects of Habitual Nutrition on Bone

Both dietary acid loads and higher protein intakes result in elevated urinary excretion rates of calcium. These renal calcium losses have long been thought to remain unbalanced by compensatory increases in intestinal calcium absorption so that calcium release from bone had to fill the gap, not only increasing circulating calcium levels but concurrently titrating the noncarbonic acids endogenously produced by higher nutritional acid loads [11]. Frassetto et al. stated the view that this might result in a kind of vicious circle in that – although the kidney daily excretes the bulk of the diet net acid load – the body retains a small fraction of acidity sufficient to induce a persisting low-grade metabolic acidosis. This may ensure continued titration of the alkaline salts of bone, with attendant loss of calcium and phosphorus in urine, since impairment of renal calcium reabsorptive efficiency is a characteristic of metabolic acidosis [12]. The corresponding bone catabolic effects should become discernible in negative calcium balances, that is, in lower daily intestinal net calcium absorption than in daily renal calcium losses. In fact, impaired calcium balances have been observed in a few short-term studies, for example see [13]. However, other studies did not confirm negative calcium balances with higher nutritionally induced proton loads. In a recent meta-analysis performed by Fenton et al., no significant overall association between calcium balance and NAE was observed [14]. The authors concluded that their meta-analysis did not support the concept that the calciuria associated with higher NAE reflects a net loss of whole body calcium.

All together this paper of Fenton et al. [14] contributes to our understanding that calcium balance measurements are not an adequate and sensitive tool to evaluate the modest but long-term prevailing influence on bone quality or bone mineral status exerted through nutrition. In line herewith, Rafferty et al. [15] reported that healthy postmenopausal women on potassium-rich diets over many years did not demonstrate notable improvements in (*short-term-tested*) *calcium balances* since the clearly enhanced renal reabsorption of calcium (i.e., the reduced calciuria) in these females was offset by a parallel reduction in intestinal calcium absorption. Similar findings were obtained in a randomized feeding study by Hunt et al. [16] who observed that higher renal excretion rates of calcium with high protein (and concomitant low calcium) intake were almost balanced by a parallel elevation in intestinal calcium absorption (determined by ^{47}Ca isotope retention methodology). From these data it appears that a dynamically adapted response in intestinal calcium absorption does balance the ups and downs in calcium handling of the healthy kidney, provided that enough vitamin D and its activated metabolites are available and calcium intake is low to moderate (not deficient). At high calcium intakes the corresponding calcium flood (passive influx) suppresses much of the regulatory calcitriol activity, but calcium absorption is elevated per se. In any case, even at not-so-high calcium intakes occurring along with higher renal calcium losses, for example, through a higher dietary acid load, most or almost all of the calcium losses via the kidney can be compensated by intestinal upregulation of active absorption. A finally resulting net negative balance developing over the years cannot be easily identified by conventional short-term balance measurement techniques.

Sustained mildly unfavorable effects with reductions in bone quality and bone mineral content in the long run, that is, over years or even decades, require other more bone structure-specific analysis methods. In this respect, measurements of bone turnover markers – reflecting to a large degree the momentary bone resorption activity at the time of blood and/or urine collection – are not helpful as well. For example, bone accretion is highest during puberty when adolescents' growth velocity shows the typical peak height velocity pattern. At that time with the strongest increments in mineral mass, also the bone resorption marker levels reach their maximum (Fig. 9.1). For an appropriate bone gain and bone modeling, an initial bone resorption, that is, some removal of older bone, is required. Only if such remodeling takes place, formation of new

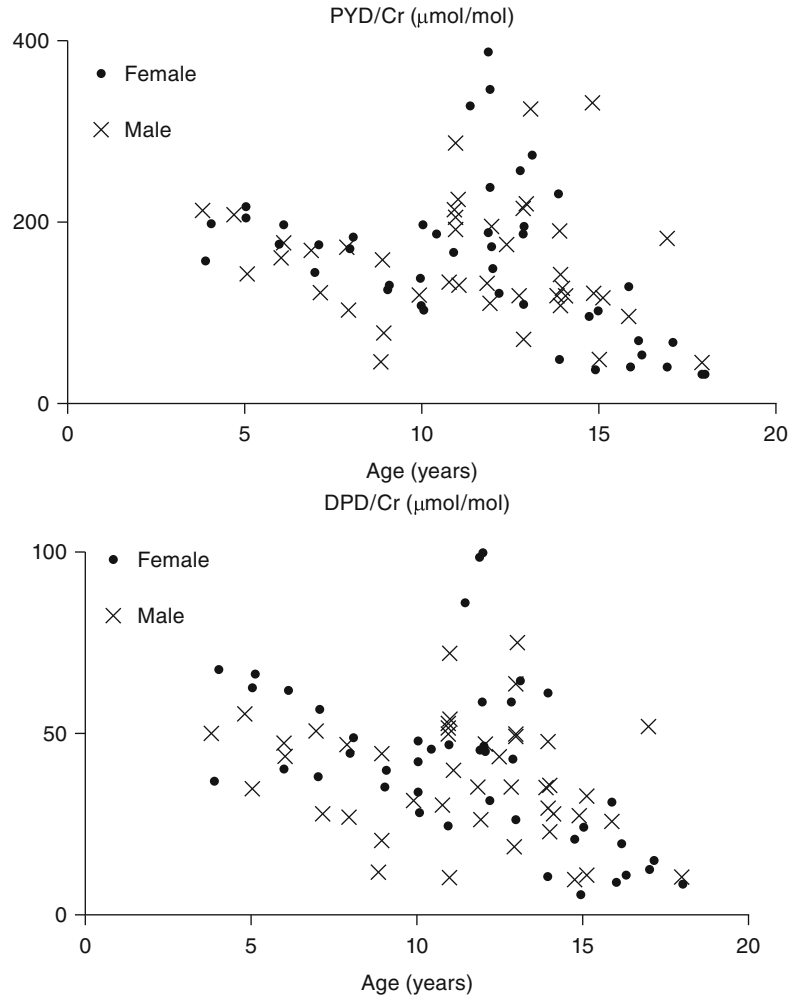

Fig. 9.1 Variation with age of 24-h excretion of pyridinoline (*PYD*) and deoxypyridinoline (*DPD*). *Cr* creatinine (Reprinted from Rauch et al. [17].With permission from Thieme Publishers)

additional mineral structures is possible, showing that bone resorption markers do by no means reflect always a bone catabolic situation. This is not only true for periods of growth, but can be found also during adulthood. For example, increases in the bone resorption marker carboxy-terminal cross-linked telopeptide of type I collagen do occur in response to exercise, that is, in a principally musculoskeletal anabolic situation as well as after growth hormone administration [18]. On the other hand, marked elevations in calcium intake have been shown to decrease specific markers of bone formation in healthy nonosteoporotic older men. These few examples show that bone resorption and bone formation markers obviously do not specifically predict subsequent bone catabolism and anabolism, respectively. For a more reliable assessment of the longer-term consequences of nutritional preventive measures like a habitual mineral-rich alkalizing diet for bone health, computer tomographic analyses of bone structures as well as other sophisticated densitometric bone measurements including high-quality dual-energy X-ray absorptiometry (DEXA) scans are obviously more expedient.

Densitometric Bone Outcomes and Dietary Acid Load in Adults

Until recently, only two randomized controlled (intervention) trials (RCTs) have been published that examined the impact of alkali supplementation for at least 1 year on acknowledged densitometric bone outcomes. A third study based on an initial examination of the influence of definite

base equivalent ingestion on renal calcium excretion [11] has recently been submitted for publication. In that study additional BMD measurements at the hip and spine had been performed, but results were not reported up to now. All three trials used DEXA to evaluate bone status and all three examined postmenopausal women. Alkali exposures were administered for 1 [19], 2 [20], or 3 [21] years. In the 2- and 3-year studies, no positive effects of alkali supplementation or additional fruit and vegetable intake [20, 21] on BMD outcomes (spine, hip) were observed. Placebo administration and potassium citrate or potassium bicarbonate ingestion at varying doses between 18 and 90 mmol/day did not yield significantly different BMD changes. Only in the 1-year study using a daily dose of 30 mmol potassium citrate, a significant improvement of spine and hip BMD was observed compared to blinded control subjects. A characteristic of all three study populations was a low baseline PRAL of around 0 mEq/day [19, 20] or −10 mEq/day [11, 21], showing a certain degree of selection bias for each participant groups. In all three trials, participating subjects did already ingest a quite low-net-acid-producing diet before the study began, strongly suggesting a considerable degree of health and nutrition consciousness. What differed between the three examined cohorts was the bone status: bone health was normal in both studies that showed no skeletal improvement with the oral alkali supplement, whereas osteopenia was present in those postmenopausal women who responded with a clear improvement in BMD.

These findings led to the following conclusions:

- Alkalization, for example, with potassium citrate or bicarbonate for at least 1 year does not show relevant bone-anabolic effects in healthy (postmenopausal) subjects with normal bone status (no osteopenia) who already at baseline consume “healthy” low-PRAL diets.
- However, if bone status is impaired, a supplemental alkali load appears to improve skeletal mineral content within a year, even if initial (habitual) dietary acid load is relatively low. It appears that in healthy adults (with normal BMDs) already on a rather alkalizing diet, a further reduction in dietary PRAL might not provide additional benefit with regard to bone status at least at hip and spine.

Apart from the RCTs also four prospective observational studies (evidence level II) have been performed in adults aged 45–97 years, showing positive associations between alkaline nutrient ingestion and BMD changes over 4–7 years in two studies [22, 23], but no association in two other studies [24, 25]. In one of these latter studies done with peripheral quantitative computer tomography (pQCT) [25], a reanalysis of the data – now focusing on the outcome parameter bone area – yielded a significant association of bone area increases with lower dietary PRAL intakes [26]. This reanalysis suggesting a possible bone-anabolic influence with reduced dietary PRALs was done by the authors in response to a letter [27] that we sent to the editor of the publication journal.

Densitometric Bone Outcomes and Dietary Acid Load in Children

For healthy children no randomized controlled trials (evidence level I for epidemiological or (medical) research studies) are available that have examined the bone health–dietary acid load relationship. However, prospective observational long-term findings have been published for healthy children and adolescents and significant negative associations between diaphyseal bone parameters by pQCT (bone mineral content or cortical area) and dietary as well as urinary PRAL determined over a 4-year observation period before bone analysis were observed [8, 28]. Importantly, in these studies not only the more bone structure-specific pQCT analysis (compared to DEXA) [6] was used but also repeated 3-day weighed dietary records, considered to belong to the most valid dietary assessment tools. Furthermore, the findings of a more bone-anabolic low-PRAL nutrition could be confirmed and substantiated by repeated urinary PRAL biomarker measurements.

Figure 9.2 and the results with respect to low- and high-urinary PRAL levels are presented

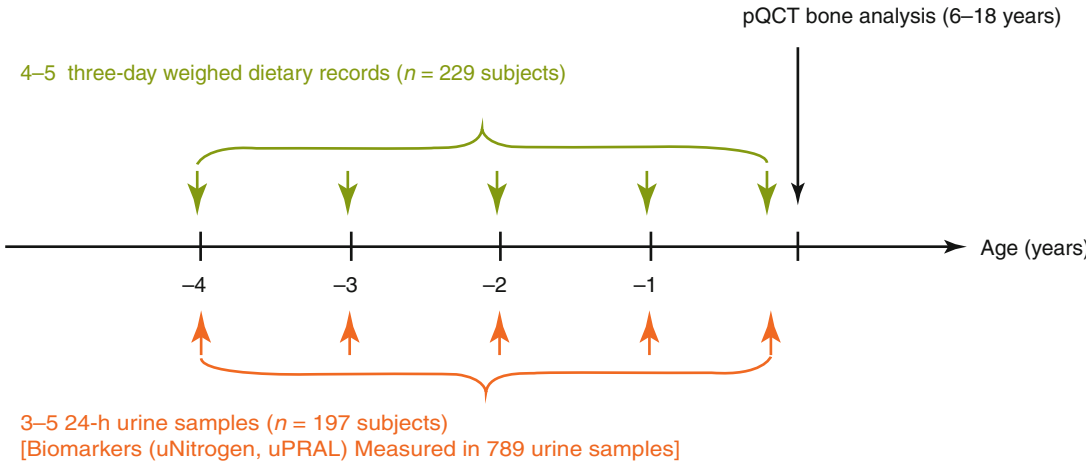

Fig. 9.2 Schematic study design of two prospective observational examinations with measured diaphyseal bone parameters by pQCT as outcomes in healthy children and adolescents using repeated 3-day weighed dietary records [28] as well as 24-h urinary biomarker measurements (PRAL) [8] determined over a 4-year observation period before bone analysis

stratified for subgroups of comparably high and comparably low protein intakes (Fig. 9.3). It is discernible that the basically known [3] bone-anabolic effect of higher protein intakes becomes particularly prominent, if children are on a habitual low-PRAL diet, that is, if they favor a fruit- and vegetable-rich diet along with higher protein intakes.

Although prospective and longitudinal observational studies are not definite proof of a causal relationship, the above findings provide a high level of plausibility because of the (i) prospective study design; (ii) computer tomographic bone analysis; (iii) repeatedly collected, highly reliable weighed dietary records; and (iv) confirmation by biomarker measurements in separate sample material (24-h urines). For ethical and practical reasons, such kind of investigation on the impact of habitual diet-related net proton loads on the skeletal system is not possible as a RCT in healthy children. Accordingly, at least for children, substantially better evidence cannot be expected soon.

Taking the findings in children and adults together, the statement put forward by a few authors – there is no evidence that an alkalizing diet is protective for bone [29] – is not supported by the specific literature focusing exclusively on the most reliable outcomes hitherto available, namely, densitometric bone measurements. The correct statement should read that there is a certain degree of evidence that an alkalizing nutrition has a preventive benefit for bone health at least in particular subject groups like children, postmenopausal women with impaired bone status, and probably adults on a clearly acidifying long-term nutrition before dietary or supplemental intervention. To date, the latter can only be deduced from prospective observational studies, as for an experimental switch from a habitual high-PRAL diet to a controlled low dietary PRAL state, no randomized trial data are available. Systematic reviews and meta-analyses done on calcium balance and bone turnover markers may or even must – for physiological reasons – yield inconsistent results with regard to an alkalizing diet, but do not yield proofs for the nonexistence of a preventive–medical relationship between a habitual base-producing nutrition and long-term development of bone quality, structure, and strength.

Potential Bone Catabolic Mechanisms of High PRAL

Several interacting mechanisms may commonly underlie the bone health strengthening effect of a long-term low dietary acid load, of which here

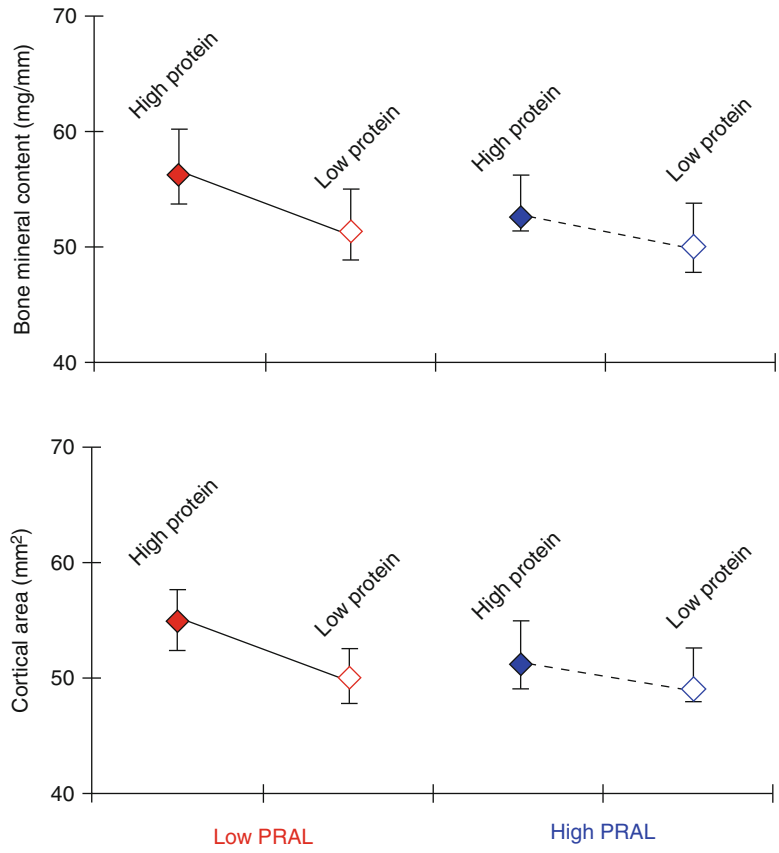

Fig. 9.3 Bone mineral content and cortical area by low (−5 mEq/d/m²) and high (14 mEq/d/m²) mean urinary PRAL levels further stratified according to high (1.5 g/kg body weight) and moderately high (1.2 g/kg body weight) protein intakes (median split)

only three will be shortly outlined: (i) GH/IGF, (ii) cortisol, and (iii) direct proton effects.

With regard to (i), impairments of the GH/IGF-1-axis with a higher proton load have been observed in vitro [30] and in animal studies [31]. In humans, induction of metabolic acidosis in healthy adults [32] as well as alkali therapy in acidotic hemodialysis patients [33] suggest a hepatocellular GH resistance and reduced IGF-1-levels under acidotic conditions. (ii) A higher glucocorticoid secretion with a higher acid load was indicated by an increased cortisol excretion in humans on experimental acidosis [34] and by reduced plasma cortisol levels and a diminished excretion of cortisol metabolites after alkali administration in healthy young adults on a rather acidifying diet [35]. Both higher IGF-1 [36] and reduced cortisol levels [37] can contribute to an improved bone health.

Apart from these indirect endocrine mechanisms that may mediate beneficial effects of a lower dietary proton load on bone health, (iii) direct effects of even moderate changes in extracellular pH or bicarbonate concentration on bone cells [38, 39] have been observed. In this context it is important to consider that small changes in systemic acid–base status, as inducible by dietary means, may result in greater changes in interstitial pH and bicarbonate because the buffer capacity of the interstitium is lower than that of plasma due to a lower interstitial albumin concentration [40].

Conclusions

In principle, a low dietary PRAL appears to be beneficial for bone in the long term. This can be deduced from several prospective observational (epidemiological evidence level II) studies in adults, although it has not been confirmed in all studies [24, 25]. Hitherto performed RCTs on healthy subjects without osteopenia could not prove the potential bone-anabolic influence of a reduction in dietary acid load as

they were all done in populations who already at baseline consumed diets yielding only minor net proton loads. For a proof of causality through a RCT, all volunteers included in that trial must have been on a high-PRAL diet for years before study onset, that is, before alkali equivalents are provided, for example, in the form of potassium citrate. However, such a study design appears not to be easily achieved, since compliance of habitual unhealthy eaters cannot be expected to be high.

In children, evidence for an influence of dietary alkalization on bone health through RCTs cannot be expected to be performed in the near future. Observational studies, however, suggest that a low-PRAL diet may be especially relevant in some subgroups, for example, in children with higher dietary protein intakes. Thus, a moderate high protein intake with a concurrent high ingestion of base-forming nutrients (i.e., a diet high in fruit and vegetables) might be most beneficial.

The mechanisms mediating bone strengthening effects of lower dietary acid loads are probable more subtle than acutely detectable positive calcium balances and may include several endocrine as well as direct bone–cell-related changes. In any case, to identify moderate alterations in bone status exerted through nutritional influences, not only accurate measurements of dietary proton load (either using carefully collected dietary data or biomarkers reflecting current nutrition) but also outcomes that are closely related to long-term bone structure may be required.

References

1. Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. Am J Clin Nutr. 1994;59(6): 1356–61.
2. Groen BB, Res PT, Pennings B, Hertle E, Senden JM, Saris WH, et al. Intragastric protein administration stimulates overnight muscle protein synthesis in elderly men. Am J Physiol Endocrinol Metab. 2012;302(1): E52–60.
3. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 2009;90(6):1674–92.
4. Kalhoff H, Manz F. Nutrition, acid–base status and growth in early childhood. Eur J Nutr. 2001;40(5): 221–30.
5. Proctor DN, Balagopal P, Nair KS. Age-related sarcopenia in humans is associated with reduced synthetic rates of specific muscle proteins. J Nutr. 1998; 128(2 Suppl):351S–5.
6. Remer T, Krupp D, Shi L. Dietary protein's and dietary acid load's influence on bone health. Crit Rev Food Sci Nutr. [in press].
7. Berkemeyer S, Remer T. Anthropometrics provide a better estimate of urinary organic acid anion excretion than a dietary mineral intake-based estimate in children, adolescents, and young adults. J Nutr. 2006; 136(5):1203–8.
8. Remer T, Manz F, Alexy U, Schoenau E, Wudy SA, Shi L. Long-term high urinary potential renal acid load and low nitrogen excretion predict reduced diaphyseal bone mass and bone size in children. J Clin Endocrinol Metab. 2011;96(9):2861–8.
9. Frassetto LA, Lanham-New SA, Macdonald HM, Remer T, Sebastian A, Tucker KL, et al. Standardizing terminology for estimating the diet-dependent net acid load to the metabolic system. J Nutr. 2007; 137(6):1491–2.
10. Fenton TR, Eliasziw M, Tough SC, Lyon AW, Brown JP, Hanley DA. Low urine pH and acid excretion do not predict bone fractures or the loss of bone mineral density: a prospective cohort study. BMC Musculoskelet Disord. 2010;11:88.
11. Frassetto L, Morris Jr RC, Sebastian A. Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. J Clin Endocrinol Metab. 2005;90(2):831–4.
12. Lemann J, Litzow JR, Lennon EJ. Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. J Clin Invest. 1967;46(8):1318–28.
13. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris Jr RC. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med. 1994;330(25): 1776–81.
14. Fenton TR, Lyon AW, Eliasziw M, Tough SC, Hanley DA. Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance. J Bone Miner Res. 2009;24(11):1835–40.
15. Rafferty K, Davies KM, Heaney RP. Potassium intake and the calcium economy. J Am Coll Nutr. 2005; 24(2):99–106.
16. Hunt JR, Johnson LK, Fariba Roughead ZK. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. Am J Clin Nutr. 2009;89(5):1357–65.
17. Rauch F, Schonau E, Woitge H, Remer T, Seibel M. Urinary excretion of hydroxy-pyridinium cross-links of collagen reflects skeletal growth velocity in normal children. Exp Clin Endocrinol. 1994;102(2):94–7.
18. Wallace JD, Cuneo RC, Lundberg PA, Rosen T, Jorgensen JO, Longobardi S, et al. Responses of

markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. J Clin Endocrinol Metab. 2000;85(1):124–33.
19. Jehle S, Zanetti A, Muser J, Hulter HN, Krapf R. Partial neutralization of the acidogenic Western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia. J Am Soc Nephrol. 2006;17(11):3213–22.
20. Macdonald HM, Black AJ, Aucott L, Duthie G, Duthie S, Sandison R, et al. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. Am J Clin Nutr. 2008;88(2):465–74.
21. Frassetto LA, Hardcastle AC, Sebastian A, Aucott L, Fraser WD, Reid DM, et al. No evidence that the skeletal non-response to potassium alkali supplements in healthy postmenopausal women depends on blood pressure or sodium chloride intake. Eur J Clin Nutr. 2012;66:1315–22.
22. Tucker KL, Hannan MT, Kiel DP. The acid–base hypothesis: diet and bone in the Framingham Osteoporosis Study. Eur J Nutr. 2001;40(5):231–7.
23. Macdonald HM, New SA, Golden MH, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr. 2004;79(1):155–65.
24. Kaptoge S, Welch A, McTaggart A, Mulligan A, Dalzell N, Day NE, et al. Effects of dietary nutrients and food groups on bone loss from the proximal femur in men and women in the 7th and 8th decades of age. Osteoporos Int. 2003;14(5):418–28.
25. Pedone C, Napoli N, Pozzilli P, Lauretani F, Bandinelli S, Ferrucci L, et al. Quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. Bone. 2010;46(4):1063–7.
26. Pedone C, Napoli N, Pozzilli P, Lauretani F, Bandinelli S, Ferrucci L, et al. Author reply – quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. Bone. 2011;48(2):416.
27. Remer T, Shi L, Alexy U. Potential renal acid load may more strongly affect bone size and mass than volumetric bone mineral density. Bone. 2011;48(2): 414–5; author reply 6.
28. Alexy U, Remer T, Manz F, Neu CM, Schoenau E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. Am J Clin Nutr. 2005;82(5):1107–14.
29. Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. Nutr J. 2011;10:41.
30. Green J, Maor G. Effect of metabolic acidosis on the growth hormone/IGF-I endocrine axis in skeletal growth centers. Kidney Int. 2000;57(6):2258–67.
31. Ordonez FA, Santos F, Martinez V, Garcia E, Fernandez P, Rodriguez J, et al. Resistance to growth hormone and insulin-like growth factor-I in acidotic rats. Pediatr Nephrol. 2000;14(8–9):720–5.
32. Brungger M, Hulter HN, Krapf R. Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: new cause of growth hormone insensitivity in humans. Kidney Int. 1997;51(1):216–21.
33. Wiederkehr MR, Kalogiros J, Krapf R. Correction of metabolic acidosis improves thyroid and growth hormone axes in haemodialysis patients. Nephrol Dial Transplant. 2004;19(5):1190–7.
34. Sicuro A, Mahlbacher K, Hulter HN, Krapf R. Effect of growth hormone on renal and systemic acid–base homeostasis in humans. Am J Physiol. 1998;274(4 Pt 2):F650–7.
35. Maurer M, Riesen W, Muser J, Hulter HN, Krapf R. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am J Physiol Renal Physiol. 2003;284(1):F32–40.
36. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, et al. Circulating levels of IGF-1 directly regulate bone growth and density. J Clin Invest. 2002;110(6):771–81.
37. Canalis E, Delany AM. Mechanisms of glucocorticoid action in bone. Ann N Y Acad Sci. 2002; 966:73–81.
38. Arnett T. Regulation of bone cell function by acid–base balance. Proc Nutr Soc. 2003;62(2):511–20.
39. Geng W, Hill K, Zerwekh JE, Kohler T, Muller R, Moe OW. Inhibition of osteoclast formation and function by bicarbonate: role of soluble adenylyl cyclase. J Cell Physiol. 2009;220(2):332–40.
40. Street D, Nielsen JJ, Bangsbo J, Juel C. Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. J Physiol. 2005;566(Pt 2):481–9.

The Effect of Alkaline Potassium Salts on Calcium and Bone Metabolism

10

Deborah E. Sellmeyer

Abstract

Diets in industrialized nations are no longer based predominately on potassium rich fruit and vegetables, resulting in substantially lower potassium intakes as well as the alkaline anions such as bicarbonate and citrate that accompany potassium in fruit and vegetables. The reduction in potassium and alkali intake while maintaining adequate dietary protein intake leads to an imbalance between the acid producing and the base producing components of the diet. This dietary net acid load is theorized to require mobilization of skeletal base to aid in acid neutralization, leading to ongoing skeletal resorption to maintain systemic acid–base homeostasis. Short-term calcium balance studies suggest that supplementing the diet with alkaline potassium salts lowers urine calcium losses with no increase in stool calcium, resulting in a net improvement in calcium balance. The majority of studies examining potassium supplements also suggest bone resorption is reduced by potassium citrate or bicarbonate. However, not all studies show a reduction in bone turnover and the two existing bone density trials provide conflicting results. In particular, controversy remains over whether the benefits to calcium metabolism demonstrated in the short-term calcium balance studies persist. To address this controversy, we conducted a randomized, placebo controlled trial in 52 men and women (mean age 65.2 + 6.2 years) who were randomly assigned to potassium citrate 60, 90 mmol, or placebo daily with measurements of bone turnover markers, net acid excretion, and calcium metabolism including intestinal fractional calcium absorption and calcium balance at baseline and 6 months. At 6 months, 24-h urine calcium was significantly reduced in both potassium treatment groups and fractional calcium absorption was

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not changed by potassium citrate supplementation. In subjects randomized to potassium citrate 90 mmol/day, net calcium balance was significantly improved compared to placebo. Serum C-telopeptide, a marker of bone resorption, decreased significantly in both potassium citrate groups compared to placebo, while bone specific alkaline phosphatase did not change. Our study supports the hypothesis that supplementation with alkaline potassium salts such as potassium citrate has the potential to improve skeletal health. Studies with definitive outcomes such as bone density and fracture are needed.

Keywords

Potassium • Acid–base • Osteoporosis • Calcium balance • Protein • Citrate Bicarbonate • Bone • Diet

Introduction

Osteoporosis and fractures pose a substantial health burden for older men and women. One in six Caucasian women will experience a hip fracture in her lifetime with mortality after hip fracture reaching 26 % in the first year [1]. Men are also significantly affected, experiencing one-third of all hip fractures and an even higher mortality rate than women [2]. Inexpensive and safe interventions that can be implemented on a population basis are very limited. While effective, strategies such as weight bearing exercise and calcium and vitamin D have modest effects on skeletal health, and substantial fracture risk remains even after optimization of these approaches [3]. It has long been hypothesized that optimizing dietary acid–base balance may provide an effective strategy to further improve skeletal health. Typical Western diets tend to be limited in fruit and vegetable content, leading to deficiencies in potassium and the alkaline salts that accompany dietary potassium, such as bicarbonate and citrate. While potassium and base intake typically are limited, intake of acid precursors, the sulfur containing amino acids in dietary proteins and cereal grains, remains abundant. The imbalance between the acid-producing and base-producing components of the diet results in a chronic low-level metabolic acidosis that worsens with age as renal function declines [4–7]. Long-term exposure to chronic dietary acid may mobilize alkaline salts from the skeleton to help mitigate the diet-induced acidosis. Supplementation of the diet with alkaline potassium salts such as potassium bicarbonate or potassium citrate has been investigated as a way to neutralize dietary acid while maintaining adequate protein intake. While studies with definitive skeletal outcomes have yet to be completed, investigation of the effects of alkaline potassium salts on calcium and bone metabolism provides intriguing evidence that this approach may ultimately be able to improve skeletal health.

Effects of Alkaline Potassium Salts on Calcium and Bone Metabolism

Effects on Short-Term Calcium Balance and Intestinal Calcium Absorption

Two studies have examined the effects of potassium bicarbonate on calcium balance, the difference between calcium intake and calcium excretion via urine and stool while on a controlled metabolic diet with standardized nutrient intake. The first study compared potassium bicarbonate to sodium bicarbonate, each given as 20 mmol three times per day in nine healthy men between the ages of 23 and 46 years [8] consuming controlled diets containing from approximately 200–800 mg/day of calcium. During the 18-day intervention period, urine calcium significantly decreased in the potassium bicarbonate group while remaining unchanged

in the sodium bicarbonate group. Fecal calcium was unchanged by either intervention, leading to an improvement in calcium balance of approximately 40 mg/day. Similar results were demonstrated in 18 postmenopausal women consuming controlled diets containing approximately 650 mg/day of calcium [9]. During 18 days of treatment with 60–120 mmol/day potassium bicarbonate, urine calcium was significantly reduced with no impact on fecal calcium, resulting in an improvement in calcium balance of approximately 56 mg/day. In both of these studies, phosphorus balance was also significantly improved by potassium bicarbonate administration. The unchanged fecal calcium values in these short-term studies suggest intestinal calcium absorption is not affected by potassium bicarbonate administration. Intestinal calcium absorption was directly measured by the dual stable calcium isotope technique in 18 postmenopausal women treated with 40 mmol/day of potassium citrate for 2 weeks [10] while consuming controlled diets containing 400 mg/day of calcium. Compared to placebo, fractional intestinal calcium absorption was unaffected by potassium citrate supplementation. During ingestion of low and high protein diets for 10 days along with consumption of approximately 1,200 mg/day of calcium, intestinal calcium absorption as measured by the dual isotope technique was higher in subjects randomized to concurrent treatment with potassium bicarbonate at doses up to 90 mmol/day [11] compared to placebo. It has been demonstrated that the urine calcium lowering effect of alkaline potassium compounds persists to at least 3 years [12]. However, others have questioned whether, in the long term, intestinal adaptation to alkaline potassium salts may occur, leading to a reduction in intestinal calcium absorption so that even though urine calcium is reduced, there is no net improvement in the calcium economy on a long-term basis [13].

Effects on Markers of Bone Turnover and Calcitropic Hormones

The effect of alkaline potassium salts on markers of bone turnover, serum and urine proteins that reflect increased bone resorption and bone formation, has been mixed. Osteocalcin, a marker of bone formation, was significantly increased in one study [9], decreased in two studies [14, 15], and unchanged in one study [16]. Other markers of bone formation have also been examined. Bone specific alkaline phosphatase was not affected by potassium citrate in two studies [10, 16] and N-terminal propeptide of type 1 collagen was unchanged in one study [17]. Markers of bone resorption including urinary *N*-telopeptide, hydroxyproline, and deoxypyridinoline have been reduced by alkaline potassium salt administration in five studies [9, 14, 15, 18, 19] and unchanged in three studies [10, 16, 17]. The magnitude of reduction in net acid excretion induced by treatment with alkaline potassium salts was associated with the level of reduction in urinary *N*-telopeptide in a 3 month trial [15], but no dose response was demonstrated in a 2 year study that included a 55.5 mmol/day and a 18.5 mmol/day treatment group.

Intact parathyroid hormone and 1,25 dihydroxyvitamin D have been unaffected in nearly all trials [8, 10, 14–16, 18] with a single study showing an increase in parathyroid hormone during potassium bicarbonate treatment [9].

Effects on Bone Mineral Density

Two longer-term trials examining the effects of potassium citrate on bone density have been reported. While one was positive and one was negative, both studies have limitations precluding definitive conclusions. In the first trial, 161 postmenopausal women were randomized to 30 mmol/day of either potassium citrate or potassium chloride [16]. After 12 months of treatment, bone density, measured by dual energy X-ray absorptiometry (DXA), was 1.9 and 2.0 % higher at the lumbar spine and total hip, respectively, in the potassium citrate group than the potassium chloride group. Interpretation of these results is somewhat limited by the lack of a placebo group. It is unknown whether the difference between the treatment groups represents a beneficial effect of the potassium citrate or an adverse effect of the potassium chloride. The second bone density

study randomized 276 postmenopausal women to 55.5 mmol/day of potassium citrate, 18.5 mmol/day of potassium citrate, or sufficient fruit and vegetables to provide 18.5 mmol/day of potassium citrate [17]. At the end of 2 years of treatment, there were no differences in bone density at either the lumbar spine or hip among the treatment groups. However, there was also no reduction in urine calcium excretion with potassium citrate supplementation except at a single timepoint in the 55.5 mmol/day group, raising questions about adherence to or effectiveness of the preparation utilized in this study.

Longer-Term Effects on Calcium Balance and Skeletal Metabolism

We recently conducted a 6 month trial to determine whether the improvements in calcium balance demonstrated in the previously published short-term balance studies described above persist and specifically whether there is intestinal adaptation with time that offsets a decrease in urine calcium induced by treatment with an alkaline potassium salt.

Subjects for the study were men and women over the age of 55 years recruited through population based direct mailings. As lowering urine calcium excretion is likely an important component to the mechanism of action of alkaline potassium salts, we recruited subjects who, on their free living diets, had a urine calcium excretion above the median value seen in previous studies (120 mg/day women, 140 mg/day men). We additionally excluded women who were within 5 years past menopause to avoid the rapid bone turnover state that occurs following menopause as well as subjects with medical conditions or on medications that would predispose them to gastrointestinal intolerance of oral potassium or development of hyperkalemia. Subjects were also excluded for known metabolic bone disease or use of skeletally active medications such as osteoporosis therapies or glucocorticoids. After telephone and in-person screening, 52 individuals were ultimately eligible to participate in the study and randomized to treatment. The study protocol was approved by the UCSF Institutional Review Board and written informed consent was provided by each study participant.

The subjects' calcium intake was standardized to a total intake of 1,230 mg/day. They were counseled by the study dietitian to achieve approximately 600 mg of calcium per day in their diet during the parts of the study where they were consuming a self selected diet. In addition, subjects were given daily supplements containing calcium citrate (630 mg elemental calcium) and vitamin D3 400 IU; all other vitamin and mineral supplementation were discontinued for the duration of the study.

After 4 weeks total of standardized calcium and vitamin D intake, subjects completed baseline serum and urine studies including minerals, parathyroid hormone, vitamin D, bone turnover markers, and net acid excretion (the sum of the excretion rates of titratable acid and ammonium minus that of bicarbonate). They additionally completed a calcium balance study, consuming a controlled diet containing 600 mg/day of calcium for 12 days. During days 7-12 of the 12 day period, all urine was collected in 24-h increments and each stool was collected individually. In addition, each subject underwent assessment of intestinal fractional calcium absorption, using dual stable calcium isotopes [20, 21]. After completing their baseline measurements, subjects began their randomized treatment assignments: placebo ($n=18$), potassium citrate 60 mmol/day ($n=17$), or potassium citrate 90 mmol/day ($n=17$). Subjects were monitored for adherence and side effects with regular study visits and, after 6 months of potassium citrate or placebo supplements, underwent all measurements again including calcium balance and intestinal calcium absorption.

Data were analyzed by examining the mean changes in the endpoints using a two-sample *t*-test comparing the change from baseline to the 6 month measurement. Kruskal-Wallis and Wilcoxon-Mann–Whitney tests were used to analyze the bone turnover marker data as they were not normally distributed.

At baseline, there were no significant differences in subject characteristics or laboratory parameters among study groups; approximately

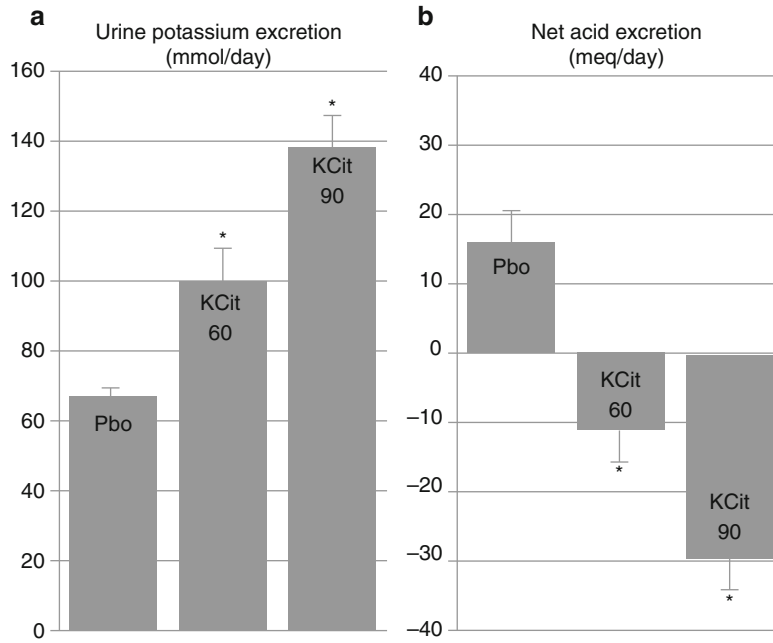

Fig. 10.1 Urine potassium and net acid excretion after 6 months of potassium citrate supplementation. Panel (**a**): Twenty-four-hour urine potassium excretion at the end of study. Panel (**b**): Twenty-four-hour net acid excretion at the end of study. *Pbo* placebo, *KCit60* potassium citrate 60 mmol/day, *KCit90* potassium citrate 90 mmol/day. *$p<0.05$ vs. placebo. Values are mean ± SE

two-thirds of the participants were Caucasian women. Subjects were 64.5 ± 4.9 years of age with baseline 25 hydroxyvitamin D levels averaging 32.7 ± 9.8 ng/mL. With potassium supplementation, urine potassium excretion significantly increased and net acid excretion significantly decreased, commensurate with the ingested dose of potassium and citrate (Fig. 10.1). Urine potassium excretion in both potassium-treated groups was significantly higher than placebo. In addition, subjects on potassium citrate 90 mmol/day had significantly higher urine potassium values than those on 60 mmol/day (138.6 ± 13.6 mmol/day vs. 100.6 ± 9.6 mmol/day, respectively; $p=0.05$). One participant developed mild hyperkalemia to 5.4 mmol/L and was removed from the study after approximately 4 weeks of potassium supplementation. As the potassium dose was initiated at 10 mmol/day and escalated to the full study dose over 9 weeks, this subject was consuming 40 mmol/day at the time of study withdrawal. In the remainder of the subjects, serum potassium remained unchanged at any dose of potassium citrate or placebo at 6 months, averaging 4.2 ± 0.1 mmol/L in the placebo group, 4.3 ± 0.1 mmol/L in the 60-mmol/day group, and 4.3 ± 0.1 mmol/L in the 90-mmol/day group.

Both potassium groups demonstrated a significant reduction in 24-h urine calcium (Table 10.1). Twenty-four-hour urine calcium excretion in participants taking potassium citrate 60 mmol/day was reduced by 46 ± 15.9 mg/day, and among participants taking 90 mmol/day urine, calcium was reduced by 59 ± 31.6 mg/day compared to baseline ($p<0.005$ for both comparisons). There were no significant changes in fractional calcium absorption with potassium citrate 60 or 90 mmol/day when compared with placebo. Participants taking 90 mmol/day of potassium citrate for 6 months had a significant improvement in calcium balance compared with participants taking placebo (142 ± 80 mg/day in the 90 mmol/day treatment group vs. −80 ± 54 mg/day in the placebo group; $p<0.05$, Table 10.1). Calcium balance improved by 33 ± 66 mg/day in those subjects on potassium citrate 60 mmol/day, but this did not reach statistical significance ($p=0.18$). Intact PTH significantly decreased in subjects taking potassium citrate 90 mmol/day ($p=0.01$), but there were no significant changes in 1,25-dihydroxy vitamin D levels during potassium citrate treatment (Table 10.1).

Serum C-telopeptide, a marker of bone resorption, decreased significantly in both potassium

Table 10.1 Change in calcium metabolism and calcitropic hormones from baseline to 6 months during treatment with placebo ($n=18$), potassium citrate 60 mmol/day ($n=17$), or potassium citrate 90 mmol/day ($n=17$) in 52 men and women over age 55 years

	Pbo	KCit60	KCit90
Change in urine Ca (mg/day)	25.0 ± 12.9	−46.0 ± 15.9[a]	31.6
Change in fractional calcium absorption (%)	1.5 ± 3.0 %	−0.2 ± 2.0 %	−0.1 ± 2.2 %
Change in calcium balance	−80 ± 53.7	33.4 ± 65.5	141.5 ± 79.8[b]
Change in iPTH (pg/mL)	2.4 ± 2.3	−2.6 ± 4.4	−8.3 ± 3.2[a]
Change in 1,25 OH D (pg/mL)	−5.5 ± 3.4	4.5 ± 4.5	−3.3 ± 3.7

Values are mean ± SE. Statistical significance indicated by superscripted letters. *Pbo* placebo, *KCit60* potassium citrate 60 mmol/day, *KCit90* potassium citrate 90 mmol/day. [a]$p<0.01$ vs. placebo. [b]$p=0.02$ vs. placebo

Table 10.2 Change in bone turnover markers from baseline to 6 months for the 52 men and women over age 55 years randomized to placebo ($n=18$), potassium citrate 60 mmol/day ($n=17$), or potassium citrate 90 mmol/day ($n=17$)

	Placebo	K Citrate 60 mmol/day	K Citrate 90 mmol/day
Change in bone specific alkaline phosphatase (μg/L)	−0.95 ± 0.8	−1.1 ± 0.9	−1.8 ± 0.8
Change in serum C-telopeptide (ng/L)	44.0 ± 27.6	−71.6 ± 40.7[a]	−34.6 ± 39.1[a]

Values are mean ± SE. Statistical significance indicated by superscripted letters. [a]$p<0.05$ vs. placebo

citrate treated groups, while bone specific alkaline phosphatase, a marker of bone formation, was unchanged (Table 10.2).

Discussion

Nutrition interventions such as calcium and vitamin D have been shown to beneficially impact skeletal health [22]. Altering the acid–base composition of the diet represents another potentially beneficial nutrition intervention. Nutrition interventions such as these are attractive given the low cost, wide availability, and safety of these nutrients. The relationship between the dietary components that generate acid and the skeleton is complex. Much of the epidemiologic research examining the potential role of dietary acid has focused on the role of dietary protein or specifically animal protein. For example, the Nurse's Health Study found a 21 % increase in forearm fracture over 12 years of follow-up among women aged 35-59 in the highest quintile of both total and animal protein [23]. Worldwide, per capita consumption of animal protein is associated with an increased risk of hip fracture in women over age 50 [24]. However, the relationship between dietary protein intake and the skeleton is complex. The metabolism of dietary protein provides dietary acid, but adequate protein is critically important for achieving peak bone mass and maintaining skeletal health. Additionally, hunter-gatherer diets are net base producing despite containing two to three times as much protein as typical Western diets because these paleolithic style diets also contain much higher amounts of vegetable foods and lack cereal grains [25]. It is the net balance between the acid and base generating components of the diet that determines the net dietary acid load. To examine both the acid and base aspects of dietary intake rather than simply protein intake, we examined the ratio between animal and vegetable protein and skeletal outcomes in a previous study. In this

cohort of postmenopausal women, we demonstrated that diets with high animal to vegetable protein intake ratios are associated with an increased rate of femoral neck bone loss and hip fracture [26], supporting the hypothesis that the balance between acid and base generating components of the diet can have a long-term impact on the skeleton.

A low acid diet could be generated by either decreasing the dietary acid precursors or by increasing dietary base precursors. Protein is a major constituent of bone and has known anabolic effects on bone [27]. Additionally, protein supplements have been shown to speed recovery after hip fractures [28]. However, these effects may or may not be realized depending on the magnitude of the countervailing catabolic influences on bone from the prevailing diet net acid load, which depend not only on the protein intake but on relative total intakes of acid and base precursors from all dietary sources. Thus, the preferred strategy to lowering the net dietary acid load would be to increase intake of base rather than to reduce dietary protein.

Short-term studies over the past several decades have demonstrated beneficial effects on calcium balance and skeletal metabolism, but substantial controversy remains. Two short-term calcium balance studies both demonstrated a reduction in urine calcium with no change in fecal calcium, resulting in a net improvement in calcium balance [8, 9]. While neither of these balance studies directly measured intestinal fractional calcium absorption, fractional calcium absorption as measured by dual stable calcium isotopes was not affected by potassium citrate supplementation in a separate study [10]. As the studies to date have been small and short term, the net effect of potassium-containing foods (fruits, vegetables, dairy) on calcium metabolism in the long term has remained controversial. In a cross-sectional study of women consuming diets similar to their typical self selected diet, dietary potassium intake was associated with lower urine calcium excretion, but also with lower intestinal calcium absorption [13], leading to speculation that adaptation to increased dietary potassium intake occurs with time. Under this theory, while urine calcium remains lowered, calcium absorption is also decreased, resulting in no significant net benefit on the calcium economy in the long term. Of note, in this cross-sectional analysis, however, dietary potassium intake was from meat and milk sources rather than fruits and vegetables. Thus, dietary potassium intake may not have reflected base intake in this study, unlike the short-term balance studies that utilized alkaline potassium salts.

In our recent trial, we demonstrated that oral administration of potassium citrate results in a long-term, positive effect on calcium balance. Even after 6 months of supplementation, urine calcium was significantly reduced in both potassium treated groups while intestinal calcium absorption was unchanged, leading to a net improvement in calcium balance in both treatment groups that reached statistical significance in the potassium citrate 90 mmol/day group. Further, net acid excretion was significantly lower among participants taking potassium citrate 90 mmol/day compared to those taking 60 mmol/day. These metabolic findings would suggest that potassium citrate 90 mmol/day may be the best dose to study in trials examining definitive skeletal outcomes such as bone density and fracture.

Markers of bone resorption were also reduced in our trial, similar to previous studies [9, 15, 18, 29]; however, this finding is not universal in the literature and several studies of alkaline potassium salts have not shown effects on markers of bone resorption [10, 16, 17]. Overall, our study supports the hypothesis that alkaline potassium salts can have a sustained beneficial impact on the skeleton. Previous studies have demonstrated a persistence of the urine calcium lowering effects to at least 3 years [12]. In our trial, improvements in calcium balance were also maintained through 6 months, supporting the need for long-term studies with bone density as the outcome.

Despite two previous trials, one positive and one negative, that had bone density as an out-

come, no definitive conclusions can be made. The first study utilized an active comparator and did not have a placebo group, making it difficult to ascertain the effect of the potassium citrate intervention [16]. The second study showed no difference in bone density after 24 months of potassium citrate or fruit and vegetable supplementation compared to placebo in postmenopausal women [17]. However, urine calcium excretion in this study was not decreased by potassium supplementation in this study. The ability of alkaline potassium salts to lower urine calcium is a well described and reproducible finding, raising questions regarding the effectiveness of the specific potassium compound used in this study. Additionally, the two bone density studies done to date utilized doses of potassium citrate (18.5–55.5 mmol/day) that, based on our study, may have been too low to impact calcium balance in the long term. Our study demonstrated a dose response for potassium citrate for many parameters and, importantly, the improvement in calcium balance at 6 months was statistically significant only in the 90 mmol/day group.

Similar to previous studies, in our study, potassium citrate was well tolerated with no gastrointestinal intolerance. One study participant in our trial became mildly hyperkalemic on a relatively small dose of potassium citrate, only 40 mmol/day. We have conducted numerous studies utilizing potassium supplements with no previous episodes of hyperkalemia. Nonetheless, this does emphasize that, in future studies, participants' serum potassium will require monitoring with potassium citrate supplementation.

One important limitation to our study is that subjects were specifically recruited for baseline higher calcium values. We selected these individuals as interventions such as potassium citrate that lower urine calcium excretion may be of limited utility in individuals who already have a low urine calcium excretion. However, this does limit the generalizability of these findings, and selection criteria for larger, long-term studies with definitive skeletal outcomes will have to be selected carefully.

Consistent with previous short-term calcium balance studies, our recent trial demonstrates that persistent benefits to calcium metabolism occur during longer-term potassium citrate supplementation. After 6 months of supplementation, urine calcium remained lowered compared to placebo with no evidence of a compensatory decrease in intestinal calcium absorption. Our findings of longer-term improvements in calcium balance and reduction in bone resorption markers suggest that long-term utilization of potassium supplementation could lead to sustained benefits for the skeleton. Like calcium and vitamin D, potassium citrate is widely available, inexpensive, and has an excellent safety profile. In addition, the amounts of potassium citrate provided in our study are readily consumed in the form of increased fruit and vegetable consumption, making this potential intervention applicable on a population-wide basis. There have been two previous bone density studies with conflicting results and significant limitations. Our data support the development of a long-term potassium citrate intervention trial with a sufficient dose of potassium citrate and definitive skeletal outcomes such as bone density, bone structure, and fracture. Alkalinizing potassium salts have the potential to significantly impact the increasing threat osteoporosis poses to aging men and women consuming the typical net acid producing diets of Western societies.

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References

1. Cooper C. The problem – health impact of osteoporosis. Scand J Rheumatol. 1996;25 Suppl 103:3–5.
2. Campion JM, Maricic MJ. Osteoporosis in men. Am Fam Physician. 2003;67(7):1521–6.
3. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004;291(16):1999–2006.
4. Adler S, Lindeman RD, Yiengst MJ, et al. Effect of acute acid loading on urinary acid excretion by the aging human kidney. J Lab Clin Med. 1969;72:278–89.
5. Agarwal BN, Cabebe FG. Renal acidification in elderly subjects. Nephron. 1980;26:291–5.
6. Frassetto L, Morris Jr RC, Sebastian A. Effect of age on blood acid–base composition in adult humans: role of age-related renal functional decline. Am J Physiol. 1996;271:1114–22.
7. Hilton Jr JG, Goodbody M, Kruesi OR. The effect of prolonged administration of ammonium chloride on the blood acid–base equilibrium of geriatric subjects. J Am Geriatr Soc. 1955;3:697–703.
8. Lemann Jr J, Gray RW, Pleuss JA. Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney Int. 1989;35:688–95.
9. Sebastian A, Harris ST, Ottaway JH, et al. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med. 1994;330:1776–81 [see comments].
10. Sakhaee K, Maalouf NM, Abrams SA, Pak CY. Effects of potassium alkali and calcium supplementation on bone turnover in postmenopausal women. J Clin Endocrinol Metab. 2005;90(6):3528–33.
11. Ceglia L, Harris SS, Abrams SA, et al. Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption. J Clin Endocrinol Metab. 2009;94(2):645–53.
12. Frassetto L, Morris Jr RC, Sebastian A. Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. J Clin Endocrinol Metab. 2005;90(2):831–4.
13. Rafferty K, Davies KM, Heaney RP. Potassium intake and the calcium economy. J Am Coll Nutr. 2005;24(2):99–106.
14. Marangella M, Di SM, Casalis S, et al. Effects of potassium citrate supplementation on bone metabolism. Calcif Tissue Int. 2004;74(4):330–5.
15. Dawson-Hughes B, Harris SS, Palermo NJ, et al. Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women. J Clin Endocrinol Metab. 2009;94(1):96–102.
16. Jehle S, Zanetti A, Muser J, et al. Partial neutralization of the acidogenic Western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia. J Am Soc Nephrol. 2006;17(11):3213–22.
17. MacDonald HM, Black AJ, Aucott L, et al. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. Am J Clin Nutr. 2008;88(2):465–74.
18. Sellmeyer DE, Schloetter M, Sebastian A. Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. J Clin Endocrinol Metab. 2002;87:2008–12.
19. Karp HJ, Ketola ME, Lamberg-Allardt CJ. Acute effects of calcium carbonate, calcium citrate and potassium citrate on markers of calcium and bone metabolism in young women. Br J Nutr. 2009;102(9):1341–7.
20. Yergey AL, Abrams SA, Vieira NE, et al. Determination of fractional absorption of dietary calcium in humans. J Nutr. 1994;124:674–82.
21. Lee W, McCabe GP, Martin BR, Weaver CM. Validation of a simple isotope method for estimating true calcium fractional absorption in adolescents. Osteoporos Int. 2011;22(1):159–66.
22. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet. 2007;370(9588):657–66.
23. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. Protein consumption and bone fractures in women. Am J Epidemiol. 1996;143:472–9.
24. Abelow BJ, Holford TR, Insogna KL. Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. Calcif Tissue Int. 1992;50:14–8.
25. Sebastian A, Frassetto LA, Sellmeyer DE, et al. Estimation of the net acid load of the diet of ancestral preagricultural *Homo sapiens* and their hominid ancestors. Am J Clin Nutr. 2002;76(6):1308–16.
26. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. Am J Clin Nutr. 2001;73(1):118–22.
27. Dawson-Hughes B. Interaction of dietary calcium and protein in bone health in humans. J Nutr. 2003;133(3):852S–4S.
28. Bonjour JP. Protein intake and bone health. Int J Vitam Nutr Res. 2011;81(2–3):134–42.
29. Lin PH, Ginty F, Appel LJ, et al. The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults. J Nutr. 2003;133(10):3130–6.

The Effects of Protein Supplementation on Bone Mass in Chinese Postmenopausal Women

11

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Abstract

Background: Sufficient calcium intake is essential for the maintenance of bone health in older people. However, the effect of dietary protein on bone mass of older women has been controversial. To the best of our knowledge, there has been no clinical trial evaluating the effect of protein supplementation on bone mass in older Chinese women.

Objective: To evaluate the effect of 1-year protein and calcium supplementation on bone mass in older Chinese women compared to calcium supplementation alone.

Design: A 1-year randomized controlled trial was conducted in 283 Chinese postmenopausal women aged 68.1 ± 0.5 years (range 60–86 years). Study participants were randomized to receive either protein powder containing 30 g soybean protein and 1,000 mg calcium as calcium carbonate (Pro + Ca group, $n = 142$) or only 1,000 mg calcium per day (Ca group, $n = 141$). Measurements performed include dietary intakes by 1-year food-frequency questionnaire, physical activity by International Physical Activity Questionnaire (IPAQ)-Short Form, and areal bone mineral density (aBMD) at hip, lumbar spine (L2–L4), and total body by DXA at baseline and 1 year later.

Results: There were no significant differences between the two groups in baseline characteristics. With supplementation, both groups had significantly higher calcium intake compared to the baseline (1,647 ± 53 mg/

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day vs. 879 ± 30 mg/day, $P = 0.01$), and the average dietary protein intake was significantly higher in the Pro + Ca group compared to the Ca group (107.8 ± 4.6 g/day vs. 75.7 ± 3.1 g/day, $P < 0.001$).

After 1-year supplementation, there was a slight but significant increase in aBMD at total body, femoral neck, trochanter, and total hip in both groups after adjusting for baseline age, BMI, calcium intake, physical activity level, and serum 25(OH)D level (time effect, all $P < 0.05$). There were no significant time effects on lumbar spine aBMD in either group.

The Pro + Ca group had significantly greater increase in total-body aBMD (9.5 mg/cm2) compared to the Ca group (0.4 mg/cm^2) after 1 year of supplementation before adjustment for covariates (time × group interaction, $P < 0.05$). There were no significant effects of protein supplementation on aBMD of other sites.

Conclusion: Higher intake of dietary protein might have a positive effect on total-body bone mass in Chinese postmenopausal women when calcium intake is sufficient.

Keywords
Protein • Bone mineral density • Calcium • Postmenopausal women

Introduction

The prevalence of osteoporosis has been increasing in recent years because of increases in life expectancy and the number of older individuals in most countries, especially in China. For example, in 2004, osteoporosis and low bone density affected about 12 % of the total population in China [1].

Balanced diet is a basic strategy for the prevention of osteoporosis. Protein is a major component of bone matrix. However, data on the effect of dietary protein on bone mass has been controversial, especially in elderly. A few earlier studies indicated a negative effect of high protein intakes on bone mass [2], whereas most observation studies in elderly population have shown that relatively high protein intakes could reduce bone loss [3–5] and reduce the risk of hip fracture in elderly women [6].

Moreover, it was observed that the effect of protein on bone health could be related to calcium intake. A cross-sectional study showed that the highest quartile of protein intake (mean intake = 72 g/day) was associated with higher BMD in elderly women only when the calcium intake exceeded 408 mg/day [7].

Most studies evaluating the effect of protein intake on bone mass were carried out in western populations. It is well known that calcium intakes and bone structure are different to some extent between Chinese and westerners. To the best of our knowledge, there has been no published clinical trial with protein supplementation in Chinese older women. Thus, the objective of this study was to evaluate the effect of protein supplementation on bone mineral density in Chinese postmenopausal women who also received 1,000 mg calcium per day.

Subjects and Methods

Study Design

A 1-year, randomized, placebo-controlled trial of calcium and calcium plus protein in Chinese older women.

Participants

The study subjects were 283 Chinese women aged over 65 years. They were recruited by mail or poster in community in Beijing, China. The inclusion criteria were (A) Chinese women over

the age of 60 years and (B) living in Beijing during intervention period. The exclusion criteria were (A) fracture within 6 months of screening; (B) patient with bone disease in the last 12 months; (C) previous osteoporosis treatments in the last 12 months except for calcium supplementation; (D) patient with liver or kidney disorder or other endocrinosis, such as diabetes; (E) significant prior neuromuscular disorders that impair balance; (F) oral corticosteroids in the last year; (G) patients with serious, uncontrolled disease likely to interfere with the study and/or likely to cause death within the study duration; and (H) participation in another clinical trial during the last half year. The investigator explained benefits and risks of participation in the study to each subject during the interview. A written informed consent form was signed by each subject prior to the initiation of nonroutine tests at baseline survey. The study was approved by the ethics committees of the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Protein Supplements

A researcher who was not part of the study group was in charge of randomization. The randomization used block size of ten using random numbers. The 283 subjects were randomized into two groups:

- Calcium group, $n=142$, supplied with three calcium carbonate tablets, containing total of 1,000 mg calcium per day
- Calcium + protein group, $n=141$, supplied with two packets of powder containing total of 30 g soybean protein and 1,000 mg calcium per day

All supplementation were produced and packaged by the same factory. A researcher outside of the study group dispensed calcium tablets in bottles or protein and calcium powder in satchels to study participants every 3 months. The supplement was instructed to be taken with meal. A record form for taking supplementation was also delivered to all subjects with supplementation. During the supplementation period, the study staff contacted each subject every month to record the number of supplements taken and to promote compliance. The remaining bottles and satchels were collected and counted at the end of the study to calculate compliance.

Bone Measurements

Bone mineral density of spine (L2–L4), proximal femur (femoral neck, trochanter, and total hip), and total body were measured by dual-energy X-ray absorptiometry, using a Norland XR-46 densitometer in pencil-beam mode (Norland Medical Systems, Inc., Fort Atkinson, WI, USA) with software version 3.94 at baseline and the end of the study. Two experienced technicians performed the measurements throughout the study. The DXA had a variation in precision of <1.0 % for the measured bone sites at standard speed. A daily quality assurance test was performed with a manufacturer-supplied hydroxyapatite phantom, and the accuracy error was <1.0 %.

Biochemistry

Serum 25-hydroxyvitamin D [25(OH)D] concentration in fasting blood samples was determined by radioimmunoassay (RIA, DiaSorin Inc., Stillwater, MN, USA) in duplicates. All samples were measured in one laboratory. The interassay coefficient was 7.80 % at 18.9 ng/ml, with interassay and intra-assay coefficients of variation (CVs) of 11.1 and 8.8 %, respectively.

Dietary Intakes

Dietary intakes were assessed by using a 51-item food-frequency questionnaire, which was adopted from the questionnaire used in the 2002 China National Nutrition and Health Survey [8]. Nutrient intakes were calculated using the Chinese Food Composition Tables published in 2002 [9] and 2004 [10].

Other Assessments

Anthropometry measurements, including height and weight, were performed with subjects in light clothes and without shoes. Physical activity (PA) level was estimated using the Chinese version of the International Physical Activity Questionnaire (IPAQ)-Short Form. Total physical activity level (METs/week) was calculated and divided as high (score = 3), moderate (score = 2), and low level (score = 1).

Statistical Analysis

The repeated measurement for variables was analyzed by Mixed Linear Model for continuous variables (such as bone mineral density) in SAS program, version 9.0, with or without adjustment for body mass index (BMI), calcium intake, serum 25(OH)D, age, and other potential confounders. The supplement × time interaction was tested, and significant interaction indicates significant effect of supplementation. *P* value less than 0.05 was considered as statistically significant difference.

Results

In 283 subjects who participated in the protein intervention trial, 190 subjects were reexamined at the end of the intervention. No significant difference in dropout rates was observed between the calcium plus protein group and the calcium group (36.9 and 28.9 %, respectively, $P=0.40$). At baseline, there were no significant differences in age, height, BMI, protein and other nutrients intakes, and bone measures between the subjects who completed the study and those who did not (data were not shown).

Baseline characteristics are given in Table 11.1 and were not significantly different between the protein plus calcium and the calcium group ($P>0.1$ for all). Their mean calcium intake (879 ± 30 mg/day) was below the recommended intake level of 1,000 mg/day, and protein intake (72.0 ± 2.2 g/day) was slightly higher than the recommended intake level of 65 g/day published by Chinese Nutrition Society [11]. With supplementation, both groups had significantly higher calcium intake compared to the baseline (1,647 ± 53 mg/day, $P=0.01$), and the average dietary protein intake was significantly higher in the Pro + Ca group compared to the Ca group (107.8 ± 4.6 g/day vs. 75.7 ± 3.1 g/day, $P<0.001$).

There were no significant differences between groups in the bone mineral density (BMD) of total body, femoral neck, trochanter, total hip, and lumbar spine at baseline ($P>0.05$ for all). After 1-year supplementation, aBMD at total body, femoral neck, trochanter, and total hip increased slightly but significantly in both groups after adjusting for baseline age, BMI, calcium intake, physical activity level, and serum 25(OH) D level (time effect, all $P<0.05$). There were no significant time effects on lumbar spine aBMD before and after adjustment (Table 11.2).

The Pro + Ca group had significantly greater increase in total-body aBMD (9.5 mg/cm^2) compared to the Ca group (0.4 mg/cm^2) after 1 year of supplementation before adjustment and still after adjustment for covariates (time × group interaction, all $P<0.05$). There were no significant effects of protein supplementation on aBMD of other sites (Table 11.2).

Table 11.1 Characteristics of participants in calcium and calcium plus protein groups at baseline and 1 year[a]

	Baseline		1 year	
	Calcium	Calcium + protein	Calcium	Calcium + protein
	($n=142$)	($n=141$)	($n=101$)	($n=89$)
Age (year)	68.1 ± 0.5	68.2 ± 0.5	69.0 ± 0.6	69.2 ± 0.5
Height (cm)	153.8 ± 0.5	154.1 ± 0.4	154.0 ± 0.5	154.1 ± 0.5
Weight (kg)	61.7 ± 0.8	62.9 ± 0.8	61.3 ± 1.0	63.3 ± 1.0
BMI (kg/m^2)	26.1 ± 0.3	26.5 ± 0.3	25.8 ± 0.4	26.7 ± 0.4
Dietary intake				
Protein (g/day)	71.9 ± 2.4	72.0 ± 2.2	75.7 ± 3.1[b]	107.8 ± 4.6[b,c]
Calcium (mg/day)	853 ± 31	906 ± 29	1589 ± 48.0[b]	1713 ± 56.5[b,c]
Serum 25(OH)D (ng/ml)	15.9 ± 0.89	15.6 ± 0.56	14.5 ± 0.73	16.8 ± 1.05
PA score	2.6 ± 0.05	2.8 ± 0.04^	2.5 ± 0.06	2.8 ±0.05^

[a]Data were Mean ± SE and analyzed with linear mixed model
[b]Significant difference from the baseline, $P<0.05$
[c]Significant difference from that of calcium group, $P<0.05$

Table 11.2 Estimated marginal means of bone mineral density at baseline and 1 year (g/cm2) adjusted for age, BMI, calcium intake, physical activity, serum 25(OH)D[a]

	Baseline		1 year	
	Calcium	Calcium + protein	Calcium	Calcium + protein
	($n = 142$)	($n = 141$)	($n = 101$)	($n = 89$)
Total body	0.824 ± 0.007	0.829 ± 0.009	0.828 ± 0.009	0.852 ± 0.012[b,c]
Total hip	0.767 ± 0.001	0.775 ± 0.011	0.771 ± 0.012[b]	0.781 ± 0.014[b]
Femoral neck	0.679 ± 0.009	0.688 ± 0.010	0.682 ± 0.010[b]	0.699 ± 0.013[b]
Trochanter	0.569 ± 0.009	0.579 ± 0.009	0.579 ± 0.01[b]	0.588 ± 0.011[b]
Lumbar spine	0.894 ± 0.014	0.932 ± 0.017	0.893 ± 0.017	0.964 ± 0.022

[a]Data were mean ± SE and analyzed with linear mixed model
[b]Significant difference from the baseline, $P < 0.05$
[c]Significant interaction between group and time, $P < 0.05$

Discussion

The present study showed that after protein and calcium supplementation for 1 year, protein supplementation with calcium led to a significant increase in total-body BMD, compared to subjects who consumed only calcium supplement. These results suggest that at similar high level of calcium intake, higher protein intake contributed to a higher total-body BMD.

Protein could provide amino acids as substrates for building bone matrix; therefore, adequate protein intake is important for the maintenance of bone mass in the elderly. A number of cross-sectional and longitudinal studies with older subjects have shown that relatively high protein intakes were associated with reduced bone loss [2, 3, 5]. For example, the Framingham Osteoporosis Study showed that participants in the two lowest quartiles of protein intake (<67 g/day) had greater bone loss at the femoral neck compared to those in the highest quartile (>84 g/day) [3]. In this intervention study in Chinese postmenopausal women, a higher protein intake with calcium had positive effect on total-body BMD after 1 year. Our results extend the findings of observational studies of postmenopausal women that suggest beneficial effects of higher protein intake on BMD.

In a recent study in older Western Australian women, 30 g extra protein per day did not affect change in bone density or strength over 2 years [12]. A possible reason for the lack of effect in older Western Australian women was their relatively high usual dietary protein intake, 1.1 g/kg body weight/day at baseline, which is well above the Australian EAR of 0.75 g/kg body weight/day for older women [13]. In the present study in Chinese older women, the mean protein intake at baseline was 1.1 g/kg body weight/day, which is slightly less than the RNI for Chinese older people of 1.27 g/kg body weight/day [11]. The protein RNI for Chinese is higher than that for western countries because Chinese consume more plant-based protein.

Several studies have indicated that the effect of protein intake on bone mass in the elderly could be influenced by calcium intake [7, 14]. A prospective study showed that higher protein intake was significantly associated with a favorable 3-year change in total-body BMD in the supplemented group with a mean calcium intake of 1,346 mg/day but not in the placebo group with a mean calcium intake of 871 mg/day [14]. Therefore, sufficient calcium intake is important for the beneficial effect of protein on bone mass.

A limitation of the present study was that the assessment of bone mass with dual-energy X-ray absorptiometry, which cannot evaluate the changes in bone geometry and volumetric density. Another limitation of the present study was that we did not have another control group with low calcium and high protein intake, so we cannot evaluate the effect of higher protein intake on bone health in Chinese older women with lower calcium intake.

In conclusion, the present study showed that 1-year protein supplementation with sufficient calcium intake would benefit total-body bone mineral density, but not other skeletal sites in

Chinese postmenopausal women. Both calcium and protein nutrition are important for bone health in Chinese postmenopausal women.

References

1. Zhao D, Wu H, Liu Z. The epidemiology of osteoporosis in China. Chin J Osteoporos. 2004;10:614–8.
2. Pannemans D, Schaafsma G, Westerterp K. Calcium excretion, apparent calcium absorption and calcium balance in young and elderly subjects: influence of protein intake. Br J Nutr. 1997;77:721–9.
3. Hannan M, Tucker K, Dawson-Hughes B, Cupples L, Felson D, Kiel D. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000;15:2504–12.
4. Promislow J, Goodman-Gruen D, Slymen D, Barrett-Connor E. Protein consumption and bone mineral density in the elderly: the Rancho Bernardo Study. Am J Epidemiol. 2002;155:636–44.
5. Devine A, Dick I, Islam A, Dhaliwal S, Prince R. Protein consumption is an important predictor of lower limb bone mass in elderly women. Am J Clin Nutr. 2005;81:1423–8.
6. Wengreen H, Munger R, Cutler D, Corcoran C, Zhang J, Sassano N. Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. J Bone Miner Res. 2004;19:537–45.
7. Rapuri P, Gallagher J, Haynatzka V. Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. Am J Clin Nutr. 2003;77:1517–25.
8. Wang DL. Report of China National Nutrition and Health Survey in 2002 I: total report. Beijing: Public Health; 2005.
9. Yang Y, Wang G, Pan X. China food composition 2002. Beijing: Peking University Medical Press; 2002.
10. Yang Y. China food composition 2004-book 2. Beijing: Peking University Medical Press; 2004.
11. Chinese Nutrition Society. Chinese DRIs. Beijing: Chinese Light Industry Press; 2000.
12. Zhu K, Meng X, Kerr D, Devine A, Solah V, Binns C, et al. The effects of a two-year randomised controlled trial of whey protein supplementation on bone structure, IGF-I and urinary calcium excretion in older postmenopausal women. J Bone Miner Res. 2011;26(9):2298–306.
13. National Health and Medical Research Council. Dietary guidelines for protein intake affects bone mass 1427. Canberra: Australian Government Publishing Service; 1991.
14. Dawson-Hughes B, Harris S. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr. 2002;75:773–9.

The Negative Effect of a High-Protein–Low-Calcium Diet

12

Peter Burckhardt

Abstract

The interdependent influence of the protein and the calcium intake on bone health has been conclusively studied. For obtaining a positive bone effect from nutritional protein, an adequate calcium intake is required, and vice versa. A high-protein intake was first considered as potentially negative for bone, but this fear was not defendable anymore when it became evident that a high-protein intake increases urinary calcium excretion because it stimulates calcium absorption. Protein deficiency was shown to be detrimental to bone, not a high-protein intake. However, some large follow-up studies demonstrated that the combination of a high-protein intake with a low-calcium diet increases fracture risk. This particular nutritional profile seems to be rare, but some cross-sectional studies seem to confirm that.

Keywords

Protein intake • Protein/calcium ratio • Fracture risk

Introduction

At the first International Symposium on Nutritional Aspects of Osteoporosis (ISNAO) in 1992, E.S. Orwoll stated "… weather a relatively high intake of protein influences mineral and bone metabolism remains controversial," and "… commonly consumed diets, replete in phosphate but low in calcium, may be associated with potentially harmful metabolic changes" [1]. Although the eventual negative bone effect of a high protein stemmed mainly from animal studies, the doubt remained, but was already linked to a low-calcium diet. At the sixth ISNAO in 2006, R.P. Heaney recalled that "… protein-related benefit is dependent upon an adequate calcium intake" [2], and at the seventh ISNAO in 2009, A.L. Darling summarized her review on protein effects with the alarming sentence "high calcium intakes may offset any detriment caused by high protein intake, and low calcium intakes may make protein-induced detriment worse" [3].

This was formulated as a hypothesis. The purpose of this study is to gather the evidence for

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this statement. It will not discuss the various effects of dietary protein, such as providing substrates for bone matrix, stimulating IGF-1, and increasing calcium absorption and urinary calcium excretion [4], and it will not review the evidence for the various benefits of an adequate protein intake, such as higher BMD, slower bone loss, and smaller fracture risk in postmenopausal women and elderly people. Nor will it discuss the still controversial observation that high-protein intake may affect bone via acid load [5]. But it will analyze the literature in search of an answer to the question if a high-protein–low-calcium diet is detrimental to bone.

Definition of a High-Protein Intake

The impressive number of studies on the bone effects of dietary protein intake reviewed by [6] allows comparing the protein intakes recorded in the various studies (Fig. 12.1). For this comparison, all values were indicated in g/kg. When the total intake was indicated in g/day and the body weight was not given, a body weight (BW) of 70 kg was assumed. It appeared in general the protein intake was about 1 g/kg in most of the studies, with a lowest value at 0.8 g/kg, although the RDA is in general set at 0.8 g/kg. The three studies close or below 0.8 g/kg concerned vegetarians [7], or subjects with a high intake of vegetable proteins and for that with a relatively low total protein intake [8], and a study based on non-dairy proteins only [9]. Since there is no definition of a high-protein intake, the upper limit of "normal" or "adequate" could be arbitrarily fixed, for reasons of symmetry, at 1.2 g/kg, considering an intake above this figure as high, not as abnormal or inadequate.

Definition of the Optimal Calcium/Protein Ratio

In several studies, the ratio calcium intake/protein intake has been used for evaluating the combined effect of calcium and of protein on bone. However, there is no definition of a normal range for the calcium/protein ratio, although this ratio is used as a parameter in many studies. In order

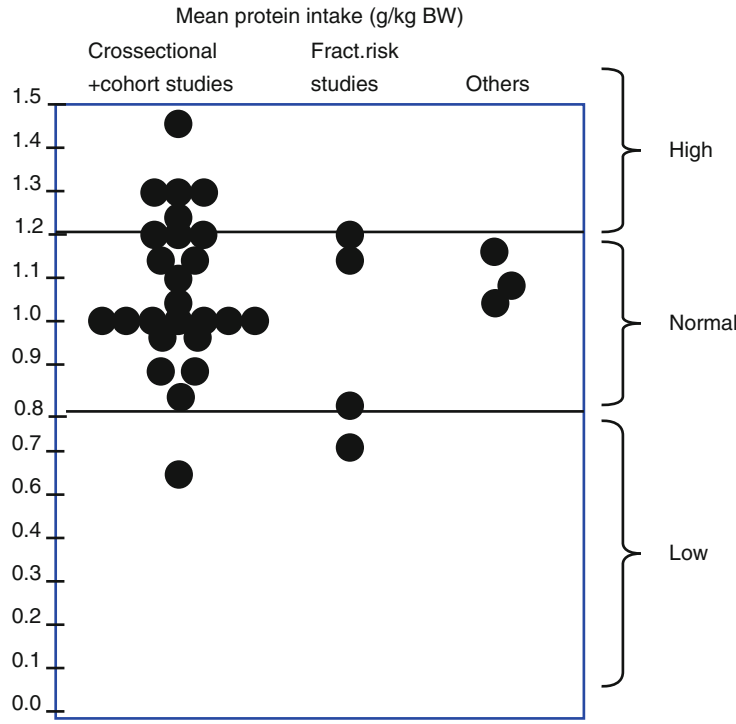

Fig. 12.1 Mean protein intake from the studies analyzed by Darling et al. When protein intake was given only in g/day, a mean body weight (BW) of 70 kg was taken for calculation of g/kg BW (Based on data from Darling et al. [6])

to define a minimal ratio, one could implement a calcium intake of 800 mg/day and a protein intake of 1.2 g/kg/day, which would result in a calcium/protein ratio of 11.1 mg/g for a BW of 60 kg and 8.3 mg/g for a BW of 80 kg. A ratio of eight could then be considered as the lowest acceptable value, but to the knowledge of this author, no official recommendation has been formulated. For assessing this ratio, one can calculate it for taller and for smaller subjects. For a young white US or a Dutch man with a BMI of 23 kg/m^2, both with a median height of 182 cm, an intake of 1.2 g protein/kg and of 800 mg calcium would give a calcium/protein ratio of 8.8, while the same calculation for a Japanese or Portuguese man (median height 172, resp. 171 cm) would give a ratio of 9.8, resp. 9.9. These values give the wrong impression to be close, but they should also be valid for taller and for smaller subjects. This is not the case. For historical reasons, the calcium recommendations are given in absolute values, while the recommendations for protein intake are adapted to BW. This explains why the same calculation using the same intake of calcium and of protein, performed for a tall US man at percentile 95, results in a ratio of 8.1 and for a small Japanese women at percentile 5 in a ratio of 13.4. This important variation of the ratio, which depends on BW, makes the ratio unfit for scientific use, unless it is applied to a homogenous population as seen, e.g., in rat experiments. To illustrate this statement, the reported or calculated calcium/protein ratios of the studies reviewed by [6] are presented on a diagram (Fig. 12.2). It becomes evident that the ratio cannot be used as a parameter which helps to evaluate the effects of various protein and calcium intakes.

Analysis of Published Studies in Search of a Negative Effect of a High-Protein–Low-Calcium Intake

Studies with Calcium Isotopes

Four studies from two groups demonstrated that a high-protein intake enhances calcium absorption [10–13]. All used very-high-protein intakes (average values of 1.6–2.1 g/kg), but the average calcium intakes were not or only moderately low (average values 600–800 mg/day). Therefore, no information on the effect of a high-protein–low-calcium intake could be drawn from these studies. But one study [13] came to the conclusion that the increase in calcium absorption might "nearly" compensate the increase in urinary calcium excretion. By that it evokes the possibility

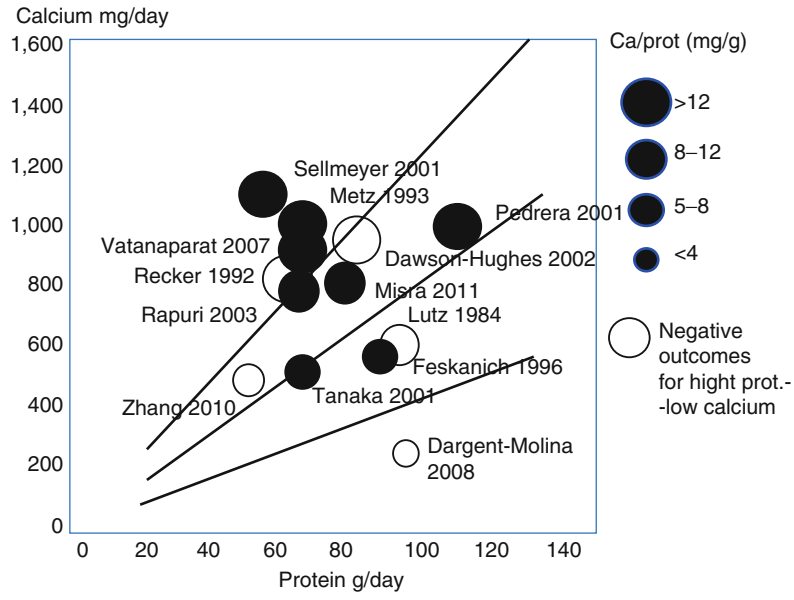

Fig. 12.2 The calcium/protein ratio taken from the studies analyzed by Darling et al. (Based on data from Darling et al. [6])

that a high-protein intake might lead to a negative calcium balance when the calcium intake is inadequately low.

Cross-Sectional and Cohort Studies

None of the numerous studies reviewed by [6] studied specifically the effect of a high-protein–low-calcium diet, neither the more recent study on the relation between protein intake and fracture risk by Misra et al. [14].

A special look on the five studies of Darling's meta-analysis with a protein intake above 1.2 g/kg [15–19] also did not reveal data of subgroup analysis with high-protein–low-calcium intakes, although the three Japanese studies among them reported rather low-calcium intakes (average values 458–660 mg/day). However, one of these studies [19] found a surprising negative correlation between protein intake and radial bone density. Calcium intake was high (±1,001 mg/day), and there was a positive correlation between the calcium/protein ratio and BMC.

The Exception of the Growing Bone

In children and adolescents too, adequate protein intake is essential for bone growth and strength, and the positive response to calcium supplementation is also influenced by the protein intake [20]. But the requirements seem to be different, since the three studies on young adults or peripubertal girls reported a positive correlation between the calcium/protein ratio and BMC or BMD [19, 21, 22]. All three studies showed a negative correlation between protein intake and bone measurements. Since the physiology of growth and development of peak bone mass is different from that of bone loss after menopause and in advanced age, it also can be assumed that the nutritional needs for building up bone are not the same as for preventing bone loss after menopause or in advanced age.

This is also demonstrated in a study of 15–17-year-old female ballet dancers ($N=127$) [23], where food intake was compared with BMC. The intake of nondairy proteins, assessed by portions (servings), was negatively correlated by bivariate analysis with femoral neck BMC ($p=0.008$, coeff. −0.089) and by multivariate analysis ($p=0.045$, coeff. −0.62), while the intake of dairy products was positively correlated with femoral neck BMC by bivariate analysis ($p=0.049$, coeff. 0.083) and by multivariate analysis ($p=0.067$, coeff. 0.069). In addition, dairy products were also positively correlated with lumbar spine BMC by bivariate analysis ($p=0.008$, coeff. 1.84) and by multivariate analysis ($p=0.015$, coeff. 1.69). The multivariate analysis ($p<0.02$) corrected for BW, pubertal stage, years since menarche, and hours of dancing. Even when the Caucasians and the Asians were analyzed separately by Spearman correlations, significant negative coefficients were found between nondairy protein intake and BMAD of the lumbar spine in Caucasians (−0.303, $p<0.05$) and with BMAD of the femoral neck in Asians (−0.301, $p<0.05$). Positive coefficients were found in Caucasians between the intake of dairy products and BMAD of the spine (+0.323, $p<0.01$) and the femoral neck (+0.249, $p<0.05$), while in Asians the coefficient was −0.305 ($p<0.05$) with the lumbar spine.

When the highest tertile of nondairy protein intake was combined with the lowest tertile of dairy intake, the mean Z-scores of lumbar BMD were −1.61 in Caucasians and −1.12 in Asians, compared to −0.57, resp. −004, with the highest tertile of dairy products ($p<0.02$, resp. <0.04). These results show again that in the growing skeleton and in young adults, a high-protein intake combined with a low-calcium intake is in negative correlation to bone mineral content.

Intervention Studies

Intervention studies, where the protein intake was modified, also could be a source of information on the effect of a high-protein–low-calcium diet. Most intervention studies showed no detrimental effect of a high-protein intake on bone metabolism, even when the calcium intake was low [24–28]. But these studies were probably too short for detecting a negative bone effect of a

high-protein–low-calcium intake. Spencer et al. [29] approached this question in her early study but could not deliver statistics on such a particular subgroup from the small number of subjects studied. But Lutz [30] observed that calcium balance became more negative when the protein intake was doubled while the calcium intake was kept low at ±500 mg/day.

Cross-Sectional and Follow-Up Studies with Analysis of High-Protein–Low-Calcium Intake

The studies with and without calcium supplements [31] and the studies with specific analysis of the calcium intake [20, 32] pointed to the importance of an adequate calcium intake for developing a positive effect of protein on bone. In the study of Dawson-Hughes [31], calcium supplementation revealed a positive effect of the protein intake on bone loss in elderly men and women over 3 years, although calcium intake was not low without supplementation (±940 mg/day) and the highest tertile of protein intake was not very high (±87.6 g/day) [32]. Rapuri et al. concluded in their 3 years' follow-up study that high-protein intake was associated with higher BMD only when the calcium intake exceeded 408 mg/day. But whether the combination of high-protein with low-calcium intake was detrimental was not examined. Vatanparast et al. [20] made a similar conclusion, since in their study on young adults, protein intake predicted TB-BMC only in females who had a calcium intake >1,000 mg/day. They even stated "in the absence of sufficient calcium, protein doesn't confer as much benefit to bone" without showing the figures and without examining the issue of a high-protein–low-calcium intake.

Follow-Up Studies with Subgroup Analysis of High-Protein–Low-Calcium Diet

There are finally five studies which approached the question of the effect on bone of a high-protein–low-calcium diet (Table 12.1). The first one [33], based on NHANES 1999–2000, compared the fracture risk in nine groups of postmenopausal women: three tertiles of protein intake and three tertiles of calcium intake. The highest tertile of protein intake (>70 g) with the lowest calcium intake (<400 mg/day) did not show an increased fracture risk, but this group consisted in 43 subjects, which obviously was too small to obtain a reliable estimate of the odds ratio for fractures.

The study of Feskanich et al. [34] based on the Nurses' Health Study, showed in a 12-year follow-up an increase of the risk for forearm fractures by 31 % in the tertile with the highest protein intake (>90 g/day) combined with the lowest tertile of calcium intake (<541 mg/day), but this result was not significant.

In the large French epidemiologic study of Dargent-Molina et al. [35] in more than 35,000 subjects and a mean follow-up of more than 8 years, there was 51 % increase in the RR for fractures in the quartile with highest protein intake combined with quartile of the lowest calcium intake and of 46 % when the protein intake was weight adjusted. This subgroup had a calcium intake of <210 mg and a protein intake of >99.6 g, resp. 1.71 g/kg (extrapolated figures), which lets us assume that these extreme values only concern a small percentage of the population.

The large Norwegian follow-up study in 2,302 men and women over 10–12 years [9] also showed an increased hip fracture risk (+96 %, sign.) in women in the highest quartile of protein intake (>20.6 g nondairy animal protein) and the lowest calcium intake (<435 mg/day), while the same analysis in men (>21.6 g nondairy animal protein and <623 mg calcium) showed an increased RR of 1.67, which however was not significant. Here the proportion of subjects was indicated – 7.4 % of the women and 8.4 % of the men.

The last study, based on the Framingham offspring cohort, came to the same conclusion [36]. This 12-year follow-up study on 2,697 men and women showed that in the subjects with a calcium intake of >800 mg/day, a high-protein intake (tertile median 60 g/day animal protein, calcium intake 1,096 mg/day) lowered the fracture risk by 70 %, while in the subjects with a calcium intake

Table 12.1 Increased fracture risk with high-protein and low-calcium intake

Author	Ref.	Sex Mean age	Follow-up		Calcium mg/day	Protein g/day	 g/kg BW	Ca/prot mg/g	Outcome
Feskanich et al. (1996)	[34]	F 46.5 years	12 years	Tertiles	<541?	>90	>1.15?	<6.0	Forearm fractures
Dargent-Molina et al. (2008)	[35]	F 56 years	8.4 years	Quartiles	<210	>99.9	>1.71	<2.1	Any low-impact fracture
Meyer et al. (1997)	[9]	M+F 47 years	11.4 years	Quartiles	<435	>20.6 nondairy	n.a.	n.a.	Hip fractures
Sahni et al. (2010)	[36]	M+F 55 years	33 years	Tertiles	±517	±60 animal	n.a.	8.6	Hip fractures

of <800 mg (±578 mg/day), the fracture risk was doubled (RR 2.02) in the tertile with the highest protein intake (median 60 g/day). This means that in presence of a sufficient calcium intake, a high-protein diet was protective, while in the presence of a low-calcium diet, a high-protein intake increased the fracture risk.

Conclusion

Normal protein intake is essential for preventing osteoporosis and decreasing fracture risk, especially hip fracture in advanced age. High-protein intake has never been shown to have a negative effect on bone in humans when integrated in an otherwise equilibrated diet. But several large and long-term follow-up studies demonstrated that a high-protein diet combined with a low-calcium intake is detrimental to bone, leading to an elevated fracture risk. Some cross-sectional studies, which analyzed this phenomenon, seem to confirm that. This particular nutritional profile is rare, but its negative impact should be known.

References

1. Orwoll ES. The effect of dietary protein insufficiency and excess on skeletal health. In: Burckhardt P, Heaney RP, editors. Nutritional aspects of osteoporosis. Serono symposia. New York: Raven; 1991. p. 355–70.
2. Heaney RP. Effects of protein on the calcium economy. In: Burckhardt P, Heaney RP, Dawson-Hughes B, editors. Nutritional aspects of osteoporosis 2006, B.V. Internet congress series, vol. 1297. Amsterdam: Elsevier; 2007. p. 191–7.
3. Darling AL, Lanham-New SA. Dietary protein and bone health: the urgent need for large-scale supplementation studies. In: Burckhardt P, Dawson-Hughes B, Weaver C, editors. Nutritional influences on bone health. London: Springer; 2010. p. 17–26.
4. Heaney RP, Layman DK. Amount and type of protein influences bone health. Am J Clin Nutr. 2008; 87(Suppl):1567S–70.
5. Barzel US, Massey LK. Excess dietary protein can adversely affect bone. J Nutr. 1998;128:1051.
6. Darling AL, Millward JD, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 2009;90:1674.
7. Lau EMC, Kwok T, Ho SC. Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. Eur J Clin Nutr. 1998;52:60–4.
8. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Am J Clin Nutr. 2001;73:118–22.
9. Meyer HE, Pedersen JI, Laken EB, Meyer AT. Dietary factors and the incidence of hip fracture in middle-aged Norwegians. A prospective study. Am J Epidemiol. 1997;145:117–23.
10. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein affects intestinal calcium absorption. Am J Clin Nutr. 1998;68:859–65.
11. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab. 2005; 90:26–31.
12. Roughead ZK, Johnson LK, Lykken GI, Hunt JR. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. J Nutr. 2003;133:1020–6.
13. Hunt JR, Johnson LK, Roughead ZKF. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. Am J Clin Nutr. 2009;89:1357–65.
14. Misra D, Berry SD, Broe KE, McLean RR, Cupples LA, Tucker KL, et al. Does dietary protein reduce hip

fracture risk in elders? The Framingham osteoporosis study. Osteoporos Int. 2011;22:345–9.
15. Pedreraa JD, Canala ML, Postigoa S, Lavadoa J, Hernandez ER, Rico H. Phalangeal bone ultrasound and its possible correlation with nutrient in an area of high protein intake. Ann Nutr Metab. 2001;45:86–90.
16. Tanakaa M, Itoha K, Abea S, Imai K, Masuda T, Koga R, et al. Relationship between nutrient factors and osteo-sono assessment index in calcaneus of young Japanese women. Nutr Res. 2001;21:1475–82.
17. Nakamura K, Hori Y, Nashimoto M, Okuda Y, Miyazaki H, Kasai Y, et al. Dietary calcium, sodium, phosphorus, and protein and bone metabolism in elderly Japanese women: a pilot study using the duplicate portion sampling method. Nutrition. 2004;20:340–5.
18. Lacey JM, Anderson JJB, Fujita T, Yoshimoto Y, Kukase M, Tsuchie S, et al. Correlates of cortical bone mass among premenopausal and postmenopausal Japanese women. J Bone Miner Res. 1991;6(7):651–9.
19. Metz JA, Anderson JB, Gallagher Jr PN. Intakes of calcium, phosphorus, and protein, and physical activity level are related to radial bone mass in young adult women. Am J Clin Nutr. 1993;58:537–42.
20. Vatanparast H, Bailey DA, Baxter-Jones ADG, Whiting SJ. Effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. J Nutr. 2007;137:2674–9.
21. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA. 1992;268:2403–8.
22. Zhang Q, Ma G, Greenfield H, Zhu K, Du X, Foo LH, et al. The association between dietary protein intake and bone mass accretion in pubertal girls with low calcium intakes. Br J Nutr. 2010;103:714–23.
23. Burckhardt P, Wynn E, Krief M-A, Bagutti C, Faouzi M. The effects of nutrition, puberty and dancing on bone density in adolescent ballet dancers. J Dance Med Sci. 2011;15(2):51.
24. Shapses S, Robins SP, Schwartz EI, Chowdhury H, et al. Short-term changes in calcium but not protein intake alter the rate of bone resorption in healthy subjects as assessed by urinary pyridinium cross-link excretion. J Nutr. 1995;125:2814–21.
25. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am J Clin Nutr. 2003;78(Suppl):584S–92.
26. Kerstetter JE, Wall DE, O'Brien KO, Caseria DM, Insogna KL. Meat and soy protein affect calcium homeostasis in healthy women. J Nutr. 2006;136(7):1890–5.
27. Maalouf NM, Moe OW, Adams-Huet B, Sakhaee K. Hypercalciuria associated with high dietary protein intake is not due to acid load. J Clin Endocrinol Metab. 2011;96(12):3733–40.
28. Cao JJ, Johnson LK, Hunt JR. A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. J Nutr. 2011;141:391–7.
29. Spencer H, Kramer L, Osis D, Norris D. Effect of a high protein (meat) intake on calcium metabolism in man. Am J Clin Nutr. 1978;31:2167–80.
30. Lutz J. Calcium balance and acid–base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. Am J Clin Nutr. 1984;39:281–8.
31. Dawson-Hughes B, Harris SH. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr. 2002;75:773–9.
32. Rapuri PB, Gallagher JC, Haynatzka V. Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. Am J Clin Nutr. 2003;77:1517–25.
33. Zhong Y, Okoro CA, Balluz LS. Association of total calcium and dietary protein intakes with fracture risk in postmenopausal women: the 1999–2002 National Health and Nutrition Examination Survey (NHANES). Nutrition. 2009;25:647–54.
34. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. Protein consumption and bone fractures in women. Am J Epidemiol. 1996;143:472–9.
35. Dargent-Molina P, Sabia S, Touvier M, Kesse E, Bréart G, Clavel-Chapelon F, et al. Proteins, dietary acid load, and calcium and risk of post-menopausal fractures in the E3N French women prospective study. J Bone Miner Res. 2008;23:1915–22.
36. Sahni S, Cupples A, Mclean RC, Tucker LL, Broe KE, Kiel DP, et al. Protective effect of high protein and calcium intake on the risk of hip fracture in the Framingham offspring cohort. J Bone Miner Res. 2010;25:2770–6.

Prebiotics, Probiotics, Polyunsaturated Fatty Acids, and Bone Health

13

Marlena C. Kruger and Magdalena Coetzee

Abstract

Improvement of peak bone mass in younger age and reducing bone loss in aging are two strategies to reduce the risk for developing osteoporosis. Modulating intestinal calcium absorption by modifying the diet can contribute to improvement of bone mass, and reduction of inflammation during menopause can help reduce the risk of bone loss. Calcium absorption takes place via an active process in the duodenum, modulated by active vitamin D, or by passive paracellular absorption that can take place throughout the intestine. Prebiotics are nondigestible carbohydrates which promote bacterial growth in the colon. Fermentation by the bacteria results in the production of organic acids which reduce the pH in the large intestine and may improve solubility of minerals increasing passive diffusion via the paracellular pathway. Increased cell proliferation and hypertrophy of the colon wall have also been reported, while some authors also report increased expression of calbindin-D9k, the protein responsible for carrying calcium through the intestinal cell. While the mechanism by which probiotics improve calcium absorption has not been proven, it is possible that the mechanism is similar to that of the prebiotics. Another dietary component that can affect intestinal calcium absorption is long-chain polyunsaturated fatty acids (LCPUFA). These have been shown to improve calcium absorption by modulating the action of vitamin D in the intestine, modulating intestinal membrane composition and thereby increasing activity of the membrane pumps responsible for transport of minerals across the basolateral membranes. The omega 3 LCPUFAs also have

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specific effects on bone cells and reduce inflammation which may be of benefit to bone especially during menopause. In addition, LCPUFAs may have a prebiotic effect, modulating gut microflora. The possible contribution of these dietary components to calcium absorption and bone maintenance in rats and younger as well as older adults is presented.

Keywords
Calcium absorption • Prebiotics • Probiotics • Gut microflora • Long-chain polyunsaturated fatty acids • Bone density • Rats • Humans

Introduction

Maximizing peak bone mass during adolescence, in addition to minimizing bone resorption in old age, may be the key to postponing and possibly preventing bone fractures due to osteoporosis, later in life. A key way to accomplish this is through increased calcium intake [1]. Normally, only about 30 % of the dietary calcium is absorbed by the body and deposited in the bones. Improved calcium absorption in the body could raise the balance of calcium retained in the body and could therefore have important consequences on the occurrence of osteoporosis and bone fractures. Calcium absorption can be manipulated using foods to influence the bacterial population in the large intestine, for example, or change transporters in the epithelial cells. The following chapter provides an overview of the role that prebiotics, probiotics, and long-chain polyunsaturated fatty acids (LCPUFA) may play in optimizing intestinal calcium absorption and improving bone health.

Calcium Absorption

The majority of calcium, up to 90 %, is absorbed in the small intestine. There are two distinct processes involved, transcellular uptake which takes place mainly in the upper part of the small intestine and paracellular uptake which takes place throughout the small intestine and can also take place in the colon [2]. When calcium intake is normal or high, the relative amount of calcium absorbed in the duodenum is low with the largest amount being absorbed in the lower half of the small intestine, particularly in the ileum. Transcellular active absorption is upregulated when calcium intake is low and is also high in growing children. With a low dietary calcium intake, the amount absorbed in the duodenum may be larger than with the paracellular pathway [3].

The transcellular pathway requires metabolic energy, and it is dependent on vitamin D. The process can be divided into three steps: entry, intracellular diffusion, and extrusion. The entry across the brush border utilizes several transport proteins, such as transient receptor potential vanilloid (TRPV6). The entry is down an electrochemical gradient, and the channels are not voltage gated [4]. The rate-limiting step is the diffusion of calcium across the intestinal cell. This process is vitamin D dependent and uses calbindin-D_{9k}, which is induced by the active form of vitamin 1,25 dihydroxyvitamin D (1,25 (OH) D_3). The transport of the calcium across the basolateral membrane is via the calcium-magnesium ATPase (PMCA1b), which is also vitamin D dependent and an active process. There is a sodium-calcium exchanger located in the basolateral membrane, but it plays a small role in calcium absorption [2, 3, 5].

Passive calcium absorption, or paracellular diffusion down a chemical gradient, takes place throughout the small intestine. This transport is responsible for most of the calcium absorption when intake is high due to downregulation or saturation of active absorption [2, 3, 5]. Calcium absorption in the colon could contribute up to 10 % of the total calcium being absorbed. In the rat, up to 11 % of calcium absorbed could be via

the colon [3]. Some active calcium absorption can also take place in the colon, as calbindin-D_{9k} is also found in the rat cecum and large intestine [3].

Several studies in rats and humans have been published over the past decade which support an effect of indigestible fiber (prebiotics), intestinal microflora (probiotics), and long-chain polyunsaturated fatty acids (LCPUFA) on intestinal calcium absorption.

Prebiotics

Prebiotics are nondigestible food ingredients, mostly carbohydrates, and these promote bacterial growth primarily in the colon. The fermentation is thought to provide health benefits which include increased mineral absorption. Chicory inulin and oligofructose are the most studied prebiotics [6]. Inulin belongs to the fructan family, which is an important storage carbohydrate found in noticeable amounts in chicory, artichokes, onions, and asparagus. Inulin-type fructans are composed of beta-D-fructofuranoses attached by beta-2-1 linkages. The first monomer of the chain is either a beta-D-glucopyranosyl or beta-D-fructopyranosyl residue. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetable sources [7]. Generally a product with a degree of polymerization (DP) from 2 to 60+ is labeled as inulin, whereas oligofructose which is produced by partially enzymatic hydrolysis of inulin is defined by a DP < 10 [6]. Fructooligosaccharides (FOS) stimulate the growth of bifidobacteria. The luminal bacteria in the large intestine ferment indigestible carbohydrates, and various authors suggested an important correlation between increases in the absorption of minerals and the fermentation of indigestible carbohydrates in the large intestine [5, 8].

Several hypotheses about the mechanisms of action of the prebiotics have been proposed. Indigestible oligosaccharides reach the large intestine intact and are fermented by bacteria in the intestinal lumen. The result is an increase in the production of organic acids such as acetate, propionate, and butyrate, also known as short-chain fatty acids (SCFA) [9]. Due to the synthesis of these acids, the luminal pH drops which may help dissolve insoluble calcium salts in the luminal content and accelerate the passive diffusion of minerals via the paracellular pathway. It is also possible that these short-chain fatty acids (SCFA) contribute directly to the enhancement of calcium absorption via a cation exchange mechanism, by increased exchange of cellular H^+ for luminal Ca^{2+}. Chonan et al. [10] showed that in rats fed with galacto-oligosaccharides (GOS), cecal pH dropped and this was correlated with the highest uptake and retention of calcium. Raschka and Daniel [9] reported that feeding inulin and short-chain FOS lowered cecal pH and increased total cecal calcium, soluble calcium, and ionized calcium. Secondly, absorption of SCFA is accompanied by absorption of minerals. SCFA, especially butyrate, could serve as a fuel for mucosal cells and stimulate cell proliferation, leading to hypertrophy of the colon wall which can lead to enhanced capacity for absorption of minerals [11]. Perez-Conesa et al. [12] reported increased cell density and crypt depth in the distal colon in rats fed with a diet high in galacto-oligosaccharides (GOS) and showed that these parameters correlated with measured calcium absorption. Raschka and Daniel [9], using the Ussing chamber model, reported similar calcium absorption rates in various intestinal segments from control and inulin + FOS-fed rats, but the prebiotic diet did increase cecal surface and wall weight. More recent work reported increased cecal wall weight and reduced cecal pH in rats fed with varying levels of GOS for 8 weeks [13].

Another suggested mechanism is upregulation of active calcium absorption. Raschka and Daniel [9] reported changes in mRNA levels between control and inulin + FOS-fed rats for genes that may be involved in calcium absorption. Increased transcript levels were reported for calbindin as well as the sodium-calcium exchanger, but no effect was observed on the TRPV6. Ohta et al. [14] showed that in rats, FOS diets increased colorectal and cecal calbindin D_{9k}. Figure 13.1 provides a summary of these suggested mechanisms of action by prebiotics.

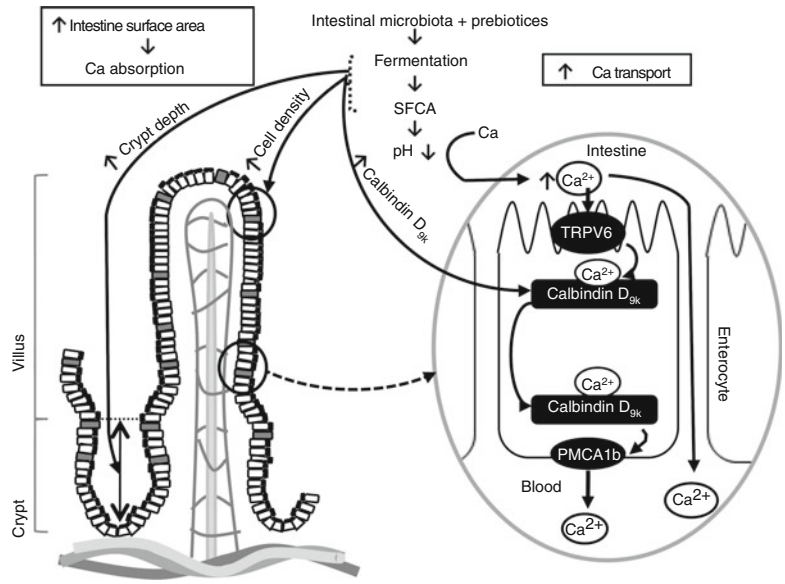

Fig. 13.1 The hypothesized mechanisms of the positive prebiotic effect on calcium absorption. *SCFA* short-chain fatty acid (Reprinted from Parks and Weaver [5]. With permission from Decker Publishing Inc.)

There have been many studies done in rats and in humans investigating the effect of prebiotics on calcium absorption, calcium retention, and bone properties. Some of these are summarized below.

Animal Studies

With regard to calcium balance and absorption, several fibers have been studied: oligofructose [15–21] and several others including inulin [22, 23], galacto-oligosaccharides (GOS) [13, 24, 25], lactulose [26], or resistant starch [27, 28]. In general inulin-type fructans exhibit a dose-dependent effect on calcium absorption. Some studies however have shown a similar increase of about 60 % in apparent calcium absorption in the presence of 5 or 10 % inulin or FOS [26, 29].

Various bone parameters have changed/improved when rats were fed with FOS or inulin. One study in rats showed that the various fructans could have different effects. In this small study, rats were fed with either short-chain FOS (DP2-8), inulin (DP>23), or a mixture of these (92 % inulin/8 % FOS) at 5 % of the diet for 4 weeks. While the fibers did not have a significant effect on calcium balance, ex vivo femur bone density was significantly higher in the group fed with inulin. Urinary excretion of C telopeptide of type I collagen, a marker of bone resorption, was also reduced significantly in the inulin-fed group [23]. Feeding intact rats with 5 % of either GOS or FOS improved bone mineral content (BMC) [25], enhanced bone volume [15], and increased whole-body bone mineral density (BMD) [20]. In ovariectomized rats, FOS prevented osteopenia [15] and improved bone ash weight, calcium content and bone microarchitecture [21].

GOS at varying levels (2–8 % of the diet) significantly decreased cecal pH and increased wall weight and content weight in a dose-dependent manner. Calcium absorption, femur calcium uptake, calcium retention, and femur strength were significantly improved [13]. Feeding GOS increased the relative proportion of bifidobacteria in the large intestine, thereby changing the colonic bacterial community structure. Weaver et al. [30] compared eight different novel fibers to cellulose over 12 weeks in rats. The rats were fed with the fibers at 4–5 % of their diets. Two resistant starches, soluble fiber dextrin and polydextrose, increased bone calcium content, while soluble corn fiber and soluble fiber dextrin improved whole-body BMC, BMD, including having a specific effect on bone structure. Cortical thickness and area, as well as bone strength, were also improved. Soluble fiber dextrin as well as a mixture of inulin and FOS

Table 13.1 The effect of 5 % dietary soluble corn fiber on various bone parameters in the growing rat

Bone parameter	Positive change (%)
Total body BMC (g)	7.6
Total body BMD (g/cm^2)	2.9
Femur	
Volumetric bone density (g/cm^2)	8.3
Cortical area (mm^2)	19.6
Cortical thickness (mm)	22.4
Peak breaking force (N)	8.8

Based on data from Ref. [30]

significantly improved zinc retention, while soluble corn fiber improved magnesium retention (Table 13.1).

Human Studies

Studies in humans at various ages have shown some effects of fermentable carbohydrates on calcium absorption and retention. In adolescents, 5 g of FOS per day in orange juice improved fractional calcium absorption by 10 % [31]. Griffin et al. (2002) supplemented young girls with 8 g/day of FOS or a mixture of FOS and inulin on top of a sufficient calcium intake of 1,200–1,300 mg/day for 3 weeks [32]. The FOS alone did not have a significant effect, but the mixture enhanced calcium absorption by 18 % in comparison to the control group. Urinary calcium did not change, so it may be assumed that the absorbed calcium was retained. A further study by Griffin et al., in girls aged 10–15 years, showed that 8 g of a mixture of FOS and inulin per day over 4 weeks improved calcium absorption from 33 to 36 %. The most significant effect was found in those girls with a lower habitual calcium absorption [33]. Coudray et al. in 1997 showed that 40 g chicory inulin per day stepwise increased for 26 days improved calcium absorption from 21 to 33 %, a 58 % increase [34]. In contrast, a study in young adults by Van den Heuvel et al. [35], where the diets were supplemented with 15 g inulin per day for 21 days, and a study by Martin et al. [36] using 9 g/day of a FOS/inulin mixture had no effect on calcium absorption.

Griffin et al. (2002) speculated that if the additional calcium obtained from the rise in calcium absorption was retained daily over 2 years during maximum bone mineralization as would take place during adolescence, a net increase of 65 g relating to 5.5 % gain in bone mass could take place [32]. In 9- to 13-year-old children, 1 year of supplementation, with 8 g/day of inulin, resulted in a significant increase in whole-body bone mineral content (245 ± 11 vs. 210 ± 10 g) and BMD, compared to the control group [37].

Tahiri et al. [38] investigated the effect of 10 g/day of FOS for 5 weeks on calcium absorption in older women. No significant effect was found, but sub-analysis indicated that this dose may have affected absorption in women more than 6 years past menopause. In another study conducted in older women, more than 5 years past menopause, 10 g/day of lactulose for 9 days and 10 g of a mixture of FOS and inulin for 6 weeks significantly increased mean calcium absorption (by 5 and 7 %, respectively) [39–41]. In women who were a minimum of 10 years past menopause, 10 g/day of a mixture of FOS/inulin for 6 weeks significantly increased true fractional calcium absorption compared to the control group [39].

Data on specific bone effects in older adults are sparse. Bone turnover markers do not seem to respond well to prebiotics. Tahiri et al. [38] failed to measure an effect on osteocalcin, a marker of bone turnover, and urinary excretion of deoxypyridinoline, a marker of bone resorption. Holloway et al. [39] showed a change in biomarkers but only in the women in whom calcium absorption was significantly improved.

Coxam [42] summarized the various human studies and commented on the possible impact of prebiotic supplementation at various ages. Studies by Van der Heuvel et al., Griffin et al., and by Abrams et al. [31–33, 37, 43] indicated that prebiotics could affect peak bone mass accrual and help optimize peak bone mass. Calcium absorption in older adults could be improved, [34] while in women with postmenopausal osteoporosis, several studies were inconclusive. In elderly women, studies by Tahiri et al., Van der Heuvel et al., Kim et al., and Holloway et al. [38–40, 44] reported improved calcium absorption, with only

two reporting an effect on bone metabolism [41, 44]. Long-term effects of prebiotics on bone health and risk of fracture therefore need further investigation.

Probiotics

The endogenous bacterial population can be manipulated by introducing exogenous bacteria into the colonic microflora, and these exogenous bacteria are called probiotics. Probiotics may be defined as viable microorganisms that (when ingested) have a beneficial effect on the health and metabolism of their host. The most popular strains are represented by the following genera: Lactobacillus, Streptococcus, and Bifidobacterium [7, 45, 46].

The mechanisms by which probiotics exert biological effects are not clear, and research is still required to confirm the possible mechanisms. One suggestion is that the indigenous anaerobic flora limits the concentration of potentially pathogenic flora in the digestive tract. Although probiotic bacteria are thought to mediate their effects by using some of the same mechanisms as the native intestinal flora, probiotics may also work through other modes of action such as supplying enzymes or influencing enzyme activity in the gastrointestinal tract or synthesizing vitamins [7, 45–48]. Other suggested mechanisms of action include degradation by probiotics of the mineral complexing phytic acid and stimulation of calcium uptake by the enterocytes [46].

Only few studies have been published on the effect of probiotics on mineral absorption. In 1994 one of the first studies to be done using probiotics reported that feeding *Bifidobacterium longum* BB536 to rats, with or without lactulose, increased breaking strength of the bones [49]. When rats were fed with yogurt containing probiotics, short-chain fatty acid production was increased and calcium absorption improved [50]. Perez-Conesa et al. [51] fed functional follow-on infant formulae containing pre- and/or probiotics to rats and found that calcium and magnesium absorption was enhanced and bone calcium improved in groups fed with synbiotics, a mixture of pre- and probiotics. The formula containing only probiotics improved tibial calcium content significantly compared to the control diet devoid of pre- and probiotics. These results suggested that probiotics even in the absence of prebiotics may affect mineral balance.

Narva et al. [52] studied the effect of a bioactive peptide, valyl-prolyl-proline (VPP), in water or *Lactobacillus helveticus*-fermented milk in the ovariectomized (OVX) female rat. In this model *Lactobacillus helveticus*-fermented milk containing VPP attenuated bone loss due to OVX by 16 % compared to water plus VPP. The fermented milk also significantly increased tibial moment of inertia. These results should be interpreted with caution as the calcium intake between the OVX control group and the OVX group receiving the fermented milk was significantly different. The authors did consider differences in nutritional intakes and concluded that the difference in calcium intake was probably too small to affect bone density significantly. They do point out that other nutrients in the fermented milk also could have had an effect.

Perez-Conesa et al. [53] found that *Bifidobacterium bifidum* and *Bifidobacterium longum* significantly increased femoral and tibial calcium content in weanling rats when fed for 30 days. These authors showed that probiotics increase crypt depth in the colon and lowered colon pH compared to a control diet with no probiotics. Calcium absorption was correlated with the pH of the colonic contents. *Lactobacillus rhamnosus* HN001 improved calcium and magnesium retention in growing male rats, but due to differences in food intake, the results were inconclusive. In the female OVX rat, HN001 reduced the rate of bone loss and improved bone density after 12 weeks of feeding in comparison to the OVX control (Fig. 13.2). HN001 had no effect on the bone resorption marker, CTX-1 [54]. Scholz-Ahrens et al. [46] tested prebiotics alone and in combination with *Lactobacillus acidophilus* NCC90 using the female OVX rat model. In this model the prebiotics lowered cecal pH over 16 weeks of feeding, as reported before, and increased cecal content weight, but the probiotic strain used had no effect on cecal pH or weight.

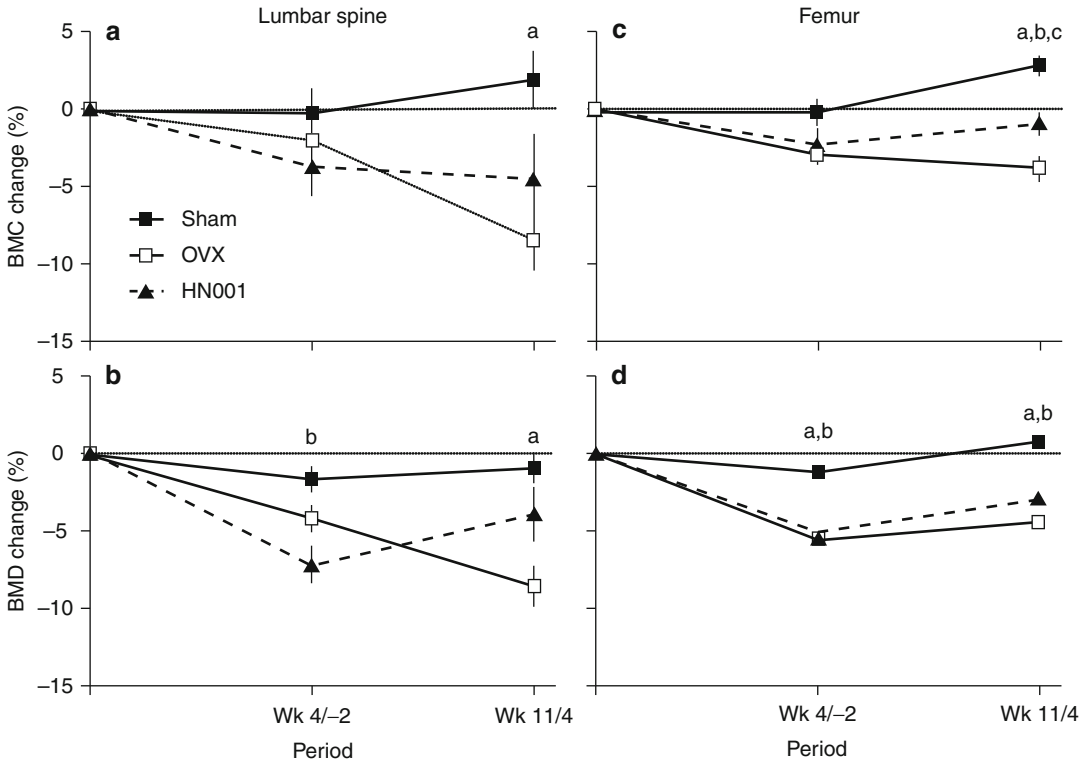

Fig. 13.2 The percentage change of lumbar spine and femur bone mineral content (**a**, **c**) and density (**b**, **d**) of female Sprague Dawley rats between weeks 4 and 2 and weeks 11 and 4. The rats were fed a casein-based diet based on AIN93. The experimental group received 10^9 CFU HN001 per day for 12 weeks. An "a" represents $P<0.05$ for Sham vs. OVX, a "b" for $P<0.05$ for Sham vs. HN001, and a "c" for $P<0.05$ for OVX vs. HN001 (Reprinted from Kruger et al. [54]. With permission from Dairy Science Technology)

While calcium balance was improved by the combination of pre- and probiotics (synbiotics), only the prebiotics improved calcium balance significantly. Bone parameters such as bone calcium content were improved by the synbiotics, but bone structure was only improved by the prebiotic diets.

Narva et al. [55] reported results of a small study where they compared the effect of milk fermented with *Lactobacillus helveticus* compared to a control on acute changes in calcium metabolism in postmenopausal women. He reported a reduction in serum parathyroid hormone and a raise in serum calcium but no effect on a marker of bone resorption. Cheung et al. [56] in a randomized crossover study compared calcium absorption from fortified soy milk in using normal and fermented milk. The fermented milk was inoculated with *Lactobacillus acidophilus.* No difference was observed between groups.

The calcium absorptive and bone-enhancing effects of the pre- and probiotics are affected by the experimental conditions and the physiological characteristics of the target group. Dietary conditions include the calcium level of the diets and the level of pre- or probiotics provided. In humans the physiological age, which will determine the calcium need, higher for growing adolescents, or after menopause, would affect the outcome. The postmenopausal stage is also important, as it seems as if women more than 6 years past menopause may benefit more by adding pre- or probiotics to their diets. And lastly, calcium absorptive capacity will affect the level to which pre- and probiotics could stimulate absorption.

Long-Chain Polyunsaturated Fatty Acids

Dietary lipids influence bone density by altering the efficiency of intestinal calcium absorption as well as by regulating the processes of bone remodeling and mineralization. Several epidemiological studies have demonstrated a relationship between fat intake and bone health. NHANES III (National Health and Nutrition Examination Survey (1988–1994)) is an ongoing nationally representative survey conducted in the United States involving a large number of men and women of different ages. Data from 14,850 participants were analyzed to determine the relationship between diet and bone density. Total fat intake was negatively associated with hip bone mineral content and density. The effect was evident in both sexes but was more pronounced in men than in women and most profound when total saturated fat intake was compared to bone mineral measurements [57]. More recent data from the Women's Health Initiative also reported a negative association between hip fracture risk and saturated fat intake [58]. Total lower fracture risk was associated with higher intakes of monounsaturated fatty acids and PUFAs, whereas a higher total fracture risk was associated with a high intake of omega 3 fats specifically [58]. Results from the Framingham Osteoporosis study found that higher intakes of fish (>3 servings per week) were associated with maintenance of femoral neck BMD in men and women; in women higher intakes of omega 3 fats had a higher baseline femoral neck BMD [59]. Data from the same study also indicated that blood phospholipid PUFA levels may be associated with risk of fracture [60]. In contrast, data from the Cardiovascular Health Study reported no association between intakes of LCPUFA and fracture risk, and only small differences in BMD were detected when data were analyzed based on fish and PUFA intake [61].

Humans lack the ability to synthesize fatty acids with a double bond past the carbon-9 position; therefore, these fatty acids must be obtained from the diet and hence are termed "essential." Essential fatty acids (EFAs) are classified into one of two families designated as omega 3 and omega 6 depending on the location of the first unsaturated carbon from the methyl terminus [62]. Alpha-linolenic acid (ALA) (18:3) is the parent compound for the omega 3 series of fatty acids, and linoleic acid (LA) (18:2) is the parent compound for the omega 6 series. The most common and widely recognized dietary source of omega 3 EFAs is fish oil, although α-linolenic acid is present in the chloroplasts of green leafy vegetables and also in some plant oils such as canola. Omega 6 EFAs are found in many edible oils as linoleic acid is found in the seed oils of most plants [63]. Evening primrose oil and most of the vegetable oils used to make margarine contain omega 6 EFAs. The EFAs are elongated and desaturated by endogenous enzymes to form longer-chain PUFAs. The most important of the longer-chain fatty acids are arachidonic acid (AA) of the omega 6 series and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of the omega 3 series. The latter two fatty acids are found in high levels in oily fish, such as tuna and salmon, and fish oils. The longer-chain PUFAs are precursors for various eicosanoids such as prostaglandins and leukotrienes which have a regulatory role in the body.

EFA deficiency reduces intestinal mucosal mass [64]. Dietary EFAs appear to have a trophic effect on the intestinal mucosa and may aid in promoting mucosal recovery after surgery or injury [65]. The fluidity of intestinal cell membranes also increases as the level of membrane lipid unsaturation increases. Aside from changing the physical structure of the intestinal mucosa, dietary fat can also influence the release of enterotrophic peptides. Bolus dosing of long-chain essential fatty acids has been reported to significantly increase the release of peptide tyrosine-tyrosine and enteroglucagon [66].

Dietary intake of omega 3 and omega 6 EFAs is correlated with increased duodenal ion transport. Several mechanisms have been proposed to explain the effect of EFAs on intestinal calcium transport. One possibility is that enhanced membrane fluidity as a result of increased incorporation of EFAs into phospholipids alters the physical environment of membrane-bound enzymes,

thereby enhancing their activity [67]. Another possibility is that EFAs may mimic, or facilitate, the action of vitamin D in promoting calcium absorption. Ca^{2+}-ATPase is the rate-limiting enzyme in active calcium transport. It is upregulated by the calcium-binding protein calmodulin. Unsaturated fatty acids bind with Ca^{2+}-ATPase mimicking the action of calmodulin and stimulating Ca^{2+}-ATPase activity [68]. DHA (but not EPA or AA) has been reported to increase Ca^{2+}-ATPase activity in the absence of calmodulin [68]. In addition, vitamin D receptor (VDR) availability, which is increased by ovariectomy in female rats, is reduced after EFA supplementation [69]. This is possibly indicative of increased vitamin D binding to the receptor.

Several intervention studies involving feeding rats with different combinations of omega 3 and omega 6 EFAs have been conducted. The majority of these studies have utilized OVX rats. Positive results in terms of decreased levels of bone resorption markers, increased levels of bone formation markers, and/or increased bone density measured by DEXA have generally been obtained. It appears that both omega 3 and omega 6 EFAs are required for maximal inhibition of loss of bone density post-ovariectomy. Synergism may exist between the two EFA families; however, the optimal ratio of omega 3/omega 6 EFAs or the effects of individual omega 3 and omega 6 EFAs are yet to be determined.

Few human studies using LCPUFA supplementation and measuring bone outcomes have been conducted. Fish oil supplementation (4 g/day) or a mixture of fish oil and evening primrose oil for 16 weeks in elderly, osteoporotic women increased levels of serum calcium as well as levels of the bone formation marker osteocalcin [70]. Supplementation of elderly, osteoporotic/osteopenic women who had habitually low dietary calcium intakes with 6 g of high PUFA oil in conjunction with 600 mg calcium carbonate per day for 18 months resulted in maintenance of lumbar spine bone density compared to a 3.2 % decrease in lumbar spine density in the control subjects. LCPUFA supplementation for a further 18-month period resulted in an increase of 3.1 % in lumbar spine bone density [71]. PUFAs, therefore, appear to be beneficial in treating senile osteoporosis which is often caused by low dietary calcium intake, a decreased ability to absorb dietary calcium, and decreased vitamin D status as a result of lifestyle and metabolic factors associated with aging [63, 67]. More recent studies supplementing omega 3 fats reported a significant effect by 900 mg omega 3 PUFAs on a bone resorption marker over 6 months [72], improvement of bone formation markers after supplementing with a PUFA, and vitamin-fortified milk for 1 year [73], but in a shorter study, no effect on the bone resorption marker CTx after 3 months of supplementing with 1.48 g omega 3 fats was observed [74]. Exercise combined with 1,000 mg/day of omega 3 fats reduced inflammatory markers and CTx in postmenopausal women and improved femoral neck BMD over 6 months [75].

A recent study by Järvinen et al. [76] reports a positive relationship between dietary PUFAs and BMD at the lumbar spine in 554 women older than 60 years. These findings were significant only in those women who did not use hormone replacement therapy. These authors conclude that PUFAs may be especially important for bone integrity and maintenance in older women. Poulsen et al. [77] suggested that there may be an increased need in women for LCPUFAs after menopause. The fatty acid composition of adipose tissue changes with age, with an increase in adipose tissue content of AA, docosapentaenoic acid (DPA), and DHA [78]. Serum phospholipid composition as well as fatty acid composition of membranes changes after menopause [79], and higher serum levels of DHA in women of this age were reported compared to men [80]. Estrogen may also increase the synthesis of AA and DHA from their precursors [80].

Aging and menopause may lead to a reduction in the ability of endogenous enzymes to convert ALA and LA into the longer-chain metabolites. In addition, intake of omega 6 EFAs increases PGE_2 which in turn stimulates synthesis of proinflammatory cytokines such as interleukin −1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) [62]. Estrogen deficiency also results in elevation of the levels of these cytokines,

and all three cytokines are known to promote bone resorption [81]. Omega 3 EFAs downregulate production of all prostaglandins but particularly those derived from omega 6 EFAs such as PGE_2. As a result, omega 3 EFAs act as anti-inflammatory agents, inhibiting cytokine synthesis [82]. Estrogen deficiency results in increased bone resorption as well as formation, although the increase in rate of formation is less than that of resorption. This leads to reduced bone density and bone loss. Modulating the dietary ratio of omega 6:omega 3 EFAs may be a means of at least partially compensating for the effects of estrogen deficiency postmenopause. The evidence to date from cross-sectional as well as intervention studies suggests that during estrogen deficiency, a higher consumption of omega 3 EFAs may be beneficial in reducing bone density losses. A recent review summarizes the suggested mechanisms of action of the long-chain PUFAs on bone cells [83]. These actions involve modulation of fatty acid metabolites such as resolvins and protectins, several signaling pathways, cytokines, and growth factors. Omega 3 PUFAs may be protective as they could reduce synthesis of PGE_2, suppress inflammatory cytokines, and give rise to lipid mediators which are anti-inflammatory and thereby reduce bone resorption [83].

It is feasible that the PUFAs may also act similar to a prebiotic agent and affect intestinal microbiota and calcium absorption. In gnotobiotic pigs, omega 3 supplementation affected adhesion of *Lactobacillus paracasei* to the jejunal mucosa and increased the number by 12 % in comparison to the control group. In this particular study, the authors did not measure any mineral uptake activity but inferred that the improvement in adhesion of the lactobacilli could inhibit digestive tract pathogens [84]. Andersen et al. [85] supplemented infants with 5 mL fish oil or sunflower oil per day from 9 to 18 months of age and collected stool samples. Molecular fingerprints of the bacterial DNA were obtained by terminal restriction fragment length polymorphism (T-RFLP). These profiles indicated a few new T-RFs became more dominant, together with an overall increase in diversity of the microbiota. Profiles were also affected if the children were breast-fed during the oil supplementation. Breastfeeding limited the gut response to the oils. In 10-month-old infants, fish oil supplementation could only affect gut microbiota in children fed with cow's milk and not in those fed with infant formula [86]. Finally, free fatty acids, LA, AA, ALA, and DHA, were added to growth medium in physiological concentrations to assess their effects on growth and adhesion of *Lactobacillus GG, Lactobacillus casei,* and *Lactobacillus bulgaricus* [87]. Concentrations at 10–40 μg PUFA/mL inhibited growth and mucus adhesion of all tested strains. The PUFA also altered adhesion sites on Caco-2 cells, and an improvement in adhesion and growth was not consistently measured. These observations should be investigated further as adhesion to mucosal surfaces is necessary to support the health-promoting effects of the probiotics.

Summary

Evidence is presented that addition of prebiotics or probiotics to the diet may improve calcium absorption by modulating intestinal microbiota, increasing SCFA synthesis, and solubilizing calcium. The intestinal wall may also be affected by the pre- and probiotics increasing absorptive surface. In addition, LCPUFA may modulate intestinal membrane composition and pumps, thereby affecting active calcium absorption. LCPUFA however may also affect intestinal microflora, having a prebiotic effect. No research has been done linking a prebiotic effect by LCPUFAs to calcium metabolism. Further research is required.

References

1. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus A, Matkovic V, Weaver C. Peak bone mass. Osteoporosis Int. 2000;11:985–1009.
2. Bronner F. Recent developments in intestinal calcium absorption. Nutr Rev. 2009;67(2):109–13.
3. Bronner F, Pansu D. Nutritional aspects of calcium absorption. J Nutr. 1999;129:9–12.
4. Bronner F. Calcium. In: O'Dell BL, Sunde RA, editors. Handbook of nutritionally essential mineral elements. New York: Marcel Dekker; 1997. p. 13–61.

5. Park CY, Weaver CM. Calcium and bone health: influence of prebiotics. Funct Food Rev. 2011;3(2): 62–72.
6. Roberfroid M, Slavin J. Nondigestible oligosaccharides. Crit Rev Food Sci Nutr. 2000;40(6):461–80.
7. Roberfroid MB. Prebiotics and synbiotics: concepts and nutritional properties. Br J Nutr. 1998;80 Suppl 2:S197–202.
8. Roberfroid MB. Concepts of functional foods: the case of inulin and oligofructose. J Nutr. 1999;129: 1398S–401.
9. Raschka L, Daniel H. Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. Bone. 2005;37:728–35.
10. Chonan O, Takahashi R, Watnuki M. Role of activity of gastrointestinal microflora in absorption of calcium and magnesium in rats fed beta-4 linked galactooligosaccharides. Biosci Biotechnol Biochem. 2001;65:1872–5.
11. Blottiere HM, Buecher B, Galmiche JP, Cherbut C. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc Nutr Soc. 2003;62:101–6.
12. Pérez-Conesa D, López G, Ros G. Effects of probiotic, prebiotic and symbiotic follow-up infant formulas on large intestine morphology and bone mineralisation in rats. J Sci Food Agric. 2007;87:1059–68.
13. Weaver CM, Martin BR, Nakatsu CH, Armstrong AP, Clavijo A, McCabe LD, et al. Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. J Agric Food Chem. 2011;59:6501–10.
14. Ohta A, Motohashi Y, Ohtsuki M, Hirayama M, Adachi T, Sakuma K, et al. Dietary fructooligosaccharides change the concentration of calbindin-d9k differently in the mucosa of the small and large intestine of rats. J Nutr. 1998;128:934–9.
15. Takahara S, Morohashi T, Sano T, Ohta A, Yamada S, Sasa R. Fructooligosaccharide consumption enhances femoral bone volume and mineral conversion in rats. J Nutr. 2000;130:1792–5.
16. Ohta A, Ohtuki M, Takizawa T, Inaba H, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of magnesium and calcium by cecectomised rats. Int J Vitam Nutr Res. 1994;64:316–23.
17. Ohta A, Ohtsuki M, Baba S, Adachi T, Sakata T, Sakaguchi EI. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. J Nutr. 1995;125:2417–24.
18. Taguchi A, Ohta A, Abe M, Baba S, Ohtsuki M, Takizawa T, et al. The influence of fructooligosaccharides on the bone of model rats with ovariectomised osteoporosis. Sci Rep Meija Seika Kaisha. 1994; 33: 34–43.
19. Delzenne N, Aertsens J, Verplaetse H, Roccaro M, Roberfroid M. Effect of fermentable fructooligosaccharides on mineral, nitrogen and energy digestive balance in the rat. Life Sci. 1995;57:1579–87.
20. Roberfroid MB, Cumps J, Devogelaer JP. Dietary chicory inulin increases whole-body bone mineral density in growing male rats. J Nutr. 2002;132:3599–602.
21. Scholz-Ahrens K, Acil Y, Schrezenmeier J. Effect of oligofructose or dietary calcium on repeated calcium and phosphorus balances, bone mineralisation and trabecular structure in ovariectomised rats. Br J Nutr. 2002;88:365–77.
22. Levrat MA, Remesy C, Demigne C. High propionate acid fermentation and mineral accumulation in the caecum adapted to different levels of inulin. J Nutr. 1991;121:1730–7.
23. Kruger MC, Brown KE, Collett G, Layton L, Schollum LM. The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. Exp Biol Med. 2003;228:683–8.
24. Chonan O, Matsumoto K, Watanuki M. Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomised rats. Biosci Biotechnol Biochem. 1995;59:236–9.
25. Chonan O, Watanuki M. The effect of 6-galactooligosaccharides on bone mineralisation of rats adapted to different levels of dietary calcium. Int J Vitam Nutr Res. 1996;66:244–9.
26. Brommage R, Binacua C, Antille S, Carrie AL. Intestinal calcium absorption in rats is stimulated by dietary lactulose and other resistant sugars. J Nutr. 1993;123:2186–94.
27. Schulz AGM, Von Amelsvoort JMM, Beynen AC. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. J Nutr. 1993;123:1724–31.
28. Younes H, Coudray C, Bellanger J, Demigne C, Rayssiguier Y, Remesy C. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. Br J Nutr. 2001;86:479–85.
29. Roberfroid M. Dietary fibers, inulin and oligofructose, a review comparing their physiological effects. Crit Rev Food Sci Nutr. 1993;33:103–48.
30. Weaver CM, Martin BR, Story JA, Hutchinson I, Sanders L. Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. J Agric Food Chem. 2010;58:8952–7.
31. Van der Heuvel EGHM, Muys T, Van Dokkum W, Schaafsma G. Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr. 1999;69:544–8.
32. Griffin IJ, Davila PM, Abrams SA. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. Br J Nutr. 2002;87(S2): S187–91.
33. Griffin IJ, Hicks PMD, Heaney RP, Abrams SA. Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. Nutr Res. 2003;23:901–9.
34. Coudray C, Bellanger J, Castiglia-Delavaud C, Remesy C, Vermorel M, Rayssiguier Y. Effect of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr. 1997;151:375–80.
35. Van der Heuvel EGHM, Schaafsma G, Muys T, Van Dokkum W. Nondigestible oligosaccharides do not

interfere with calcium and nonheme iron absorption in young healthy men. Am J Clin Nutr. 1998;67: 445–51.
36. Martin BD, Braun MM, Wigertz K, Bryant R, Zhao Y, Lee WH, et al. Fructo-oligosaccharides and calcium absorption and retention in adolescent girls. J Am Coll Nutr. 2010;29(4):382–6.
37. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005;82: 471–6.
38. Tahiri M, Tressol JC, Arnaud J, Bornet FRJ, Bouteloup-Demange C, Feillet-Coudray C, et al. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. Am J Clin Nutr. 2003;77:449–57.
39. Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, Friedlander AL. Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. Br J Nutr. 2007;97:365–72.
40. Coxam V. Inulin type fructans and bone health: state of the art and perspectives in the management of osteoporosis. Br J Nutr. 2005;93(Suppl): S111–23.
41. De Vrese M. Health benefits of probiotics and prebiotics in women. Menopause Int. 2009;15:35–40.
42. Coxam V. Current data with inulin-type fructans and calcium, targeting bone health in adults. J Nutr. 2007;137:2527S–33.
43. Abrams SA, Griffin IJ, Hawthorne KM. Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. J Nutr. 2007;137: 2524S–6.
44. Kim YY, Jang KH, Lee EY, Cho Y, Kang SA, Ha WK, et al. The effect of chicory fructan fiber on calcium absorption and bone metabolism in Korean postmenopausal women. Nutr Sci. 2004;7:151–7.
45. Chow J-MC. Probiotics and prebiotics: a brief overview. J Renal Nutr. 2002;12(2):76–86.
46. Scholz-Ahrens KE, Ade P, Marten B, Weber P, Timm W, Asil Y, et al. Prebiotics, probiotics and symbiotic affect mineral absorption, bone mineral content, and bone structure. J Nutr. 2007;137:838S–46.
47. Beynen AC, Baas JC, Hoekemeijer PE, Kappert HJ, Bakker MH, Koopman JP, et al. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. J Anim Physiol Anim Nutr (Berl). 2002;86:298–305.
48. Minamida K, Sujaya IN, Tamura A, Shigematsu N, Sone T, Yokota A, et al. The effects of di-D-fructofuranose-1,2:2,3-dianhydride (DFA III) administration on human intestinal microbiota. J Biosci Bioeng. 2004;98(4):244–50.
49. Igarashi M, Liyama Y, Kato R, Tomita M, Asami N, Ezawa I. Effect of *Bifidobacterium longum* and lactulose on the strength of bone in ovariectomised osteoporosis rat model. Bifidus. 1994;7:139–47.
50. Ghanem KZ, Badawy IH, Abdel-Salam AM. Influence of yoghurt and probiotic yoghurt on the absorption of calcium, magnesium, iron and bone mineralisation in rats. Milk Sci Int. 2004;59(9–10):472–5.
51. Perez-Conesa D, Lopez G, Ros G. Effects of probiotic, prebiotic and symbiotic follow-up infant formulas on large intestine morphology and bone mineralisation in rats. J Sci Food Agric. 2007;87(6): 1059–68.
52. Narva M, Rissanen J, Jussi H, Heiki V, Kalervo V, Riita K. Effects of bioactive peptide, valyl-prolyl-proline (VPP), and *Lactobacillus helveticus* fermented milk containing VPP on bone loss in ovariectomized rats. Ann Nutr Metab. 2007;51:65–74.
53. Perez-Conesa D, Lopez G, Abellan P, Ros G. Bioavailability of calcium, magnesium and phosphorous in rats fed probiotic, prebiotic and symbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. J Sci Food Agric. 2006;86:2327–36.
54. Kruger MC, Fear A, Chua W-H, Plimmer GG, Schollum LM. The effect of *Lactobacillus rhamnosus* HN001 on mineral absorption and bone health in growing male and ovariectomised female rats. Dairy Sci Technol. 2009;89:219–31.
55. Narva M, Nevala R, Poussa T, Korpela R. The effect of *Lactobacillus helveticus* fermented milk on acute changes in calcium metabolism in postmenopausal women. Eur J Nutr. 2004;43(2):61–8.
56. Cheung ALTF, Wilcox G, Walker KZ, Shah NP, Strauss B, Ashton JF, et al. Fermentation of calcium-fortified soya milk does not appear to enhance acute calcium absorption in osteopenic post-menopausal women. Br J Nutr. 2011;105:283–6.
57. Corwin RL, Hartman TJ, Maczuga SA, Graubard BI. Dietary saturated fat intake is inversely associated with bone density in humans: analysis of NHANES III. J Nutr. 2006;136:159–65.
58. Orchard TS, Cauley JA, Frank GC, Neuhouser ML, Robinson JG, Snetselaar FT, et al. Fatty acid consumption and risk of fracture in the Women's Health Initiative. Am J Clin Nutr. 2010;92:1452–60.
59. Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr. 2011;93:1142–51.
60. Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker L. Plasma phosphatidylcholine concentrations of polyunsaturated fatty acids are differentially associated with hip bone mineral density and hip fracture in older adults: the Framingham Osteoporosis Study. J Bone Miner Res. 2012;27(5): 1222–30.
61. Virtanen JK, Mozaffarian D, Cauley JA, Mukamal K, Robins J, Siscovick DS. Fish consumption, bone mineral density, and risk of hip fracture among older

adults: the Cardiovascular Health Study. J Bone Miner Res. 2010;25(9):1972–9.
62. Fernandes G, Lawrence R, Sun D. Protective role of n-3 lipids and soy protein in osteoporosis. Prostaglandins Leukot Essent Fatty Acids. 2003;68: 361–8.
63. Albertazzi P, Coupland K. Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention? Maturitas. 2002;42:13–8.
64. Maxton DG, Cynk EU, Jenkins AP, Thompson RP. Effect of dietary fat on the small intestinal mucosa. Gut. 1989;30:1252–5.
65. Hart MH. Essential fatty acid deficiency and postsection mucosal adaptation in the rat. Gastroenterology. 1988;94:682–8.
66. Jenkins AP, Ghatai MA, Bloom SR, Thompson RP. Effects of bolus doses of fat on small intestinal structure and release of gastrin, cholecystokinin, peptide tyrosine-tyrosine, and enteroglucagon. Gut. 1992;33: 218–23.
67. Kruger MC, Horrobin DF. Calcium metabolism, osteoporosis and essential fatty acids: a review. Prog Lipid Res. 1997;36:131–51.
68. Haag M, Magada NO, Claassen N, Bohmer LH, Kruger MC. Omega-3 fatty acids modulate ATPases involved in duodenal Ca absorption. Prostaglandins Leukot Essent Fatty Acids. 2003;68:423–9.
69. Leonard F, Haag M, Kruger MC. Modulation of intestinal vitamin D receptor availability and calcium ATPase activity by essential fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2001;64:147–50.
70. Van Papendorp DH, Coetzer H, Kruger MC. Biochemical profile of osteoporotic patients on essential fatty acid supplementation. Nutr Res. 1995;15:325–34.
71. Kruger MC, de Winter R, Van Papendorp DH, Gericke GJ. Calcium, gamma-linolenic acid and eicosapentaenoic acid supplementation in senile osteoporosis. Aging Clin Exp Res. 1998;10:385–94.
72. Salari P, Rezaie A, Larijani B, Abdollahi M. A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. Med Sci Monit. 2008;14(3): RA37–44.
73. Martin-Bautista E, Muñoz-Torres M, Fonolla J, Quesada M, Poyatos A, Lopez-Huertas E. Improvement of bone formation biomarkers after 1-year consumption with milk fortified with eicosapentaenoic acid, docosahexaenoic acid, oleic acid, and selected vitamins. Nutr Res. 2010;30:320–6.
74. Appleton KM, Fraser WD, Rogers PJ, Ness AR, Tobias JH. Supplementation with a low dose-moderate dose of n-3 long chain PUFA has no short-term effect on bone resorption in human adults. Br J Nutr. 2011; 105:1145–9.
75. Tartibian B, Maleki BH, Kanaley J, Sadeghi K. Long-term aerobic exercise and omega-3 supplementation modulate osteoporosis through inflammatory mechanisms in post-menopausal women: a randomized, repeated measures study. Nutr Metab. 2011;8:71–83.
76. Järvinen R, Tuppurainen M, Erkkila AT, Penttinen P, Kärkkäinen M, Salovaara K, et al. Associations of dietary polyunsaturated fatty acids with bone mineral density in elderly women. Eur J Clin Nutr. 2012;66: 496–503.
77. Poulsen RC, Moughan PJ, Kruger MC. Long-chain polyunsaturated fatty acids and the regulation of bone metabolism. Exp Biol Med. 2007;232:1275–88.
78. Bolton-Smith C, Woodward M. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. Eur J Clin Nutr. 1997;51:619–24.
79. Weiss LA, Barrett-Connor E, von Muhlen D. Ration of n-6 to n-3 fatty acids and bone mineral density in older adults. The Rachero Bernardo study. Am J Clin Nutr. 2005;81:934–8.
80. Giltay EJ, Gooren LJG, Toorians A, Katan MB, Zock PL. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. Am J Clin Nutr. 2004;80:1167–74.
81. McLean RR. Proinflammatory cytokines and osteoporosis. Curr Osteoporos Rep. 2009;7:134–9.
82. Miggiano GA, Gagliardi L. Diet, nutrition and bone health. Clin Ther. 2005;156:47–56.
83. Kruger MC, Coetzee M, Haag M, Weiler H. Long-chain polyunsaturated fatty acids: selected mechanisms of action on bone. Prog Lipid Res. 2010;49: 439–49.
84. Bomba A, Nemcová R, Gancarciková S, Herich R, Pisti J, Révajová V, et al. The influence of omega-3 polyunsaturated fatty acids (ometa-3 pufa) on lactobacilli adhesion to the intestinal mucosa and on immunity in gnotobiotic piglets. Berl Munch Tierarztl Wochenschr. 2003;116(7–8):312–6.
85. Andersen AD, Mølbak L, Michaelsen KF, Lauritzen L. Molecular fingerprints of the human fecal microbiota from 9 to 18 months old and the effect of fish oil supplementation. J Pediatr Gastroenterol Nutr. 2011; 53(3):303–9.
86. Nielsen S, Nielsen DS, Lauritzen L, Jakobsen M, Michaelsen KF. Impact of diet on the intestinal microbiota in 10-month-old infants. J Pediatr Gastroenterol Nutr. 2007;44:613–8.
87. Kankaanpää PE, Salminen SJ, Isolauri E, Lee YK. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microbiol Lett. 2001;194:149–53.

Comparison of Natural Products for Effects on Bone Balance

14

Connie M. Weaver and Emily E. Hohman

Abstract

Dietary compounds from natural products are the subject of investigation for their beneficial effects on bone. Natural products may be safer and better tolerated by consumers than current therapies for treatment of osteoporosis. Soy isoflavones have been the most studied but results are mixed. Whole soy food consumption in Asian women is associated with reduced fracture incidence in observational studies. However, purified isolated soy isoflavones in randomized controlled trials in postmenopausal Western women are not protective of bone loss. Polyphenolic compounds in plum and berries have both anabolic effects and the ability to suppress bone resorption. These effects occur through antioxidation and anti-inflammatory cell signaling pathways. Rapid screening approaches using urinary excretion of calcium tracers from labeled bone can be used to compare doses and types of natural products for their effect on bone calcium balance.

Keywords

Natural products • Soy isoflavones • Plum • Berry • Bone turnover

Introduction

Loss of estrogen at menopause is a major contributing factor to bone loss in women. Up to 20 % of bone density may be lost in the 5–7 years following menopause [1]. Estrogen replacement therapy is effective at reducing bone loss [2], but has fallen out of favor following the discovery of potential adverse effects in the Women's Health Initiative study [3]. Many natural products, mostly from plant sources, have been investigated as potential alternative therapies. Isoflavones have been the most studied natural product for their effect on menopausal bone loss. Higher fruit and vegetable intake has been associated with greater bone mass in postmenopausal Chinese women [4] and elderly US men and women [5]. Our laboratory has been investigating the efficacy of some plant-derived constituents for their

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ability to improve bone balance following estrogen deficiency associated with menopause.

Measuring Effects on Bone

The most common traditional method for assessing efficacy of interventions on preventing bone loss associated with estrogen deficiency is by measuring bone mineral density (BMD) changes over time measured by dual energy X-ray absorptiometry (DXA). The ovariectomized (OVX) rodent is an approved model of bone loss following menopause. BMD is the most used method because it is predictive of fracture. Fracture studies are not feasible for dietary studies.

Bone turnover rates are also predictive of fracture. Urinary excretion of bone-seeking tracers can be used to monitor bone turnover in relatively short intervention periods and, thus, are useful to compare several interventions in a crossover design. When the intervention is an antiresorptive agent, reduction in excretion of tracer indicates the extent of the antiresorptive agent. Reduction in excretion of tracer indicates the extent of the antiresorptive capacity of the intervention. In a comparison of urinary excretion of a bone-seeking tracer compared to full calcium kinetics in postmenopausal women, it best predicted net bone balance [6].

Muhlbauer et al. [7] surveyed a number of foods for their antiresorptive capacity using tritiated tetracycline (^{3}H-TC) as a bone-seeking label. Rats were given ^{3}H-TC to pre-label their bones and were then randomized to diets with a specific fruit, vegetable, or other food component. Urinary ^{3}H-TC excretion in response to these diets was compared to urinary tetracycline excretion in rats fed a control diet to determine the antiresorptive capacity of the foods. Using this method, Muhlbauer's group found that several vegetables, mushrooms, fruits, and red wine reduced bone resorption.

We have developed a similar method utilizing ^{45}Ca as a bone-seeking label. ^{45}Ca incorporates into bone with higher efficiency than ^{3}H-TC [8]. We previously compared ^{3}H-TC and ^{45}Ca kinetics simultaneously in OVX rats. We developed a 9-compartment model to fit both tracers simultaneously, including two bone compartments with different turnover rates. Bone resorption rates from both bone compartments did not differ between ^{3}H-TC and ^{45}Ca, suggesting that the two tracers may be used interchangeably to measure bone resorption [9]. Therefore, ^{45}Ca is preferred over ^{3}H-TC in animal studies because ^{3}H-TC is more expensive and is a surrogate for calcium in bone mineral matrix.

In humans, neither ^{3}H-TC and ^{45}Ca are practical choices for a bone-seeking label because of safety and the short half-life of ^{45}Ca. ^{41}Ca, a long-lived radioisotope ($t_{1/2}$ = ~10^5 year), can be used as alternative tracer. The long half-life of ^{41}Ca allows it to behave more like a stable isotope, minimizing radiation exposure for subjects and allowing long-term monitoring of bone turnover. ^{41}Ca can be measured in urine of dosed subjects by accelerator mass spectrometry (AMS). The sensitivity of this instrument allows ^{41}Ca excretion to be determined for years following the initial dose. This methodology can be used to screen multiple therapies in a crossover design in the same subject [10]. Figure 14.1 illustrates how changes in urinary ^{41}Ca can be used to determine the efficacy of an intervention. ^{41}Ca correlates with traditional biomarkers of bone turnover including serum

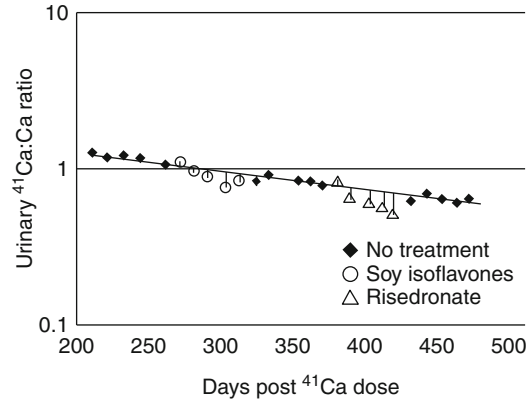

Fig. 14.1 Urinary ^{41}Ca for one subject over multiple treatments. The baseline and washout periods, where no treatment occurs, are used to generate a prediction equation. The difference between this prediction line and the observed ^{41}Ca during a treatment, which is represented by the *vertical lines*, is used to calculate the effect of treatment on net bone turnover

osteocalcin and urinary NTx [11], but is more specific to bone mineral matrix, is more sensitive, and has a greater precision than biomarkers, allowing for reduced sample size.

Soy Isoflavones

Isoflavones have been the most studied natural product. Isoflavones, also known as phytoestrogens, are naturally occurring plant compounds that bind to estrogen receptors in humans and animals. The predominant source of isoflavones is soy, but they are also found in red clover and kudzu. The primary isoflavones found in soy are genistein, daidzein, and glycitein [12].

Epidemiological evidence suggests a link between soy consumption and reduced risk of fracture. In the Shanghai Women's Health Study, a cohort of 75,000 Chinese women aged 40–70 years, there was an inverse relationship between soy isoflavone intake, adjusted for age, calorie intake, and other covariates, and risk of fracture [13]. The Singapore Chinese Health Study, a prospective cohort study of over 63,000 Chinese men and women, showed a significant association between soy intake and hip fracture risk in women but not in men. After adjusting for covariates, women in the second through fourth quartiles of soy intake had 21–36 % lower risk of hip fracture than those in the lowest quartile [14]. The relationship between soy isoflavone intake and fracture risk has not been clearly established in non-Asian populations, largely due to low consumption of isoflavones. Greendale et al. [15]. used data from the Study of Women's Health Across the Nation, an ethnically diverse US community-based cohort, to examine the relationship between genistein intake and bone mineral density. Higher genistein intake was associated with higher spine and femoral neck BMD in premenopausal Japanese women. However, no relationship between genistein intake and BMD was observed in postmenopausal Japanese women or in Chinese women, and genistein intakes in Caucasian and African-American women were too low to pursue analyses. These epidemiological studies are summarized in Table 14.1.

In contrast to epidemiological evidence for postmenopausal women consuming whole soy foods in Asian countries, randomized controlled trials focusing on the effect of isoflavone supplementation on bone mineral density have had largely negative results (Table 14.1). The soy isoflavones for reducing bone loss (SIRBL) study, 3-year randomized, placebo-controlled trial of two doses of isoflavones, 80 and 120 mg/day, found no protective effect on BMD with the exception of a modest effect at the femoral neck [16]. Similarly, Tai et al. observed no effect on BMD following 2 years of supplementation with 300 mg/day isoflavone in Taiwanese

Table 14.1 Epidemiological studies of soy isoflavone consumption and bone health

Reference	Location	Population *n*, characteristics	Findings
Zhang et al. [13]	Shanghai, China	*n* = 24, 403 Chinese women aged 40–70 years	Soy protein and soy isoflavone consumption was associated with reduced risk of fracture; effect was strongest in women within 10 years of menopause.
Koh et al. [14]	Singapore	*n* = 63, 257 Chinese men and women aged 45–74 years	Soy intake associated with reduced risk of fracture among women, but not men.
Greendale et al. [15]	USA	*n* = 2, 413 African-American, Caucasian, Chinese, and Japanese women aged 42–52 years	Higher genistein intake associated with higher spine and femoral neck BMD in premenopausal Japanese women, but not in Chinese women or postmenopausal Japanese women. African-American and Caucasian women had too low of genistein intakes to pursue analysis.

Table 14.2 Randomized, controlled trials of soy isoflavones for postmenopausal bone loss

Reference	Population *n*, average age	Intervention	Duration	Primary outcomes	Effect of isoflavone intervention
Alekel et al. [16]	*n* = 255, 54 years	80 or 120 mg/day soy isoflavones or placebo	3 years	Lumbar spine, proximal femur, and total body BMD	No effect except a modest benefit at the femoral neck with 120 mg
Levis et al. [18]	*n* = 248, 53 years	200 mg/day soy isoflavones or placebo	2 years	Lumbar spine, total hip, femoral neck BMD	No effect
Tai et al. [17]	*n* = 431, 56 years	300 mg/day soy isoflavones or placebo	2 years	Lumbar spine, proximal femur BMD	No effect
Wong et al. [19]	*n* = 403, 55 years	80 or 120 mg/day soy hypocotyls aglycone isoflavones or placebo	2 years	Whole body, lumbar spine, total hip, femoral neck, and trochanter BMD and BMC	120 mg/day reduced whole-body bone loss but no effect at regional sites

women [17]. Levis et al. [18]. observed no effect on BMD or menopausal symptoms following 2 years of supplementation with 200 mg/day isoflavones. In a 2-year multicenter trial, Wong et al. found 120 mg/day, but not 80 mg/day, soy hypocotyl aglycone isoflavones exerted a small protective effect on total body BMD, but did not prevent bone loss at common fracture sites [19]. These recent clinical trials are summarized in Table 14.2.

One limitation of the large clinical trials that have been conducted to date is that most studies have focused only on BMD. As previously mentioned, BMD is a major predictor of fracture risk, but is not the only contributor. In a 5-year longitudinal study, Wainwright et al. found that 54 % of subjects who suffered a hip fracture would not be classified as osteoporotic based on their baseline BMD scores [20]. Additionally, changes in BMD cannot fully account for the reduction in fracture risk seen in patients using antiresorptive therapies [21]. Thus, other factors in addition to BMD are important in determining overall bone strength and resistance to fracture. Such factors may include rate of bone turnover, bone shape, size, microarchitecture, and material properties at the tissue level. There is some evidence to suggest that soy isoflavones may impact these factors instead of, or independently of, changes in BMD. Indices of bone turnover have been recognized as BMD-independent predictors of mechanical competence of bone [22]. Using ^{41}Ca methodology, we have tested several isoflavone preparations for their ability to suppress bone turnover. In one study, we investigated the ability of isoflavones from several different plant sources to reduce bone resorption [23]. In this randomized, crossover trial, 11 postmenopausal women were given a dose of ^{41}Ca and, following an equilibration period of 100 days or more, were then assigned to 50-day interventions in a randomized order. The interventions consisted of four botanical supplements from different plant sources, including soy cotyledon, soy germ, red clover, and kudzu, and a positive control treatment of either estrogen or risedronate. Urinary ^{41}Ca:Ca during pre-intervention and intervention periods was used to determine suppression of bone turnover. The positive controls, estrogen and alendronate, reduced bone resorption by 22 % and 24 %, respectively. The soy cotyledon and soy germ interventions significantly reduced net bone resorption by 9 and 5 %, respectively, while the red clover and kudzu interventions did not have a significant effect. Although the soy isoflavones were not as effective as the drugs, they may provide some benefit to protecting against bone loss for long periods in non-osteoporotic women without the serious side effects of the drugs.

Evidence from animal studies suggests that isoflavones may affect bone microarchitecture.

In OVX rats, Devareddy et al. found that an isoflavone-enriched soy protein diet restored tibial trabecular number and separation to levels seen in sham rats, but did not restore BMD or BMC [24]. Only one study has looked at the effects of soy isoflavones on bone geometry in humans. Subjects in the SIRBL study underwent pQCT scanning at baseline and at 12, 24, and 36 months of isoflavone supplementation. Scans were taken at the femoral midshaft and the distal tibia. The authors found that soy isoflavone treatment had no significant effects on geometry or volumetric BMD (vBMD) at the distal tibia and only modest effects on femoral midshaft vBMD and stress–strain index (SSI) [25]. However, pQCT is limited to peripheral sites, so whether isoflavone treatment affects bone geometry at clinically relevant fracture sites such as the hip and spine remains unknown. Isoflavones may also improve bone material properties. Vertebrae from OVX rats treated with 5 mg/kg/day genistein for 15 weeks had lower microcrack density and microcrack length, and higher maximum load, than untreated OVX controls. However, BMD and BMC did not significantly differ between the two groups [26]. A novel technology, reference point indentation, has recently made it possible to assess bone material properties at the tissue level in vivo in humans [27]. Future studies with soy isoflavones and other natural products should utilize novel technologies to assess the effect of these treatments on bone material properties, geometry, microarchitecture, and bone turnover.

One potential explanation for the inconsistent results of isoflavone intervention studies is variation in the ability to produce the isoflavone metabolite equol. Equol is a product of bacterial metabolism of daidzein in the intestine. S-equol, the naturally occurring enantiomer, has approximately 80-fold greater estrogen receptor-β binding affinity than daidzein [28], suggesting that it may be a more potent antiresorptive. Approximately 30–50 % of humans have the capacity to produce equol [29]. In a 1-year double-blind trial of 75 mg/day isoflavones in Japanese women, Wu et al. [30]. found that the capacity to produce equol significantly enhanced the effect of isoflavones. Among the women randomized to isoflavone treatment, equol producers experienced BMD changes of −0.46 % at the total hip and −0.04 % at the intertrochanteric region, while non-equol producers experienced changes of −2.28 and −2.61 % at these sites. To determine the effect of equol-producing status on the efficacy of an isoflavone intervention, we prescreened subjects for equol-producing status prior to enrollment in an isoflavone intervention trial. Subjects were categorized as equol producers or non-equol producers based on equol excretion in urine following consumption of 1 soy bar/day for 3 days. Subjects were classified as equol producers if urinary equol was greater than 10,000 nM. Nineteen subjects, including 7 equol producers and 12 non-producers, were dosed with ^{41}Ca and participated in a 50-day intervention with a commercial soy isoflavone product (Novasoy 50, ADM) containing 105 mg total isoflavones, including 46 mg genistein, 44 mg daidzein, and 15 mg glycitein. Net bone turnover decreased by 8 % with the soy treatment, with no significant difference between equol producers and non-producers, suggesting that equol-producing status did not affect the efficacy of the soy intervention [31].

Equol can also be given as a supplement itself. In OVX rats, dietary racemic equol increased femoral calcium content but also had modest uterotropic effects [32]. Tousen et al. [33]. found that supplementation with *S*-equol reduced bone resorption in non-equol-producing menopausal women. Following 12 months of supplementation with 10 mg/day equol, subjects had significantly greater whole-body BMD (but not for regional sites) as well as significantly lower urinary DPD than subjects who received the placebo.

Dried Plum

Dried plum (*Prunus domestica L.*) has been shown to suppress bone resorption, prevent and reverse bone loss, and prevent loss of mechanical strength in sex steroid deficiency female and male animal models of osteopenia [7, 34–36]. In the pre-labeled bone rat model of Mühlbauer

described above, dried plum was the most effective fruit source tested. In the 9-month-old orchidectomized, male rat model with established bone loss, feeding dried plum at 25 % of the diet for 90 days increased vertebral and femoral BMD by ~11, 50 % as effective as PTH with about 60 % of the effect of PTH on biomechanical properties [37]. Trabecular microarchitecture was restored (not observed with other dietary interventions) and cortical bone increased through periosteal expansion. Bone resorption was reduced dose-dependently by up to 60 % as determined by urinary resorption markers which is of similar magnitude to bisphosphonates [36]. Feeding dried plum to 6-month- and 18-month-old male mice increased trabecular bone at dietary levels of 25 % plum and bone gain in the younger adult male mice at dietary levels of 15 % [38]. Improvements in bone measures have been associated with a dose-dependent increase in serum IGF-1 in female [34] and male [35] rats and humans [39] which suggests one mechanism of action may be through stimulating this anabolic hormone.

The bioactive constituent(s) in dried plum is uncertain. The mechanism of action of plum on bone differs from classical estrogens, because it has no uterotrophic activity [40]. Dried plums contain high amounts of polyphenols relative to many other fruits and vegetables at 184 mg/100 g [41]. The predominant phenolic compounds are neochlorogenic and chlorogenic acids. These and other phenolics may inhibit bone resorption due to their antioxidant and inflammatory properties. In fact, dried plums have higher oxygen radical absorbance capacity than most fruits and vegetables [42]. However, it may be that specific polyphenolic compounds have potent bone resorption inhibiting potential. For example, dried plums contain 3.3 ng/100 g rutin [41]. Rutin was identified as the bioactive ingredient in onion that makes it one of the most effective plant food or ingredient tested on bone resorption [43]. However, this group later identified Γ-L-glutamyl-trans-S-1-propenyl-L-cysteine sulfoxide as the likely bioactive component of onion [44]. Still, rutin, in purified form, increased BMD in estrogen-deficient osteopenic rats [45]. Although Bu et al. [37]. claimed that the effect of dried plum was greater than the effect of rutin alone, there is no report of a direct comparison. Rutin is hydrolyzed to its aglycone, quercetin, prior to absorption and can be converted to glucuro- or sulfoconjugates during absorption. Quercetin has antioxidant activity, but also binds to ER_{β} [46]. Quercetin dose-dependently inhibited osteoclast-like cell formation, inhibited RANKL, induced tartrate-resistant acid phosphatase of preosteoclasts, and disrupted the actin rings of these precursor cells [47]. This could explain how quercetin-like compounds may reduce bone resorption. In addition to polyphenols, dried plum also contains high levels of potassium (745 mg/100 g), vitamin K (assumed to be high as fresh plums have 8 μg K_1/100 g), and boron (2.2 mg/100 g) [41]. Each of these nutrients has been associated with positive effects on bone [48–50], but they are unlikely to play a major role in reducing postmenopausal bone loss at dietary concentrations [43, 51–53].

Clinical research on dried plums and bone health is minimal. Feeding 100 g/day of dried plums for 3 months increased serum IGF-1 by 17 % and a biochemical marker of bone formation, bone-specific alkaline phosphatase, by 5.8 % in postmenopausal women, while feeding 100 g/day dried apples did not [54]. A 1-year trial of the same treatments in 160 postmenopausal women resulted in positive changes from both fruits in ulna, spine, femoral neck, total hip, and whole-body BMD with more pronounced effects with plum on spine and ulna [55]. These changes were associated with decreased markers of bone turnover.

Blueberries

Recent studies have shown an anabolic action on bone of blueberry supplemented diets. Blueberry powder (5 % w/w) prevented OVX-induced whole-body BMD loss in 6-month-old Sprague–Dawley rats, but BMD of tibia, femur, and vertebrae were not significantly different [56]. Osteoblastogenesis and mineral apposition rate

were increased in vivo associated with increased expression of Runx2 in bone following activation of Wnt-β catenin signaling and increased phosphorylation of MAP kinase p38 [57].

Blueberry extracts have the highest antioxidant capacity of fruits [58], and blueberry juice was surpassed only by the lingonberry juice for total oxidant scavenging capacity among 14 juices [59]. Blueberries are rich in phenolic compounds with established antioxidant activity. Total polyphenolics ranged from 399 to 556 mg/100 g of Georgia blueberries [60]. They include phenolic acids (≤259 mg/100 g gallic acid, ≤104 mg/100 g p-hydroxybenzoic acid, ≤16 mg/100 p-coumaric, ≤6.3 mg/100 g caffeic, ≤17 mg/100 g ferulic, and ≤6.7 mg/100 g ellagic acids) and flavonoids (≤114 mg/100 g anthocyanins, including delphinidin and malvidin glucosides and galactosides, ≤387 mg/100 g catechin, ≤130 mg/100 g epicatechin, ≤15 mg/100 g quercetin, ≤3.7 mg/100 g kaempferol, and ≤10 mg/100 g myricetin). Antioxidant activity as measured by Trolox equivalent antioxidant capacity (TEAC) ranged from 8.11 to 38.29 μM TEAC/g in the Georgia blueberries which correlated with phenolic content ($r^2 = 0.98$) and, to a lesser degree, anthocyanin content ($r^2 = 0.60$). A mixture of the phenolic acid metabolites in serum after feeding blueberries (hippuric acid, phenylacetic and hydroxybenzoic acids) induced the same effects on Wnt signaling and osteoblastogenesis as blueberries [60]. The effect of blueberries on bone health in humans has not been reported.

Future Research

There is great promise for plant bioactives to help protect against bone loss associated with menopause. Most of the work has focused on in vitro or preclinical models. We likely need to move beyond BMD as the main outcome measure to better understand their impact on bone strength through influencing bone turnover and bone quality. Better understanding of the mechanisms of action of bioactive compounds in the diet can provide insights for what to measure.

The pathogenesis of osteoporosis has moved from an estrogen-centric to a perspective of aging and oxidative stress [61], which extends the potential role of diet in ameliorating bone loss beyond compounds that interact with estrogen receptors. Antioxidants retard reactive oxygen species (ROS) which influences cells involved in bone turnover. The interaction of redox systems with sex steroids via nonnuclear MAP kinase regulated pathways may be the molecular targets for nutritional interventions. MAP kinase activation results in downstream actions on a number of cellular redox systems to increase antioxidant capacity and inhibit formation of ROS. ROS production in mesenchymal stem cells inhibits osteoblastogenesis. Estrogens, and presumably phytochemicals, antagonize ROS actions in bone cells via upregulation of glutathione reductase and a number of antioxidant systems. Plant sources of bioactives that merit more research for bone health include plum berries, grapes, oranges, mushrooms, and many herbs.

References

1. National Osteoporosis Foundation. Fast facts. http://www.nof.org/node/40. Accessed 10 Feb 2011.
2. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroiz AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB, Women's Health Initiative Investigators. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA. 2003;290:1729–38.
3. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288:321–33.
4. Chen Y, Ho SC, Woo JLF. Greater fruit and vegetable consumption is associated with increased bone mass among postmenopausal Chinese women. Br J Nutr. 2006;96:745–51.
5. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr. 1999;69:727–36.

6. Lee W-H, Wastney ME, Jackson GS, Martin BR, Weaver CM. Interpretation of ^{41}Ca data using compartmental modeling in post-menopausal women. Anal Bioanal Chem. 2011;399:1613–22.
7. Muhlbauer RC, Lozano A, Reinli A, Wetli H. Various selected vegetables, fruits, mushrooms, and red wine residue inhibit bone resorption in rats. J Nutr. 2003;133:3592–7.
8. Cheong JMK, Gunarata N, McCabe GP, Jackson GS, Weaver CM. Bone seeking labels as markers for bone turnover: effect of dosing schedule on labeling various bone sites in rats. Calcif Tissue Int. 2009;85:444–50.
9. Zhao Y, Cheong JMK, Lee WH, Wastney M, Martin BR, Weaver CM. Tetracycline and calcium kinetics are comparable for estimating bone resorption in rats. J Nutr. 2010;140:1704–9.
10. Cheong JMK, Martin BR, Jackson GS, Elmore D, McCabe GP, Nolan JR, Barnes S, Peacock M, Weaver CM. Soy isoflavones do not affect bone resorption in postmenopausal women: a dose–response study using a novel approach with ^{41}Ca. J Clin Endocrinol Metab. 2007;92:577–82.
11. Jackson G, Lee WH, Martin B, Weaver C. Correlations of urinary 41Ca with biomarkers and minerals in postmenopausal women. J Bone Miner Res. 2011;26: S473.
12. The North American Menopause Society. The role of soy isoflavones in menopausal health: report of the North American Menopause Society/Wulf H. Utian Translational Science Symposium in Chicago, IL (October 2010). Menopause. 2011;18:732–53.
13. Zhang X, Shu X-O, Li H, Yang G, Li Q, Gao Y-T, Zheng W. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. Arch Intern Med. 2005;165:1890–5.
14. Koh W-P, Wu AH, Wang R, Ang L-W, Heng D, Yuan J-M, Yu MC. Gender-specific associations between soy and risk of hip fracture in the Singapore Chinese Health Study. Am J Epidemiol. 2009;170:901–9.
15. Greendale GA, FitzGerald G, Huang M-H, Sternfeld B, Gold E, Seeman T, Sherman S, Sowers M. Dietary soy isoflavones and bone mineral density: results from the study of women's health across the nation. Am J Epidemiol. 2002;155:746–54.
16. Alekel DL, Van Loan MD, Koehler KJ, Hanson LN, Stewart JW, Hanson KB, Kurzer MS, Peterson CT. The Soy Isoflavones for Reducing Bone Loss (SIRBL) Study: a 3-y randomized controlled trial in postmenopausal women. Am J Clin Nutr. 2010;91:218–30.
17. Tai TY, Tsai KS, Tu ST, Wu JS, Chang CI, Chen CL, Shaw NS, Peng HY, Wang SY, Wu CH. The effect of soy isoflavone on bone mineral density in postmenopausal Taiwanese women with bone loss: a 2-year randomized double-blind placebo-controlled study. Osteoporos Int. 2012;23:1571–80.
18. Levis S, Strickman-Stein N, Ganjei-Azar P, Xu P, Doerge DR, Krischer J. Soy isoflavones in the prevention of menopausal bone loss and menopausal symptoms. Arch Intern Med. 2011;171:1363–9.
19. Wong WW, Lewis RD, Steinberg FM, Murray MJ, Cramer MA, Amato P, Young RL, Barnes S, Ellis KJ, Shypailo RJ, Fraley JK, Konzelmann KL, Fischer JG, Smith EO. Soy isoflavone supplementation and bone mineral density in menopausal women: a 2-y multicenter clinical trial. Am J Clin Nutr. 2009;90:1433–9.
20. Wainwright SA, Marshall LM, Ensrud KE, Cauley JA, Black DM, Hillier TA, Hochberg MC, Vogt MT, Orwoll ES, Study of Osteoporotic Fractures Research Group. Hip fracture in women without osteoporosis. J Clin Endocrinol Metab. 2005;90:2787–93.
21. Delmas P, Seeman E. Changes in bone mineral density explain little of the reduction in vertebral or nonvertebral fracture risk with anti-resorptive therapy. Bone. 2004;34:599–604.
22. Garnero P, Delmas PD. Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. J Musculoskelet Neuronal Interact. 2004;4: 50–63.
23. Weaver CM, Martin BR, Jackson GS, McCabe GP, Nolan JR, McCabe LD, Barnes S, Reinwald S, Boris ME, Peacock M. Antiresorptive effects of phytoestrogens supplements compared with estradiol or risedronate in postmenopausal women using 41Ca methodology. J Clin Endocrinol Metab. 2009;94:3798–805.
24. Devareddy L, Khalil DA, Smith BJ, Lucas EA, Soung DY, Marlow DD, Arjmandi BH. Soy moderately improves microstructural properties without affecting bone mass in an ovariectomized rat model of osteoporosis. Bone. 2006;38:686–93.
25. Shedd-Wise KM, Alekel DL, Hofmann H, Hanson KB, Schiferl DJ, Hanson LN, Van Loan MD. The Soy Isoflavones for Reducing Bone Loss (SIRBL) Study: three year effects on pQCT bone mineral density and strength measures in postmenopausal women. J Clin Densitom. 2011;14:47–57.
26. Dai R, Ma Y, Sheng Z, Jin Y, Zhang Y, Fang L, Fan H, Liao E. Effects of genistein on vertebral trabecular bone microstructure, bone mineral density, microcracks, osteocyte density, and bone strength in ovariectomized rats. J Bone Miner Metab. 2008;26:342–9.
27. Diez-Perez A, Guerri R, Nogues X, Caceres E, Pena MJ, Mellibovsky L, Randall C, Bridges D, Weaver JC, Proctor A, Brimer D, Koester KJ, Ritchie RO, Hansma PK. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. J Bone Miner Res. 2010;25:1877–85.
28. Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, Katzenellenbogen BS, Helferich WG, Katzenellenbogen JA. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. Bioorg Med Chem. 2004;12:1559–67.
29. Setchell KDR, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002;132:3577–84.

30. Wu J, Oka J, Ezaki J, Ohtomo T, Ueno T, Uchimaya S, Toda T, Uehara M, Ishimi Y. Possible role of equol status in the effect of isoflavone on bone and fat mass in postmenopausal Japanese women: a double-blind, randomized, controlled trial. Menopause. 2007;14: 866–74.
31. Wiersma J, Martin B, McCabe G, McCabe L, Jackson G, Peacock M, Barnes S, Simon J, Weaver C. Equol-producing status does not predict the antiresorptive effects of soy isoflavone supplements. J Bone Miner Res. 2009; 24:Abst SU0412.
32. Legette LL, Martin BR, Shahnazari M, Lee WH, Helferich WG, Qian J, Waters DJ, Arabshahi A, Barnes S, Welch J, Bostwick DG, Weaver CM. Supplemental dietary racemic equol has modest benefits to bone but has mild uterotropic activity in ovariectomized rats. J Nutr. 2009;139:1908–13.
33. Tousen Y, Ezaki J, Fujii Y, Ueno T, Nishimuta M, Ishimi Y. Natural S-equol decreases bone resorption in postmenopausal, non-equol-producing Japanese women: a pilot, randomized, placebo-controlled trial. Menopause. 2011;18:563–74.
34. Deyhim F, Stoecher BJ, Brusewitz GH, Devareddy L, Arjmandi B. Dried plum reverses bone loss in an osteopenic rat model of osteoporosis. Menopause. 2005;12:755–62.
35. Arjmandi BH, Lucas EA, Juma S, Soliman A, Stoecker BJ, Khalil DA, Smith BJ, Wang C. Prune prevents ovariectomy-induced bone loss in rats. JANA. 2001;4:50–6.
36. Franklin M, Bu SY, Lerner MK, Lancaster EA, Bellmer D, Marlow D, Lightfoot SA, Arjmandi BH, Brackett DJ, Lucas EA, Smith BJ. Dried plum prevents bone loss in a male osteoporosis model via IGF-1 and RANK pathway. Bone. 2006;39:1331–42.
37. Bu SY, Lucas EA, Franklin M, Marlow D, Brachett DJ, Boldrin EA, Devareddy L, Arjmandi BH, Smith BJ. Comparison of dried plum supplementation and intermittent PTH in restoring bone in osteopenic orchidectomized rats. Osteoporos Int. 2007;189: 931–42.
38. Holloran BP, Wronski TJ, VonHerzen DC, Chu V, Xia X, Pingel JE, Williams AA, Smith BJ. Dietary dried plum increases bone mass in adult and aged male mice. J Nutr. 2010;143:1781–7.
39. Arjmandi BH, Khalil DA, Lucas EA, Georgies A, Stoeker BJ, Hardin C, Payton ME, Wild RA. Dried plums improve indices of bone formation in postmenopausal women. J Womens Health Gend Based Med. 2002;11:61–8.
40. Hooshmand S, Arjmandi BH. Viewpoint: dried plum, an emerging functional food that may effectively improve bone health. Ageing Res Rev. 2009;8:122–7.
41. Stacewicz-Saputzakis M, Bowen PE, Hussain EA, Damayanti-Wood BI, Farnsworth NR. Chemical composition and potential health effects of prunes: a functional food? Crit Rev Food Sci Nutr. 2001;41: 25–286.
42. McBride J. Can foods forestall aging? Agri Res. 1999;47:14.
43. Mühlbauer RC, Lozano A, Reinli A. Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanisms independent of their base excess. J Bone Miner Res. 2002;17: 1230–6.
44. Weltli HA, Brenneisen R, Tschudi I, Langos M, Bigler P, Sprang T, Schurch S, Mühlbauer RC. A Γ-glutamyl peptide isolated from onion (Allium cepa L.) by bioassay-guided fractionation inhibits resorption activity of osteoclasts. J Agric Food Chem. 2005;53: 3408–14.
45. Horcajada-Moltini MN, Crespy V, Coxam V, Davicco M-J, Remesy C, Barlet J-P. Rutin inhibits ovariectomy-induced osteopenia in rats. J Bone Miner Res. 2000;15:2251–8.
46. Caltagirone S, Ranelletti FO, Rinelli A, Muggiano N, Colasante A, Musiani P, Aiello FB, Piantelli M. Interactions with type II estrogen-binding sites and antiproliferative activity of tamoxifen and quercetin in human non-small cell lung cancer. Am J Respir Cell Mol Biol. 1997;17:51–9.
47. Woo J-T, Nakogawa H, Notoya M, Yoneqawa T, Udagawa N, Lee I-S, Ohnishi M, Hagiwara H, Nagui K. Quercetin suppresses bone resorption by inhibiting the differentiation and activation of osteoclasts. Biol Pharm Bull. 2004;27:504–9.
48. MacDonald HM, New SA, Fraser WD, Campbell MK, Reid DM. Low dietary potassium intakes and high dietary estimates of net endogenous acid production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. Am J Clin Nutr. 2005;81:923–33.
49. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris Jr RC. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med. 1994;330:1776–81.
50. Nielsen FH, Hunt CD, Mullen L, Hunt JR. Effect of dietary boron on minerals, estrogen, and testosterone metabolism in postmenopausal women. FASEB J. 1987;1:394–7.
51. Rafferty K, Davies MK, Heaney RP. Potassium intake and the calcium economy. J Am Coll Nutr. 2005;24:99–106.
52. Booth SL, Dallal G, Shea MK, Gunderberg C, Peterson JW, Dawson-Hughes B. Effect of vitamin K supplementation on bone loss in elderly men and women. J Clin Endocrinol Metab. 2008;93:1217–23.
53. Cheung AM, Tile L, Lee Y, Tomlinson G, Hawker G, Scher J, Hu H, Vieth R, Thompson L, Jamal S, Josse R. Vitamin K supplementation in postmenopausal women with osteopenia (ECKO trial): a randomized controlled trial. PLoS Med. 2008;14(10):e196.
54. Arjamandi BH, Khalil DA, Lucas EA, Georgis A, Stoecker BJ, Hardin C, Payton ME, Wild RA. Dried plums improve indices of bone formation in postmenopausal women. J Womens Health Gend Based Med. 2006;11:61–8.
55. Hooshmand S, Chai SC, Saadat RL, Payton ME, Brummel-Smith K, Arjmandi BH. Comparative

effects of dried plum and dried apply on bone in postmenopausal women. Br J Nutr. 2011;106:923–30.
56. Devareddy L, Hooshmand S, Collins JK, Lucas EA, Chai SC, Arjmandi BH. Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. J Nutr Biochem. 2008;19(10):694–9.
57. Chen JR, Lazarenko OP, Wu X, Kang J, Blackburn ML, Shankar K, Badyer TM, Ronis MJJ. Diet induced serum phenolic acids promote bone growth via p38 MAPK/β-catenin canonical Wnt signaling. J Bone Miner Res. 2010;25:2399–411.
58. Prior RL, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J Agric Food Chem. 2002;46:2686–93.
59. Lichtenthäler R, Marx F. Total oxidant scavenging capacities of common European fruit and vegetable juices. J Agric Food Chem. 2005;53:103–10.
60. Sellappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J Agric Food Chem. 2002;50:2432–8.
61. Manolagas S. From estrogen – centric to aging and oxidation stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31: 266–300.

Citrus Flavanones and Bone Health

15

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Abstract

Flavanones are a class of flavonoids found mainly in citrus fruits, the most common ones being hesperidin and naringin. These compounds exist as glycoside conjugates in the fruits at relatively abundant concentrations. Despite the extensive metabolism, the flavanones circulate in blood in the low μM range, which we therefore consider as the "physiological" concentration as opposed to more pharmacological concentrations (50–100 μM) often used in cell culture studies.

Among the various biological effects attributed to the flavanones, the more consistent ones are anti-inflammatory and lipid-lowering properties. In particular, the anti-inflammatory effects of the flavanones have been shown to be mediated through the inhibition of the NF-κB pathway.

Concerning the effects of flavanones on bone metabolism, the *in vivo* evidence in preclinical studies mimicking postmenopausal or senile bone loss shows an effect of hesperidin on preservation of bone mineral density (BMD) associated with reduced collagen breakdown. In the first placebo-controlled, randomized, double-blind clinical trial to be performed on the ability of hesperidin (500 mg daily for 2 years) to attenuate bone loss in postmenopausal women, we found that hesperidin did not significantly alter the 1–2 % BMD loss/year generally observed in this population. However, the subjects consuming hesperidin presented a better balance in bone metabolism as reflected by the bone turnover index.

Any nutritional approach to bone health should include the main bone micronutrients (calcium, vitamin D) already shown to improve BMD in osteopenic situations. However, further bioactive nutrients in the diet, such as the flavanones, may play a further role in modulating bone turnover and help protect against bone loss in at-risk populations. The question remains

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as to how best to measure such effects and show their relevance to bone quality, whether it is via bone biomarkers, bone architecture, or bone mineral density in combination or not with calcium and vitamin D.

Keywords
Flavanones • Hesperidin • Naringin • Bioavailability • Inflammation • Osteoblasts • Bone mineral density • Bone turnover

Introduction

Flavanones are a class of flavonoids found mainly in citrus fruits, although minor amounts have also been detected in herbs, red wine, and tomatoes [1].

In citrus fruits, flavanones account for approximately 95 % of the total flavonoids [2, 3].

However, the flavanone content varies depending on the part of the fruit. The solid parts of the fruit, particularly the albedo (the white spongy portion) and the membranes separating the segments, are richest in flavanones compared to juice vesicles (pulp), which explains the higher content of flavanones in the whole fruit than in the juice.

Hesperetin and naringenin (aglycones) are the most common flavanones in citrus fruits, and they are usually conjugated to a glucose-rhamnose disaccharide, typically rutinose or neohesperidose (Fig. 15.1). In the edible fraction of fruits, glycoside content (hesperidin and narirutin) ranges from 35 to 147 mg/100 g (oranges) [3, 4], and from 44 to 106 mg/100 g for naringenin glycoside (naringin and narirutin) in the edible fraction of grapefruits [1, 2].

Hesperetin and naringenin conjugates are the most studied flavanones with regard to metabolism and bioavailability. However, hesperidin (hesperetin-7-*O*-rhamnoglucoside) remains the most studied flavanone with respect to bone health. Apart from soy isoflavonoids [5], there are no controlled clinical trials and only a few preclinical studies that have examined the specific effects of other flavonoids such as flavanones on bone metabolism.

The influence of both flavanones has been examined primarily in animal models of bone loss (predominantly in female rats) and/or bone cell culture systems [6]. Most of these preclinical studies revealed multiple beneficial actions such as promoting osteoblast function, restoring bone mass and bone strength after an ovariectomy-induced bone loss, or preventing bone mineral density decrease in senescent animals.

This chapter therefore focuses on the impact of hesperidin and naringin on bone metabolism and their biological properties/mechanisms of action that may contribute directly or indirectly to the effect on bone metabolism. We report on nutritional approaches that have been investigated using preclinical models. In vitro data are also included since they provide valuable knowledge when investigating novel ingredients and when looking into tissue-level effects and mechanisms of action. Finally, the main results from the only clinical study available on the ability of hesperidin to protect against bone loss in postmenopausal women are reported.

Intake, Bioavailability, and Metabolism of Flavanones

Flavanones are highly consumed in Western countries. In the adult Spanish population, intake may reach 50 mg/day, or around 17 % of the estimated total daily flavonoid intake, which ranks them as the most consumed flavonoid subclass after proanthocyanidins [7]. Flavanone intakes of 14.4, 20.4, 22, 33.5, and 34.7 mg/day have also been reported for the USA, UK, Finland, Greece, and Italy, respectively [7]. Oranges (as whole fruits and juices) appear to be the main contributor to flavanone intake [7]. Even though the daily intake of hesperidin has not been precisely evaluated in different populations, it is arguably relatively high due to worldwide consumption of

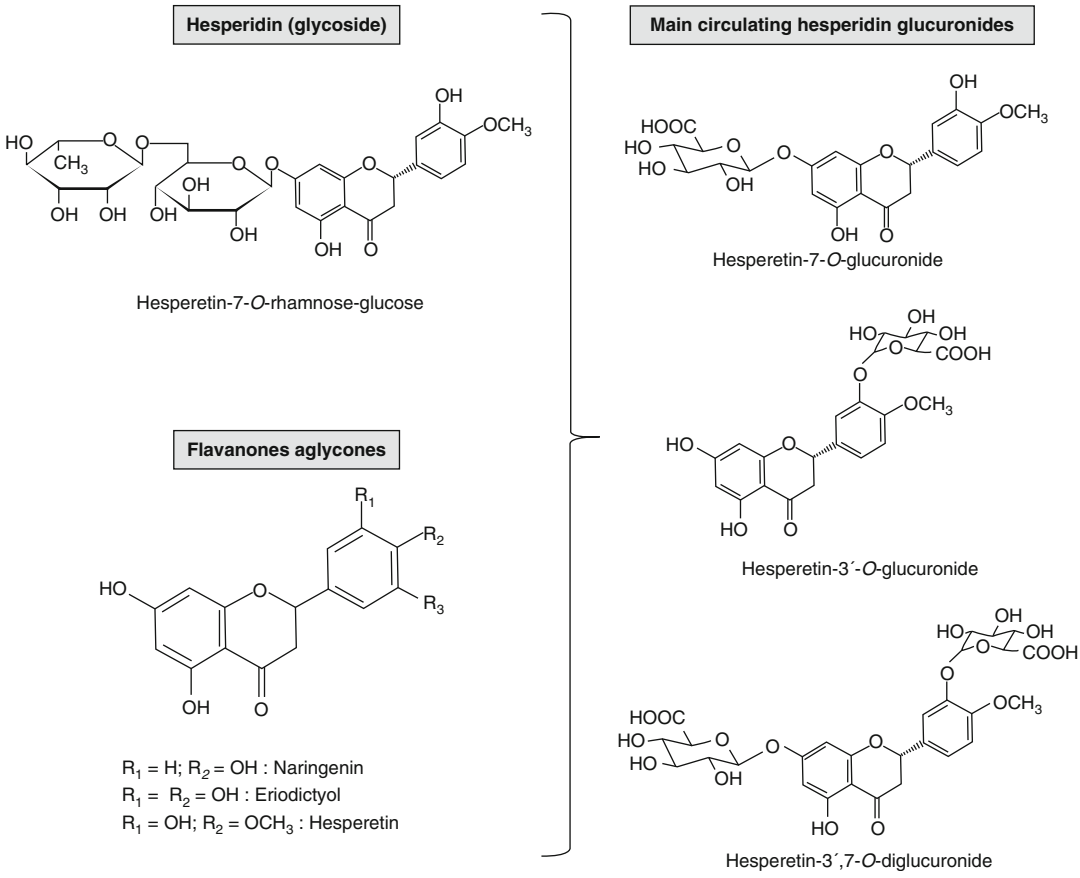

Fig. 15.1 Chemical structures of flavanone aglycones, hesperidin, and its main metabolites

citrus products such as citrus fruits and juices (e.g., in Western countries, intakes of oranges range from 35 to 50 kg per person per year).

Due to their abundance in citrus fruit, hesperetin and naringenin conjugates are the most studied flavanones with regard to metabolism and bioavailability. There is an extensive amount of literature available concerning the in vitro metabolism and bioactivity of flavanones. In spite of the value of in vitro data, in vivo studies on metabolism and bioavailability are the most crucial for knowledge of the flavanone forms to which tissues are exposed and the magnitude and time scales of this exposure [8].

Flavanones are mainly present in foods as diglycosides. It has long been known that such glycosides cannot be absorbed in their native form in the small intestine but must by hydrolyzed by intestinal microflora before absorption of their aglycone moieties in the colon. Moreover, the nature of the sugar moiety has been shown to be an important determinant of the mode of absorption. The free aglycones released are then taken up and conjugated by phase II enzymes in both the intestine and the liver. As a result, flavanones exist in the plasma mainly as sulfated and glucuronidated metabolites [8]. However, glucuronidation appears to be the principal biotransformation of hesperetin. After consumption of orange juice, the metabolites identified in the plasma were hesperetin-7-glucuronide, hesperetin-3′- glucuronide, hesperetin-3′-sulfate, naringenin-4′-glucuronide, and naringenin-7-glucuronide [9, 10]. The mean peak plasma concentrations of flavanones have been shown to be between 0.1 and 1 μM for intakes ranging from a 150 g orange to 500 mL of orange juice [9–16]. Furthermore, it was shown that peak plasma

concentrations of 0.47 and 0.6 μM were achieved after intake of 230 g of orange fruit or 550 mL of orange juice (both providing 115 mg of aglycones), respectively. The similarity between the plasma concentrations of flavanones, observed after administration of whole citrus fruits or juices, suggests that the food matrix did not significantly affect the bioavailability of the flavanones [17]. Urinary excretion of flavanones mainly occurs during the 24 h following ingestion, peaking between 6 and 12 h. The rate of urinary excretion after consumption of an orange (as juice or whole fruit) is between 1.7 and 6.4 % [10, 14, 15], indicating that flavanones are among the most bioavailable dietary polyphenols [18].

Thus, even if flavanones may appear to have limited bioavailability in humans, they may still be able to reach target cells or organs at sufficiently high concentrations to exert biological effects, in both animals and humans. Different biological activities have been reported for flavanones (e.g., lipid-lowering effects, veinotonic, anticarcinogenic); however, in the past years, more emphasis has been brought to their antioxidant and anti-inflammatory properties.

Impact of Flavanones on Oxidative Stress and Inflammation

Preclinical Data

The underlying mechanisms by which flavanones may antagonize inflammation and oxidative stress have not yet been fully elucidated. However, recent literature indicates that flavanones may also act on oxidative stress and inflammation. In both dyslipidemic and diabetic rodent models, dietary flavanone supplementation was reported to increase the activity of antioxidant enzymes, such as SOD and glutathione peroxidase [19–21]. Several in vivo studies have shown that flavanones can reduce vascular or systemic levels of chemokines as well as inflammatory and adhesion molecule expressions [21–25] that are under proinflammatory factor NF-κB control. As an example, Kim et al. showed in rats fed with hesperidin (6- and 24-month-old) a suppressed age-related NF-κB activation and age-related gene expression of proinflammatory mediators in the kidneys of the animals, thus a protective effect through a downregulation of NF-κB signaling [26]. Recently we also showed a significantly decreased IL-6 concentration and NO production in 20-month-old gonad-intact male rats receiving a diet supplemented with 0.5 % hesperidin for 3 months [27].

Potential anti-inflammatory actions for flavanones have also been explored in various cell types. Some studies have reported a decrease in TNF-α production after exposure of activated macrophages to 50 μM hesperetin [28, 29]. Macrophages exposed to physiological concentrations of naringenin, e.g., 5 μM, decreased prostaglandin E2 production and reduced the expression of COX2 [30]. Moreover, it was shown that naringenin (30–100 μM) inhibited NF-κB activation in inflammation-stimulated macrophages [31, 32]. However, hesperetin (100 μM) did not have such an effect, suggesting that other anti-inflammatory pathways could be involved [28]. Indeed, in endothelial cells exposed to inflammatory stress, hesperidin and naringin reduced VCAM-1 expression without affecting ICAM-1 or E-selectin [33, 34]. Particularly, hesperidin has been shown to reduce TNF-α-induced VCAM-1 expression through the regulation of the Akt and PKC pathways [34]. Globally, in TNF-α-stimulated endothelial cells, some studies reported a reduction of the expression of adhesion molecules and/or of monocyte adhesion to endothelial cells after exposure to aglycones (effects observed with 1–50 μM hesperetin or naringenin)[22, 33], whereas others did not observe this effect [35]. Finally, it was also reported that hesperidin can inhibit cytokines production in human mast cell line. The stimulation of interleukin (IL)-1β, IL-8, and TNF-α levels were significantly inhibited by hesperidin exposure [36]. Another in vitro study showed that treatment with hesperetin (1–10 μM) significantly inhibited IL-1β-induced production of inflammatory mediators (MMP-3 and IL-6) in human synovial cells [37].

Taken together, these preclinical data suggest that flavanones may exert anti-inflammatory

activities in the various cell types, partly through the inhibition of the NF-κB pathway. However, it is important to underline the fact that most in vitro data were obtained with pharmacological concentrations of aglycone compounds or flavanone glycosides (thus not the physiological circulating forms), thus leading to limited biological relevance.

Clinical Studies

In healthy men with cardiovascular risk factors, no changes were observed in the plasma antioxidant capacity following hesperidin supplementation (292 mg) for 4 weeks [38]. This result, which does not support a direct antioxidant effect of flavanones in vivo, may be explained by both the low antioxidant capacity of the native flavanone structure, further reduced by the conjugation process, and the low circulating levels of conjugated metabolites. Jung et al. [39] reported a significant increase in erythrocyte catalase and superoxide dismutase (SOD) activities in hypercholesterolemic subjects after 8 week-supplementation with 400 mg of naringin. This suggests that flavanones may improve endogenous antioxidant defense systems in dyslipidemic subjects.

With regard to inflammation, in subjects with metabolic syndrome, supplementation with 500 mg hesperidin was shown to reduce the plasma levels of two inflammatory biomarkers, C-reactive protein (CRP) and serum amyloid A (SAA) [40]. In healthy, middle-aged, moderately overweight men, despite no effect on circulating inflammatory markers [38], hesperidin intake (292 mg/day for 4 weeks) tended to modulate gene expression in white blood cells toward an anti-inflammatory profile [41]. In this study, the supplementation notably affected the expression of genes involved in processes such as adhesion, chemotaxis, and cell proliferation.

Some studies have also been conducted with citrus juices rich in these polyphenols. For instance, it was shown that consumption of orange and black currant juice (250 mL of each one per day during 28 days) reduces the level of the inflammatory markers CRP (C-reactive protein) and fibrinogen in patients with peripheral arterial disease compared to sugar-containing beverage [42]. Finally, a cross-sectional study with 2,115 women aged 43–70 years showed that the highest intakes of citrus fruits were associated with a 20 % lower levels of IL-18, compared with women with the lowest average intakes [43], suggesting a potential role of flavanones in the inflammation process. Thus, these findings suggest that flavanones could be one of the bioactive compounds responsible for citrus anti-inflammatory properties.

In summary, further clinical studies in different populations using isolated compounds are needed to clarify the effect of flavanones on inflammation in humans. Moreover, it would be particularly important to correlate the magnitude of the observed changes with flavanones metabolites plasma concentrations achieved. Furthermore, to improve the physiological relevance of the preclinical and in vitro data, additional studies investigating the anti-inflammatory impact of nutritional doses in vivo and nutritionally achievable concentrations of metabolites in vitro are required. These studies will be crucial to identify the underlying cellular and molecular mechanisms of the protective effects of flavanones.

Impact of Flavanones on Bone Metabolism

Anti-inflammatory properties are of great interest in the context of degenerative diseases, including cardiovascular, cancer, as well as osteoporosis [44], where it is well acknowledged that oxidative stress and inflammation are key players. For instance, the possible antiresorptive action of hesperetin and naringin we report below could be linked to modulation of nuclear factor NF-κB. Indeed, NF-κB is a key signaling factor in osteoclast proliferation and differentiation [45] and involves an inflammatory component through the response from and regulation of several cytokines (e.g., IL-6, TNF-alpha) and nitric oxide (NO) which is significantly increased in the inflammatory process [46, 47]. In addition,

high levels of TNF-alpha and NO are potent osteoclastogenic stimulators [47, 48], and age-associated increase in IL-6 has been linked to osteopenia and osteoporosis development [49].

Thus, beside the importance of adequate calcium and vitamin D intakes for bone health which is well established, it is now agreed that the human diet contains also a complex array of non-nutrient natural bioactive molecules, such as flavanones, that may influence and protect bone. It is now well acknowledged that these compounds can regulate a number of physiological functions in mammalian systems, and thus, they may offer beneficial effects in slowing down or protecting against chronic diseases.

Therefore, these compounds may be implicated in some of the positive links found between fruit and vegetable intake and higher bone mineral density in adults and children [50, 51]. Nevertheless, no long-term intervention studies in humans have investigated the effect of specific phytochemicals on the prevention of bone loss in postmenopausal women, except for phytoestrogens. In addition, some data in animal models of bone loss and flavanones efficacy are available. Moreover, in vitro experiments with bone cells have reported cellular and molecular mechanisms of flavanones involved in bone metabolism. However, only one human clinical trial assessing the long-term ability of isolated hesperidin to protect against bone loss in postmenopausal women has been conducted (unpublished data).

Preclinical Evidence for Citrus Flavanones

A study by Mühlbauer et al. showed that oranges were able to significantly inhibit bone resorption in young male rats fed with a semi-purified diet to which oranges were added at a dosage per rat of 1 g/day [52]. Hesperidin (0.5 % in the diet) was shown in Chiba's study to inhibit femoral bone loss when administrated in 2-month-old ovariectomized mice [53]. Similarly, consumption of hesperidin (0.5 % in the diet) inhibited ovariectomy-induced bone mineral density loss in female rats after 3-month duration [54]. Furthermore, we conducted a study to compare the efficacy of hesperidin in young (3-month-old) and adult (6-month-old) rats on bone metabolism. Animals were sham-operated (SH) or ovariectomized (OVX) and then pair-fed for 90 days a casein-based diet supplemented or not with 0.5 % hesperidin. In older rats, hesperidin intake led to a partial inhibition of OVX-induced bone loss, whereas a complete inhibition was obtained in younger animals. At both ages, while plasma osteocalcin concentrations were unchanged, urinary excretion of deoxypyridinoline was reduced by hesperidin intake, suggesting that hesperidin was able to slow down bone resorption. Unexpectedly, in intact young rats, hesperidin consumption resulted in a significant increase in bone mineral density (BMD). Indeed, 6-month-old intact animals fed with hesperidin had a similar BMD to 9-month-old non-treated intact adult rats, suggesting an accelerated bone mass gain in the young rats. In contrast, in intact adult rats, hesperidin did not further increase BMD but did improve their bone strength. The results of this study showed a protective effect of hesperidin on bone loss in OVX rats of both ages without uterine stimulation and accompanied by a lipid-lowering effect. [55] A further study examining hesperetin-7-glucoside, an intestinal metabolite of hesperidin which is more bioavailable than hesperidin itself, also demonstrated a greater efficiency than hesperidin in inhibiting bone loss due to ovariectomy in 6-month-old rats. The minimum effective dose was 0.25 % hesperidin in the diet [56]. Recently we compared the effect of hesperidin and naringin on the regulation of bone metabolism in male senescent rats. 20-month-old gonad-intact male Wistar rats received a casein-based diet supplemented or not with either 0.5 % hesperidin, 0.5 % naringin or a mix of both flavanones (Hp+Nar, 0.25 % each). After 3 months, daily hesperidin intake significantly improved femoral bone integrity as reflected by improvements in total and regional bone mineral density (BMD) and trabecular bone volume fraction improvement at the femur compared with control group. In contrast, naringin exerted site-specific effects on BMD (improvement at the distal metaphyseal area), and no

further benefit to bone mass was observed with the mix of flavanones. Bone resorption (DPD) was significantly attenuated by hesperidin and naringin given alone but not by the mixture of the two. All treatments significantly reduced expression of inflammatory markers to a similar extent (IL-6 and NO) compared to control. Bone formation did not appear to be strongly affected by any of the treatments (no effect on osteocalcin levels, modest modulation of tibial BMP-2 mRNA). However, as previously reported, plasma lipid-lowering effects were observed with hesperidin and naringin alone (lower total cholesterol and triglycerides compared to control) or together. Surprisingly the plasma circulating level of naringin (8.15 μM) was fivefold higher than that of hesperidin (1.44 μM) at equivalent doses (0.5 %), and a linear reduction in plasma levels was observed upon coadministration (0.25 % each) indicating absence of competition for their intestinal absorption sites and metabolism. The higher efficacy of hesperidin at a lower plasma concentration than naringin as well as the identification of the major circulating metabolite of hesperidin (hesperetin-7-*O*-glucuronide) underlines the importance of flavanone bioavailability and metabolism in their biological efficacy and suggests a structure-function relationship in the mechanism of action of the active metabolites [27]. Taken together, these preclinical studies with pure isolated hesperidin and naringin suggest that these flavanones may be two of the components responsible for the findings by Deyhim et al. that citrus juice or orange pulp modulates bone strength and decreases bone resorption in male orchidectomized senescent rats [57, 58]. Identically, these compounds may be involved in the restoration of the bone microarchitecture and decreased bone resorption in the same animal model fed with orange pulp for 4 months [59].

The beneficial effects of hesperidin and naringin on bone mass in rodent models have been mainly related to a slowdown in bone resorption (urinary free deoxypyridinoline). However, as first suggested by Chiba et al., hesperidin could not only modulate bone resorption but also affect bone formation [53, 60].

The mechanism of action of flavanones on bone metabolism is not yet elucidated and may involve several indirect and direct effects on cellular signaling and bone metabolism. For instance, hesperidin as well as naringin can act similarly to statins in reducing the activity of HMG-CoA reductase which results in lower plasma and hepatic cholesterol as well as hepatic triglycerides in rats [61]. Since statins also increase bone formation by stimulating the growth factor bone morphogenic protein-2 (BMP-2) [62, 63], one hypothesis for the mechanism of action of these flavanones is that they may act on bone in a similar way. Indeed, the mechanisms of action of hesperetin and its main circulating form, hesperetin-7-*O*-glucuronide, were assessed in rodent primary osteoblast cells. Osteoblasts were exposed to physiological concentrations of 1 or 10 μM hesperetin (aglycone). Neither proliferation nor mineralization was affected by hesperetin at either dose. However, differentiation was enhanced (significant increase in alkaline phosphatase activity). The effect of hesperetin on ALP was inhibited by addition of noggin protein, suggesting a possible action of this flavanone through the bone morphogenetic protein (BMP) pathway. Indeed, hesperetin significantly upregulated expression of genes involved in this signaling pathway (i.e., BMP-2, BMP-4, Runx2, and Osterix) [64].

In the same model, we examined the effect of hesperetin-7-*O*-glucuronide (Hp7G) on proliferation and differentiation. The impact of Hp7G on specific bone signaling pathways was explored with physiological concentrations of 1 and 10 μM of conjugate. The glucuronide did not affect proliferation but enhanced differentiation by significantly increasing alkaline phosphatase (ALP) activity. Hp7G significantly induced mRNA expression of ALP, Runx2, and Osterix. Moreover, phosphorylation of Smad1/5/8 was enhanced by Hp7G, while ERK1/2 remained unchanged. Hp7G decreased RANKL gene expression. These results suggest that Hp7G may regulate osteoblast differentiation through Runx2 and Osterix stimulation, as the aglycone form, but also might be implicated in the regulation of osteoblast/osteoclast communication [64, 65].

In the same way, an osteogenic effect of naringin on the collagen matrix of rabbit bones was associated with enhanced BMP-2 production by bone-forming cells [66]. Wong et al. investigated the effect of naringin in UMR 106 osteoblastic cell line [67]. The experimental group consisted of cells cultured with different concentrations of naringin (0.001, 0.01, and 0.1 μM) for 24, 48, and 72 h. Results for the naringin group showed a dose-dependent increase in total protein content. Naringin at 0.1 μM significantly enhanced ALP activity by up to 20 %.

Another potential and important mechanism of flavanones on bone cell signaling to be explored could be through their anti-inflammatory activities, as previously suggested by downregulation of NF-κB signaling in rats fed hesperidin [26] and decreased levels of IL-6 and NO in old senescent rats [27]. However, other potential mechanisms whereby hesperidin and naringin may protect skeletal health include modulation of fat-bone interactions [68, 69] and antioxidative status [70] and still need to be further evaluated.

In summary, the currently available in vitro data suggests that flavanones can exert interesting anabolic bone effects. However, a number of preclinical studies have also confirmed an effect on bone mineral density through mainly antiresorptive effects. Thus, after isoflavones, the flavanones are the subgroup of polyphenols with most in vivo preclinical evidence for improved bone metabolism. Whether or not the accompanying systemic anti-inflammatory and lipid-lowering effects of the flavanones play a direct or indirect role in the bone modulatory properties of these flavanones remains to be established. Moreover, further studies particularly in humans would be helpful to confirm these effects. Indeed, to date, only one clinical trial has been conducted in postmenopausal women to assess efficacy of hesperidin on bone loss.

Bone Clinical Data for Hesperidin: Results from a Two-Year Randomized Controlled Trial in Postmenopausal Women

Only one clinical study has been conducted on the ability of hesperidin to protect against postmenopausal bone loss [71] (unpublished data). This study was a parallel, double-blind, placebo-controlled, 2 years randomized intervention trial. The study aimed at obtaining clinical evidence of the efficacy of hesperidin to maintain bone mineral density (BMD) in healthy postmenopausal women.

The secondary objectives were (1) to relate the treatment-induced changes in BMD to changes in biochemical markers of bone remodeling and (2) to study the effects of hesperidin supplementation on changes in anthropometry and body composition of postmenopausal women.

The study was performed in healthy postmenopausal women (with a mean age of 57.3 years and mean time since menopause of 6.4 years) not taking any hormone replacement therapy. Volunteers (55 French Caucasian postmenopausal women per group) on the active treatment were required to consume 500 mg/day of hesperidin in the form of two biscuits, each weighing 6 g and containing 250 mg of hesperidin. Subjects on the placebo treatment received the same biscuits considering taste, smell, appearance, but without hesperidin. Subjects were asked to consume one biscuit in the morning at breakfast and the other in the evening. The randomization criteria were the daily calcium intake (mg/day) and BMD (total left hip T-score) evaluated at screening. Volunteers were assigned to either a hesperidin (500 mg hesperidin/day in two biscuits) or placebo group (same biscuits without hesperidin). Subjects kept dietary records and minimized their citrus-rich food intake during the study period.

Usual descriptive statistics and robust measures were computed. For outcome variables (BMD and bone biomarkers), the normality and/or lognormality of the data were assessed using the Kolmogorov-Smirnov test. To determine the efficacy of the treatment, mixed models were estimated. The main effects were group (A vs. B) and time (0, 3, 6, 9, 12, 15, 18, 21, and 24 months). A group × time interaction was also introduced in the model. The random component of the model is an intercept variable specific to each randomized subject. Additionally, several covariates were introduced in the model: 25(OH)-vitamin D at screening, calcium intake at baseline (V0), the baseline value of the dependent variable, and time

Table 15.1 Demographics and baseline characteristics

Characteristic	Group placebo ($n=55$)	Group hesperidin ($n=53$)	p-value
Age, years	57.3 (3.3)	57.3 (3.7)	0.9862
Weight, kg	62.5 (8.9)	64.9 (9.9)	0.1848
Height, m	1.62 (0.06)	1.63 (0.06)	0.5245
BMI, kg/cm^2	23.7 (2.6)	24.4 (3.1)	0.1977
Time since menopause, years	6.3 (2.0)	6.5 (2.5)	0.6521
Surgical menopause, %	7.3	7.6	1.000
Calcium intake, g/day	884.7 (276.2)	887.7 (246.9)	0.9521
25(OH)-vit D, nmol/l	82.0 (38.3)	79.5 (34.1)	0.7181
BMD (g/cm^2)			
Total hip	0.873 (0.088)	0.892 (0.100)	0.2875
Femoral neck	0.731 (0.075)	0.756 (0.097)	0.1337
Lumbar spine (L1–L4)	0.972 (0.099)	0.978 (0.110)	0.7837
Whole body	1.055 (0.079)	1.075 (0.076)	0.1995

Values are means (SD)

since menopause at baseline. When variables were distributed in a lognormal fashion (e.g., bone biomarkers), mixed models were estimated using the log-transformed data for the dependent variable. When randomized subjects had a missing value for one or more point(s) in time for the interest variable (ITT), a "last-observation carried forward" approach was used. The treatment effect was considered to be statistically significant if the p value was lower than 5 %.

No demographic and baseline characteristics between groups were observed, especially concerning bone mineral density (whatever bone site measured), bone biomarkers, and plasma 25(OH)$_2$D (Table 15.1). Globally, whatever the group of treatment, volunteers showed a well-balanced dietary pattern and a moderate level of physical activity (<10 h/week). Concerning daily calcium intake evaluated at baseline, a mean of ~890 mg of calcium/day was consumed without any significant difference between the two groups.

The completion rate of the study was similar in the two groups (87.5 % in the placebo group and 91 % in hesperidin group).

Concerning bone mineral density, regardless of bone site (total hip, femoral neck, or lumbar spine), no statistically significant difference was found between groups (main effect and interaction with time) based on mixed models with or without covariates in an ITT analysis. The pattern of significance remains unchanged when the data were analyzed according to a per-protocol approach or when separate analyses were performed in groups determined according to time since menopause (threshold = 6 years). In the placebo group, from baseline to 24 months, we observed a mean % change in bone mineral density between −2.48 and −1.15 % depending on the bone site (ITT analysis). In the hesperidin group, changes ranged from −2.85 to −1.43 % (ITT analysis). The yearly rates of bone loss during the follow-up were equivalent in the two groups and in accordance with the epidemiological and clinical data obtained in large populations (postmenopausal bone mineral loss usually average 1–2 % per year in cortical bone and 2–3 % per year in trabecular bone such as in lumbar spine and femoral neck).

For calciuria, no statistically significant difference was seen between groups (main effect and interaction with time) based on mixed models with or without covariates in an ITT analysis. However, a significant interaction group × time was detected for the bone turnover index b-ALP/CTX-1 (Fig. 15.2) and P1NP/CTX-1. This significant effect is mainly attributable to a higher value of the ratio at 18 and 21 months in the hesperidin group than in the placebo group. The loss of significance at 24 months could be partly explained by the significant difference in compliance between groups for this period: 98.6 % in placebo group vs. 96.7 % in hesperidin group; p-value = 0.014.

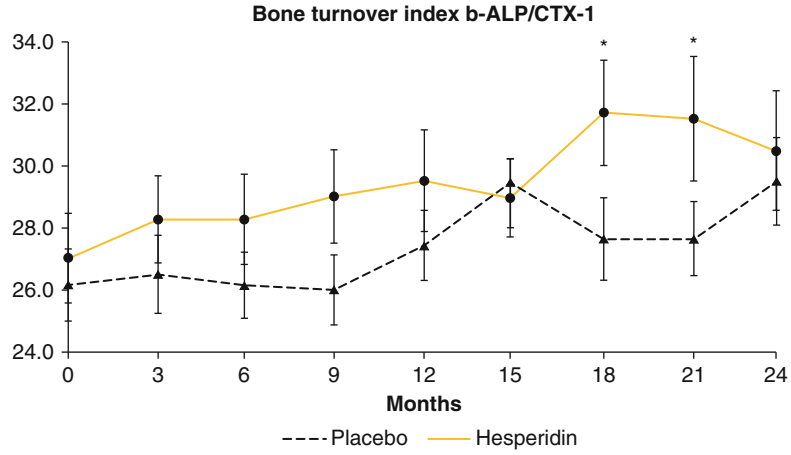

Fig. 15.2 Changes in bone turnover index from baseline to 24 months. Values are means ± SEM (ITT – LOCF) (*$p < 0.05$ vs placebo)

Finally, concerning the effect of the two types of biscuits on the evolution of body composition, we observed in the hesperidin group a trend to a lower gain in fat mass and a better protection of lean mass.

In summary, the yearly rates of bone loss (1–2 %) were equivalent in groups receiving hesperidin or not. Evolution in BMD during the 2 years was not statistically different between the two groups. However, the subjects consuming hesperidin presented a better balance in bone metabolism, as reflected by the bone turnover index, during the second year of follow-up at the 18- and/or 21-month time points [71]. These results on bone turnover index should be further strengthen using more sensitive technology to assess bone resorption, such as ^{41}calcium methodology, as previously used by Weaver et al. with phytoestrogens [72]. Moreover, even if biomarkers reflect bone turnover, and BMD bone quantity, it would have been very interesting to have measures on bone quality at the micro-architecture level.

Conclusion

Broader than flavanones effects on bone metabolism, findings from further clinical trials with plant-derived compounds are obviously required to give a stronger scientific support to current nutritional strategies highlighting the role of phytonutrients. However, combination of plant bioactive compounds together with calcium and vitamin D should be preferred to bring strong nutritional support in the prevention of age-related bone loss. In that context, it is important to highlight that human trials which include not only outcome measures related to bone quantity (BMD) but also bone quality (e.g., bone microarchitecture, bone turnover) and bone strength (using finite element analysis) should be emphasized to gain a more comprehensive outlook on how bone responds to various nutritional factors.

References

1. Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database. 2010;2010:bap024. doi:10.1093/database/bap024.
2. Peterson JJ, Beecher GR, Bhagwat SA, Dwyer JT, Gebhardt SE, Haytowitz DB, et al. Flavanones in grapefruit, lemons, and limes: a compilation and review of the data from the analytical literature. J Food Composition Anal. 2006;19(Suppl):S74–80.
3. Peterson JJ, Dwyer JT, Beecher GR, Bhagwat SA, Gebhardt SE, Haytowitz DB, et al. Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature. J Food Composition Anal. 2006;19(Suppl):S66–73.
4. Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, Yano M, Ohta H. Flavonoid composition of fruit tissues of citrus species. Biosci Biotechnol Biochem. 2006;70(1):178–92.
5. Setchell KDR, Lydeking-Olsen E. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. Am J Clin Nutr. 2003;78(3):593S–609.

6. Habauzit V, Horcajada MN. Phenolic phytochemicals and bone. Phytochem Rev. 2008;7(2):313–44 [review].
7. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventós RM, Berenguer T, Jakszyn P, Barricarte A, et al. Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc. 2010;110(3):390–8.
8. Urpi-Sarda M, Rothwell JA, Morand C, Manach C. Bioavailability of flavanones. Flavonoids and related compounds. In: Spencer J, Crozier A, editors. Bioavailability and function. Boca Raton: CRC Press; 2012. p. 1–65.
9. Brett GM, Hollands W, Needs PW, Teucher B, Dainty JR, Davis BD, et al. Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. Br J Nutr. 2009;101(5):664–75.
10. Vallejo F, Larrosa M, Escudero E, Zafrilla MP, Cerdá B, Boza J, et al. Concentration and solubility of flavanones in orange beverages affect their bioavailability in humans. J Agric Food Chem. 2010;58(10):6516–24.
11. Bredsdorff L, Nielsen ILF, Rasmussen SE, Cornett C, Barron D, Bouisset F, et al. Absorption, conjugation and excretion of the flavanones, naringenin and hesperetin from-rhamnosidase-treated orange juice in human subjects. Br J Nutr. 2010;103(11):1602–9.
12. Erlund I, Silaste ML, Alfthan G, Rantala M, Kesäniemi YA, Aro A. Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. Eur J Clin Nutr. 2002;56(9):891–8.
13. Gardana C, Guarnieri S, Riso P, Simonetti P, Porrini M. Flavanone plasma pharmacokinetics from blood orange juice in human subjects. Br J Nutr. 2007;98(1):165–72.
14. Manach C, Morand C, Gil-Izquierdo A, Bouteloup-Demange C, Rémésy C. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two dose of orange juice. Eur J Clin Nutr. 2003;57(2):235–42.
15. Mullen W, Archeveque MA, Edwards CA, Matsumoto H, Crozier A. Bioavailability and metabolism of orange juice flavanones in humans: impact of a full-fat yogurt. J Agric Food Chem. 2008;56(23):11157–64.
16. Nielsen ILF, Chee WSS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, et al. Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. J Nutr. 2006;136(2):404–8.
17. Chanet A, Milenkovic D, Manach C, Mazur A, Morand C. Citrus flavanones: what is their role in cardiovascular protection? J Agric Food Chem. 2012;60(36):8809–22.
18. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1 Suppl):230S–42.
19. Jung UJ, Lee MK, Park YB, Kang MA, Choi MS. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. Int J Biochem Cell Biol. 2006;38(7):1134–45.
20. Lee MK, Bok SH, Jeong TS, Moon SS, Lee SE, Park YB, et al. Supplementation of naringenin and its synthetic derivative alters antioxidant enzyme activities of erythrocyte and liver in high cholesterol-fed rats. Bioorg Med Chem. 2002;10(7):2239–44.
21. Sharma AK, Bharti S, Ojha S, Bhatia J, Kumar N, Ray R, et al. Up-regulation of PPARγ, heat shock protein-27 and-72 by naringin attenuates insulin resistance, β-cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. Br J Nutr. 2011;106(11):1713–23.
22. Chanet A, Milenkovic D, Deval C, Potier M, Constans J, Mazur A, et al. Naringin, the major grapefruit flavonoid, specifically affects atherosclerosis development in diet-induced hypercholesterolemia in mice. J Nutr Biochem. 2012;23(5):469–77.
23. Choe SC, Kim HS, Jeong TS, Bok SH, Park YB. Naringin has an antiatherogenic effect with the inhibition of intercellular adhesion molecule-1 in hypercholesterolemic rabbits. J Cardiovasc Pharmacol. 2001;38(6):947–55.
24. Lee CH, Jeong TS, Choi YK, Hyun BH, Oh GT, Kim EH, et al. Anti-atherogenic effect of citrus flavonoids, naringin and naringenin, associated with hepatic ACAT and aortic VCAM-1 and MCP-1 in high cholesterol-fed rabbits. Biochem Biophys Res Commun. 2001;284(3):681–8.
25. Pu P, Gao DM, Mohamed S, Chen J, Zhang J, Zhou XY, et al. Naringin ameliorates metabolic syndrome by activating AMP-activated protein kinase in mice fed a high-fat diet. Arch Biochem Biophys. 2012;518(1):61–70.
26. Kim JY, Jung KJ, Choi JS, Chung HY. Modulation of the age-related nuclear factor-kappaB (NF-kappaB) pathway by hesperetin. Aging Cell. 2006;5(5):401–11.
27. Habauzit V, Sacco SM, Gil-Izquierdo A, Trzeciakiewicz A, Morand C, Barron D, et al. Differential effects of two citrus flavanones on bone quality in senescent male rats in relation to their bioavailability and metabolism. Bone. 2011;49(5):1108–16.
28. Comalada M, Ballester I, Bailón E, Sierra S, Xaus J, Gálvez J, et al. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. Biochem Pharmacol. 2006;72(8):1010–21.
29. Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. J Pharmacol Exp Ther. 2001;296(1):181–7.
30. Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. Life Sci. 2001;68(8):921–31.

31. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007;2007:45673.
32. Tsai SH, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NFκB in macrophages by resveratrol. Br J Pharmacol. 1999;126(3):673–80.
33. Choi JS, Choi YJ, Park SH, Kang JS, Kang YH. Flavones mitigate tumor necrosis factor-α-induced adhesion molecule upregulation in cultured human endothelial cells: role of nuclear factor-κB. J Nutr. 2004;134(5):1013–9.
34. Nizamutdinova IT, Jeong JJ, Xu GH, Lee SH, Kang SS, Kim YS, et al. Hesperidin, hesperidin methyl chalone and phellopterin from Poncirus trifoliata (Rutaceae) differentially regulate the expression of adhesion molecules in tumor necrosis factor-α-stimulated human umbilical vein endothelial cells. Int Immunopharmacol. 2008;8(5):670–8.
35. Lotito SB, Frei B. Dietary flavonoids attenuate tumor necrosis factor α-induced adhesion molecule expression in human aortic endothelial cells: structure-function relationships and activity after first pass metabolism. J Biol Chem. 2006; 281(48):37102–10.
36. Choi IY, Kim SJ, Jeong HJ, Park SH, Song YS, Lee JH, et al. Hesperidin inhibits expression of hypoxia inducible factor-1 alpha and inflammatory cytokine production from mast cells. Mol Cell Biochem. 2007;305(1–2):153–61.
37. Choi EM, Lee YS. Effects of hesperetin on the production of inflammatory mediators in IL-1β treated human synovial cells. Cell Immunol. 2010;264(1): 1–3.
38. Morand C, Dubray C, Milenkovic D, Lioger D, Martin JF, Scalbert A, et al. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J Clin Nutr. 2011;93(1):73–80.
39. Jung UJ, Kim HJ, Lee JS, Lee MK, Kim HO, Park EJ, et al. Naringin supplementation lowers plasma lipids and enhances erythrocyte antioxidant enzyme activities in hypercholesterolemic subjects. Clin Nutr. 2003;22(6):561–8.
40. Rizza S, Muniyappa R, Iantorno M, Kim JA, Chen H, Pullikotil P, et al. Citrus polyphenol hesperidin stimulates production of nitric oxide in endothelial cells while improving endothelial function and reducing inflammatory markers in patients with metabolic syndrome. J Clin Endocrinol Metab. 2011;96(5):E782–92.
41. Milenkovic D, Deval C, Dubray C, Mazur A, Morand C. Hesperidin displays relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human volunteers: a randomized controlled crossover study. PLoS One. 2011;6(11):e26669.
42. Dalgård C, Nielsen F, Morrow JD, Enghusen-Poulsen H, Jonung T, Hørder M, et al. Supplementation with orange and blackcurrant juice, but not vitamin E, improves inflammatory markers in patients with peripheral arterial disease. Br J Nutr. 2009;101(2):263–9.
43. Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, et al. Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. J Nutr. 2011;141(4):618–25.
44. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr. 2005;45(4):287–306.
45. Asagiri M, Takayanagi H. The molecular understanding of osteoclast differentiation. Bone. 2007;40(2):251–64.
46. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J. 1992;6(12):3051–64.
47. Wimalawansa SJ. Nitric oxide and bone. Ann N Y Acad Sci. 2010;1192:391–403.
48. Nanes MS. Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. Gene. 2003;321:1–15.
49. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med. 2000;51:245–70.
50. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr. 2000;71(1):142–51.
51. New SA, Millward DJ. Calcium, protein, and fruit and vegetables as dietary determinants of bone health. Am J Clin Nutr. 2003;77(5):1340–1.
52. Muhlbauer RC, Lozano A, Reinli A, Wetli H. Various selected vegetables, fruits, mushrooms and red wine residue inhibit bone resorption in rats. J Nutr. 2003; 133(11):3592–7.
53. Chiba H, Uehara M, Wu J, Wang XX, Masuyama R, Suzuki K, et al. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. J Nutr. 2003;133(6):1892–7.
54. Horcajada M, Coxam V. Hesperidin, a citrus flavanone, improves bone acquisition and prevents skeletal impairment in rats. In: Burckardt P, Dawson-Hughes B, Heaney RP, editors. Nutritional aspects of osteoporosis. London: Elsevier; 2004. p. 103–20.
55. Horcajada MN, Habauzit V, Trzeciakiewicz A, Morand C, Gil-Izquierdo A, Mardon J, et al. Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. J Appl Physiol. 2008;104(3):648–54.
56. Habauzit V, Nielsen IL, Gil-Izquierdo A, Trzeciakiewicz A, Morand C, Chee W, et al. Increased bioavailability of hesperetin-7-glucoside compared with hesperidin results in more efficient prevention of bone loss in adult ovariectomised rats. Br J Nutr. 2009;27:1–9.
57. Deyhim F, Garica K, Lopez E, Gonzalez J, Ino S, Garcia M, et al. Citrus juice modulates bone strength in male senescent rat model of osteoporosis. Nutrition. 2006;22(5):559–63.

58. Deyhim F, Villarreal A, Garcia K, Rios R, Garcia C, Gonzales C, et al. Orange pulp improves antioxidant status and suppresses lipid peroxidation in orchidectomized male rats. Nutrition. 2007;23(7–8):617–21.
59. Morrow R, Deyhim F, Patil BS, Stoecker BJ. Feeding orange pulp improved bone quality in a rat model of male osteoporosis. J Med Food. 2009;12(2):298–303.
60. Uehara M. Prevention of osteoporosis by foods and dietary supplements. Hesperidin and bone metabolism. Clin Calcium. 2006;16(10):1669–76.
61. Bok SH, Lee SH, Park YB, Bae KH, Son KH, Jeong TS, et al. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J Nutr. 1999;129(6):1182–5.
62. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. Science. 1999;286(5446):1946–9.
63. Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S. Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun. 2000;271(3):688–92.
64. Trzeciakiewicz A, Habauzit V, Mercier S, Lebecque P, Davicco MJ, Coxam V, et al. Hesperetin stimulates differentiation of primary rat osteoblasts involving the BMP signalling pathway. J Nutr Biochem. 2010;21(5):424–31.
65. Trzeciakiewicz A, Habauzit V, Mercier S, Barron D, Urpi-Sarda M, Manach C, et al. Molecular mechanism of hesperetin-7-O-glucuronide, the main circulating metabolite of hesperidin, involved in osteoblast differentiation. J Agric Food Chem. 2010;58(1):668–75.
66. Wong RWK, Rabie ABM. Effect of naringin collagen graft on bone formation. Biomaterials. 2006;27(9):1824–31.
67. Wong RWK, Rabie ABM. Effect of naringin on bone cells. J Orthop Res. 2006;24(11):2045–50.
68. Reid IR. Relationships between fat and bone. Osteoporos Int. 2008;19:595–606.
69. Wolf G. Energy regulation by the skeleton. Nutr Rev. 2008;66(4):229–33.
70. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, et al. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev. 2009;8(1):18–30.
71. Habauzit V, Offord E, Chee W, Jafrelo L, Ameye L, Williamson G, et al. Effect of two years dietary hesperidin supplementation on bone metabolism in postmenopausal women. Annual meeting of the American Society for Bone and Mineral Research. San Diego; 2011.
72. Weaver CM, Martin BR, Jackson GS, McCabe GP, Nolan JR, McCabe LD, et al. Antiresorptive effects of phytoestrogen supplements compared with estradiol or risedronate in postmenopausal women using 41Ca methodology. J Clin Endocrinol Metab. 2009;94(10):3798–805.

Intake of B Vitamins and Carotenoids in Relation to Risk of Hip Fracture in Elderly Chinese

16

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Abstract

Experimental evidence has implicated that homocysteine and oxidative stress may be involved in the pathogenesis of osteoporosis. In accord, observational studies among Caucasian populations have also suggested that homocysteine-related B vitamins (vitamins B2, B6, B12, and folate) and carotenoids with antioxidant functions may be beneficial to bone health and therefore protect against osteoporotic fractures. Incidence of hip fracture is rising in Asia, but there is paucity among Asian populations on dietary factors. We prospectively examined the associations of dietary intakes of B vitamins and carotenoids with hip fracture risk among elderly Chinese in the Singapore Chinese Health Study. Cox proportional hazards model was applied to determine the strength of association after adjusting for potential confounders. Our results showed a dose-dependent inverse relationship

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between vitamin B6 intake and hip fracture risk among women (p for trend=0.002). Conversely, no protective association was found in men. Dietary intakes of vitamins B2, B12, and folate were not related to hip fracture risk in either gender. Vegetables are main sources of carotenoid intake in this population. In men, we found a dose-dependent inverse relationship between consumption of total vegetables and hip fracture risk (p for trend=0.004). Similarly, higher intakes of total carotenoids, α- and β-carotene, were associated with lower hip fracture risk in men (all p for trend <0.05). Consumption of vegetables or carotenoids did not show an association with hip fracture risk in women. The gender-specific results in the protective roles of dietary B vitamins and carotenoids suggest that the mechanistic pathway for osteoporosis may be different between men and women.

Keywords
B vitamins • Hip fracture • Osteoporosis • Pyridoxine • Carotenoids • Chinese

Introduction

Incidence of osteoporotic hip fracture is rising in Asia, with up to 50 % of total cases projected to happen in this part of the world by 2050 [1, 2]. Malnutrition and low intakes of nutrients have been found to be more prevalent and severe among hip fracture patients compared to the general elderly populations [3, 4]. Hence, there is an urgent need to evaluate dietary risk factors for hip fracture among Asian populations, particularly in the context that the existing epidemiologic evidence on dietary factors is primarily available among Caucasian populations. Experimental evidence has suggested that homocysteine may promote bone resorption and consequently disturb cross-link collagen and reduce bone quality [5], and elevated blood levels of homocysteine have been associated with decreased bone mineral density and increased hip fracture risk in observational studies [6–8]. Thus, low status of the B vitamins that are involved in homocysteine metabolism may affect hip fracture risk. Yet, evidence on B vitamins and osteoporotic hip fracture risk remains inconsistent [9–13]. Another speculation in the pathophysiology of osteoporosis is that increased oxidative stress may play a role in age-related bone loss and consequent osteoporotic fractures [14, 15]. Free radicals, which are markers of oxidative stress, may increase bone resorption by promoting osteoclastic differentiation and hence increase risk of osteoporotic fractures [16, 17]. Carotenoids with antioxidant function from fruit and vegetables have been reported to reduce oxidative stress in bones [18–20] and protect against osteoporosis [21–26]. However, such data are mainly from Western populations. In the present study, we examined the relationship of dietary intakes of B vitamins and carotenoids with the risk of hip fracture among Chinese men and women in an Asian population living in Singapore.

Methods

We prospectively examined the associations of dietary intakes of B vitamins and carotenoids with hip fracture risk among elderly Chinese in the Singapore Chinese Health Study, which enrolled 63,257 middle-aged or elderly men and women between 1993 and 1998. Baseline assessment was conducted through a face-to-face structured interview during the initial enrollment that included demographics, medical history, diet, tobacco smoking, alcohol consumption, physical activity, and detailed menstrual and reproductive history (women only). Dietary intakes were recorded using a previously validated food frequency questionnaire which incor-

porated common and distinct food items in Singapore [27]. After a mean follow-up of 13.8 years, 1,630 hip fracture incident cases were identified up to December 31, 2010 via record linkage to the nationwide database that captures all hospital admissions in Singapore. Cox proportional hazards model was applied to measure the strength of association between dietary intakes of B vitamins/carotenoids and hip fracture risk after adjustment for potential confounders.

Results

Women accounted for 72.4 % of all hip fractures. The baseline characteristics showed that fracture cases for both genders were older at recruitment, had lower BMI, and more likely to have smoking habits and to report history of diabetes or stroke as compared to non-fracture cases. Table 16.1 summarized the main findings of the current study. After adjusting for the covariates and other nutritional factors such as calcium and soy intakes, we observed a statistically significant and dose-dependent inverse relationship between vitamin B6 intake and hip fracture risk among women (p for trend = 0.002). Compared to women in the lowest quartile intake (<0.6 mg/1,000 kcal/day), women in the highest quartile intake (≥0.8 mg/1,000 kcal/day) had a 22 % reduction in hip fracture risk. Conversely, we did not find such a protective effect in hip fracture risk for men. However, dietary intakes of vitamins B2, B12, and folate were not associated with hip fracture risk in either gender (data not showed). In terms of association with carotenoid intake, we first examined the association of dietary intake of vegetables with the risk of hip fracture, as vegetables are the primary sources of carotenoids (76 % of carotenoid intake) in this population. In men, we found a statistically significant, dose-dependent, protective relationship between intakes of total vegetables and hip fracture risk (p for trend = 0.004). In contrast, we did not see a protective association in women with increasing intake of vegetables. Fruit intake did not show any association with hip fracture risk in both men and women. Similarly, we found a protective effect against hip fracture with increasing intakes of carotenoids in men only. Intakes of total carotenoids, α- and β-carotene, showed a dose-dependent inverse relationship with hip fracture risk in men (p for trend <0.05) but not in women (Table 16.1).

Discussion

In this study, using prospective data from the Singapore Chinese Health Study, we examined the intakes of B vitamins related to homocysteine pathway and found a protective association between vitamin B6 and hip fracture risk in women but not in men. Conversely, the risk reduction in hip fracture with increasing intake of total carotenoids, α- and β-carotene, was limited to men. The difference in the protective roles of dietary B vitamins and carotenoids suggests that the pathophysiology of bone degradation may be different between men and women.

The lack of association with other homocysteine-related B vitamins such as B2, folate, and B12 suggests that protective effect of vitamin B6 against hip fracture in women may not involve the homocysteine mechanism. Indeed, experimental studies have shown that vitamin B6 may have a direct effect on bone integrity, such as its effect on collagen cross-linking and bone formation [28, 29]. In contrast to the association between vitamin B6 and hip fracture risk in women, statistically significant inverse associations between dietary intakes of vegetables and carotenoids, and the risk of hip fracture were observed only in men. We speculate that oxidative stress may play a greater role in osteoporosis in men than in women, which could explain a stronger protection through the antioxidant property from carotenoids in men than in women. Furthermore, other constituents in vegetables, especially carotenoid-rich vegetables, could be responsible for the observed inverse relation between vegetable intake and hip fracture risk [30].

Although women are generally about twice as susceptible to fracture compared to men [4, 31], mortality related to fractures has been shown to

Table 16.1 Dietary intake of vitamin B6, vegetables, and carotenoids in relation to hip fracture risk (The Singapore Chinese Health Study, 1993–2010)

	Men ($n=27,913$)			Women ($n=35,241$)		
Characteristics	Cases	HR	95 % CI	Cases	HR	95 % CI
B6 (pyridoxine) (mg/1,000 kcal/day)						
Q1 (<0.6)	118	1.00		417	1.00	
Q2 (0.6–0.7)	117	1.23	0.95–1.59	285	0.83	0.71–0.96
Q3 (0.7–0.8)	97	1.11	0.85–1.47	249	0.79	0.67–0.93
Q4 (≥0.8)	118	1.29	0.99–1.68	229	0.78	0.66–0.93
p for trend	0.115			0.002		
Total vegetables (g/1,000 kcal/day)						
Quartile 1 (<48.7)	186	1.00		285	1.00	
Quartile 2 (48.7–66.6)	121	0.90	0.71–1.14	294	0.92	0.80–1.11
Quartile 3 (66.6–89.3)	89	0.79	0.61–1.02	326	1.00	0.85–1.17
Quartile 4 (≥89.3)	54	0.65	0.48–0.89	275	0.82	0.69–0.98
p for trend	0.004			0.079		
Total carotenoids (μg/1,000 kcal/day)						
Quartile 1 (<2314.5)	177	1.00		329	1.00	
Quartile 2 (2314.5–3283.0)	122	0.89	0.70–1.13	310	0.94	0.81–1.10
Quartile 3 (3283.0–4577.1)	93	0.87	0.67–1.13	288	0.93	0.79–1.09
Quartile 4 (≥4577.1)	58	0.72	0.53–0.99	253	0.86	0.73–1.03
p for trend	0.046			0.106		
α-carotene (μg/1,000 kcal/day)						
Quartile 1(<59.8)	166	1.00		273	1.00	
Quartile 2 (59.8–114.4)	117	0.84	0.66–1.07	303	0.90	0.76–1.06
Quartile 3 (114.4–212.3)	107	0.91	0.71–1.16	309	0.94	0.80–1.11
Quartile 4 (≥212.3)	60	0.65	0.48–0.88	295	0.91	0.77–1.08
p for trend	0.014			0.424		
β-carotene (μg/1,000 kcal/day)						
Quartile 1 (<850.6)	183	1.00		282	1.00	
Quartile 2 (850.6–1235.5)	134	1.00	0.76–1.21	319	0.99	0.84–1.16
Quartile 3 (1235.5–1772.8)	76	0.68	0.52–0.90	291	0.91	0.77–1.08
Quartile 4 (≥1772.8)	57	0.70	0.52–0.96	288	0.89	0.75–1.06
p for trend	0.003			0.136		

Hazard ratios (HRs) were adjusted for age at recruitment (years), year of recruitment (1993–1995, 1996–1998), dialect group (Hokkien, Cantonese), body mass index (BMI; <20, 20–24, 24–28, ≥28), level of education (no formal education, primary, secondary or higher), smoking status (never, ex-smoker, current smoker), total energy intake (kcal/day), physical activity (none, 2–3 h weekly, ≥4 h weekly), physician-diagnosed history of diabetes or stroke (yes, no) at recruitment, quartile intake of calcium and soy isoflavones, and use of hormone replacement therapy at recruitment (women only; yes, no)
CI confidence interval

be higher in men [32, 33]. Hip fracture prevention is essentially important in both genders. Our findings reveal that adequate intake of vitamin B6 and carotenoids may be beneficial in the prevention of osteoporotic hip fracture in the elderly population. The gender-specific results in the protective roles of dietary B vitamins and carotenoids suggest that the mechanistic pathway for osteoporosis may be different between men and women, and warrants further studies to substantiate or refute this hypothesis.

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References

1. Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. Osteoporos Int. 1997;7(5): 407–13.
2. Sambrook P, Cooper C. Osteoporosis. Lancet. 2006;367(9527):2010–8.
3. Bonjour JP, Schurch MA, Rizzoli R. Nutritional aspects of hip fractures. Bone. 1996;18(3 Suppl): 139S–4444.
4. Jordan KM, Cooper C. Epidemiology of osteoporosis. Best Pract Res Clin Rheumatol. 2002;16(5):795–806.
5. Lubec B, Fang-Kircher S, Lubec T, Blom HJ, Boers GH. Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. Biochim Biophys Acta. 1996;1315(3):159–62.
6. Gerdhem P, Ivaska KK, Isaksson A, Pettersson K, Vaananen HK, Obrant KJ, et al. Associations between homocysteine, bone turnover, BMD, mortality, and fracture risk in elderly women. J Bone Miner Res. 2007;22(1):127–34.
7. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Gjessing HK, et al. Plasma total homocysteine level and bone mineral density: the Hordaland Homocysteine Study. Arch Intern Med. 2006;166(1):88–94.
8. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Meyer HE, Tell GS. Plasma homocysteine, folate, and vitamin B 12 and the risk of hip fracture: the Hordaland homocysteine study. J Bone Miner Res. 2007;22(5): 747–56.
9. Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. J Bone Miner Res. 2005;20(6): 921–9.
10. McLean RR, Jacques PF, Selhub J, Fredman L, Tucker KL, Samelson EJ, et al. Plasma B vitamins, homocysteine, and their relation with bone loss and hip fracture in elderly men and women. J Clin Endocrinol Metab. 2008;93(6):2206–12.
11. Ravaglia G, Forti P, Maioli F, Servadei L, Martelli M, Brunetti N, et al. Folate, but not homocysteine, predicts the risk of fracture in elderly persons. J Gerontol A Biol Sci Med Sci. 2005;60(11):1458–62.
12. Stone KL, Bauer DC, Sellmeyer D, Cummings SR. Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: a prospective study. J Clin Endocrinol Metab. 2004;89(3):1217–21.
13. Yazdanpanah N, Zillikens MC, Rivadeneira F, de Jong R, Lindemans J, Uitterlinden AG, et al. Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: the Rotterdam study. Bone. 2007;41(6):987–94.
14. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31(3): 266–300.
15. Sheweita SA, Khoshhal KI. Calcium metabolism and oxidative stress in bone fractures: role of antioxidants. Curr Drug Metab. 2007;8(5):519–25.
16. Bai XC, Lu D, Liu AL, Zhang ZM, Li XM, Zou ZP, et al. Reactive oxygen species stimulates receptor activator of NF-kappaB ligand expression in osteoblast. J Biol Chem. 2005;280(17):17497–506.
17. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest. 1990;85(3):632–9.
18. Mackinnon ES, Rao AV, Rao LG. Dietary restriction of lycopene for a period of one month resulted in significantly increased biomarkers of oxidative stress and bone resorption in postmenopausal women. J Nutr Health Aging. 2011;15(2):133–8.
19. Rao LG, Krishnadev N, Banasikowska K, Rao AV. Lycopene I–effect on osteoclasts: lycopene inhibits basal and parathyroid hormone-stimulated osteoclast formation and mineral resorption mediated by reactive oxygen species in rat bone marrow cultures. J Med Food. 2003;6(2):69–78.
20. Rao LG, Mackinnon ES, Josse RG, Murray TM, Strauss A, Rao AV. Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. Osteoporos Int. 2007;18(1):109–15.
21. Kim L, Rao AV, Rao LG. Lycopene II–effect on osteoblasts: the carotenoid lycopene stimulates cell proliferation and alkaline phosphatase activity of SaOS-2 cells. J Med Food. 2003;6(2):79–86.
22. Maggio D, Polidori MC, Barabani M, Tufi A, Ruggiero C, Cecchetti R, et al. Low levels of carotenoids and retinol in involutional osteoporosis. Bone. 2006;38(2): 244–8.
23. Park CK, Ishimi Y, Ohmura M, Yamaguchi M, Ikegami S. Vitamin A and carotenoids stimulate differentiation of mouse osteoblastic cells. J Nutr Sci Vitaminol (Tokyo). 1997;43(3):281–96.
24. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. J Bone Miner Res. 2009;24(6):1086–94.
25. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Inverse association of carotenoid intakes with 4-y change in bone mineral density in elderly men and women: the Framingham Osteoporosis Study. Am J Clin Nutr. 2009;89(1):416–24.
26. Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Ando F, Shimokata H, et al. Dietary patterns of antioxidant vitamin and carotenoid intake associated with bone mineral density: findings from post-menopausal Japanese female subjects. Osteoporos Int. 2011;22(1): 143–52.
27. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer. 2001; 39(2):187–95.

28. Bird TA, Levene CI. Lysyl oxidase: evidence that pyridoxal phosphate is a cofactor. Biochem Biophys Res Commun. 1982;108(3):1172–80.
29. Saito M. Elevated plasma concentration of homocysteine, low level of vitamin B6, pyridoxal, and vitamin D insufficiency in patients with hip fracture: a possible explanation for detrimental cross-link pattern in bone collagen. Clin Calcium. 2006;16(12):1974–84.
30. Stahl W, Ale-Agha N, Polidori MC. Non-antioxidant properties of carotenoids. Biol Chem. 2002;383(3–4):553–8.
31. Cooper C, Campion G, Melton 3rd LJ. Hip fractures in the elderly: a world-wide projection. Osteoporos Int. 1992;2(6):285–9.
32. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet. 1999;353(9156):878–82.
33. Seeman E, Bianchi G, Khosla S, Kanis JA, Orwoll E. Bone fragility in men–where are we? Osteoporos Int. 2006;17(11):1577–83.

Dietary Anthocyanidins and Bone Health

17

Claire J. Macdonald-Clarke and Helen M. Macdonald

Abstract

There is evidence to suggest that fruit and vegetable consumption is beneficial for bone health. Anthocyanins are bioactive flavonoid compounds found mostly in fruits, particularly berries, some vegetables, and wine. A diet rich in anthocyanidins is thought to have multiple health benefits, and recent studies have shown that high intakes of anthocyanidins are associated with markers of bone health. However, much is still unknown about the variation in composition of anthocyanidins in foods worldwide, and therefore it is difficult to quantify dietary intakes with certainty.

To date there have been no human intervention studies looking at anthocyanidins in relation to bone health, but anthocyanidin-rich dried fruits have shown positive effects on markers of bone health in both human observational and animal studies.

The metabolism, bioavailability, and absorption of anthocyanidins vary greatly between people, partly due to differences in gut microflora which metabolizes anthocyanidins into products such as phenolic acids. However, the processes involved with the metabolism, bioavailability, and absorption of anthocyanidins are not fully understood. Cellular studies have shown that it is the anthocyanidin metabolites and not the intact anthocyanidins that affect osteoblast differentiation. Neither anthocyanidins nor their metabolites have been studied directly on osteoclasts in vitro.

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Preliminary data suggests that an anthocyanidin-rich diet may have positive effects on bone health which may in future have consequences for dietary guidelines. However, much is still to be investigated in this area particularly with regard to metabolism and cellular mechanism of action. An intervention study quantifying the effects of anthocyanidins on markers of bone health is recommended. This chapter explores the current evidence for the role of anthocyanidins in bone health.

Keywords
Anthocyanidins • Anthocyanins • Polyphenols • Flavonoids • Osteoporosis
Bone • Bone turnover • Bone mineral density

Abbreviations

ALP	Alkaline phosphatase
APOSS	Aberdeen Prospective Osteoporosis Screening Study
BALP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
CRP	C-reactive protein
DPD	Deoxypyridinoline
FFQ	Food frequency questionnaire
HP	Helical peptide
HPLC	High-performance liquid chromatography
HRT	Hormone replacement therapy
IGF-1	Insulin-like growth factor
NF-κB	Nuclear factor-κB
OC	Osteocalcin
ORX	Orchidectomized
OVX	Ovariectomized
PTH	Parathyroid hormone
PYD	Pyridinoline
TbN	Trabecular number
TbSp	Trabecular separation
TRAP	Tartrate-resistant acid phosphate
USDA	US Department of Agriculture

Introduction

High intakes of fruit and vegetables in the diet have been shown to be beneficial for bone health [1–4]. One hypothesis to explain the association is via the restoration of acid–base balance, as alkaline salts produced by fruit and vegetables are thought to neutralize acid generated by excessive protein intake in the Western diet and thereby prevent bone loss [5]. However, there is conflicting opinion about the importance of acid–base balance. The longest trial performed to date showed no such change in bone turnover or bone mineral density (BMD) over 2 years [6], and a recent systematic review and meta-analysis concluded that an alkaline-producing diet does not protect bone health [7].

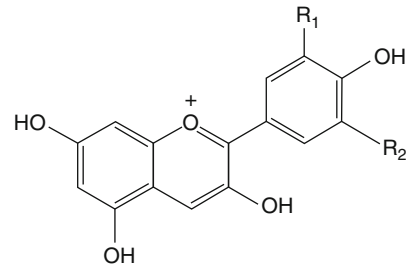
Fruit and vegetables are a source of many vitamins such as vitamins C and K and minerals such as magnesium, potassium, and calcium which are known to contribute to bone health [8].

In 2002, Muhlbauer and Li found that in rats, a mixture of 14 different dried vegetable extracts significantly reduced bone resorption to a greater extent than one extract alone [9]. There is accumulating evidence that fruit- and vegetable-derived bioactive compounds, such as flavonoids, may play a role in reducing bone resorption and improving bone health. Recent studies have found associations between flavonoid intakes and bone health [10, 11], and interest is growing in a particular group of flavonoids, the anthocyanidins. This chapter examines the evidence for the role of anthocyanidins in bone health.

Anthocyanidin Structure

Anthocyanidins are colorful, water-soluble flavonoid compounds responsible for the red, blue, and purple colors of fruit and vegetables.

Fig. 17.1 Anthocyanidin structure

Anthocyanidin	Basic structure	R1	R2
Cyanidin		H	OH
Delphinidin		OH	OH
Malvidin		OCH_3	OCH_3
Pelargonidin		H	H
Peonidin		H	OCH_3
Petunidin		OH	OCH_3

Anthocyanidins are found in food primarily as glycoside or acylglycoside anthocyanins of their respective aglycone anthocyanidins. Anthocyanidins are unstable compounds but can be quantified accurately in foods by acid hydrolysis high-performance liquid chromatography (HPLC) and conversion of anthocyanins to simplified anthocyanidins [12]. Anthocyanidins are polyphenolic C6-C3-C6 compounds. There are six major dietary anthocyanidins with slightly different structures, as shown in Fig. 17.1.

Anthocyanidins in the Diet

The richest sources of anthocyanidins to the diet are red wine and fruit, particularly berries. Foods can have one or a variety of anthocyanidins, and the concentration is influenced by environmental conditions, ripeness, cultivar, cultivation site, processing, and storage. Freezing may be the best method of preserving anthocyanidins in foods so as the consumption can continue throughout the year, even when particular foods such as berries are not in season [13].

In 1976, estimated intakes in the USA were thought to be around 180 mg/day in winter and 215 mg/day in summer [14]. However, more recent estimates suggest that consumption is around 12.5 mg/day in the USA [13] and 82 mg/day in Finland [15]. These differences are explained by both cultural differences such as the high consumption of berries in the traditional Finnish diet [15] and advances in techniques for more accurate analysis [12]. Our recent findings from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort using data from the recently updated US Department of Agriculture (USDA) database [16] show that the average consumption of total anthocyanidins was 16 (11) mg/day (mean (SD)). The greatest contributor to the diet was cyanidin (32 %) followed by malvidin (22 %), pelargonidin (19 %), delphinidin (12 %), petunidin (9 %), and peonidin (6 %) (peer-reviewed manuscript in preparation).

The majority (63 %) of the anthocyanidins in the diet of the APOSS cohort were from fruit and vegetables. The remainder (37 %) were consumed in wine. The fruit and vegetables contributing the greatest quantity of anthocyanidins to the diet in this population were salad vegetables, pears, grapes, apples, onions, and berries.

Bioavailability, Metabolism, and Absorption

The bioavailability, metabolism, and absorption of anthocyanidin compounds are not fully understood. A review of anthocyanin bioavailability studies shows that they are generally present in the plasma quickly after consumption at around 15–60 min [17]. The elimination half-life of anthocyanins is thought to be between 0.5 and 3 h [18].

Urinary excretion is reported to be around 0.01–3 % [18], therefore showing that only a small proportion of intact dietary anthocyanins are absorbed. For example, Mazza et al. found that in humans, when 1.2 g anthocyanins were ingested

from freeze-dried blueberry powder, approximately 12 μg/L was present as anthocyanins in serum after 3 h [19]. This is the equivalent of approximately 0.002 % absorption of intact anthocyanins. More recent studies have shown similar findings: in a population of elderly women, Milbury et al. showed that from a dose of cranberry juice containing 94 mg of anthocyanins, only 0.79–0.90 % of the dose was absorbed intact [20].

It is thought that the majority of anthocyanins are metabolized, similarly to other flavonoid compounds, to aglycone anthocyanidins; methylated, glucuronated, or sulfated anthocyanidins; or phenolic acids [19, 21–24].

Bioavailability, absorption, and metabolism are influenced by many factors including the particular aglycone and sugar moieties [24] and the gut microflora [25, 26]. Bacteria in the colon are able to metabolize anthocyanidins into various products such as phenolic acids, and therefore variations in the gut flora may explain the considerable differences in absorption, metabolism, and bioavailability of anthocyanidins and metabolites between people.

Anthocyanidins and Bone Health

Anthocyanidins have been implicated to have many health protective roles including cardioprotective [27, 28], chemoprotective [29, 30], and antidiabetic [31, 32] effects in animal and cellular models and in human epidemiological and clinical intervention studies, as discussed in reviews [27–31] and a separate epidemiological study [32]. Anthocyanidins have also been shown to have anti-inflammatory [33–35] effects in animal and cellular models and in human epidemiological and clinical intervention studies and neuroprotective [36, 37] and anti-obesity [38–40] effects in cellular and animal models. There is growing evidence to suggest that a diet rich in anthocyanidins may also be associated with better bone health.

Human Observational Studies

Hardcastle et al. showed positive associations between flavonoid intakes and BMD and negative associations between flavonoid intakes and markers of bone resorption in the APOSS cohort, although anthocyanidins were not included in this study [10]. We recently found that, in the APOSS cohort, higher dietary anthocyanidin intake of dietary was associated with higher BMD and lower markers of bone turnover. These associations were still significant after adjustment for confounding factors (age, height, weight, physical activity level, national deprivation category, current smoking status, calcium intake, alcohol intake, vitamin D intake, menopausal status, and hormone replacement therapy (HRT) use).

In agreement with our findings, Welch et al. (2012) showed that both total flavonoid and anthocyanidin intakes were positively associated with BMD in women in the TwinsUK cohort (population of women aged 18–79 years) [11]. Wine consumption was also shown to be positively associated with spine BMD in a subset of postmenopausal women also from the TwinsUK cohort [41], and it is possible that the anthocyanidins in the wine may be the compounds responsible for this association.

Human Intervention Studies

There have been no human intervention studies to date that have examined the effect of anthocyanidins on bone, but there are a handful which have examined the effects of dried plum on markers of bone health. A 3-month trial, in which 58 postmenopausal women, not using HRT, consumed either 100 g dried plum or 75 g dried apple, showed that only the dried plum was associated with increased bone formation markers. Neither diet had an effect on bone resorption markers [42]. A recent study by Hooshmand et al. also showed reduced bone turnover and increased BMD in postmenopausal women consuming dried plum over 12 months compared with those consuming dried apple [35]. Although dried plum is known to be a rich source of anthocyanidins, neither study quantified the anthocyanidin content of the dried plums consumed or the concentrations of anthocyanidins or metabolites in the serum of the

participants, and so the results cannot be related back to anthocyanidins.

The next section describes the effect of anthocyanidins on bone health in animal models. It is clear that a randomized controlled trial to study the effects of an anthocyanidin-rich diet or supplement on markers of bone health in humans would be extremely valuable in moving this research forward.

Animal Studies

A number of animal studies have also shown a protective effect of anthocyanidin-rich foods and bone health. Arjmandi et al. showed that anthocyanidin-rich dried plum lowered markers of bone resorption, increased markers of bone formation, and prevented bone loss in ovariectomized (OVX) rats [43]. In other studies, dried plum has been shown to reverse bone loss in an osteopenic rat model of osteoporosis [44] and prevent bone loss in a male rat model of osteoporosis [45]. In a study by Devareddy et al., a blueberry-rich diet was shown to prevent BMD loss in a rat model of osteoporosis [46]. Chen et al. also showed that a blueberry-rich diet increased bone formation without increasing overall growth in young rats, and serum from the blueberry-fed young rats was able to increase osteoblast differentiation and reduce osteoclastogenesis in vitro [47]. Zhang et al. showed that rats fed with blueberries at an early age had significantly reduced OVX-induced bone loss; pre-osteoblasts treated with the serum from the blueberry-fed young rats were found to maintain osteoblast development and differentiation in vitro [48]. Kaume et al. found that in OVX rats, a diet containing 5 % freeze-dried blackberry was protective against BMD loss [49]. However, interestingly, no beneficial effect was seen with 10 % blackberry diet on bone, therefore suggesting that there may be an optimum dose of anthocyanidin-rich foods for the beneficial effects seen on bone.

Recently Arjmandi et al. have shown an enhanced effect of anthocyanin-rich dried plum in reversing OVX-induced bone loss in rats when given in combination with prebiotic fructooligosaccharide suggesting again that the bioavailability and metabolism of anthocyanin-derived compounds may be influenced by gut microflora [50].

The results of the major human and animal studies looking at the effects of anthocyanidins and bone outcomes are shown in Tables 17.1 and 17.2.

Cellular Studies

It is unclear whether anthocyanins themselves or their metabolites or breakdown compounds are responsible for the beneficial effects observed on bone health. Previous data has shown that the antioxidant capacity of blueberry correlates more strongly with anthocyanidin composition than other phenolic compounds suggesting the effect is due to the anthocyanidins [51]. Conversely, Chen et al. [47] suggested that the effects of blueberry may be due to the phenolic acid metabolites since a similar mixture of the phenolic acids present in the serum of the blueberry-fed rats was able to increase osteoblast differentiation in vitro. We recently studied the effects of a number of individual anthocyanin and anthocyanidin compounds and found that they had no effect on mouse calvarial osteoblast differentiation in vitro, over 3 days, as measured by alkaline phosphatase activity (see Fig. 17.2) (data not published). These novel data suggest that the effect of dietary anthocyanidins on bone may be explained by anthocyanidin metabolites, such as phenolic acids, rather than the pure compounds although further analysis is required.

To date, anthocyanidins have not been studied directly on osteoclasts in vitro. Some flavonoid compounds, similar to anthocyanidins, such as quercetin, kaempferol, myricetin, isorhamnetin, and curcumin have been shown to inhibit osteoclastogenesis in vitro [52].

Further work is required to study the effects of anthocyanidins and their metabolites on osteoclast cells in vitro and to investigate the specific compounds responsible for the effects of anthocyanidin-rich diets on bone.

Table 17.1 Summary of anthocyanidin studies in humans

Author	Subjects	Duration	Food source	Anthocyanidins	Outcomes
Human observational					
Fairweather-Tait et al. [41]	TwinsUK cohort 2,464 postmenopausal women	–	Wine	Measured by food frequency questionnaire (FFQ)	Positive association with spine BMD
Macdonald-Clarke et al. (manuscript in preparation)	APOSS cohort 3,214 middle-aged women	–	Habitual diet	Quartiles of anthocyanidin intake measured by FFQ	Positive associations with hip BMD, spine BMD, and bone formation marker P1NP. Negative association with bone resorption markers DPD and PYD
Welch et al. [11]	TwinsUK cohort 3,160 women aged 18–79	–	Habitual diet	Quartiles of anthocyanidin intake measured by FFQ	Positive association with hip BMD but not spine BMD
Human intervention					
Arjmandi et al. [42]	58 postmenopausal women not using HRT	3 months	100 g dried plum	Unknown	Increase in serum bone formation marker IGF-1.No change in bone resorption markers TRAP, DPD, or HP
Hooshmand et al. [35]	236 postmenopausal women not using HRT	3,6, and 12 months	100 g dried plum	Unknown	Increase in ulna and spine BMD. Decrease in BALP, OC, TRAP5a, and inflammation marker CRP

TRAP tartrate-resistant acid phosphatase, *DPD* deoxypyridinoline, *PYD* pyridinoline, *IGF-1* insulin-like growth factor-1, *HP* helical peptide, *BALP* bone-specific alkaline phosphatase, *OC* osteocalcin, *CRP* C-reactive protein

Table 17.2 Summary of anthocyanidin studies in animals

Author	Subjects	Duration	Food source	Outcomes compared with controls
Arjmandi et al. [43]	48 female rats (OVX or sham)	45 days	5 % (low) and 25 % (high) dried plum	Increase in formation marker IGF-1 with both diets. No change in formation marker ALP. Lower bone resorption marker TRAP in high diet than low diet but not different to OVX control. Prevention of OVX-induced loss of trabecular bone area with high diet
Deyhim et al. [44]	60 female rats (OVX or sham)	60 days	5 % (low), 15 % (medium), 25 % (high) dried plum	All plum diets increased BMD
Franklin et al. [45]	60 male rats (ORX or sham)	90 days	5 % (low), 15 % (medium), 25 % (high) dried plum	All plum diets increased BMD. Increase in TbN and TbSp. No change in bone formation markers OC or ALP. Prevention of ORX-induced increase in bone resorption marker DPD
Devareddy et al. [46]	30 rats female (OVX or sham)	100 days	5 % dried blueberry	Increase in BMD and bone formation marker ALP.
				Decrease in bone formation marker OC. No change in bone resorption marker DPD
Chen et al. [47]	20 young rats (postnatal day 20) (10 male, 10 female)	14 and 34 days	10 % dried blueberry	Increased BMD. Increased bone formation markers ALP and OC. No change in bone formation marker IGF-1 or bone resorption marker RatLaps
Zhang et al. [48]	Female rats (fed blueberry before OVX or sham)	14 and 40 days	10 % dried blueberry	Increase in BMD with both short- and long-term diets. Increased bone formation markers OC and ALP. Decreased bone resorption marker RatLaps

OVX ovariectomized, *IGF-1* insulin-like growth factor-1, *ALP* alkaline phosphatase, *TRAP* tartrate-resistant acid phosphatase, *ORX* orchidectomized, *TbN* trabecular number, *TbSp* trabecular separation, *OC* osteocalcin, *DPD* deoxypyridinoline

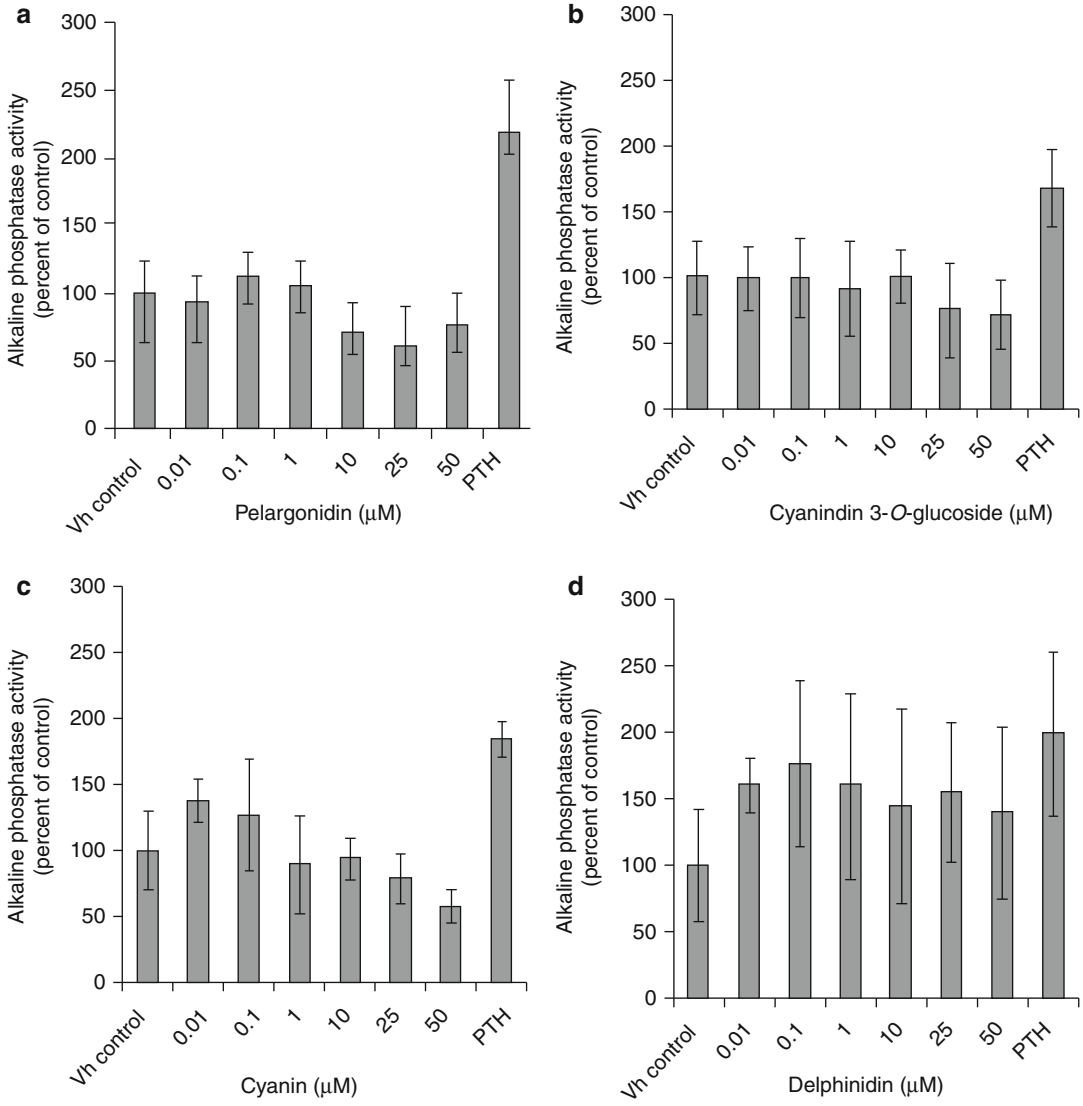


Fig. 17.2 The effects of anthocyanidins on mouse osteoblast differentiation in vitro ($n=3$) (mean +/− SEM). To investigate the effects of anthocyanidins on osteoblast differentiation, mouse calvarial osteoblasts were isolated and cultured with 0.01–50 μM specific anthocyanins or anthocyanidins for 3 days. Vehicle control (dH_2O) and a positive control, 40 nM parathyroid hormone (PTH) were also included in the experiment. After 3 days, cell viability was assessed by incubation with Alamar Blue to measure cell viability, and cells were lysed and assayed with p-nitrophenyl phosphate to quantify differentiation by alkaline phosphatase activity. The results are shown for pelargonidin, cyanidin 3-*O*-glucoside, cyanidin, and delphinidin in **a**, **b**, **c**, and **d**, respectively, and show alkaline phosphatase activity, adjusted for cell viability expressed as percent of the vehicle control. Anthocyanidin compounds were also tested (data not shown). None of the anthocyanidin or anthocyanin compounds tested showed statistically significant differences (one-way ANOVA) in osteoblast differentiation compared with control

Mechanism of Action on Bone

The precise mechanism or mechanisms of action of anthocyanidins on bone are unknown. There are multiple reported and hypothesized mechanisms that could explain the potential effects of anthocyanidins on bone including the following: antioxidant, anti-inflammatory, cell signaling, gene transcription, and binding with estrogen or cannabinoid receptors.

Oxygen-derived free radicals have been shown to stimulate osteoclast-mediated bone resorption. Anthocyanins are potent antioxidants [53]. Other dietary antioxidants such as carotenoids have been associated with less bone loss and decreased risk of hip fracture [54, 55]. Therefore, it is possible that anthocyanidins, known potent antioxidants, may inhibit bone resorption and improve bone health similarly to carotenoids. However, it has been suggested that the anthocyanins may be absorbed at such a low concentration that they are unlikely to have an antioxidant effect [20]. Even so, at these concentrations, anthocyanins may still be able to affect signal transduction [33] or gene expression [56]. A 3-week placebo-controlled trial showed that supplementation with 300 mg anthocyanidin per day could suppress of nuclear factor-κB (NF-κB) transactivation and decrease plasma concentrations of proinflammatory chemokines, cytokines, and inflammatory mediators [33].

Recently, Hooshmand et al. showed that anthocyanidin-rich dried plum was able to significantly lower serum C-reactive protein (CRP), a marker of inflammation, after 3 months in postmenopausal women [35]. Since higher circulating serum CRP has been associated with osteoporosis, it could be inferred that anthocyanidins may reduce inflammation and thereby reduce bone turnover and osteoporosis.

Cellular studies have reported effects of anthocyanidin metabolites on cell signaling, for example, serum from blueberry-fed young rats was found to stimulate osteoblast differentiation and reduce osteoclastogenesis through P38 MAP kinase/β-catenin canonical Wnt signaling [47]. Also, Zhang et al. found that blueberry powder delayed osteoblast senescence through regulation of the Runx2 gene [48].

In addition, anthocyanins have also been shown to have low binding affinity with estrogen and cannabinoid receptors [57] therefore suggesting yet more possible mechanisms of action.

It is conceivable that anthocyanidins and their metabolites may exert their effects by a number of mechanisms which may be dependent on the frequency and quantity of anthocyanidin consumption.

Conclusions

The evidence reviewed in this chapter indicates that anthocyanidins may play a role in bone health. Further work is required to determine which metabolites are responsible and the cellular mechanism of action. The few human studies undertaken have investigated the role of anthocyanidin-rich foods on bone loss but lack information on the anthocyanidin composition of the foods that have been tested or have not measured biomarkers of anthocyanidin intake. A randomized controlled trial investigating the role of anthocyanidins on bone health is required to confirm whether these compounds prove to be a cost-effective means of attenuating bone loss. The role of dietary anthocyanidins in peak bone mass in humans is unknown.

References

1. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr. 1997;65(6):1831–9.
2. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr. 1999;69(4):727–36.
3. Macdonald HM, New SA, Golden MHN, Campbell MK, Reid DM. Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr. 2004;79(1):155–65.
4. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, et al. Fruit and vegetable intakes and bone mineral status: a cross-sectional study in 5 age and sex cohorts. Am J Clin Nutr. 2006;83(6):1420–8.
5. Arnett T. Regulation of bone cell function by acid–base balance. Proc Nutr Soc. 2003;62:511–21.
6. Macdonald HM, Black AJ, Aucott L, Duthie G, Duthie S, Sandison R, et al. Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial. Am J Clin Nutr. 2008;88(2):465–74.
7. Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review and meta-analysis applying Hill's epidemiologic criteria for causality. Nutr J. 2011;10:41.

8. Hamidi M, Boucher BA, Cheung AM, Beyene J, Shah PS. Fruit and vegetable intake and bone health in women aged 45 years and over: a systematic review. Osteoporos Int. 2011;22(6):1681–93.
9. Muhlbauer RC, Li F. Effect of vegetables on bone metabolism. Nature. 1999;401(6751):343–4.
10. Hardcastle AC, Aucott L, Reid DM, Macdonald HM. Associations between dietary flavonoid intakes and bone health in a Scottish population. J Bone Miner Res. 2011;26(5):941–7.
11. Welch A, Macgregor A, Jennings A, Fairweather-Tait S, Spector T, Cassidy A. Habitual flavonoid intakes are positively associated with bone mineral density in women. J Bone Miner Res. 2012;27(9):1872–8.
12. Zhang Z, Kou X, Fugal K, McLaughlin J. Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. J Agric Food Chem. 2004;52(4):688–91.
13. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006;54(11):4069–75.
14. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet. 1976;24:117–91.
15. Heinonen M. Antioxidant activity and antimicrobial effect of berry phenolics – a Finnish perspective. Mol Nutr Food Res. 2007;51(6):684–91.
16. Bhagwat SA, Haytowitz DB, Holden JM. USDA database for the flavonoid content of selected foods: release 3. 2011. http://www.ars.usda.gov/sp2UserFiles/Place/12354500/Data/Flav/Flav_R03.pdf. Accessed 7th November 2011.
17. McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res. 2007;51(6):702–13.
18. Kay CD. Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans. Nutr Res Rev. 2006;19(1):137–46.
19. Mazza G, Kay CD, Cottrell T, Holub BJ. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. J Agric Food Chem. 2002;50(26):7731–7.
20. Milbury PE, Vita JA, Blumberg JB. Anthocyanins are bioavailable in humans following an acute dose of cranberry juice. J Nutr. 2010;140(6):1099–104.
21. Tsuda T, Horio F, Osawa T. Absorption and metabolism of cyanidin 3-O-beta-D-glucoside in rats. FEBS Lett. 1999;449:179–82.
22. Miyazawa T, Nakagawa K, Kudo M, Muraishi K, Someya K. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. J Agric Food Chem. 1999;47(3):1083–91.
23. Felgines C, Talavera S, Texier O, Gil-Izquierdo A, Lamaison J, Remesy C. Blackberry anthocyanins are mainly recovered from urine as methylated and glucuronidated conjugates in humans. J Agric Food Chem. 2005;53(20):7721–7.
24. Wu X, Pittman III HE, Mckay S, Prior RL. Aglycones and sugar moieties alter anthocyanin absorption and metabolism after berry consumption in weanling pigs. J Nutr. 2005;135(10):2417–24.
25. Fleschhut J, Kratzer F, Rechkemmer G, Kulling SE. Stability and biotransformation of various dietary anthocyanins in vitro. Eur J Nutr. 2006;45(1):7–18.
26. Aura AM, Martin-Lopez P, O'Leary KA, Williamson G, Oksman-Caldentey KM, Poutanen K, et al. In vitro metabolism of anthocyanins by human gut microflora. Eur J Nutr. 2005;44(3):133–42.
27. de Pascual-Teresa S, Moreno DA, Garcia-Viguera C. Flavanols and anthocyanins in cardiovascular health: a review of current evidence. Int J Mol Sci. 2010;11(4):1679–703.
28. Wallace TC. Anthocyanins in cardiovascular disease. Adv Nutr. 2011;2(1):1–7.
29. Stoner GD, Wang LS, Casto BC. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. Carcinogenesis. 2008;29(9):1665–74.
30. Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer Lett. 2008;269(2):281–90.
31. Sancho RAS, Pastore GM. Evaluation of the effects of anthocyanins in type 2 diabetes. Food Res Int. 2012;46(1):378–86.
32. Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, et al. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr. 2012;95(4):925–33.
33. Karlsen A, Retterstol L, Laake P, Paur I, Kjolsrud-Bohn S, Sandvik L, et al. Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. J Nutr. 2007;137(8):1951–4.
34. Xie C, Kang J, Ferguson ME, Nagarajan S, Badger TM, Wu X. Blueberries reduce pro-inflammatory cytokine TNF-alpha and IL-6 production in mouse macrophages by inhibiting NF-kappaB activation and the MAPK pathway. Mol Nutr Food Res. 2011;55(10):1587–91.
35. Hooshmand S, Chai SC, Saadat RL, Payton ME, Brummel-Smith K, Arjmandi BH. Comparative effects of dried plum and dried apple on bone in postmenopausal women. Br J Nutr. 2011;106(6):923–30.
36. Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM, Joseph JA. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci. 2005;8(2):111–20.
37. Shih PH, Wu CH, Yeh CT, Yen GC. Protective effects of anthocyanins against amyloid beta-peptide-induced damage in neuro-2A cells. J Agric Food Chem. 2011;59(5):1683–9.
38. Jayaprakasam B, Olson LK, Schutzki RE, Tai M, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in cornelian cherry (Cornus mas). J Agric Food Chem. 2006;54(1):243–8.
39. Kaume L, Gilbert WC, Brownmiller C, Howard LR, Devareddy L. Cyanidin 3-O-β-d-glucoside-rich blackberries modulate hepatic gene expression, and anti-

obesity effects in ovariectomized rats. J Funct Foods. 2012;4(2):480–8.
40. Tsuda T. Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. J Agric Food Chem. 2008;56(3):642–6.
41. Fairweather-Tait SJ, Skinner J, Guile GR, Cassidy A, Spector TD, MacGregor AJ. Diet and bone mineral density study in postmenopausal women from the TwinsUK registry shows a negative association with a traditional English dietary pattern and a positive association with wine. Am J Clin Nutr. 2011;94(5):1371–5.
42. Arjmandi BH, Khalil DA, Lucas EA, Georgis A, Stoecker BJ, Hardin C, et al. Dried plums improve indices of bone formation in postmenopausal women. J Womens Health Gend Based Med. 2002;11(1):61–8.
43. Arjmandi B, Lucas E, Juma S, Soliman A, Stoecker B, Khalil D, et al. Dried plums prevent ovariectomy-induced bone loss in rats. JANA. 2001;4(1):50–6.
44. Deyhim F, Stoecker BJ, Brusewitz GH, Devareddy L, Arjmandi BH. Dried plum reverses bone loss in an osteopenic rat model of osteoporosis. Menopause. 2005;12(6):755–62.
45. Franklin M, Bu SY, Lerner MR, Lancaster EA, Bellmer D, Marlow D, et al. Dried plum prevents bone loss in a male osteoporosis model via IGF-I and the RANK pathway. Bone. 2006;39(6):1331–42.
46. Devareddy L, Hooshmand S, Collins JK, Lucas EA, Chai SC, Arjmandi BH. Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. J Nutr Biochem. 2008;19(10):694–9.
47. Chen JR, Lazarenko OP, Wu X, Kang J, Blackburn ML, Shankar K, et al. Dietary-induced serum phenolic acids promote bone growth via p38 MAPK/beta-catenin canonical Wnt signaling. J Bone Miner Res. 2010;25(11):2399–411.
48. Zhang J, Lazarenko OP, Blackburn ML, Shankar K, Badger TM, Ronis MJ, et al. Feeding blueberry diets in early life prevent senescence of osteoblasts and bone loss in ovariectomized adult female rats. PLoS One. 2011;6(9):e24486.
49. Kaume L, Gadang V, Gilbert W, Wang L, Smith BJ, Devareddy L. Dose-dependant effects of blackberries in the prevention of bone loss in ovariectomized rats. J Bone Miner Res. 2010;25 Suppl 1:SU0431.
50. Arjmandi BH, Johnson CD, Campbell SC, Hooshmand S, Chai SC, Akhter MP. Combining fructooligosaccharide and dried plum has the greatest effect on restoring bone mineral density among select functional foods and bioactive compounds. J Med Food. 2010;13(2):312–9.
51. Wang J, Mazza G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-gamma-activated RAW 264.7 macrophages. J Agric Food Chem. 2002;50(4):850–7.
52. Yamaguchi M, Hamamoto R, Uchiyama S, Ishiyama K. Effects of flavonoid on calcium content in femoral tissue culture and parathyroid hormone-stimulated osteoclastogenesis in bone marrow culture in vitro. Mol Cell Biochem. 2007;303(1–2):83–8.
53. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest. 1990;85(3):632–9.
54. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Inverse association of carotenoid intakes with 4-y change in bone mineral density in elderly men and women: the Framingham Osteoporosis Study. Am J Clin Nutr. 2009;89(1):416–24.
55. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. J Bone Miner Res. 2009;24(6):1086–94.
56. DeFuria J, Bennett G, Strissel KJ, Perfield JW, Milbury PE, Greenberg AS, et al. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. J Nutr. 2009;139(8):1510–6.
57. Korte G, Dreiseitel A, Schreier P, Oehme A, Locher S, Hajak G, et al. An examination of anthocyanins' and anthocyanidins' affinity for cannabinoid receptors. J Med Food. 2009;12(6):1407–10.

Chapter 18 Relative Effects of Vitamin D_3 and Calcifediol

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Abstract

This chapter evaluates differences between oral supplementation with calcifediol (25-hydroxyvitamin D) and vitamin D3 (cholecalciferol). Oral supplementation with calcifediol results in an immediate and sustained increase in serum 25-hydroxyvitamin D concentrations. This may be relevant in clinical care as higher circulating level of 25-hydroxyvitamin D can be reached much faster than on the standard supplementation with vitamin D3. However, whether calcifediol has additional benefits superior to vitamin D3 will need further investigation in an equivalent dose comparison trial.

Keywords

Calcifediol • Vitamin D_3 • 25(OH)D • Cholecalciferol • Bone health Nonskeletal endpoints

Introduction

Most recent recommendations on vitamin D intake agree that many people are vitamin D deficient and need vitamin D supplements to meet their vitamin D requirement [1–3]. The most common form of dietary supplementation used today is cholecalciferol or vitamin D_3, and most healthy adults reach a target range of 20 ng/ml with 600–800 IU vitamin D per day. However, the target range of at least 30 ng/ml, which is desirable for optimal fracture prevention [1–4], is less well defined with respect to vitamin D_3 requirements, and doses between 1,600 and 4,000 IU vitamin D_3 per day have been proposed [5, 6].

As an alternative strategy to enhance 25-hydroxyvitamin D (25(OH)D) levels, supplementation with the 25(OH)D_3 metabolite itself (calcifediol) has been suggested [7–14]. Potential advantages are its immediate, potent, and linear increase in serum 25(OH)D levels combined with documented benefits on bone metabolism and nonskeletal endpoints from small clinical trials as reviewed in this chapter [8, 9, 12–17]. Whether the benefit of calcifediol can be confirmed in a larger trial of fracture prevention and whether its bone effects are superior

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to vitamin D_3 within the same target serum 25-hydroxyvitamin D range cannot be answered to date.

Half-Life, Dose Response, and Safety

Compared to vitamin D_3 (half-life about 20 days [18]), calcifediol has hydrophilic properties, has a much shorter half-life of 8–11 h after oral administration, and causes a rapid increase in serum 25(OH)D levels (see Table 18.1 and Fig. 18.1). Also in contrast with vitamin D_3 supplementation, which shows a curvilinear dose–response curve for serum 25(OH)D increase [6], calcifediol results in a linear increase in serum 25(OH)D across a dose range [7]. With respect to potency of shifting mean 25(OH)D serum levels, two recent trials compared a daily dose of 20 μg of calcifediol to 20 μg of vitamin D_3, and both trials (Bischoff-Ferrari et al. [13]. and Cashman et al. [14]. – see Table 18.1) suggest that calcifediol is about twice as potent to increase mean 25(OH)D levels if compared to the same dose of vitamin D_3. With respect to safety, several studies report no incidence of hypercalcemia with 20 μg [13, 14] and up to 150 μg [12] calcifediol per day.

Bone Effects

Four smaller trials reported a benefit on bone density among groups of cardiac and kidney transplant patients, patients with glucocorticoid-induced osteopenia, and elderly hip fracture patients [8, 16, 19, 20], while this was not confirmed in a larger trial among 438 seniors [15] where no benefit of calcifediol was documented on bone mineral density (BMD) or fracture reduction if compared with calcium supplementation or placebo (see Table 18.1). However, the treatment dose of calcifediol in the larger trial was low with 15 μg per day [15]. Further, while human data are missing, one laboratory fracture healing study among old rats found that calcifediol improved mechanical strength of the fractured bone [20].

Nonskeletal Effects

One small RCT compared calcifediol with vitamin D_3 with respect to nonskeletal endpoints of vitamin D including lower extremity function, blood pressure, and innate immunity. Comparing 20 μg of calcifediol with 20 μg of vitamin D_3, the trial among healthy postmenopausal women showed that calcifediol treatment for 4 months not only results in a rapid increase in 25(OH)D levels (see Fig. 18.1) but may also be associated with a significant 2.8-fold higher odds of maintained or improved lower extremity function, an immediate and sustained decrease in systolic blood pressure by 5.6 mmHg, and a significantly more pronounced suppression of four out of seven markers of innate immunity (eotaxin, IL-12, MCP-1, MIP-1Beta). Notably, however, both calcifediol and vitamin D_3 contributed to a decrease in markers of innate immunity, indicating a benefit from both substances.

Participants treated with calcifediol (compared to the participants in the vitamin D_3 group) reached the 25(OH)D threshold of 30 ng/ml within 35 days of treatment and experienced a significant reduction of systolic blood pressure by 5.7 mmHg in the same time frame (see Fig. 18.2). In fact, the blood pressure reduction occurred in the early treatment phase and stabilized at this level for the remainder of the 4 months follow-up period. Systolic blood pressure did not change in the vitamin D_3 group which may be explained by the much slower and smaller increase in 25(OH)D levels in the vitamin D_3 group, leaving 50 % of the participants below 30 ng/ml even at 4 months of treatment (see Fig. 18.2).

Summary

In summary, available trials outlined in Table 18.1 show that oral supplementation with calcifediol results in an immediate and sustained increase in 25(OH)D levels. A higher circulating level of 25(OH)D can be reached much faster than on the standard supplementation with vitamin D_3 and may explain the benefit of 20 μg calcifediol per

Table 18.1 Intervention trials that tested calcifediol

Author/year	Calcifediol dose/control	N of subjects Age Follow-up/duration	Change in serum 25(OH)D (mean) with oral calcifediol (peak) Other endpoints
Acute administration of 25-hydroxycholecalciferol in man (*Haddad et al. 1976* [7])	Bolus dose of 1.5/5/10 μg calcifediol per kg body weight *No control*	Healthy volunteers *N*=27 Age 21–40 years Hourly in first 24 h, then days 2,3,4,5,7,10	At 24 h 1.5 μg per kg: 100 ng/ml 5 μg per kg: 65 ng/ml 10 μg per kg: 30 ng/ml (peak 4–8 h) Other endpoints: no
Comparison of oral 25-hydroxycholecalciferol, vitamin D, and UV light as determinants of circulating 25-hydroxyvitamin D (*Stamp et al. 1977* [9])	Daily dose of 15–20/40/80 μg calcifediol *Control:* UVB/1,800/10,000/20,000/40,000 IU vitamin D_3	Patients with rickets, osteomalacia, osteoporosis, hypoparathyroidism *N*=200 Daily short-term (4 weeks) and long-term treatment (6+ weeks)	At 4 weeks 15–20 μg: 25 ng/ml 40 μg: 75 ng/ml 80 μg: 150 ng/ml Other endpoints: compared with vitamin D_3 9–12 x more potent
Altered mineral metabolism in glucocorticoid-induced osteopenia (*Hahn et al. 1979* [8])	Daily mean dose of 42.3 μg calcifediol+500 mg calcium per day *Control:* 100 mg calcium per day	*N*=17 patients with glucocorticoid-induced osteopenia *N*=15 matched controls Mean age 46 years 12 months	At all time points (3, 6, 9, 12 months) 20 μg: 45 ng/ml 40 μg: 90 ng/ml 60 μg: 130 ng/ml Other endpoints Calcifediol compared with control – Significant benefit on forearm bone mass – Bone histology: significant less osteoclasts

(continued)

Table 18.1 (continued)

Author/year	Calcifediol dose/control	N of subjects Age Follow-up/duration	Change in serum 25(OH)D (mean) with oral calcifediol (peak) Other endpoints
Calcium malabsorption in the elderly: effect of treatment with oral 25-hydroxyvitamin D_3 (*Francis et al. 1983* [12])	Daily dose of 5/10/20/40/80/120 μg calcifediol	Hospitalized in acute geriatric care *N*=48 adults Mean age 80 years 7 days	After treatment (day 7) 5 μg: 39 ng/ml 10 μg: 59 ng/ml 20 μg: 62 ng/ml 40 μg: 101 ng/ml 80 μg: 210 ng/ml 120 μg: 502 ng/ml Other endpoints: no patient developed hypercalcemia, and plasma creatinine stayed in the normal range
Absorption, dosage, and effect on mineral homeostasis of 25-hydroxycholecalciferol in premature infants: comparison with 400 and 800 IU vitamin D2 supplementation *(Hillman et al. 1985* [21])	Daily dose of 1/2 μg per kg calcifediol *Control:* 400/800 IU vitamin D_2 per day	Premature infants *N*=67 Mean age 33.5 weeks 4 weeks	At 4 weeks 1 μg/kg: 25 ng/ml 2 μg/kg: 27 ng/ml Other endpoints Compared with 400 IU vitamin D_4 – Significantly higher target 25(OH)D levels at 4 weeks (16 ng/ml) – Significantly lower PTH – No improvement in mineralization (x-ray based)
Efficiency of preventive treatment of glucocorticoid-induced osteoporosis with 25(OH)D3 and Ca in kidney transplant patients *(Talalaj et al. 1996* [20])	Daily dose of 40 μg calcifediol + 3 g calcium carbonate *Control:* No preventive treatment	Kidney transplant patients *N*=77 Age 15–63 years 12 months	At 12 months 40 μg: 27 ng/ml Other endpoints Compared with control – Significantly higher BMD at the lumbar spine, femoral neck, and total skeleton – Significantly lower PTH

Calcitonin, etidronate, and calcidiol treatment in bone loss after cardiac transplantation (*Garcia-Delgado et al. 1997* [19])	Weekly dose of 800 µg calcifediol + 1 g calcium *Control/comparison:* Calcitonin 100 IU/day (nasal route)/etidronate cyclic treatment (400 mg orally for 2 weeks every 3 months) + 1 g calcium in controls	Cardiac transplant patients *N* = 40 Mean age 53 18 months	25(OH)D level not reported Other endpoints Compared with both controls – Significant increase in lumbar spine BMD
Vitamin D and its major metabolites: serum levels or graded oral dosing in men (*Barger-Lux et al. 1998* [17])	Daily dose of 10/20/50 µg calcifediol *Control/comparison:* Vitamin D_3 (1,000/10,000/50,000 IU per day) 1,25-dihydroxyvitamin D (0.5/1/2 µg per day)	Healthy men *N* = 116 Mean age 28 4 weeks	At 4 weeks 10 µg: + 16 ng/ml 20 µg: + 30 ng/ml 50 µg: + 82 ng/ml Other endpoints – PTH suppression was significant with 50 µg of calcifediol, the two higher doses of vitamin D_3, and 1,25-dihydroxyvitamin D
The effect of 25-hydroxyvitamin D on the bone mineral metabolism of elderly women with hip fracture (*Sosa et al. 2000* [16])	Weekly dose of 266 µg calcifediol + 1 g calcium per day *Control:* 1 g calcium per day	Women with osteoporosis and proximal femur fracture *N* = 58 Mean age 78 12 months Open design	25(OH)D level not reported Other endpoints – PTH suppression was significant – Significant improvement in hip BMD – No difference in fracture risk

(continued)

Table 18.1 (continued)

Author/year	Calcifediol dose/control	N of subjects Age Follow-up/duration	Change in serum 25(OH)D (mean) with oral calcifediol (peak) Other endpoints
Effect of calcium or 25(OH) vitaminD_3 dietary supplementation on bone loss at the hip in men and women over the age of 60 (*Peacock et al. 2000* [15])	Daily dose of 15 μg calcifediol *Controls:* 1 g calcium per day placebo	316 women, 122 men, Age 60+ *N*=438 Mean age 74 years 48 months Double-blind RCT	At 12 months 15 μg: 48 ng/ml Other endpoints – No difference in incidence of nonvertebral and vertebral fractures – Loss in BMD at the hip similar to placebo
Oral supplementation with 25(OH)D vs vitamin D_3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity (*Bischoff-Ferrari et al. 2011* [13])	Daily dose of 20 μg calcifediol *Controls:* 800 IU vitamin D_3 per day	Healthy postmenopausal women Mean age 62 years *N*=20 4 months (14 visits) Double-blind RCT	At 4 months Calcifediol 20 μg: 70 ng/ml Vitamin D3 800 IU: 31 ng/ml Other endpoints – Significant 2.8-fold increased odds of maintained or improved lower extremity function – Significant increase in knee extensor strength – Significant 5.7 mmHg reduction in systolic blood pressure – No participant developed hypercalcemia – Innate immunity markers: more pronounced reduction for four out of seven markers (eotaxin, IL-12, MCP-1, MIP-1Beta)
Relative effectiveness of oral 25-hydroxyvitamin D_3 and vitamin D_3 in raising wintertime serum 25-hydroxyvitamin D in older adults (*Cashman et al. 2012* [14])	Daily dose of 7/20 μg calcifediol *Controls:* 800 IU vitamin D_3 per day	25 men, 31 women *N*=56 Mean age 57 10 weeks Double-blind RCT	At 10 weeks Calcifediol 7 μg: 28 ng/ml Calcifediol 20 μg: 54 ng/ml Vitamin D_3 800 IU: 28 ng/ml Other endpoints – Significant reduction in PTH – No participant developed hypercalcemia

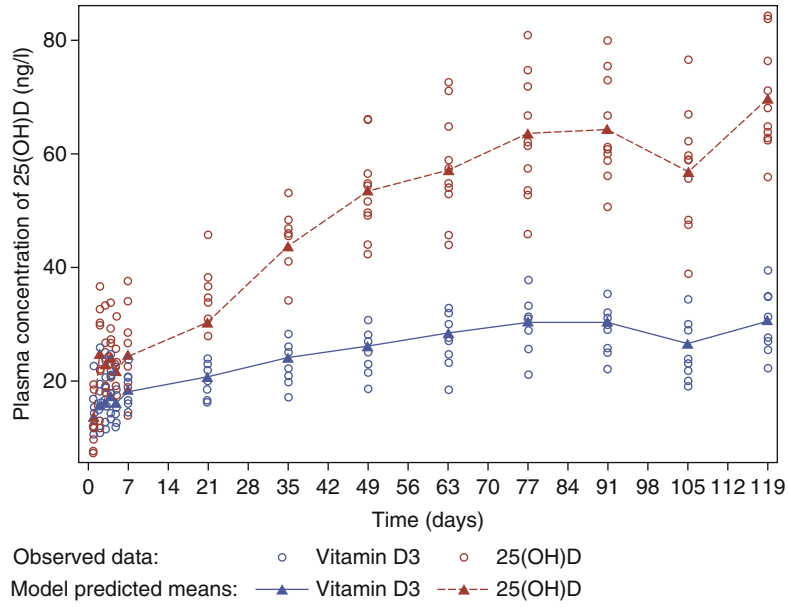

Fig. 18.1 Change in serum 25(OH)D level – comparison of a daily dose of 20 μg of calcifediol with 20 μg of vitamin D_3. *Dashed line* indicates the 30 ng/ml (75 nmol/l) threshold of 25(OH)D serum concentration. Graph shows individual and model-predicted means of serum 25(OH)D levels across 14 clinical visits by treatment. Predicted means are adjusted for age, BMI, and baseline 25(OH)D levels. The 25(OH)D levels shifted to above 30 ng/ml in all participants of the calcifediol group (25(OH)D) at 35 days of follow-up, while in the vitamin D_3 group, about 50 % of participants remained below this threshold throughout the 4-month follow-up (Adapted from Bischoff-Ferrari et al. [13]. With permission from John Wiley and Sons, Inc.)

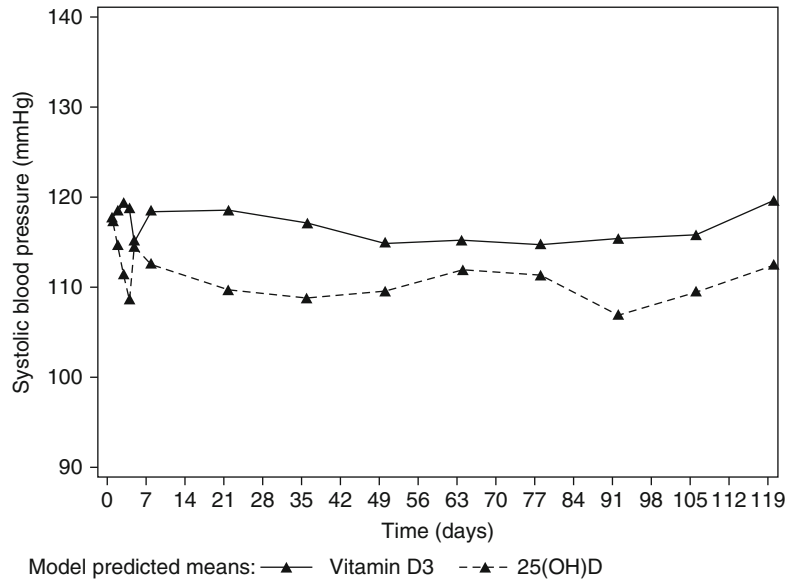

Fig. 18.2 Change in systolic blood pressure – comparison of a daily dose of 20 μg of calcifediol with 20 μg of vitamin D_3. Graph shows model-predicted means of systolic blood pressure across 120 days of follow-up (14 clinical visits) by treatment. Predicted means are adjusted for age, BMI, and baseline systolic blood pressure. Systolic blood pressure decreased in the calcifediol (25(OH)D) group progressively for the first 3 weeks of follow-up (up to day 21) and then stabilized at a lower level for the remainder of follow-up; in the vitamin D_3 group, on the other hand, systolic blood pressure remained virtually constant for the entire 4-month follow-up (Adapted from Bischoff-Ferrari et al. [13]. With permission from John Wiley and Sons, Inc.)

day on systolic blood pressure reduction, improvement in lower extremity function, and the more pronounced reduction in several markers of immunity among healthy postmenopausal women in one small trial [13]. Notably, however, none of the available trials tested an equivalent dose of calcifediol and vitamin D_3. Therefore, benefits documented by calcifediol with respect to skeletal and nonskeletal benefits summarized in this chapter may likely be caused by its rapid increase in 25(OH)D level. Alternatively, calcifediol may have additional benefits superior to vitamin D_3, which will need further investigation. Finally, the findings of the largest trial performed to date by Peacock and colleagues suggest that 15 μg calcifediol per day may be insufficient to increase bone mineral density and reduce fracture risk with calcifediol in the senior population.

References

1. Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement: vitamin D recommendations for older adults. Osteoporos Int. 2010;21:1151–4.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911–30.
3. Bischoff Ferrari HA, Burckhardt P, Quack-Loetscher K, et al. Vitamin D deficiency: evidence, safety, and recommendations for the Swiss population Report written by a group of experts on behalf of the Federal Commission for Nutrition (FCN) 2012 Approved by the Federal Commission for Nutrition on November 3, 2011. http://www.bag.admin.ch/themen/ernaehrung_bewegung/05207/13246/index.html?lang=en. Last accessed 16 Oct 2012.
4. Bischoff-Ferrari HA, Orav EJ, Willett WC, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367(1):40–9.
5. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, Willett WC. Benefit-risk assessment of vitamin D supplementation. Osteoporos Int. 2010;21:1121–32.
6. Gallagher JC, Sai A, Templin 2nd T, Smith L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Ann Intern Med. 2012;156:425–37.
7. Haddad Jr JG, Rojanasathit S. Acute administration of 25-hydroxycholecalciferol in man. J Clin Endocrinol Metab. 1976;42:284–90.
8. Hahn TJ, Halstead LR, Teitelbaum SL, Hahn BH. Altered mineral metabolism in glucocorticoid-induced osteopenia. Effect of 25-hydroxyvitamin D administration. J Clin Invest. 1979;64:655–65.
9. Stamp TC, Haddad JG, Twigg CA. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet. 1977;1:1341–3.
10. Colodro IH, Brickman AS, Coburn JW, Osborn TW, Norman AW. Effect of 25-hydroxy-vitamin D3 on intestinal absorption of calcium in normal man and patients with renal failure. Metabolism. 1978;27:745–53.
11. Stamp TC. Intestinal absorption of 25-hydroxycholecalciferol. Lancet. 1974;2:121–3.
12. Francis RM, Peacock M, Storer JH, Davies AE, Brown WB, Nordin BE. Calcium malabsorption in the elderly: the effect of treatment with oral 25-hydroxyvitamin D3. Eur J Clin Invest. 1983;13:391–6.
13. Bischoff-Ferrari HA, Dawson-Hughes B, Stöcklin E, et al. Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure and markers of innate immunity. J Bone Miner Res. 2011; [Epub ahead of print].
14. Cashman KD, Seamans KM, Lucey AJ, et al. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. Am J Clin Nutr. 2012;95:1350–6.
15. Peacock M, Liu G, Carey M, et al. Effect of calcium or 25OH vitamin D3 dietary supplementation on bone loss at the hip in men and women over the age of 60. J Clin Endocrinol Metab. 2000;85:3011–9.
16. Sosa M, Lainez P, Arbelo A, Navarro MC. The effect of 25-dihydroxyvitamin D on the bone mineral metabolism of elderly women with hip fracture. Rheumatology (Oxford). 2000;39:1263–8.
17. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos Int. 1998;8:222–30.
18. Smith JE, Goodman DS. The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. J Clin Invest. 1971;50:2159–67.
19. Garcia-Delgado I, Prieto S, Gil-Fraguas L, Robles E, Rufilanchas JJ, Hawkins F. Calcitonin, etidronate, and calcidiol treatment in bone loss after cardiac transplantation. Calcif Tissue Int. 1997;60:155–9.
20. Talalaj M, Gradowska L, Marcinowska-Suchowierska E, Durlik M, Gaciong Z, Lao M. Efficiency of preventive treatment of glucocorticoid-induced osteoporosis with 25-hydroxyvitamin D3 and calcium in kidney transplant patients. Transplant Proc. 1996;28:3485–7.
21. Hillman LS, Hollis B, Salmons S, et al. Absorption, dosage, and effect on mineral homeostasis of 25-hydroxycholecalciferol in premature infants: comparison with 400 and 800 IU vitamin D2 supplementation. J Pediatr. 1985;106:981–9.

Physical Performance, Muscle Strength, Falls, and Vitamin D

19

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Abstract

Vitamin D status is associated with muscle strength, physical performance, and falls as has been observed in many epidemiological studies. When serum 25-hydroxyvitamin D increases from very low levels to 50 nmol/l, physical performance increases and plateaus with higher levels of serum 25(OH)D. Randomized controlled clinical trials were performed with vitamin D alone or with vitamin D and calcium with the endpoint falls. Eight of thirteen studies showed a significant decrease of fall incidence, and in six of seven significant double-blind studies, vitamin D was combined with calcium and compared with double placebo. The decrease of fall incidence ranged from −19 to −70 %. One study with vitamin D3 dose of 500,000 IU once per year showed an increased fall incidence in the vitamin D group compared with the placebo group. The increased fall incidence was observed in the first 3 months after the high vitamin D dose. Eight meta-analyses have been performed on the effects of vitamin D on fall incidence. One may conclude from these that vitamin D3 is effective in doses of 800 IU/day or more and preferably combined with calcium. Vitamin D may influence muscle strength through genomic and non-genomic pathways. The active metabolite 1,25-dihydroxyvitamin D binds to the nuclear vitamin D receptor and can activate more than 300 genes, and it may also bind to a membrane receptor thus activating second messengers leading to fast calcium influx. Vitamin D may influence muscle

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fiber proliferation and differentiation, and calcium influx and calcium transport to the sarcoplasmic reticulum. There is still some debate on the presence of the vitamin D receptor in human muscle tissue because it was demonstrated by some but not by other investigators.

In conclusion, vitamin D can influence muscle strength, balance, and prevent falls.

Keywords

Physical performance • Muscle strength • Falls • Vitamin D • Vitamin D receptor • Genomic and non-genomic pathways • Meta-analyses • Prevention of falls

Introduction

Vitamin D is necessary for calcium absorption and mineralization of new bone matrix, the osteoid. Vitamin D deficiency results in rickets in children and osteomalacia in adults. Long-term less severe vitamin D deficiency leads to secondary hyperparathyroidism and bone loss and contributes to osteoporosis. More recently, a relationship has been observed between vitamin D status, muscle strength, physical performance, and falls. Vitamin D and calcium supplementation can decrease the incidence of hip fractures and other non-vertebral fractures as was shown by Chapuy et al. [1]. The effect of supplementation on the incidence of other non-vertebral fractures was already visible within 6 months, leading to the suggestion that vitamin D and calcium might prevent falls. In this chapter, the relationship between vitamin D and physical performance, muscle strength, and falls will be discussed. Subsequently, the effects of supplementation in randomized clinical trials on fall prevention will be described. These trials have led to several meta-analyses on the effects of vitamin D on the incidence of falls. Finally, the mechanistic basis of the described relationships will be reviewed with an emphasis on genomic and non-genomic pathways. The chapter will end with a conclusion and research agenda.

Epidemiological Association Studies

A relationship between physical performance tests and vitamin D status was observed in the National Health and Nutrition Examination Survey (NHANES III). The timed walking test and the timed chair stands (five chair stands without using hands) showed a fast improvement when serum 25 hydroxyvitamin D increased from below 20 to 50 nmol/l. With higher levels, the needed time tended to plateau [2]. Similar data were obtained in the Longitudinal Aging Study Amsterdam, an epidemiological study in a representative sample of the Dutch population. Almost 50 % of the participants of 65 years and older were vitamin D deficient or insufficient (serum 25(OH)D < 25 or 25–50 nmol/l, respectively). In this study physical performance tests and a fall follow-up were done. The physical performance score consisting of a walking test, five chair stands, and tandem stand was scored on a scale from 0 to 12. When serum 25(OH)D increased from below 10 to 50 nmol/l, physical performance increased 5 points, and following adjustment for age, sex, chronic diseases, and BMI, the increase with improving vitamin D status still was more than 2 points on the scale of 12 [3]. A plateau of the physical performance score vs. vitamin D status was seen above 50 nmol/l. In the LASA study vitamin D deficiency could predict the loss of muscle strength and muscle mass during 3 years of follow-up. Loss of muscle strength was more than twice as frequent when serum 25(OH)D was below 25 nmol/l than with higher values [4]. In the LASA study, a fall follow-up was done every 3 months during 3 years with a fall calendar. When serum 25(OH)D was lower than 25 nmol/l, the risk ratio for two falls or three falls was more than five in comparison with the participants with a serum 25(OH)D higher than 25 nmol/l. This was apparent below 75 years of age, but above

75 years the relationship was no longer significant [5]. An Australian study [6] showed similar risk factors for falling. Higher serum 25(OH)D levels and higher weight were protective for falling, while antipsychotic treatment, cognitive decline, a past Colles fracture, and being a wanderer all increased the risk for falling.

Randomized Clinical Trials

An intervention study [7] in 148 older women with vitamin D3 800 IU/day vs. placebo for 8 weeks reduced sway by 9 % and reduced the fall incidence. This group repeated this study in 242 community-dwelling seniors, and the fall incidence decreased 39 % ($p<0.01$) in the vitamin D group in comparison to the placebo group [8]. An Australian study comparing vitamin D 1,000 IU/day vs. placebo in 540 older persons in residential care showed a decrease of fall incidence ($p<0.05$) and a lower fracture incidence in the vitamin D group, but the latter was not significant [9]. Altogether at least 13 studies have been performed, and 8 of these showed a significant decrease of fall incidence [10–18, 20] (Table 19.1). In six of seven double-blind studies, vitamin D was combined with calcium and compared with double placebo. The decrease of fall incidence ranged from −19 to −70 %. In four studies the change was not significant, and one Australian study with a large dose of 500,000 IU once per year showed an increased fall incidence in the vitamin D group compared with the placebo group. In this study [18], the fall incidence was 15 % higher in the vitamin D group, and the fracture incidence was higher, but this was only borderline significant. It turned out that the increase in fall and fracture incidence was visible in the first 3 months after the high vitamin D dose. During these 3 months mean serum 25(OH)D increased to levels higher than 120 nmol/l. This led to the conclusion that a high dose once per year is not the way to administer vitamin D, and the high peak levels of serum 25(OH)D may be deleterious. We performed a multicenter study comparing the effect of 8,400 IU/week vs. placebo, but there was no effect on the physical performance tests and sway. However, when baseline sway was high, a decrease of sway was seen in the vitamin D group [19]. Recently, a short-term intervention study with protein and vitamin D in malnourished older adults showed a decrease of fall incidence in the intervention group compared with the control group [20]. It is difficult to deduct a threshold serum 25(OH)D necessary for fall

Table 19.1 The effect of vitamin D on fall incidence: results of randomized clinical trials

Reference	Pat N	Vit D dose	Calcium	Baseline 25(OH)D[a]	Posttreatment 25(OH)D[a]	Outcome *n* of fallers
Graafmans et al. [10]	330	400 IU/day	–	27	55	NS
Pfeifer et al. [7]	148	800 IU/day	1,200 mg/day	26	66	−40 %
Latham et al. [11]	243	300,000 IU	–	38	61	NS
Harwood et al. [12]	150	800 IU/day	1,000 mg/day	29	50	−52 %
Flicker et al. [9]	625	1,000 IU/day	600 mg/day	<40		−27 %
Bischoff-Ferrari et al. [13]	445	700 IU/day	500 mg/day	70	104	−46 %(women)
Law et al. [14]	223	100,000 IU/3 m	–	47	82	NS (men)
Broe et al. [15]	124	800 IU/day	–	53	75	−72 %
Prince et al. [16]	302	1,000 IU/day	1,000 mg/day	45	60	−19 %
Pfeifer et al. [8]	242	800 IU/day	1,000 mg/day	55	84	−27 %
Kärkkäinen et al. [17]	1,645	800 IU/day	1,000 mg/day	50	75	NS[b]
Sanders et al. [18]	2,256	500,000 IU/year	–	49	120[c]	+15 %
Neelemaat et al. [20]	210	576 IU/day	500 mg/day[d]	40	65	−59 %

[a]25(OH)D nmol/l
[b]30 % less multiple falls
[c]After 1 month 120 nmol/l, after 3 months 90 nmol/l
[d]The intervention group received a protein-rich diet in addition

Table 19.2 The effect of vitamin D on fall incidence: results of meta-analyses

Reference	*N*	RR	Remarks
Latham et al. [23]	4	0.99 (0.89–1.11)	
Bischoff-Ferrari et al. [24]	5	0.78 (0.64–0.92)	incl 2 alphacal[b]
Jackson et al. [25]	5	0.88 (0.78–1.00)	
Richy et al. [26]	11	0.92 (0.86–0.99)	incl 2 alphacal[b]
Cochrane, community [27]		0.96 (0.92–1.01)	
Cochrane, nursing care [27]		0.98 (0.89–1.09)[a]	
Bischoff-Ferrari et al. [28]	8	0.87 (0.77–0.99)	Dose split (high/low)
Kalyani et al. [29]	10	0.86 (0.79–0.93)	Dose 800 (IU/day)

[a]Rate of falls 0.72 (0.55–0.95)
[b]These meta-analyses include two studies done with the active vitamin D metabolite alphacalcidol

prevention. It may be around 50 or 60 nmol/l, near the recommendation of the Institute of Medicine [21]. A vitamin D dose of 800 IU/day will increase serum 25(OH)D to above 50 nmol/l in most older persons.

Meta-analysis

A meta-analysis of studies on the effect of vitamin D on muscle strength, gait, and balance showed a significant improvement of sway and the timed up and go test. An increase of lower extremity strength or improvement of distance walked (gait) was not obtained [22]. At least eight meta-analyses have been performed on the effect of vitamin D on fall incidence. The outcomes of the meta-analyses were different. Two meta-analyses contained studies with an active vitamin D metabolite (alphacalcidol), and one meta-analysis of Bischoff-Ferrari compared lower and higher doses showing a decreased fall incidence only with the higher dose. Another meta-analysis only included studies with vitamin D3 800 IU or more [23–29] (Table 19.2). One may conclude from these meta-analyses that vitamin D3 only is effective in higher doses of 800 IU/day or more and particularly in combination with calcium.

Mechanistic Studies

Vitamin D may influence muscle strength through genomic and non-genomic pathways [30]. The active vitamin D metabolite, 1,25-dihydroxyvitamin D, binds to the nuclear vitamin D receptor after entering the cell and can activate more than 300 genes. The alternative is binding to a membrane receptor, thus activating second messengers leading to a fast calcium influx. The active vitamin D metabolite, 1,25-dihydroxyvitamin D, may influence muscle fiber proliferation and differentiation. It may influence calcium influx and phosphate transport and especially calcium transport to the sarcoplasmic reticulum. On the other side, 1,25-dihydroxyvitamin D may also influence the metabolism of neurotransmitters or stimulate neural growth factor in the nervous system and thus indirectly influence muscle activity. Abnormal skeletal muscle development has been shown in the vitamin D receptor null mouse, and the mean size of muscle fibers was smaller in this mouse than in the wild-type mouse [31].

The vitamin D receptor was demonstrated in human muscle tissue by immunologic methods, and it turned out that the number decreased with aging [32]. However, the debate on the vitamin D receptor in muscle tissue continues with positive and negative studies showing vitamin D receptor in muscle tissue or not [33, 34].

Conclusion

Clinical and epidemiological studies suggest that vitamin D influences muscle strength and balance. Clinical trials show that vitamin D with calcium (and maybe without) can improve balance and prevent falls. Clinical trials and meta-analyses suggest that vitamin D is more effective in frail or institutionalized elderly; however, a high once yearly dose of vitamin D

may increase the fall incidence. There are several explanations why vitamin D could influence muscle function.

Research Agenda

The mechanism why vitamin D can prevent falls should be further clarified. It is not known which dose of vitamin D is optimal to prevent falls and whether calcium always should be added. It is not known which groups should most likely profit from vitamin D to prevent falls. Polymorphisms of vitamin D-related genes may influence fall incidence and the effect of vitamin D treatment.

References

1. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ. Vitamin D3 and calcium to prevent hip fractures in the elderly women. New Engl J Med. 1992;327:1637–42.
2. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, Dawson-Hughes B. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged>or=60 y. Am J Clin Nutr. 2004;80:752–8.
3. Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P. Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab. 2007;92:2058–65.
4. Visser M, Deeg DJH, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab. 2003;12:5766–72.
5. Snijder MB, van Schoor NM, Pluijm SMF, van Dam RM, Visser M, Lips P. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. J Clin Endocrinol Metab. 2006;91:2980–5.
6. Flicker L, Mead K, MacInnes RJ, Nowson C, Scherer S, Stein MS, Thomasx J, Hopper JL, Wark JD. Serum vitamin D and falls in older women in residential care in Australia. J Am Geriatr Soc. 2003;51:1533–8.
7. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C. Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. J Bone Miner Res. 2000;15:1113–8.
8. Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H. Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. Osteoporos Int. 2009;20:315–22.
9. Flicker L, MacInnes RJ, Stein MS, Scherer SC, Mead KE, Nowson CA, Thomas J, Lowndes C, Hopper JL, Wark JD. Should older people in residential care receive vitamin D to prevent falls? Results of a randomized trial. J Am Geriatr Soc. 2005;53:1881–8.
10. Graafmans WC, Lips P, Ooms ME, van Leeuwen JPTM, Pols HAP, Uitterlinden AG. The effect of vitamin D supplementation on the bone mineral density of the femoral neck is associated with vitamin D receptor genotype. J Bone Miner Res. 1997;12:1241–5.
11. Latham NK, Andersson CS, Lee A, Bennett DA, Moseley A, Cameron ID, Fitness Collaborative Group. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). J Am Geriatr Soc. 2003;51:291–9.
12. Harwood RH, Sahota O, Gaynor K, Masud T, Hosking DJ, Nottingham Neck of Femur (NONOF) Study. A randomised, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: the Nottingham Neck of Femur (NONOF) Study. Age Ageing. 2004;33:45–51.
13. Bischoff-Ferrari HA, Orav EJ, Dawson-Hughes B. Effect of cholecalciferol plus calcium on falling in ambulatory older men and women: a 3-year randomized controlled trial. Arch Intern Med. 2006;166:424–30.
14. Law M, Withers H, Morris J, Andersson F. Vitamin D supplementation and the prevention of fractures and falls: results of a randomised trial in elderly people in residential accommodation. Age Ageing. 2006;35:482–6.
15. Broe KE, Chen TC, Weinberg J, Bischoff-Ferrari HA, Holick MF, Kiel DP. A higher dose of vitamin D reduces the risk of falls in nursing home residents: a randomized, multiple-dose study. J Am Geriatr Soc. 2007;55:234–9.
16. Prince RL, Austin N, Devine A, Dick IM, Bruce D, Zhu K. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. Arch Intern Med. 2008;168:103–8.
17. Kärkkäinen MK, Tuppurainen M, Salovaara K, Sandini L, Rikkonen T, Sirola J, Honkanen R, Arokosi J, Alhava E, Kroger H. Does daily vitamin D 800 IU and calcium 1000 mg supplementation decrease the risk of falling in ambulatory women aged 65–71 years? A 3-year randomized population-based trial (OSTPRE-FPS). Maturitas. 2010;65:359–65.
18. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010;303:1815–22.
19. Lips P, Binkley N, Pfeifer M, Recker R, Samanta S, Cohn DA, Chandler J, Rosenberg E, Papanicolaou DA. Once-weekly dose of 8400 IU vitamin D3 compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. Am J Clin Nutr. 2010;91:985–91.

20. Neelemaat F, Lips P, Bosmans JE, Thijs A, Seidell JC, van Bokhorst-de van der Schueren MA. Short-term oral nutritional intervention with protein and vitamin D decreases falls in malnourished older adults. J Am Geriatr Soc. 2012;60:691–7.
21. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53–8.
22. Muir SW, Montero-Odasso M. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc. 2011;59:2291–300.
23. Latham NK, Andersson CS, Reid IR. Effects of vitamin D supplementation on strength, physical performance, and falls in older persons: a systematic review. J Am Geriatr Soc. 2003;51:1219–26.
24. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB. Effect of vitamin D on falls: a meta-analysis. JAMA. 2004;291:1999–2006.
25. Jackson C, Gaugris S, Sen SS, Hosking D. The effect of cholecalciferol (vitamin D3) on the risk of fall and fracture: a meta-analysis. QJM. 2007;100:185–92.
26. Richy F, Dukas L, Schacht E. Differential effects of D-hormone analogs and native vitamin D on the risk of falls: a comparative meta-analysis. Calcif Tissue Int. 2008;82:102–7.
27. Gillespie LD, Robertson MC, Gillespie WJ, Lamb SE, Gates S, Cumming RG, Rowe BH. Interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev. 2009;15: CD007146.
28. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ. 2009;339: b3692.
29. Kalyani RR, Stein B, Valiyil R, Manno R, Maynard JW, Crews DC. Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. J Am Geriatr Soc. 2010;58:1299–310.
30. Annweiler C, Montero-Odasso M, Schott AM, Berrut G, Fantino B, Beauchet O. Fall prevention and vitamin D in the elderly: an overview of the key role of the non-bone effects. J Neuroeng Rehabil. 2010;7:50.
31. Endo I, Inoue D, Mitsui T, Umaki Y, Akaike M, Yoshizawa T, Kato S, Matsumoto T. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription. Endocrinology. 2003;144:5138–44.
32. Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stähelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res. 2004;19:265–9.
33. Wang Y, DeLuca HF. Is the vitamin D receptor found in muscle? Endocrinology. 2011;152:354–63.
34. Ceglia L, da Silva Morais M, Park LK, Morris E, Harris SS, Bischoff-Ferrari HA, Fielding RA, Dawson-Hughes B. Multi-step immunofluorescent analysis of vitamin D receptor loci and myosin heavy chain isoforms in human skeletal muscle. J Mol Histol. 2010;41:137–42.

Vitamin D Status in Relation to Veiling, Obesity, and Milk Intake in Saudi Women

20

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Abstract

Introduction: Vitamin D deficiency is a global health problem. Limited skin exposure to sunlight, obesity, and low dietary calcium intake may all impact vitamin D status and bone health. The aims of this study were to determine vitamin D status and its association with the extent of veiling and different measures of obesity and to examine the impact of milk intake on vitamin D status and bone metabolism markers in a sample of randomly selected pre- and postmenopausal healthy Saudi women.

Methods: A total of 449 women were studied. Fasting blood samples were collected for assessment of 25(OH)D status and carboxy-terminal telopeptide of type I collagen (CTX). Anthropometric parameters and total body fat (TBF) by dual-energy X-ray absorptiometry were measured. Milk intake was determined using a validated food frequency questionnaire.

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Results: A total of 85.5 % of women had vitamin D deficiency with a serum level <50 nmol/L. Women who were completely covered (both face and hands or face only) ($n=261$) were found to have a significantly lower 25(OH)D status than women who covered their heads, but not their faces and hands ($n=188$) [26.5 ± 19.6 nmol/L vs. 32.0 ± 24.4 nmol/L], respectively ($P<0.011$). A significant negative correlation between 25(OH)D and body mass index (BMI) ($r=-0.203$, $P<0.01$), TBF ($r=-0.340$, $P<0.01$), and waist circumference (WC) ($r=0.140$, $P<0.05$) was found in the postmenopausal women. A positive correlation was found between milk intake and 25(OH)D status, which remains significant after controlling for BMI and age ($r=0.193$, $P<0.001$). A trend for milk intake to be negatively associated with CTX excretion was also observed ($r=-.083$, $P\leq 0.07$) after adjustment for age and BMI.

Conclusion: Vitamin D deficiency is rather highly prevalent among healthy Saudi women. Further investigations are currently under way to explore concomitant effects of these factors on bone density in this population.

Keywords
Saudi women • Vitamin D • Veiling • Milk intake • Deficiency • Obesity

Introduction

Vitamin D deficiency is a global health problem, with a range of chronic conditions being associated with low circulating levels of 25-hydroxyvitamin D (25(OH)D) [1]. Vitamin D deficiency may lead to secondary hyperparathyroidism causing a generalized decrease in bone mineral density (BMD) and consequent increased risk of osteopenia and osteoporosis. Moreover, vitamin D insufficiency may increase postural imbalance due to increased body sway, muscle weakness, and diminished ability to respond to falls [2, 3], thus increasing the risk of fractures [4]. Serum 25(OH)D is considered to be the best indicator of vitamin D nutritional status [5, 6]. It has been suggested that serum 25(OH)D values <50 nmol/L indicate vitamin D deficiency [7] and <75 nmol/L indicate vitamin D insufficiency [5, 7, 8]. Vitamin D is synthesized in the skin after sunlight exposure or can be obtained through a balanced dietary intake [5]. However, because few foods provide a natural source of vitamin D, skin synthesis is thought to constitute the major source of vitamin D [9]. Clothing style is an important factor that influences vitamin D production [10]. Hence, a limited skin exposure to sunlight has been presumed to be one of the major causes of vitamin D deficiency. Obesity is another factor to be blamed for vitamin D deficiency [11]. This is likely due to the decreased bioavailability of vitamin D_3 from cutaneous and dietary sources because of its deposition in body fat compartments [12, 13]. Calcium and vitamin D are essential for bone growth and maintenance [14]. Among the bone-forming minerals, dietary calcium supply is close to biological requirements and may be limited in some parts of the world where there are few rich dietary sources of calcium. Milk is a rich source of calcium and, in countries where milk is fortified with vitamin D, also a contributor to dietary vitamin D intake [1]. However, in Saudi Arabia, the fortification of milk is not widespread enough to prevent vitamin D deficiency in the population [15].

Data on the effect of different lifestyle factors such as clothing styles, obesity, and poor dietary supplements on vitamin D status in Saudi women are limited. The aims of this study were (1) to determine vitamin D status in a sample of randomly selected pre- and postmenopausal healthy Saudi women, (2) to determine the effect of extent

of veiling on vitamin D status, (3) to determine the extent of obesity and the association between different measures of adiposity and 25(OH)D, and (4) to examine the impact of milk intake on vitamin D status and bone metabolism marker in these Saudi women.

Subjects and Methods

This cross-sectional study was conducted at the Center of Excellence for Osteoporosis Research (CEOR) in Jeddah city (western region of Saudi Arabia) during the year 2010. A representative sample size was calculated using the sample-size determination option in Epi Info statistical package (version 6; USD, West Park Place, Stone Mountain, GA, USA). Subjects were recruited from primary health care centers (PHCCs) scattered around the city to ensure that the average health status of the studied subjects will reflect a randomly selected adult population. After excluding women with diseases and medications known to affect bone metabolism, pregnant or lactating women, and those who declined due to personal reasons, only 449 women were eligible and agreed to participate in the present study. A total of 226 premenopausal women aging 20–39 years and 223 postmenopausal women aging 50–82 years were studied. All women included in the study exhibited normal values for renal creatinine (serum creatinine <105 umol/L), normal values for liver function tests (serum aspartate aminotransferase <30 U/L, alanine aminotransferase <30 U/L, and alkaline phosphatase between 80 and 280 U/L).

The classification of menstrual status was confirmed by measuring FSH [16]. Premenopausal women are those who had regular menses in the past 6 months and a serum follicular stimulating hormone (FSH) level <15 mIU/L. Postmenopausal women are those who had their last menstrual period at least 1 year before the date of recruitment and FSH level >15 mIU/L.

Each woman was medically examined and personally interviewed using a standardized questionnaire to collect information about age and dress style. Information about milk intake was determined using a validated food frequency questionnaire (FFQ) [17, 18]. Women were divided according to their dress style into three groups: niqab and gloves (all body parts are covered including face and hands), niqab only (all body parts are covered including face but hands exposed), and hijab (all body parts are covered except the face and hands). Body weight, height, body mass index (BMI; kilogram/square meter), and waist-to-hip ratio (WHR) were recorded. Overweight is defined as BMI ≥ 25 and obese as BMI ≥ 30 on the basis of international obesity classification [19]. Total body fat (TBF) in grams was measured using dual-energy X-ray absorptiometry (DXA) using Lunar Prodigy Model (Lunar Corp., Madison, WI, USA).

The study was approved by CEOR's Human Ethics Research Committee, and a written informed consent was obtained from all women who participated in the present study.

Specimen Collection

Fasting venous blood samples were taken in the morning between 8:30 and 11 am under standardized conditions. After being collected, the specimens were left to clot at room temperature and then underwent centrifugation for 10 min at 3,000 rpm within 1 h from collection time. The serum extracted was stored at a temperature of −80 °C until analyzed for the determination of 25(OH)D, intact PTH, CTX, and other biochemical analytes. All essays were carried out at the same point according to the manufacturer's instructions.

Measurements of 25(OH)D, Intact PTH, and CTX

Serum 25(OH)D and intact PTH were measured by direct competitive and a direct sandwich chemiluminescence immunoassays using LIASON autoanalyzer, respectively (DiaSorin Inc., Stillwater, MN, USA). The intra- and inter-assay coefficient variation (CV) were 7.8 and 3.8 % for serum 25(OH)D and 5.1 and 4.3 % for

serum-intact PTH, respectively. The bone resorption marker, C-terminal cross-linking telopeptides of type I collagen (CTX), was measured by a direct sandwich chemiluminescence immunoassay (ECLIA) using cobas e immunoassay analyzer. The intra- and inter-assay CV were 4 %.

Measurements of FSH and Other Analytes

Serum FSH was measured by commercially available immunoassays using VITROS-ECiQ autoanalyzer (Ortho-Clinical Diagnostic, Johnson & Johnson Co., USA). The intra- and inter-assay CVs were <4 %. Serum calcium (s-Ca), magnesium (s-Mg), and phosphate (s-PO_4) were measured by kits and reagents supplied by Ortho-Clinical Diagnostics, USA, using VITROS 250 Chemistry System autoanalyzer (Ortho-Clinical Diagnostic, Johnson & Johnson Co., USA).

Statistical Analysis

SPSS version 16.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Results are presented as means ± SD, and categorical variables are expressed as frequencies. The statistical differences among the two groups for different variables were analyzed by student *t*-test and among the three groups using ANOVA. Association between variables was examined by Pearson's correlation coefficient or by Spearman's correlation for variables that are not normally distributed. Partial correlation adjusting for confounding variables was also performed. Results were considered significant if $P<0.05$.

Results

Age and anthropometric characteristics of the study population according to menopausal status are presented in Table 20.1. A total of 30.7 % of the women were overweight (28.3 % pre, 33.2 % post), and 38.5 % were obese (21.2 % pre, 56.1 % post). Postmenopausal women exhibited increased BMI ($P<0.000$) and WHR ($P<0.000$) as compared to premenopausal women. Our analysis of the FFQ did not currently allow us to report food nutrients intake. However, the vitamin D supplement intake information is presented in Table 20.2. Of the women studied, 26.1 % had vitamin D supplementation (11.9 % pre, 40.4 % post).

Serum 25(OH)D and other biochemical values are presented in Table 20.3. The overall mean (±SD) serum 25(OH)D level was 22.63 ± 19.85 and 35.01 ± 22.08 nmol/L for pre- and postmenopausal women studied ($P<0.000$) with an overall mean of 28.78 ± 21.86 nmol/L. The distribution frequencies for various 25(OH)D cutoff levels are given in Table 20.4. A total of 80.5 % of women studied showed serum 25(OH)D levels <50 nmol/L. Only 3.8 % of all women were considered with adequate vitamin D status (serum 25(OH)D ≥ 75 nmol/L), 2.7 and 4.9 % being pre- and postmenopausal, respectively. About 55 % of all women exhibited severe vitamin D deficiency (serum 25(OH)D < 25 nmol/L), 70.8 and 39 % being pre- and postmenopausal women, respectively. Moreover, about 30.5 % of all women exhibited moderate vitamin D deficiency, 23 % premenopausal and 38.1 % postmenopausal (with serum 25(OH)D < 50 nmol/L). Furthermore, about 10.7 % of all women studied exhibited vitamin D insufficiency (3.5 % premenopausal and 17.9 % postmenopausal with serum 25(OH)D ≥50 to <75 nmol/L). Distribution of BMI in relation to different levels of vitamin D in pre- and postmenopausal women is presented in Figs. 20.1 and 20.2, respectively.

The mean serum 25(OH)D levels were significantly lower in women (pre- or postmenopausal) who were completely covered (both face and hands or face only) wearing a niqab than those who exposed their faces and hands wearing a hijab (serum 25(OH)D values 26.5 ± 19.6 vs. 32.0 ± 24.4 nmol/L, respectively) ($p<0.011$). A trend was also seen for a difference in 25(OH)D status between women covering their faces only ($n=247$) vs. women covering their faces and hands ($n=14$) ($p<0.086$) (Table 20.5).

There was no significant correlation between 25(OH) D and any of the obesity measurements in the premenopausal women, even after adjusting for

Table 20.1 Age and anthropometric characteristics of the studied women

	Menopausal status	
Variables	Premenopausal ($n=226$)	Postmenopausal ($n=223$)
Age (years)	29.1 ± 5.6	58.8 ± 5.9
Weight (kg)	65.1 ± 14.9	74.8 ± 14.0
Height (cm)	158.4 ± 5.5	154.1 ± 6.0
BMI (kg/m^2)	25.9 ± 5.4	31.5 ± 5.7
Waist circumference (cm)	74.9 ± 11.7	90.9 ± 11.8
WHR	0.766 ± 0.08	0.856 ± 0.08
Total body fat (g)	25.8 ± 8.4	31.7 ± 6.7

Data are presented as mean ± SD

Table 20.2 The use of vitamin D supplements in the women studied

		Menopausal status	
Vitamin D supplementation	All women ($n=449$)	Premenopausal ($n=226$)	Postmenopausal ($n=223$)
Yes	117 (26.1 %)	27 (11.9 %)	90 (40.4 %)
No	332 (73.9 %)	199 (88.1 %)	133 (59.6 %)

Values given in parenthesis are percentages out of total within the group

Table 20.3 25(OH)D, hormones, minerals, and BTM value of the studied women

	Menopausal status	
Variables	Premenopausal ($n=226$)	Postmenopausal ($n=223$)
Serum 25(OH)D (nmol/L)	22.63 ± 19.85	35.01 ± 22.08
Serum-intact PTH (pmol/L)	9.47 ± 3.98	8.86 ± 3.27
Serum FSH (IU/L)	5.21 ± 2.79	57.03 ± 26.10
Serum calcium (mmol/L)	2.49 ± 0.24	2.55 ± 0.21
Serum magnesium (mmol/L)	0.76 ± 0.06	0.78 ± 0.07
Serum phosphate (mmol/L)	1.27 ± 0.16	1.77 ± 6.47
Serum CTX (pg/ml)	335.3 ± 179	320 ± 188

Data are presented as mean ± SD

Table 20.4 Distribution of serum 25(OH)D values according to different cutoffs

		Menopausal status	
Serum 25(OH)D (nmol/L)	All women ($n=449$)	Premenopausal ($n=226$)	Postmenopausal ($n=223$)
<25	247 (55 %)	160 (70.8 %)	87 (39 %)
≥25 to <50	137 (30.5 %)	52 (23 %)	85 (38.1 %)
≥50 to <75	48 (10.7 %)	8 (3.5 %)	40 (17.9 %)
≥75	17 (3.8 %)	6 (2.7 %)	11 (4.9 %)

Values given in parenthesis are percentages out of total within the group

age. However, a significant negative correlation between 25(OH)D and BMI ($r=-0.203$, $P<0.01$), TBF ($r=-0.340$, $P<0.01$), and WC ($r=0.140$, $P<0.05$) was found in the postmenopausal women.

A positive correlation was found between milk intake and 25(OH)D levels, which remains significant after controlling for vitamin D supplement use, BMI, and age ($r=0.193$, $P<0.001$). A trend for milk intake to be negatively associated with CTX excretion was also observed ($r=-.083$, $P\leq0.07$) after adjustment for age and BMI.

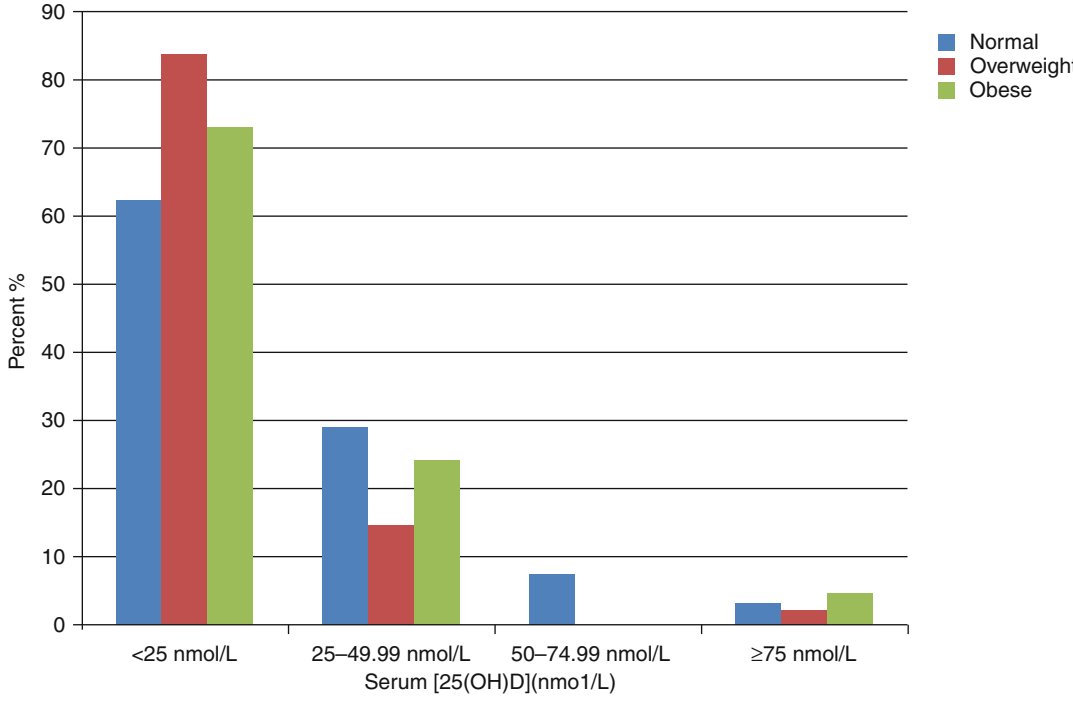

Fig. 20.1 Distribution of BMI in relation to different levels of vitamin D in the premenopausal women

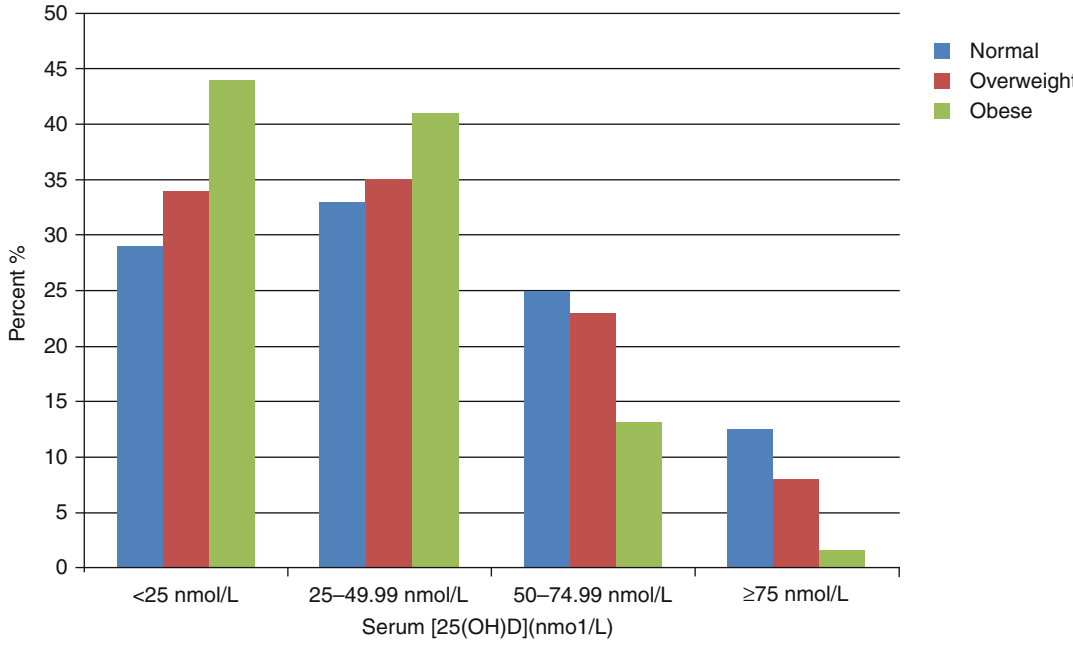

Fig. 20.2 Distribution of BMI in relation to different levels of vitamin D in the postmenopausal women

Table 20.5 Vitamin D levels in women wearing different cultural clothes style

	Subjects	25(OH)D (mean ± SD)
Niqab + gloves	14	19.2 ± 10.5
Niqab only	247	26.9 ± 19.9
Hijab	188	32.0 ± 24.4

Discussion

Despite abundant sunshine that should permit sufficient 25(OH)D synthesis all year round, this is not the case in healthy Saudi women included in this study. A high prevalence of vitamin D deficiency as defined by serum 25(OH)D levels <50 nmol/L was observed among otherwise healthy Saudi pre- (93.8 %) and postmenopausal (77.1 %) women, respectively, giving an overall deficiency rate of 85.5 %. The high prevalence of vitamin D deficiency among Arabian women was reported in previous studies [20–26] as well as in other populations [27–31]. In the present study, limited sun exposure, obesity, and poor dietary vitamin D supplementation were among the risk factors for vitamin D deficiency in healthy Saudi women. The serum level of 25(OH)D was lower in premenopausal as compared to postmenopausal women which is in agreement with previous studies [32, 33]; such differences could be attributed to vitamin D supplements' intake.

Recent evidence indicates that obesity is linked to lower serum levels of 25(OH)D [11, 12]; poor exposure to sunlight and/or decreased vitamin D bioavailability (due to the dilution of vitamin D in the high quantity of subcutaneous fat) was considered among the contributory factors to such observations in obese subjects [11, 13]. In the present study, the BMI data revealed a high prevalence of overweight and obesity in both pre- and postmenopausal women; indeed the lowest 25(OH)D was seen in the group of women with the highest BMI, confirming previous reports.

The cutaneous synthesis of vitamin D depends on several factors including age, season, latitude, time spent outdoors, skin pigmentation, skin coverage, and the use of sunscreens [1]. All Saudi women maintained a conservative clothing style while being outdoors allowing the exposure of parts of the face and/or hands to direct sunlight. In the present study, there was a significant difference in the serum levels of 25(OH)D among niqab- and hijab-dressed women. Although there is a small contribution of the exposed body areas (i.e., face and hands) to the extent of cutaneous synthesis of vitamin D, it may still explain the significant difference among these women. The results of the present study are in agreement with previous studies which showed that veiled Arabian women exhibited decreased serum 25(OH)D levels as compared to non-veiled women [21, 22, 34, 35] but in contrast with others [23, 26, 32]. Differences in the sun exposure index, which was not measured in this study, may explain the discrepancy between different studies.

Although the frequency of milk consumption tended to be low, it was positively related to 25(OH)D concentrations even after adjusting for age and BMI among women in this study. We were not able to fully adjust for vitamin D intake from the totality of their diet, and this is an area for further research. The results of the present study are in contrast to previous study which showed that calcium supplements do not alter the 25(OH)D response to vitamin D supplementation [36]. It is possible that these results show a "healthy cohort" effect. These results indicate an impact of dietary intake on vitamin D and bone health, which needs further investigation.

In conclusion, this study identifies population groups that are likely to have lower concentrations of vitamin D and factors associated with vitamin D status. The factors related to vitamin D insufficiency and/or deficiency are limited skin exposure to sun light, obesity, and poor dietary intake. Vitamin D supplements and fortification of food on a national basis with vitamin D will help to overcome such low levels as the styles of clothing and other lifestyle factors are not expected to be changed in the present time. Further investigations are currently under way to explore concomitant effects of these factors on bone density in this population.

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References

1. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87:1080S–6.
2. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA. 2005; 293:2257–64.
3. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ. 2009;339: b3692.
4. Garnero P, Munoz F, Sornay-Rendu E, Delmas PD. Associations of vitamin D status with bone mineral density, bone turnover, bone loss and fracture risk in healthy postmenopausal women. Bone. 2007;40:716–22.
5. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.
6. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80: 1689S–96.
7. Institute Of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press; 2010.
8. Hansen K, Jones AN, Lindstrom MJ, Davis LA, Engelke J, Shafer M. Vitamin D insufficiency: disease or no disease? J Bone Miner Res. 2008;23:1052–60.
9. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. CMAJ. 2002;166:1517–24.
10. Tsiaras WG, Weinstock MA. Factors influencing vitamin D status. Acta Derm Venereol. 2011;91:115–24.
11. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3.
12. Snijder MB, Van-Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. J Clin Endocrinol Metab. 2005;90:4119–23.
13. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin d status of obesity. Obesity (Silver Spring). 2012;20:1444–8.
14. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies; 2011.
15. Sedrani SH. Extraordinarily low vitamin D content of fortified milk, milk based infant formula and powder milk. J Clin Nutr. 2005;14:S94.
16. World Health Organ Scientific Group on Research on the Menopause. WHO technical report 670. Geneva: WHO; 1981.
17. Bodner CH, Souter A, New SA, Scaife AR, Byres M, Henderson GD, Brown K, Godden DJ. Validation of a food frequency questionnaire for use in a Scottish population: correlation of antioxidant vitamin intakes with biochemical measures. J Human Nutr Dietetics. 1998;11:373–80.
18. New SA, Grubb DA, Bolton-Smith C, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in pre-menopausal women. Am J Clin Nutr. 1997;65:1831–9.
19. WHO. Physical status. The use and interpretation of anthropometry. World Health Organ Tech Rep Ser. 1995;854:1–452.
20. Ghannam NH, Hammami MM, Bakheet SM, Khan BA. Bone mineral density of the spine and femur in healthy Saudi females: relation to vitamin D status, pregnancy and lactation. Calcifi Tissue Int. 1999;65: 23–8.
21. El-Hajj Fuleihan G, Deeb M. Hypovitaminosis D in sunny country. N Engl J Med. 1999;340:1840–1.
22. Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res. 2000; 15:1856–62.
23. Mishal A. Effects of different dress styles on vitamin D levels in healthy young Jordanian women. Osteoporos Int. 2001;12:931–5.
24. Meddeb N, Sahli H, Chahed M, Abdelmoula J, Feki M, Hadj S, et al. Vitamin D deficiency in Tunisia. Osteoporos Int. 2005;16:180–3.
25. Arabi A, Baddoura R, Awada H, Salamon M, Ayoub G, El-Hajj Fuleihan G. Hypovitaminosis D osteopathy: is it mediated through PTH, lean mass, or is it a direct effect? Bone. 2006;39:268–75.
26. Ardawi M, Qari M, Rouzi A, Maimani A, Raddadi R. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre-and postmenopausal women. Osteoporos Int. 2011;22:463–75.
27. Kauppinen-Makelin R, Tahtela R, Loyttyniemi E, Karkkainen J, Valimaki MJ. A high prevalence of hypovitaminosis D in Finnish medical in-and outpatients. J Intern Med. 2001;249:559–63.
28. Isaia G, Giorgino R, Rinin GB, Bevilacqua M, Maugeri D, Adami S. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. Osteoporos Int. 2003;14:577–82.
29. Bhattoa HP, Bettembuk P, Ganacharya S, Balogh A. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women. Osteoporos Int. 2004;15:447–51.
30. Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Khatzer JT, et al. Prevalence of vitamin D inadequacy

among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab. 2005;90:3215–24.
31. Macdonald HM, Mavroeidi A, Barr RJ, Black AJ, Fraser WD, Reid DM. Vitamin d status in postmenopausal women living at higher latitudes in the UK in relation to bone health, overweight, sunlight exposure and dietary vitamin D. Bone. 2008;42:996–1003.
32. Saadi HF, Nagelkerke N, Benedict S, Qazaq HS, Zilahi E, Mohamadiyeh MK, Al-Suhaili AI. Predictors relationships of serum 25 hydroxyvitamin D concentration with bone turnover markers, bone mineral density and vitamin D receptor genotype in Emirati women. Bone. 2006;39:1136–43.
33. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living adults. Am J Med. 2002;112:659–62.
34. Mallah EM, et al. Plasma concentrations of 25-hydroxyvitamin D among Jordanians: Effect of biological and habitual factors on vitamin D status. BMC Clin Pathol. 2011;11:8.
35. El-Sonbaty MR, Abdul-Ghaffar NU. Vitamin D deficiency in veiled Kuwaiti women. Eur J Clin Nutr. 1996;50:315–8.
36. Goussous R, Song L, Dallal GE, Dawson-Hughes B. Lack of effect of calcium intake on the 25-hydroxyvitamin D response to oral vitamin D3. J Clin Endocrinol Metab. 2005;90(2):707–11.

Serum 25(OH)D and Calcium Intake Predict Changes in Hip BMD and Structure in Young Active Men

21

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Abstract

Peak bone mass (PBM) is mostly determined by genetics, but lifestyle and diet during youth may modify the final PBM. We examined changes in spine and hip bone mineral density (BMD) in a cohort of 146 males entering the US Military Academy (average age = 18.8 ± 1.1), randomly sampled from the full sample (755 males). Calcium intake was determined by a brief food frequency questionnaire. Serum 25(OH)D was measured by DiaSorin RIA, and intact (1–84) PTH was measured using the Elecsys (Abbott) on a single serum sample. BMD at the lumbar spine and total hip were measured at baseline and annually for 4 years by DXA (Lunar DPX-IQ). Hip structural analysis (HSA) was done using the methodology of Yoshikawa (JBMR 1994). Slopes of change in spine and hip BMD and HSA parameters were determined for each male. Average calcium intake

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was 1,803 mg/day (range 387–6,258). There was no significant use of calcium or vitamin D supplements. Men with calcium intake <800 mg lost 1 % of total hip BMD per year, whereas those with calcium intakes >800 mg a day gained 0.23 % BMD per year ($p<0.007$), even after controlling for race and serum 25(OH)D. There was no relationship with change in spine BMD and calcium intake. Males with serum 25(OH)D level >20 ng/ml had a greater increase in hip BMD (0.2 % gain in hip BMD/year) as compared to a 0.3 % hip BMD loss per year in the 25(OH)D<20 ng/ml group. Serum vitamin D was also correlated with hip cross-sectional area. Both relationships with 25(OH)D persisted after controlling for race and BMI ($p<0.03$). However, there was no relationship between serum 25(OH)D and change in spine BMD. We conclude that in physically active college-aged men, dietary calcium intake and vitamin D status may modify peak bone mass acquisition.

Keywords
Male • Peak bone mass • Calcium • Vitamin D • College-age

Introduction

Peak bone mass may be maximized during growth and perhaps also in the early adult years through factors, such as normal endocrine function, adequate dietary intake (particularly calcium), and adequate exercise, that can modify the genetic potential that an individual may achieve [1]. However, little is known about peak bone mass acquisition in college-aged, physically active men. Numerous dietary factors have been hypothesized to relate to accrual of peak bone mass, but the strongest evidence relates to calcium and vitamin D. It has been previously reported that in younger, adolescent boys (ages 12–14), 21.7 % of the variation in calcium retention was explained by dietary calcium intake [2]. A calcium supplementation trial in young men (mean age 17) led to increases in BMD [3]. In young prepubertal boys, serum vitamin D was not a significant predictor of calcium retention [2]. However, vitamin D has been reported to increase bone density in young girls [4–6] and males [7, 8]. Therefore, the objective of this analysis was to identify whether dietary calcium and serum vitamin D predict change in BMD in young active college-age males.

Methods

The population consisted of males entering the US Military Academy who consented to participate in a study of the predictors of stress fracture and longitudinal changes in bone mineral density (755 males or 70 % of male admissions). This study was approved by the Institutional Review Board of Keller Army Hospital. Of these 755, 146 males were randomly selected to have spine and hip bone mineral density testing and serve as the sample for this analysis.

Calcium intake was assessed using a brief food frequency questionnaire with 12 questions. These assessed intake of milk, cheese, tofu, ice cream, cheese dishes, and green leafy vegetables. The males were also asked about the use of calcium supplements, but only three reported using calcium supplements.

A single blood sample was taken in July for each male. Aliquots of serum were immediately frozen. In each serum sample, 25(OH)D level was measured by DiaSorin RIA, and intact (1–84) PTH, osteocalcin, C-telopeptide, and N-telopeptide were measured using the Elecsys (Abbott).

Lunar DPX-IQ (General Electric-Lunar, Madison, WI) was used to measure spine and hip

BMD annually for 4 years. The coefficient of variation (%CV) in vivo was 1.5 and 1.5 %, respectively, for spine and left total hip in this population. Hip structural analysis (HSA) to determine cross-sectional area (CSA) and minimum cross-sectional moment of inertia (CSMI) was done using the methodology of [9].

Slopes of change in spine and hip BMD and HSA parameters were determined for each male with general linear models. Annual rates of change in BMD and HSA parameters were estimated from the linear models. Unadjusted differences in annual rates of change between high and low calcium intake and 25(OH)D groups were assessed with *t*-tests. Differences adjusted for race, BMI, age, and/or 25(OH)D were assessed with analysis of covariance.

Results

Characteristics of the males are found in Table 21.1. Average age at entry was 18.8 ± 1.1, mean body mass index was approximately 24 ± 2.8, and the mean bone density of the spine and hip was approximately one standard deviation above the normal population (*z*-score 0.7–1.0) for age-matched males.

Mean calcium intake in these males was 1,803 mg/day with a range of 387–6,285. The majority of calcium consumed (over 97 %) was dairy calcium. We used a cut point of 800 mg a day of dietary calcium (determined from our brief food frequency questionnaire) assuming that the remaining 200 mg required would come from trace amounts in other sources. None of these men reported vitamin D supplementation, so the serum 25(OH)D levels were from diet and sunlight exposure.

The biochemical values for serum, taken during the first week of admission (July) into the academy, are shown in Table 21.2. The mean serum 25(OH)-vitamin D value for these males was 28.3 ± 8.8 ng/ml in the normal range. However, the range of 25(OH)D values was from 12 to 54, indicating that many of these young men had serum 25(OH)D levels that fell below what is considered adequate (20 ng/ml). In fact, approximately 16 % of these young men had 25(OH)D levels below 20 ng/ml. Males with serum 25(OH)D < 20 ng/ml had significantly higher PTH levels (41±5 pg/ml) versus those with serum 25OHD ≥ 20 ng/ml (33±1.4 pg/ml) [$p < 0.04$]. In those with 25OHD < 20 ng/ml, 21 % [$n = 4$ of 19] had PTH values > 65 pg/ml. In men with 25(OH)D ≥ 20 ng/ml, only 4 % [$n = 5$ of 124] had PTH levels above the normal range.

Serum C-telopeptide and N-telopeptide levels were significantly greater in those with serum 25(OH)D levels that exceeded 20 ng/ml as compared to those with 25(OH)D below 20 ng/ml. However, there was no difference in osteocalcin between these categories of 25(OH)D.

There was no association between calcium intake and either PTH or bone turnover markers.

Table 21.1 Baseline characteristics ($n = 146$)

	Mean	Standard deviation	Range
Age (years)	18.8	1.1	17.5–22.7
Height (in)	69.1	2.4	63–75
Weight (lb)	162.6	21.9	119–243
BMI	23.9	2.8	18–34
Race *n*, (%)	–	–	–
Caucasian	$n = 126$	(86 %)	–
Black	$n = 7$	(5 %)	–
Asian	$n = 13$	(9 %)	–
Spine BMD (g/cm^2)	1.3	0.1	0.977–1.648
Spine *z*-score	1.0	1.4	–
Total hip BMD (g/cm^2)	1.2	0.2	0.921–1.659
Total hip *z*-score	0.7	1.4	–

Table 21.2 Serum biochemistry ($n=146$)

	Mean	Standard deviation	Range	Normal range
25(OH)-Vitamin D (ng/mL)	28.3	8.8	12.0–54.0	13–54
Parathyroid hormone (pg/mL)	34.2	16.6	4.0–94.0	10–64
NTX (mM BCE)	18.9	7.7	7.0–47.1	5.4–24.2
CTX (pg/mL)	680.9	319.7	177.1–1748.0	16–584
Osteocalcin (ng/mL)	9.6	2.0	4.4–16.2	3.4–11.7

The annual assessment of bone density over the 4 years in the entire group indicated that there was no significant increase or decrease in either spine or total hip BMD. There was an indication of a modest decline in femoral neck over the 4 years ($p<0.01$). We then determined whether there were changes in bone density accrual or loss when evaluated by baseline 25(OH)D value or calcium intake.

Men with calcium intake <800 mg lost 1 % of total hip BMD per year, whereas those with calcium intakes ≥800 mg a day gained 0.23 % BMD per year ($p<0.007$), even after controlling for race and serum 25(OH)D. There was no relationship with change in spine BMD and calcium intake.

There was no relationship between serum 25(OH)D and change in spine BMD. Males with serum 25(OH)D level ≥ 20 ng/ml had a significantly greater increase in total hip BMD (0.2 % gain in hip BMD/year) as compared to a 0.3 % total hip BMD loss per year in the 25(OH)D < 20 ng/ml group. Changes in femoral neck BMD differed more based on 25(OH)D level. Males with serum 25(OH)D level ≥ 20 ng/ml had a significantly smaller loss of femoral neck BMD (0.22 ± 0.31 % loss in femoral neck BMD/year) as compared to a loss of 1.24 ± 0.45 % at the femoral neck per year in the 25(OH)D < 20 ng/ml group ($p<0.04$ after controlling for race and BMI). However, femoral neck bone mineral content was increased in both groups with a greater increase in the young men with 25(OH)D > 20 ng/ml ($p<0.02$ after controlling for race and BMI).

In males with 25(OH)D < 20 ng/ml, there was no change in femoral neck area, but in males with 25(OH)D ≥ 20 ng/ml, area increased ($p=0.05$ after controlling for race and BMI). Similar results were found for hip cross-sectional area ($p<0.05$, after controlling for race and BMI).

Discussion

It is possible that at age 22, peak bone mass has not been achieved in physically active males, and in fact, there was still a significant gain in the height of these males. Higher intakes of calcium (>800 mg), predominately as dairy calcium, were associated with modest increases in total hip BMD. In males with serum 25(OH)D levels above or below 20 ng/ml, (1) PTH was higher in those with low 25(OH)D, although values were still well within the normal range; (2) higher PTH was not associated with higher bone turnover; and (3) bone turnover was positively associated with 25(OH)D level. It is unclear whether bone acquisition or the high level of exercise prior to the sample collection is influencing bone turnover. Of importance, both total hip and femoral neck BMD and BMC were also positively influenced by vitamin D ≥ 20 ng/ml in physically active males.

Clearly, physical activity is important for the accrual of peak bone mass [10]. In this study, all the young men were getting physical activity through required marches and other activities. Furthermore, all of the young men were either in a varsity sport or an intramural sport. Therefore, rather than assess physical activity, we are clarifying that these results are specific to physically active males.

This study had several limitations, primarily that there was a small sample size. As with all cohort studies, there is a possibility of uncontrolled

or unmeasured confounding. This is particularly true given the limited time the academy gave us for data collection. There may have been measurement error in the recall of dietary calcium intake, and we could not ascertain dietary calcium intake in detail. Hip structural analysis is based on a derived calculated technique; therefore, edge detection problems could create error. Although all samples were collected in the summer season for vitamin D, the sample was collected in the afternoon without control for prior exercise. This may impact bone turnover markers.

In conclusion, in highly physically active college-aged men, dietary calcium intake and vitamin D status may modify peak bone mass acquisition over 4 years.

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References

1. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C. Peak bone mass. Osteoporos Int. 2000;11:985–1009.
2. Hill KM, Braun M, Kern M, Martin BR, Navalta JW, Sedlock DA, McCabe L, McCabe GP, Peacock M, Weaver CM. Predictors of calcium retention in adolescent boys. J Clin Endocrinol Metab. 2008;93:4743–8.
3. Prentice A, Ginty F, Stear SJ, Jones SC, Laskey MA, Cole TJ. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. J Clin Endocrinol Metab. 2005;90:3153–61.
4. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr. 2002;76:1446–53.
5. Moyer-Mileur LJ, Xie B, Ball SD, Pratt T. Bone mass and density response to a 12-month trial of calcium and vitamin D supplement in preadolescent girls. J Musculoskelet Neuronal Interact. 2003;3:63–70.
6. Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Molgaard C, Lamberg-Allardt C. A positive dose–response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. J Bone Miner Res. 2006;21:836–44.
7. Guillemant J, Le HT, Maria A, Allemandou A, Peres G, Guillemant S. Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D3 supplements. Osteoporos Int. 2001;12:875–9.
8. Barnes MS, Robson PJ, Bonham MP, Strain JJ, Wallace JM. Effect of vitamin D supplementation on vitamin D status and bone turnover markers in young adults. Eur J Clin Nutr. 2006;60:727–33.
9. Yoshikawa T, Turner CH, Peacock M, Slemenda CW, Weaver CM, Teegarden D, Markwardt P, Burr DB. Geometric structure of the femoral neck measured using dual-energy x-ray absorptiometry. J Bone Miner Res. 1994;9:1053–64.
10. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010;46:294–305.

22

The Comparative Effects of Vitamin D_2 Versus Vitamin D_3 Supplementation in Improving Serum 25(OH)D Status: A Review of the Evidence

Laura Tripkovic and Susan A. Lanham-New

Abstract

At present, there appears to be a degree of controversy regarding the comparative effectiveness of vitamin D_2 and D_3 in raising and maintaining serum 25-hydroxyvitamin D (25(OH)D) levels. It was previously believed that the two vitamers were comparable in metabolic function; however, it is now known that when comparing vitamin D_2 to D_3, there are a small number of structural differences in the molecular makeup of these vitamers. Thus, the area of "vitamin D_2 versus vitamin D_3" research is expanding and exploring the possible mechanistic pathways that may highlight a clear and quantifiable difference between vitamin D_2 and D_3 that could have far-reaching consequences for both future vitamin D research and public health policy alike.

With this in mind, a recent meta-analysis of the current research available has shown that while the majority of data appear to support the conclusion that vitamin D_3 appears to be more efficacious than D_2, this does not always translate across all studies. Therefore, this review explores the studies involved, the possible mechanism behind the reported differences between vitamin D_2 and D_3, and the need for future research.

Keywords

Vitamin D • Vitamin D_2 • Vitamin D_3 • Ergocalciferol • Cholecalciferol • 25-Hydroxyvitamin D • Meta-analysis • Randomized controlled trials

Abbreviations

$1,24,25(OH)_3D$	1,24,25-Trihydroxyvitamin D
1,25(OH)2D2	1,25-Dihydroxyvitamin D (calcitriol)
25(OH)D	25-Hydroxyvitamin D
AUC	Area under curve
PTH	Parathyroid hormone
VDR	Vitamin D receptor

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Introduction

Previously it has been assumed that the two forms of vitamin D available to humans – vitamin D_2 and vitamin D_3 – are equally efficacious in maintaining a healthy vitamin D status. However, it is known that although these two vitamers are very similar in chemical composition, there are a small number of distinct differences between them: vitamin D_2 has a side chain with an additional double bond at carbon 23 and a methyl group at carbon 24 [1]. It is this difference that adds weight to the question of whether vitamin D_2 and D_3 can still be considered equal in function.

Thus, evidence has been accumulating for nearly 30 years, investigating the direct comparison between the effects of vitamin D_2 versus D_3 on raising the marker of vitamin D status, 25-hydroxyvitamin D (25(OH)D). Given the evidence now available, an in-depth qualitative and quantitative analysis of the data is warranted in order to identify whether there is a tangible difference between the effectiveness of vitamin D_2 and D_3 and how this may impact on future vitamin D research strategy and public health policy.

Background: Vitamin D Metabolism

Vitamin D is an intriguing nutrient due to the availability of two distinct vitamers that both provide physiologically effective sources. Vitamin D_2 (ergocalciferol) is found in a moderate range of plants and fungi that contain ergosterol – a steroid sensitive to UV irradiation at wavelengths of 240–300 nm [2], which then forms ergocalciferol [3]. Similarly, vitamin D_3 (cholecalciferol) is also synthesized via UV irradiation. The presence of 7-dehydrocholesterol in the skin of animals allows a conversion to previtamin D_3 at UVB wavelengths of 290–320 nm, with a further thermal isomerization step then taking place to form the cholecalciferol [3, 4].

Due to the variety of possible sources of vitamin D to humans, a combination of both vitamin D_2 and D_3 is usually achieved within a typical lifestyle of outdoor activity (ambient UV exposure, vitamin D_3), a diverse diet including the consumption of vitamin D_3-rich foods such as oily fish and egg yolks in addition to fortified foods (i.e., margarine and breakfast cereals, usually vitamin D_2 fortification) and vitamin supplements (vitamin D_2 and D_3 both available) [4].

Vitamin D_2 and D_3 are both pro-hormones and thus have no biological effect systemically [4]. In order to convert vitamin D_2 and D_3 into active compounds, a two-step enzymatic hydroxylation process is required by the body. Vitamin D_2 and D_3 are transported to the liver (first site of hydroxylation) via the vitamin D binding protein (DBP). Here, vitamin D_2 and D_3 are converted to 25-hydroxyvitamin D (25(OH)D) via the action of the following 25-hydroxylases: microsomal CYP2R1 and mitochondrial CYP27A1[4]. The kidney is then the second site of activity where 1α-hydroxylase (CYP27B1) converts 25(OH)D to $1,25(OH)_2D_2$ or D_3 (calcitriol) [3, 4].

The process of the two-step enzymatic hydroxylation is controlled, in part, via circulating parathyroid hormone (PTH) levels [3], which play a crucial role in exerting homeostatic control on the complex feedback loop required to maintain the essential vitamin D activation and metabolism.

With the active form of vitamin D (calcitriol) now available, the main systemic effects center around the maintenance of serum calcium and phosphate levels which is controlled via the intestinal absorption of calcium, renal resorption of phosphate, and the release of calcium from the skeleton [3].

Yet despite this hydroxylation process being apparently identical for both vitamin D_2 and D_3, data are available to show that there may be a difference in their respective efficacies in raising serum 25(OH)D levels [5–8], with vitamin D_3 appearing to be the more effective of the two vitamers. Within the current literature, the data suggest that a number of factors may be influencing this possible difference in efficacy: vitamin D_3 may have a greater affinity for the vitamin D receptor than vitamin D_2 [9], and vitamin D_3 may be the preferred substrate for hepatic 25-hydroxylase [10] which, in turn, could lead to a greater rate of conversion of vitamin D_3 to 25(OH)D when compared to vitamin D_2.

In addition, once the two-step 25-hydroxylation process has been completed and $1,25(OH)_2D$ has been formed, a deactivation step can occur which involves 24-hydroxylation at the kidney to form $1,24,25(OH)_3D$ [9]. The deactivation of the vitamin D metabolites could hold the key to demonstrating the differentiation between vitamin D_2 and D_3 due to the fact that $1,24,25(OH)_3D_2$ has been formed (the metabolite of vitamin D_2) and it has now been deactivated and thus is now no longer available to the body [11]. However, for vitamin D_3, now $1,24,25(OH)_3D_3$, a degree of biological activity is maintained despite the 24-hydroxylation step, and it can retain its capacity to bind to the VDR to a certain degree [11]. A further side-chain oxidation is required to complete the deactivation step [11]. As noted by Horst et al. (1986), this process of 24-hydroxylation is the step that can clearly distinguish between vitamin D_2 and D_3 and their respective capacity for a biological effect [11]. Thus, it could be concluded that by vitamin D_3 requiring a further step to deactivate, it clearly retains a distinct biological advantage over vitamin D_2 which only adds weight to the hypothesis that vitamin D_3 is the preferred substrate [12]. The main issue at this moment in time is consolidating the evidence available to see if this metabolic calculation translates to meaningful results in human studies.

Meta-analysis

Included Studies

From a search of the current literature (up to November 2011), ten studies were of appropriate study design to directly compare the effects of vitamin D_2 versus vitamin D_3 on serum 25(OH)D levels in humans and so were selected for the meta-analysis [12]. As found in Tables 22.1 and 22.2, studies were conducted in a diverse manner – seven out of the ten studies completed their investigations in free-living, healthy adults [5, 7, 8, 13–16]. The remaining three studies involved participants who were either institutionalized within a residential nursing home [6], were hospital orthopedic inpatients [17], or alternatively were outpatients treated within a rheumatology clinic [18]. Only three studies chose to conduct a double-blind, placebo-controlled study [5, 7, 8] – the gold standard in nutrition research. Even though all studies were randomized, the remaining seven studies chose either to not compare against a control/placebo group or had only a single-blind or unblinded approach [6, 13–18].

Dosage and intervention time periods were another area for diversity within the collective of studies. Three studies gave a single bolus ranging from 50,000 to 300,000 IU with periods of monitoring being from as short as 28 days up to 24 weeks [6, 14, 18]. Six studies gave daily dosages of 1,000–4,000 IU with monitoring of between 2 weeks and 1 year [5, 7, 8, 13, 15, 17], and one study gave a weekly dose of 50,000 IU for 12 weeks [16].

Results

As indicated in Table 22.1, eight studies found vitamin D_3 significantly more effective at raising serum 25(OH)D when compared to vitamin D_2, as opposed to two studies who found no difference (Table 22.2). Only seven studies were eligible for the meta-analysis due to incomplete or unavailable data for three studies [13, 14, 18]. A random effects model showed a weighted mean difference (WMD) of 15.23 nmol/l (95 % CI: 6.12, 24.34; $Z=3.28$; $P=0.001$) for the overall comparison of efficacy between vitamin D_2 and D_3 in raising serum 25(OH)D levels. This result clearly shows that vitamin D_3 is significantly more effective at raising serum 25(OH)D levels than vitamin D_2 [12].

However, when a further meta-analysis was completed focusing on dosage frequency, the studies looking at the effects of bolus dosing showed that vitamin D_3 was significantly more effective than vitamin D_2 in raising serum 25(OH)D levels (WMD 34.10 nmol/l; 95 % CI:16.38, 51.83; $Z=3.77$; $P=0.0002$) [12]. Yet when looking specifically at daily dosing of vitamin D_2 and D_3, a meta-analysis found that the significant effect of vitamin D_3 on raising serum 25(OH)D

Table 22.1 Characteristics and outcomes of studies that determined vitamin D_3 more efficacious than vitamin D_2 [11]

Study and country	Intervention, dose, and frequency	*N*	Gender/age	Follow-up	Results
Armas et al. [14] USA	1. No supplement (seasonal effect acting as control) 2. 1× tablet of 50,000 IU (1.25 mg) of vitamin D_2 3. 10× tablets of 5,000 IU (125mcg) of vitamin D_3	30	All male 20–61 year	28 days	28-day AUC significantly greater for vitamin D_3 supplementation group than vitamin D_2 ($p<0.002$)
Binkley et al. [15] USA	1. 1,600 IU of vitamin D_2 daily 2. 1,600 IU of vitamin D_3 daily 3. 50,000 IU of vitamin D_2 monthly 4. 50,000 IU of vitamin D_3 monthly	64	43 F 21 M 65 year+	12 months	Vitamin D_3 found to be significantly more effective than vitamin D_2 at raising serum 25(OH)D levels for the daily dosage ($p=0.05$) and for daily/monthly dosage groups combined ($p=0.01$). NS for monthly dosage group
Glendenning et al. [17] Australia	1. Ergocalciferol 1,000 IU/day 2. Cholecalciferol 1,000 IU/day	70	Gender unknown 82–84 year	3 months	Vitamin D_3 supplementation associated with a 31 % greater increase in levels of serum 25(OH)D than vitamin D_2 supplementation ($p=0.01$)
Heaney et al. [16] USA	1. 50,000 IU vitamin D_2 capsule 2. 5×10,000 IU vitamin D_3 capsule	33	30 F 3 M	12 weeks	12-week AUC significantly greater for vitamin D_3 supplementation group than vitamin D_2 ($p<0.001$). Vitamin D_3 calculated as 87 % more potent at raising 25(OH)D
Leventis and Kiely [18] UK	Study 1: single i.m. injection of 300,000 IU D_2 Study 2: single 100 ml oral dose of 300,000 IU D_3	69	S1: 43 F/7 M S2: 15 F/4 M 23–82 year	24 weeks	Greater increases in serum 25(OH)D achieved with vitamin D_3 intervention
Romagnoli et al. [6] Italy	1. single oral dose of 300,000 IU vitamin D_3 2. single i.m. dose of 300,000 IU vitamin D_3 3. single oral dose of 300,000 IU vitamin D_2 4. single i.m. dose of 300,000 IU vitamin D_2	32	All female 66–97 year	60 days	Vitamin D_3 significantly more potent at raising serum 25(OH)D levels compared to vitamin D_2 for both oral and i.m. administration
Tjellesen et al. [13] Denmark	1. 4,000 IU vitamin D_3 2. 4,000 IU vitamin D_2	19	All female 22–49 year	8 weeks	Vitamin D_3 linked to greater increase in serum 25(OH)D compared to vitamin D_2 intervention group. No direct statistical comparison results available.
Trang et al. [5] Canada	1. Untreated 2. 4,000 IU vitamin D_2 3. 4,000 IU vitamin D_3	89	48 F 23 M 18 U	14 days	Greater increase in serum 25(OH)D levels with vitamin D_3 group compared to the vitamin D_2 group ($p=0.03$)

Reprinted from Tripkovic et al. [12]. With permission from The American Journal of Clinical Nutrition
Key: *AUC* area under curve, *IU* international unit, *vitamin* D_2 ergocalciferol, *vitamin* D_3 cholecalciferol

Table 22.2 Characteristics and outcomes of studies that determined vitamin D_2 and D_3 equally efficacious [11]

Study and country	Intervention, dose, and frequency	*N*	Gender/age	Follow-up	Results
Biancuzzo et al. [8] USA	1. Placebo capsule + placebo orange juice 2. Placebo capsule + 1,000 IU vitamin D_3 OJ 3. Placebo capsule + 1,000 IU vitamin D_2 OJ 4. 1,000 IU vitamin D_3 capsule + placebo OJ 5. 1,000 IU vitamin D_2 capsule + placebo OJ	86	59 F 27 M 18–79 year	11 weeks	No significant difference found in AUC for 25(OH) D when comparing vitamin D_2 to vitamin D_3, irrespective of intervention vehicle (capsule or juice)
Holick et al. [7] USA	1. Placebo 2. 1,000 IU vitamin D_2 capsule 3. 1,000 IU vitamin D_3 capsule 4. 500 IU D_2 + 500 IU D_3 capsule	68	47 F 21 M 18–84 year	11 weeks	At end of intervention, no significant difference in 25(OH)D levels between D_2 and D_3 groups

Reprinted from Tripkovic et al. [12]. With permission from The American Journal of Clinical Nutrition
Key: *AUC* area under curve, *i.m.* intramuscular, *IU* international unit, *vitamin* D_2 ergocalciferol, *vitamin* D_3 cholecalciferol

levels when compared to vitamin D_2, as seen previously, is now lost. However, a statistical trend towards vitamin D_3 being the more efficacious vitamer in raising serum 25(OH)D levels compared to vitamin D_2 is still apparent (WMD 4.83 nmol/l; 95 % CI: −0.98, 10.64; $Z = 1.63$; $P = 0.10$) [12].

Discussion and Further Study

Currently the data available suggest that overall vitamin D_3 is more effective at improving and sustaining vitamin D levels than vitamin D_2. However, to date, studies involving vitamin D_2 versus D_3 comparisons have been too few, limited in participant numbers and have not attempted to elucidate the mechanism behind any perceived differences to any great extent. However, a few studies of note that have provided some mechanistic data on the fate of vitamin D metabolism include the study by Armas et al. (2004) and Heaney et al. (2011). Over a time course of 28 days and 12 weeks, respectively, the data retrieved from the studies indicated that cholecalciferol induced a quicker response in the production of serum 25(OH)D which was also sustained for longer and at higher levels than ergocalciferol [14, 16].

Heaney et al. showed that with weekly doses of 50,000 IU of cholecalciferol, area under curve (AUC) values were significantly higher than they were for the ergocalciferol intervention [16]. In addition, when the intervention period ceased, serum 25(OH)D_2 (ergocalciferol) levels appeared to degrade quicker when compared to the levels of serum 25(OH)D_3 (cholecalciferol) over a 6-week time period [16]. Similarly, Armas et al. showed that a single bolus dose of 50,000 IU induced a significantly greater AUC for cholecalciferol than ergocalciferol, with serum 25(OH)D_2 levels falling rapidly back to baseline after only 14 days [14]. In contrast, serum 25(OH)D_3 levels were peaking at the same time point but had not returned to baseline at the end of the 28-day intervention [14].

Despite the obvious comparisons between the studies completed by Heaney et al. and Armas et al., the diverse approach in study design across the included studies makes comparisons difficult and can lead to high levels of heterogeneity. This effect on heterogeneity, in turn, brings in an

element of unreliability to the results. For example, the bolus studies selected for this review were not only varied in vitamin D dosage from 50,000 IU to 300,000 IU but also in route of administration (i.e., oral versus intramuscular injection). Time of follow-up, frequency of blood sampling, and detailed monitoring all impact on the degree of detail obtained from the studies and whether meaningful results are obtained; therefore, comparable approaches would undoubtedly increase the impact of the results.

This combination of factors results in a large degree of limitation when attempting to extrapolate the results towards meaningful interpretation. Therefore far larger, more robust trials are now required which not only focus on comparing the effects of vitamin D_2 and D_3 on 25(OH)D levels, but they must also tackle the mechanistic pathway behind vitamin D metabolism in order to elucidate the true efficacy of vitamin D_2 and D_3, respectively.

Conclusion

The results of this review show that overall, vitamin D_3 is more effective at raising serum 25(OH)D levels than vitamin D_2. However, further conclusions can only be drawn tentatively due to the relatively small amount of data currently available for analysis. Further study should be encouraged that focuses on the mechanistic pathways involving vitamin D metabolism. The two-step hydroxylation activation pathway, in addition to the 24-hydroxylation deactivation process, provides plentiful opportunities for further study. Encompassing whole-body responses to the two vitamers in addition to responses at the cellular level, a plethora of data on enzymatic activity and the ensuing production of vitamin D metabolites would surely help build a true picture of vitamin D metabolism and whether the perceived differences between vitamin D_2 and D_3 are, in fact, quantifiable and meaningful.

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References

1. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81.
2. Naewbanij M, Seib PA, Burroughs R, Seitz LM, Chung DS. Determination of ergosterol using thin-layer chromatography and ultraviolet spectroscopy. Cereal Chem. 1984;61(5):385–8.
3. Calman K. ed. Nutrition and bone health with particular reference to calcium and vitamin D: Report of the Subgroup on Bone Health (Working Group on the Nutritional Status of the Population) of the Committee on Medical Aspects of Food and Nutrition Policy. London: The Stationary Office; 1998.
4. Ross CA, Taylor CL, Yaktime AL, Del Valle HB. ed. Dietary reference intakes for calcium and vitamin D. Committee to review dietary reference intakes for vitamin D and calcium; Institute of Medicine. Washington, DC: National Academies Press; 2010.
5. Trang HM, Cole DEC, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D-3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D-2. Am J Clin Nutr. 1998;68(4):854–8.
6. Romagnoli E, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmo E, Carnevale V, Scillitani A, Minisola S. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;93(8):3015–20.
7. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W, Ameri A, Tannenbaum AD. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab. 2008;93(3):677–81.
8. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, Ameri A, Reitz R, Salameh W, Chen TC, Holick MF. Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. Am J Clin Nutr. 2010;91(6):1621–6.
9. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D-2) as a vitamin supplement. Am J Clin Nutr. 2006;84(4):694–7.
10. Holmberg I, Berlin T, Ewerth S, Bjorkhem I. 25-Hydroxylase activity in subcellular-fractions from

human-liver – evidence for different rates of mitochondrial hydroxylation of vitamin-D2 and vitamin-D3. Scand J Clin Lab Invest. 1986;46(8):785–90.
11. Horst RL, Reinhardt TA, Ramberg CF, Koszewski NJ, Napoli JL. 24-Hydroxylation of 1,25dihydroxyergocalciferol—an unambiguous deactivation process. J Biol Chem 1986;261:9250–6.
12. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hypponen E, Berry J, Vieth R, Lanham-New S. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;95(6):1357–64.
13. Tjellesen L, Hummer L, Christiansen C, Rodbro P. Serum concentration of vitamin-D metabolites during treatment with vitamin-D2 and vitamin-D3 in normal premenopausal women. Bone Miner. 1986;1(5):407–13.
14. Armas LAG, Hollis BW, Heaney RP. Vitamin D(2) is much less effective than vitamin D(3) in humans. J Clin Endocrinol Metabol. 2004;89(11):5387–91.
15. Binkley N, Gemar D, Engelke J, Gangnon R, Ramamurthy R, Krueger D, Drezner MK. Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or 50,000 IU monthly in older adults. J Clin Endocrinol Metabol. 2011;96(4):981–8.
16. Heaney RP, Recker RR, Grote J, Horst RL, Armas LAG. Vitamin D(3) is more potent than vitamin D(2) in humans. J Clin Endocrinol Metabol. 2011;96(3):E447–52.
17. Glendenning P, Chew GT, Seymour HM, Gillett MJ, Goldswain PR, Inderjeeth CA, Vasikaran SD, Taranto M, Musk AA, Fraser WD. Serum 25-hydroxyvitamin D levels in vitamin D-insufficient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. Bone. 2009;45(5):870–5.
18. Leventis P, Kiely PDW. The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. Scand J Rheumatol. 2009;38(2):149–53.

Vitamin D Supplementation and Changes in Vitamin D and Bone Metabolites in Children

23

Richard D. Lewis and Emma M. Laing

Abstract

Because a significant number of children and adolescents worldwide are considered vitamin D insufficient or deficient, skeletal health may be compromised leading to long-term fracture risks. For this reason, a heightened research priority has been placed on the role of vitamin D in bone metabolism during growth. Vitamin D supplementation increases 25-hydroxyvitamin D (25[OH]D) in children and the response appears to be dose-dependent, though dose–response trials are needed to confirm this. Increases in serum 1,25 dihydroxyvitamin D ($1,25[OH]_2D$) and decreases in intact parathyroid hormone (iPTH) occur with vitamin D supplementation, and these responses are more pronounced with higher doses of vitamin D and in populations considered vitamin D insufficient and deficient (<50 nmol/L). In vitamin D-sufficient groups (>50 nmol/L), iPTH suppression is minimal. The effect of vitamin D supplementation on biochemical markers of bone turnover likely depends on basal 25(OH)D concentrations and doses of vitamin D administered, with lower baseline 25(OH)D and higher vitamin D doses promoting more favorable responses, respectively. Because there is a dearth of knowledge with respect to race differences in vitamin D and bone metabolism, dose–response trials among multiple race and ethnic groups are needed.

Keywords

Vitamin D • Supplementation • 25(OH)D • $1,25(OH)_2D$ • iPTH • Bone turnover • Children

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Abbreviations

25[OH]D	25-hydroxyvitamin D
$1,25[OH]_2D$	1,25dihydroxyvitamin D
iPTH	Intact parathyroid hormone

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Introduction

A considerable number of children and adolescents worldwide have low circulating 25(OH)D concentrations, particularly those who are of nonwhite ethnicity and race (Fig. 23.1) [1–3]. In the United States of America (USA), almost 20 % of children, 6–11 years of age, and 30 % of adolescents,12–19 years of age, have serum 25(OH)D below the Institute of Medicine (IOM) cutoff of <50 nmol/L. Almost 50 % of black adolescents in the USA have 25(OH)D <50 nmol/L compared to 10 % of US white children [1]. Similarly, in Canada [2] and Europe [3], a large percentage of children fall below the 50 nmol/L cutoff for 25(OH)D with a higher prevalence for nonwhite populations.

The health consequences of insufficient 25(OH)D in children and adolescents have yet to be determined. Recent attention has focused on nonskeletal health outcomes, such as diabetes [4] and cardiovascular disease [5], likely linked to the obesity epidemic and the fact that obese children and adolescents have lower circulating 25(OH)D. However, much of what is known with respect to vitamin D and health in youth is related to skeletal outcomes. Historically, rickets has been associated with circulating 25(OH)D considered deficient (<25.0 nmol/L), though the influence of non-deficient but low concentrations of vitamin D on skeletal health outcomes is less obvious. Of the handful of randomized, double-blind, placebo-controlled vitamin D interventions that have been conducted in children examining skeletal outcomes, the findings have been mixed. Some have shown improvements in bone mass [6, 7], and others have not shown benefits of supplementation [6, 8]. The most likely causes of such diverse findings are the wide ranges of vitamin D inputs and subjects' baseline 25(OH)D. Moreover, the majority of intervention trials conducted to date have been conducted in females only and have not addressed race.

To better guide study designs for future clinical trials, the relationships between vitamin D and changes in 25(OH)D and other intermediate endpoints of vitamin D and bone metabolism (i.e., iPTH, $1,25[OH]_2D$, and biochemical markers of bone turnover) should be clarified. Study designs should also include a wide range of inputs, races, and baseline 25(OH)D concentrations. This chapter reviews what is known with respect to vitamin D supplementation and intermediate vitamin D and bone metabolites in children and adolescents, excluding calcium absorption. Table 23.1 summarizes the majority of vitamin D intervention trials performed in children to date that have reported 25(OH)D, iPTH, and/or $1,25(OH)_2D$ as outcomes.

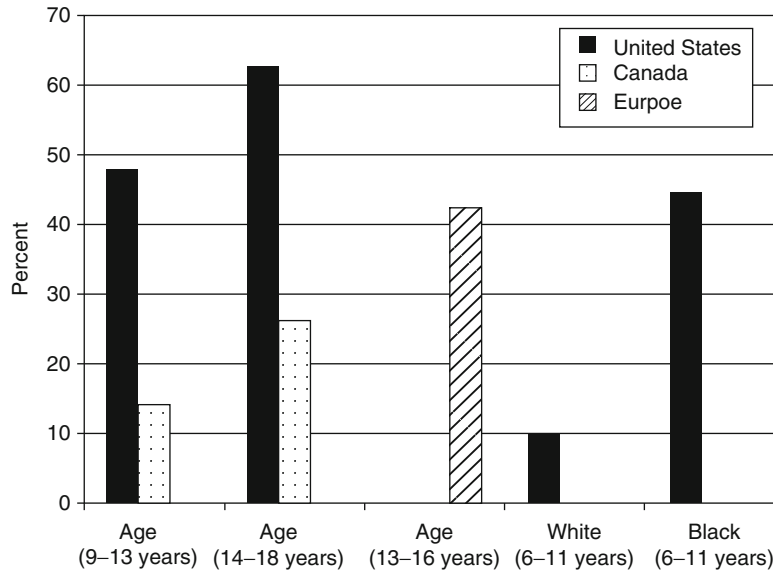

Fig. 23.1 Distribution of adolescents with serum 25(OH)D <50 nmol/L (Based on data from Refs [1–3])

Table 23.1 Vitamin D intervention studies and 25(OH)D, 1,25(OH)$_2$D, and iPTH outcomes in children and adolescents

Reference	Subjects				Dose (IU/day)		25(OH)D			1,25(OH)$_2$D			iPTH		
First author and date	Country/ ethnic origin	*N*	Sex	Age (years)	Form (D_2 or D_3)	Duration	Baseline (nmol/L)	Δ (nmol/L)	*P* for Δ	Baseline (pg/mL)	Δ (pg/ mL)	*P* for Δ	Baseline (pmol/L)	Δ (pmol /L)	*P* for Δ
Andersen et al. [9]	Denmark/ Pakistani	26	F	10–14	400 (D_3)	12 months	Median = 16.9	30.1	0.03	Not measured			Median = 3.5	−0.8	NS
					800 (D_3)	12 months	Median = 8.8	15.5	NS				Median = 4.3	−1.4	NS
Dahifar et al. [10]	Iran	7	F	11–15	~2,500 (form not specified)	20 days	Mean = 7.5	17.5	Not reported	Not measured			Mean = 11.1	−8.7	Not reported
Docio et al. [11]	Spain	21	M/F	7–10	1,600 (form not specified)	7 days	*March*: mean = 31.5	~34.0	<0.001	*March*: mean ~ 27.0	~12	<0.001	*March*: not reported	~ −0.6	<0.002
							October: mean = 74.8	~29.0	<0.001	*October*: mean ~ 9.0	~4	<0.05	*October*: not reported	~ −0.1	NS
Dong et al. [12]	USA/ Black	49	M/F	14–18	400 (D_3)	4 months	Mean = 33.3	26.5	NS	Not measured			Mean = 4.3	No change	NS
					2,000 (D_3)	4 months	Mean = 33.5	52.2	<0.001				Mean = 3.5	No change	NS
El-Hajj Fuleihan et al. [6]	Lebanon	168	F	10–17	~200 (D_3)	12 months	Mean = 35	7.5	NS	Mean = 78.0	No change	NS	Not measured		
					~2,000 (D_3)	12 months	Mean = 35	60.0	<0.001	Mean = 83.0	22	<0.001			
Ghazi et al. [13]	Iran	207	M/F	14–20	~1,600 (D_3)	5 months	Mean = 20.5	27.5	<0.001	Not measured			Mean = 2.0	−0.7	<0.001
			Data for F		~800 (D_3)	5 months	Mean = 16.3	16.3	<0.001				Mean = 2.5	−0.8	<0.001
Guillemant et al. [14]	France	58	M	13–17	~1,700 (D_3)	6 months	*March*: mean = 55.3	35.0	0.001	Not measured			*March*: mean = 3.1	1.1	0.0002
							September: mean = 53.8	7.3	NS				*September*: mean = 2.8	0.2	NS

(continued)

Table 23.1 (continued)

Reference	Subjects				Dose (IU/day)		25(OH)D			$1,25(OH)_2D$			iPTH		
First author and date	Country/ ethnic origin	*N*	Sex	Age (years)	Form (D_2 or D_3)	Duration	Baseline (nmol/L)	Δ (nmol/L)	*P* for Δ	Baseline (pg/mL)	Δ (pg/ mL)	*P* for Δ	Baseline (pmol/L)	Δ (pmol/L)	*P* for Δ
Hill et al. [15]	USA/ White and Black	302	M/F	9–13	400–4,000 (D_3)	3 months	*White*: mean = 80.0	26.3	Not reported	Not measured			Not measured		
							Black: mean = 60.3	23.5	Not reported						
Lehtonen-Veromaa et al. [16]	Finland	42	F	9–15	400 (form not specified)	3 months (f/u at 6 months)	Mean = 33.9	29.0	<0.001	Not measured			Not measured		
Maalouf et al. [17]	Lebanon	25	M/F	10–17	~2,000 (D_3)	2 months	Mean = 37.0	17.0	0.043	Not measured			Not measured		
		340	M/F		~200 (D_3)	12 months	Mean = 35.0	7.5	<0.001	Mean = 78.0	No change	NS			
			Data for F		~2,000 (D_3)	12 months	Mean = 32.5	62.5	<0.001	Mean = 83.0	21	<0.001			
Park et al. [18]	USA	11	F	12–14	1,000 (D_3)	1 month	46.8	13.3	<0.05	58.3	−1.6	NS	2.9	0.1	NS
Rajakumar et al. [19]	USA/ Black	41	M/F	6–10	400 (D_3)	1 month	*Insufficient*: mean = 38.5	19.0	<0.05	*Insufficient*: mean = 44.1	3.2	Not reported	*Insufficient*: mean = 5.0	–0.3	NS
							Sufficient: mean = 80.3	−0.75	NS	*Sufficient*: mean = 52.3	2.0	Not reported	*Sufficient*: mean = 4.0	–0.4	NS
Rajakumar et al. [20]	USA/ Black	41	M/F	6–10	400 (D_3)	1 month	*Obese*: mean = 55.5	10.0	Not reported	*Obese*: mean = 49.9	0.80	Not reported	*Obese*: mean = 4.5	–0.01	Not reported
							Non-obese: mean = 65.8	8.0	Not reported	*Non-obese*: mean = 45.8	5.1	Not reported	*Non-obese*: mean = 4.4	–0.8	Not reported
Schou et al. [21]	Denmark	19	M/F	6–13	600 (D_3)	2 months	Mean = 33.0	14.0	<0.001	Mean = 35.4	−1.7	0.38	Mean = 4.2	–0.2	0.37

Shakiba et al. [22]	Iran	96	F	12–15	~400 (D_3)	6 months	Not reported	Mean vs. control at follow up = 24.3	<0.001	Not measured	Not measured	
					~800 (D_3)	6 months	Not reported	Mean vs. control at follow up = 43.8	Not reported			
Tau et al. [23]	Argentina	18	M/F	Mean = 7.3	~800 (D_2)	6 months	73.3	*April*: 15.0 *September*: −17.3	<0.001 Not reported	Not measured	Not measured	
Viljakainen et al. [7]	Finland	228	F	11–12	200 (D_3)	12 months	46.3	5.7	<0.001	Not measured	3.5	−0.6	NS
					400 (D_3)	12 months	46.7	12.4	<0.001		3.1	−0.3	NS
Ward et al. [24]	UK	69	F	12–14	~1,600 (D_2)	12 months	18.1	37.9	<0.001	Not measured	4.8	−2.8	<0.001

25(OH)D

The 25(OH)D concentrations reflecting optimal vitamin D status for overall health in children and adolescents are unknown; however, the IOM recently established 50 nmol/L as a target value for rickets prevention, maximal calcium absorption, and positive skeletal outcomes, corresponding to recommendations of 600 IU/day [25]. Multiple vitamin D intervention trials in children and adolescents have contributed to our understanding of the serum 25(OH)D responses to a wide range of vitamin D inputs. For example, vitamin D_3 supplementation with 200 IU/day in healthy Finnish females, mean age 11.4 years, or in healthy Lebanese females [7], 10–17 years of age [6], resulted in small increases of 5.7 and 7.5 nmol/L, respectively, over 1 year. Supplementation with 400 IU/day for 1 year in Finnish girls [16] or for 1 month in 6–10-year-old black US children who had baseline values <40 nmol/L [19] increased serum 25(OH)D approximately 29 and 19 nmol/L, respectively. Higher doses of 1,600 IU/day were provided in three trials: to adolescent Iranian males and females for 5 months increasing serum 25(OH)D concentrations 27.5 nmol/L, over 1 year to adolescent females increasing concentrations by 37.9 nmol/L [24], and for 1 week to children with low wintertime baseline 25(OH)D concentrations (~31.5 nmol/L) increasing 25(OH)D concentrations to approximately 80 nmol/L [11]. The highest dose published in child intervention trials to date is 2,000 IU/day. When 2,000 IU/day was provided to US black adolescents over 16 weeks [12] or female Lebanese adolescents over 1 year [6], increases of approximately 60 nmol/L were observed in both studies.

From a range of 200–2,000 IU/day, it appears as though serum 25(OH)D concentrations respond to vitamin D supplementation in a dose-dependent manner. Two of the trials included black children and adolescents [12, 19], and it appears as though black and white children respond similarly with respect to vitamin D inputs and changes in serum 25(OH)D. However, randomized, dose–response trials, including multiple races, have not yet been published that confirm these findings. Basal circulating 25(OH)D is a key determinant of the response, with greater increases observed in those with lower concentrations. An equation to predict the changes in serum 25(OH)D based on varying vitamin D inputs has been described [26] δ: serum 25(OH)D (ng/ml) = $0.01130x + 1.13747$, where x = vitamin D (IU/d) supplementation.

$1,25(OH)_2D$

The majority of intervention trials do not report measures of serum $1,25(OH)_2D$, likely because serum $1,25(OH)_2D$ concentrations are regulated by serum calcium and iPTH concentrations, there is more variability and less reliability in the assays vs. 25(OH)D, and the metabolite has a short (~4 h) half-life. However, similar to serum 25(OH)D, $1,25(OH)_2D$ concentrations increase in response to vitamin D supplementation in children, and the response likewise appears to be dose-dependent. Intervention trials using 1,600 [11] or ~2,000 IU/day [6] reported significant increases in serum $1,25(OH)_2D$ concentrations in males and females, 6–17 years of age. In these studies, serum $1,25(OH)_2D$ increased by 12–21 pg/ml with doses of 1,600 or 2,000 IU/day, respectively. Lower doses of 200 or 600 IU/day [6, 17, 21] were not high enough to elicit significant responses. Not only was dose an important consideration, but baseline serum 25(OH)D concentrations were also of great consequence. For example, participants with lower vs. higher baseline serum 25(OH)D concentrations had greater $1,25(OH)_2D$ responses [11, 18]. It is unknown what the serum $1,25(OH)_2D$ response is to vitamin D supplementation in non-white populations, which is an important consideration since blacks vs. whites have lower 25(OH)D [15] and higher $1,25(OH)_2D$ [27].

iPTH

One of the criteria used to define optimal 25(OH)D concentrations in adults is the inflection point at which maximal suppression of serum iPTH

occurs [28]. In adults, a serum 25(OH)D concentration of approximately 80 nmol/L has been identified as a point of maximal iPTH supression [29, 30]. Most cross-sectional studies in children and adolescents have reported significant inverse linear relationships between serum 25(OH)D and iPTH [19, 31–40]; whereas, two studies identified a serum 25(OH)D inflection point. For example, nonlinear relationships were observed between iPTH and serum 25(OH)D concentrations in US black children [19] and French adolescents [39], with 25(OH)D inflection points of 75 and 83 nmol/L, respectively. Hill and colleagues [15] using pooled data from a multisite study of black and white adolescents were unable to find an inflection point for serum 25(OH)D associated with maximal suppression of iPTH and questioned the use of iPTH suppression as an outcome for determining optimal serum 25(OH)D levels in healthy children.

Few studies have reported serum iPTH responses to vitamin D supplementation, but from the limited data available, it appears that iPTH suppression, like the $1,25(OH)_2D$ responses, is minimal at lower doses of vitamin D and depends on the initial serum concentrations of 25(OH)D. When doses ranging from 200 to 600 IU/day were administered in trials of black and white children [19–21] and adolescents [7, 9], vitamin D supplementation had no significant effect on serum iPTH. In contrast, vitamin D supplementation with higher doses of vitamin D (~1,600–1,700 IU/day) in male and female children [11] and in male [14] and female adolescents [13, 24] showed significant suppression or prevented wintertime increases in iPTH (particularly among those with low basal or wintertime levels of 25(OH)D in the range of 18–43 nmol/L). An exception to these findings was the study by Dong et al. [12], in which no significant changes in serum iPTH were observed in black youth with basal levels of 25(OH)D of approximately 33 nmol/L and supplemented with either 400 or 2,000 IU/day of vitamin D_3 for 16 weeks. In order to better understand the iPTH responses to vitamin D supplementation and to determine if iPTH is a meaningful intermediate marker in children, studies should be conducted using multiple doses, administering doses higher than what has been currently tested (i.e., 2,000 IU/day), and including different races.

Biochemical Markers of Bone Turnover

Biochemical markers of bone turnover are used as intermediate functional outcome measures for determining the effectiveness of vitamin D inputs on bone metabolism. While many commercial kits are now available for measurement of bone formation and resorption, there is considerable within-subject variability due to maturational status, age, and sex, as well as assay variability. However, studies investigating the relationships between 25(OH)D concentrations and markers of bone turnover are complicated further by varying basal vitamin D concentrations [35, 41, 42].

Adolescent females who were considered vitamin D insufficient (<50 nmol/L) or severely deficient (<25 nmol/L) were reported to have significantly higher deoxypyridinoline (DPD) and bone specific alkaline phosphatase (BAP), but similar osteocalcin (OC) levels, compared to girls who had sufficient vitamin D status (>50 nmol/L) [43]. Similarly, in adolescent males [41] or in 9–15-year-old females [44] whose overall mean serum 25(OH)D concentrations were <50 nmol/L, inverse correlations were observed between serum 25(OH)D and pyridinoline/creatinine ratio ($r=-0.23$; $P<0.01$) [41] or C-terminal telopeptide (CTX; $r=-0.27$; $P<0.001$) [44]. In females from the same Finnish study with severe hypovitaminosis D, serum OC did not increase over 3 years, and the authors suggest that chronically low 25(OH)D levels may reduce bone formation and attenuate skeletal growth [44]. Alternatively, it may be that OC is not a sensitive marker of bone formation but reflects overall bone remodeling [45]. Healthy adolescent females with vitamin D insufficiency (<50 nmol/L) were reported to have higher BAP and CTX than those classified as sufficient (>50 nmol/L). However, once pubertal stage was taken into account in the statistical analysis, vitamin D did not account for any differences in the

biochemical markers of bone turnover [42]. The findings from these cross-sectional and observational studies support the notion that children and adolescents with insufficient or deficient serum 25(OH)D concentrations (<50 nmol/L) have increased bone turnover in favor of bone resorption, but the findings are not consistent. The lack of consistency may be partly related to the study of children and adolescents in various stages of sexual maturation, whereby increases in biochemical makers of bone formation and resorption occur with rapid skeletal growth and decelerate towards the cessation of peak growth.

Several intervention trials have assessed the impact of vitamin D supplementation on biochemical markers of bone turnover in children and adolescents [13, 15, 18–21, 35]. Generally, trials that administered lower doses of vitamin D did not elicit much change in bone biomarker response. Supplementation with either 400 IU/day in obese and nonobese black children 6–10 years of age [20] or 600 IU/day for 4 weeks in healthy Danish children 6–13 years of age [21] did not modify bone turnover markers, regardless of baseline 25(OH)D concentrations. In a 12-month trial, female adolescents (baseline serum 25[OH]D ~ 47 nmol/L) were supplemented with either 200 or 400 IU/day, and there was no significant effect on OC, pyridinoline, or DPD in the intent-to-treat analysis [35]. Reported in adolescents with mean serum 25(OH)D concentrations of 30 nmol/L and receiving the equivalent of either 800 or 1,600 IU/day for 5 months, serum BAP increased significantly in the girls receiving 800 IU/day and OC increased in girls and boys in both the 800 and 1,600 IU groups [13]. In a more recent vitamin D supplementation trial, Hill and colleagues [15] examined the relationships between changes in serum 25(OH)D and changes in serum BAP, OC, and urinary n-telopeptide: creatinine following 12 weeks of vitamin D supplementation in black and white boys and girls of similar maturation stage. Mean baseline serum 25(OH)D concentrations were higher than previous studies (70 nmol/L for all participants), and supplementation over 12 weeks induced changes in serum 25(OH)D ranging from −15 to 155 nmol/L. Even with these sizeable shifts in serum 25(OH)D, no significant relationships were reported for any of the bone biomarkers, regardless of sex or race.

Several points should be addressed with respect to the bone turnover marker response and vitamin D. First, vitamin D insufficiency (<50 nmol/L) seems to be associated with increased bone resorption. Second, the biomarker, OC, is included in many studies as a marker of bone formation, and in fact, it may not be specific for bone formation but for overall bone remodeling [45]. Third, the doses of vitamin D used in most of the intervention trials were likely too low to elicit significant responses, but were also confounded by differences in age, maturational stage, and basal concentrations of 25(OH)D.

Summary and Conclusion

In summary, efforts are ongoing to better understand the metabolic responses to vitamin D supplementation in children and adolescents. Vitamin D supplementation significantly increases 25(OH)D in children, and the response appears to be dose-dependent. Serum $1,25(OH)_2D$ increases, and iPTH is lowered with vitamin D supplementation; however, these responses are more noticeable with higher doses of vitamin D and in populations that would be considered vitamin D insufficient or deficient (<50 nmol/L). In vitamin D-sufficient groups (25[OH]D > 50 nmol/L), iPTH suppression is minimal regardless of dose. The effect of vitamin D supplementation on biochemical markers of bone turnover likely depends on the age and maturational status of the participants, basal 25(OH)D concentrations, and dose of vitamin D, with early puberty, lower baseline 25(OH)D, and higher vitamin D doses promoting more favorable responses. Trials assessing the impact of vitamin D supplementation on bone remodeling should be powered appropriately in order to detect significant effects of vitamin D. There is a dearth of knowledge with respect to race differences in vitamin D and bone metabolism, and dose–response trials using multiple doses are needed in populations of varying race and ethnicity.

References

1. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS Data Brief. 2011;59:1–8.
2. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: an examination in children and adults with and without supplement use. Am J Clin Nutr. 2011;94(1): 128–35.
3. Gonzalez-Gross M, Valtuena J, Breidenassel C, et al. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. Br J Nutr. 2012;107(5):755–64.
4. Williams DM, Fraser A, Sayers A, et al. Associations of 25-hydroxyvitamin D2 and D3 with cardiovascular risk factors in childhood: cross-sectional findings from the Avon Longitudinal Study of Parents and Children. J Clin Endocrinol Metab. 2012;97(5):1563–71.
5. Pollock NK, Bernard PJ, Gutin B, Davis CL, Zhu H, Dong Y. Adolescent obesity, bone mass, and cardiometabolic risk factors. J Pediatr. 2011;158(5):727–34.
6. El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab. 2006;91(2):405–12.
7. Viljakainen HT, Natri AM, Karkkainen M, et al. A positive dose–response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. J Bone Miner Res. 2006;21(6):836–44.
8. Cheng S, Lyytikainen A, Kroger H, et al. Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. Am J Clin Nutr. 2005;82(5):1115–26; quiz 1147–1118.
9. Andersen R, Molgaard C, Skovgaard LT, et al. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. Br J Nutr. 2008;100(1): 197–207.
10. Dahifar H, Faraji A, Ghorbani A, Yassobi S. Impact of dietary and lifestyle on vitamin D in healthy student girls aged 11–15 years. J Med Invest. 2006;53(3–4): 204–8.
11. Docio S, Riancho JA, Perez A, Olmos JM, Amado JA, Gonzalez-Macias J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies? J Bone Miner Res. 1998;13(4):544–8.
12. Dong Y, Stallmann-Jorgensen IS, Pollock NK, et al. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. J Clin Endocrinol Metab. 2010;95(10):4584–91.
13. Ghazi AA, Hosseinpanah F, MA E, Ghazi S, Hedayati M, Azizi F. Effects of different doses of oral cholecalciferol on serum 25(OH)D, PTH, calcium and bone markers during fall and winter in schoolchildren. Eur J Clin Nutr. 2010;64(12):1415–22.
14. Guillemant J, Le HT, Maria A, Allemandou A, Peres G, Guillemant S. Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D3 supplements. Osteoporos Int. 2001;12(10):875–9.
15. Hill KM, Laing EM, Hausman DB, et al. Bone. 2012;51(4):795–9.
16. Lehtonen-Veromaa M, Mottonen T, Irjala K, et al. Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. Eur J Clin Nutr. 1999;53(9):746–51.
17. Maalouf J, Nabulsi M, Vieth R, et al. Short-term and long-term safety of weekly high-dose vitamin D3 supplementation in school children. J Clin Endocrinol Metab. 2008;93:2693–701.
18. Park CY, Hill KM, Elble AE, et al. Daily supplementation with 25 mug cholecalciferol does not increase calcium absorption or skeletal retention in adolescent girls with low serum 25-hydroxyvitamin D. J Nutr. 2010;140(12):2139–44.
19. Rajakumar K, Fernstrom JD, Janosky JE, Greenspan SL. Vitamin D insufficiency in preadolescent African-American children. Clin Pediatr (Phila). 2005;44(8): 683–92.
20. Rajakumar K, Fernstrom JD, Holick MF, Janosky JE, Greenspan SL. Vitamin D status and response to Vitamin D(3) in obese vs. non-obese African American children. Obesity (Silver Spring). 2008; 16(1):90–5.
21. Schou AJ, Heuck C, Wolthers OD. Vitamin D supplementation to healthy children does not affect serum osteocalcin or markers of type I collagen turnover. Acta Paediatr. 2003;92(7):797–801.
22. Shakiba M, Ghadir M, Nafei Z, Akhavan Karbasi S, Lotfi MH, Shajari A. Study to evaluate two dosage regimens of vitamin D through an academic year in middle school girls: a randomized trial. Acta Med Iran. 2011;49(12):780–3.
23. Tau C, Ciriani V, Scaiola E, Acuna M. Twice single doses of 100,000 IU of vitamin D in winter is adequate and safe for prevention of vitamin D deficiency in healthy children from Ushuaia, Tierra Del Fuego, Argentina. J Steroid Biochem Mol Biol. 2007; 103(3–5):651–4.
24. Ward KA, Das G, Roberts SA, et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. J Clin Endocrinol Metab. 2010;95(10): 4643–51.
25. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.
26. Weaver CM, Laing EM, Lewis RD. Adolescence and acquisition of peak bone mass. In: Feldman D, Pike JW, Adams JS (Eds.) Vitamin D, Third Edition, San Diego, CA: Elsevier, Inc. 2011; pp 657–77.

27. Harris SS. Vitamin D and African Americans. J Nutr. 2006;136(4):1126–9.
28. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int. 2005;16(7):713–6.
29. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med. 1992;327:1637–42.
30. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338(12):777–83.
31. Cheng S, Tylavsky F, Kroger H, et al. Association of low 25-hydroxyvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. Am J Clin Nutr. 2003;78(3): 485–92.
32. Tylavsky FA, Ryder KM, Li R, et al. Preliminary findings: 25(OH)D levels and PTH are indicators of rapid bone accrual in pubertal children. J Am Coll Nutr. 2007;26(5):462–70.
33. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO. Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab. 2005;90(10):5576–81.
34. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med. 2004;158(6):531–7.
35. Viljakainen HT, Palssa A, Karkkainen M, et al. A seasonal variation of calcitropic hormones, bone turnover and bone mineral density in early and mid-puberty girls – a cross-sectional study. Br J Nutr. 2006; 96(1):124–30.
36. Outila TA, Karkkainen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. Am J Clin Nutr. 2001;74(2):206–10.
37. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS. Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. Am J Clin Nutr. 2007;86(1):150–8.
38. Harkness L, Cromer B. Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy adolescent females. Osteoporos Int. 2005;16(1):109–13.
39. Guillemant J, Taupin P, Le HT, et al. Vitamin D status during puberty in French healthy male adolescents. Osteoporos Int. 1999;10(3):222–5.
40. US Department of Health and Human Services. Effectiveness and safety of vitamin D in relation to bone health. 2007. http://archive.ahrq.gov/clinic/tp/vitadtp.htm. Last accessed 7 Aug 2012.
41. Jones G, Dwyer T, Hynes KL, Parameswaran V, Greenaway TM. Vitamin D insufficiency in adolescent males in Southern Tasmania: prevalence, determinants, and relationship to bone turnover markers. Osteoporos Int. 2005;16(6):636–41.
42. Fares JE, Choucair M, Nabulsi M, Salamoun M, Shahine CH, Fuleihan Gel H. Effect of gender, puberty, and vitamin D status on biochemical markers of bone remodeling. Bone. 2003;33(2):242–7.
43. Foo LH, Zhang Q, Zhu K, et al. Low vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. J Nutr. 2009;139(5):1002–7.
44. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr. 2002;76(6):1446–53.
45. Ferron M, Wei J, Yoshizawa T, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010;142(2):296–308.

Determinants of the 25-Hydroxyvitamin D Response to Vitamin D Supplements

24

Bess Dawson-Hughes

Abstract

Supplemental vitamin D is now widely recommended, but little guidance is provided on how or when to take vitamin D supplements. The process of vitamin D replacement is complicated by the fact that the increment in 25-hydroxyvitamin D (25OHD) in response to a given dose varies greatly from person to person. This chapter will examine the factors associated with the 25OHD response to supplementation with vitamin D, including genetic determinants, the starting 25OHD level, body mass index, and gastric acid production. It will also consider the effects of a meal and of meal composition with respect to cholesterol and phytosterol intake and the amount and type of dietary fat on the 25OHD response to supplementation.

Keywords

25-Hydroxyvitamin D • Vitamin D absorption • Genetics • Dietary fat • MUFA • PUFA

Introduction

More than 70 % of adult men and women in the USA have serum 25-hydroxyvitamin D (25OHD) levels below 75 nmol/L [1], the level considered by many to be optimal for musculoskeletal health and perhaps other health benefits [2, 3]. As a consequence of growing recognition of the prevalence and importance of vitamin D insufficiency, supplemental vitamin D is now widely recommended. Little guidance is provided on how or when to take vitamin D supplements, however, and in fact, there have been few human studies that would inform such guidance. The increment in 25OHD in response to a given dose varies greatly from person to person. This chapter will examine the

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factors associated with the 25OHD response to supplementation with vitamin D. These factors include determinants of vitamin D absorption and factors affecting the transport and metabolism of 25OHD. It is important to understand the factors affecting vitamin D absorption in order to define and standardize conditions under which patients and the general public can achieve a greater and more predictable improvement in vitamin D status. Herein we will consider genetic determinants of 25OHD levels; subject characteristics including the starting 25OHD level and body mass index (BMI, wt/ht^2); dietary/gastrointestinal factors including the presence or absence of food, cholesterol, and phytosterol intake; and the amount and type of dietary fat. Compliance is of course a critical factor in any supplement regimen, but it will not be discussed further.

Genetic Determinants of the Serum 25OHD Level and Response to Supplementation

The 25OHD response to supplementation is a function not only of how much vitamin D is absorbed but also of the affinity of 25OHD to its binding protein in the circulation and to its metabolic fate. Serum 25OHD levels are known to have a high level of heritability, with estimates ranging from 28 [4] to 80 % [5]. Since 2005, several studies have identified genetic variants that affect the measured 25OHD concentration in the circulation. The most significant of these to be recognized to date is the multifunctional plasma protein, Gc, located on chromosome 4. This gene is also known as group-specific complement gene and Gc globulin. These variants have been implicated in susceptibility to several chronic diseases including osteoporosis, type 1 diabetes, and multiple sclerosis (each of which, interestingly, has been linked to serum 25OHD levels) [6]. Gc is best known, however, for its role in encoding vitamin D-binding protein, which transports vitamin D, 25OHD, and 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) in the circulation. Lauridsen and colleagues measured the plasma concentration of the Gc proteins and examined the association of different Gc alleles with circulating 25OHD levels in a cohort of 595 Danish Caucasian postmenopausal women [7]. The mean plasma Gc concentrations of the three alleles were for Gc1-1: 5.22 ± 0.03 (SEM), for Gc1-2: 4.80 ± 0.03, and for Gc2-2: 4.35 ± 0.07 μmol/L, respectively, $P<0.001$. These declining concentrations may be attributable to more rapid degradation of the Gc2-2 proteins. Kawakami and colleagues reported the rate of metabolism of Gc1-1 to be 25 % per day and of Gc2-2 to be 33 % per day [8]. In concert with declining concentrations of Gc across the Gc1-1, Gc1-2, and Gc2-2 alleles, Lauridsen found that mean serum 25OHD levels were progressively lower (approximately 65, 55, and 45 nmol/L, respectively). The ratio of 25OHD to Gc protein, or the 25OHD index, was variable suggesting that the differences in 25OHD across the genotypes were connected to the genotype per se rather than to the Gc concentration.

Bu and colleagues did a broader search for genetic determinants of serum 25OHD by screening nine functionally important candidate genes regulating various aspects of vitamin D metabolism using 49 single nucleotide polymorphism (SNP) markers in a group of 156 healthy unrelated Caucasian women [9]. This work was followed by a replication study in a different group of 340 women [9]. They observed associations of 25OHD with variants of SNPs in the Gc gene, consistent with observations described above. They also found associations of serum 25OHD with the cytochrome P450, family 2, subfamily R, and polypeptide 1 (CPY2R1) gene. The latter is a key enzyme that hydroxylates vitamin D in the liver at the C30 position to form 25OHD [10]. This finding has been confirmed in a GWAS study in 30,000 people of European ancestry [11].

Fu and colleagues took this work a step further by determining whether Gc variants influenced the 25OHD increment in response to vitamin D supplementation [12]. They genotyped the Gc variant, T436K, in 98 healthy Caucasian adults who were randomly treated with either 600 or 4,000 IU of vitamin D_3 per day for 1 year. The distribution of these alleles in this relatively small sample of 85 subjects was TT 48, TK 31, and KK 6 subjects. Baseline serum 25OHD levels averaged 66 nmol/L and did not differ significantly in the two dose groups. Consistent with the findings of Lauridsen [7], the

baseline 25OHD levels tended to be progressively lower across the variants, TT, TK, and KK (analogous to Gc1-1, Gc1-2, and Gc2-2, respectively). In contrast, the increments in 25OHD after 1 year of supplementation with each of the two doses were graded in the opposite direction. The increases on the higher dose, expressed as percentage change from baseline, were 136±16 %, 256±58 %, and 416±52 %, respectively, across the genotypes (*P* for trend=0.003). Overall T436K accounted for 8.5 % of the variance in increment in serum 25OHD levels. For comparison, dose of vitamin D (600 vs 4,000 IU per day) accounted for 22 %. While it is well recognized that starting 25OHD levels are inversely related to increments in response to supplementation (as discussed below), this phenomenon is unlikely to explain rises in serum 25OHD of the magnitude seen in the TK and KK groups. Difference in binding capacity across the alleles is a consideration, but that too seems unlikely to explain the large step-up in serum 25OHD observed in the KK subjects, since the binding capacity was lower in this group initially and declined more over the course of the study when compared with the other two genotypes [12].

Racial differences in the Gc gene have long been recognized [13], but only recently have differences been linked to 25OHD levels. Signorello and colleagues reported Black-White differences in the contribution of selected genes to the serum 25OHD level in a cohort of 379 Blacks and 379 Whites participating in the Southern Community Cohort Study in the USA [14]. Out of the 94 SNPs evaluated, three in the Gc region and one in the CYP27B1 region predicted 25OHD levels in the Blacks, whereas none of these SNPs predicted 25OHD levels in the Whites [14]. Other investigators evaluated 30 SNPs in the Gc, VDR, and CYP27B1 regions in 513 African-Americans from 42 families and identified significant findings for only one SNP, located in the Gc region [15].

Starting 25OHD Level and BMI Influence 25OHD Response to Supplementation

It is well known that the increment in 25OHD is inversely related to the starting 25OHD level and that the association is nonlinear. Progressively higher doses are required to achieve the same increment as the 25OHD level approaches the target. From the modeling of Barger-Lux [16] and Heaney [17], the amounts of vitamin D needed to raise the blood level to 80 nmol/L are approximately 2,200 IU per day at starting levels of 20–40 nmol/L, 1,800 IU at starting levels of 40–60, and 1,160 IU at levels of 60–80 nmol/L. The same pattern was observed in the multiple-dose vitamin D intervention study of Gallagher and colleagues [18].

Both 25OHD levels and the increments in 25OHD in response to supplementation with vitamin D are inversely related to body mass index (BMI) [18–20]. Among healthy men and women, age 65 years and older, treated with 700 IU per day of vitamin D, the 1-year increment in 25OHD was 21 % smaller in obese than in normal weight subjects 22.8 ng/ml [20]. Among healthy postmenopausal women treated with vitamin D in doses ranging from 400 to 4,800 IU daily for 1 year, there was no interaction of dose with BMI. When compared with 25OHD levels in normal weight women (BMI<25 kg/m^2), levels in overweight women (BMI 25.0–29.9 kg/m^2) were 12.2 nmol/L lower, and levels in obese women were 17.7 nmol/L lower throughout the full serum 25OHD concentration range tested [18]. Drinic and colleagues performed a cross-sectional analysis of associations between indices of body size and serum 25OHD levels [21]. They concluded that dilution of ingested vitamin D (and of vitamin D produced cutaneously) in the fat mass of obese subjects fully explained their lower serum 25OHD levels and that there was no need to invoke sequestration of vitamin D in fat tissue as an explanation. This observation is consistent with the earlier finding by Blum and colleagues that concentrations of vitamin D in serum were highly correlated with those in fat tissue [22].

The Serum 24,25-Dihydroxyvitamin D [24,25(OH)2D] Level as a Predictor of the 25OHD Response to Supplemental Vitamin D

The measurable serum 25OHD level is a function of the balance between incoming vitamin D, through skin synthesis and diet, and 25OHD

metabolism. The primary product of 25OHD catabolism is 24,25$(OH)_2$D. Wagner and colleagues assessed the impact of vitamin D supplementation on the ratio of serum 24,25$(OH)_2$D to serum 25OHD concentration, testing the hypothesis that a higher ratio would predict a lower 25OHD in response to supplementation with vitamin D_3 [23]. This was in fact observed, leading them to conclude that catabolism of 25OHD increases as the serum 25OHD level rises.

Dietary/Gastrointestinal Determinants of Vitamin D Absorption

Mechanisms of Vitamin D Absorption

It has been generally accepted that vitamin D is absorbed by passive diffusion, based largely on elegant gut perfusion studies in live, unanesthetized rats performed by Hollander and colleagues in 1978 [24]. He observed a linear relationship between the absorption rate of vitamin D and its intraluminal concentration in both the jejunum and ileum. Recent evidence indicates that there is also an active component to vitamin D absorption [25]. Reboul and colleagues reasoned that since cholesterol absorption is active and cholesterol and vitamin D are structurally similar, perhaps vitamin D absorption has an active component [25]. Cholesterol absorption is promoted by several "transporters" which ferry it across enterocytes, including SR-BI (Scavenger Receptor class B type I), CD 36 (Cluster Determinant 36), and NPC1L1 (Niemann-Pick C1 Like 1). Reboul and colleagues demonstrated that transvection of human Caco-2 cells and human embryonic kidney cells with cholesterol transporters caused increased cellular uptake of vitamin D and that addition of inhibitors of cholesterol transporters blocked the uptake of vitamin D [25]. These findings suggest that genetic variations in cholesterol transporter concentrations likely contribute to the variability in vitamin D absorption. No information is currently available on whether cholesterol content of a meal influences vitamin D absorption.

Method of Assessing Vitamin D Absorption

The absorption of vitamin D is usually assessed with use of the classical absorption or tolerance test described by Lo and colleagues [26]. This test is based on the measurement of the parent vitamin D level at its peak following a single oral dose of 50,000 IU of vitamin D. Repeated 25OHD measurements over 72 h following the dose revealed that the peak level occurred consistently at 12 h after ingestion in normal subjects and also in most subjects with known malabsorption. Moreover the timing of the peak vitamin D level after ingestion of vitamin D mixed in food versus in capsule form was found to be similar [26]. The assessment of absorption has been updated recently to a method that involves coadministering 50,000 IU of vitamin D_3 with 800 IU of deuterated D_3 after an overnight fast. Blood is drawn before and 12 h after dosing and analyzed for vitamin D content by LC/MS/MS [22]. This method separates and quantifies the deuterated and undeuterated D_3. The use of deuterated D_3 increases the accuracy of the absorption measurement since deuterated D_3 is not subject to extraneous influences on the test day, such as sun exposure or inadvertent ingestion of vitamin D.

Potential Impact of Gastric Acid Production on Vitamin D Absorption

Although gastric acid production is known to influence the absorption of fats and other fat-soluble vitamins [27, 28], its specific effect on vitamin D absorption has not been defined. Proton pump inhibitors (PPIs) are among the most commonly prescribed drugs and are an effective treatment of gastroesophageal reflux. An effect of PPI use on the bioavailability of vitamin D has not been reported, but several clinical studies suggest that PPI use may impair fat absorption and, as a consequence, also potentially impair vitamin D absorption. PPIs raise gastric pH from 2 to above 5, and this greatly decreases the rate of decomposition of food particles in the stomach by reducing the activity of pepsin and other gastric enzymes [29]. The

result is not only reduced food decomposition but also delayed gastric emptying. Gastric acidification increases plasma cholecystokinin (CCK) levels, and use of PPIs over 10 days in healthy subjects has been shown to significantly reduce their plasma CCK levels after a high-fat meal [28]. This is potentially important for fat and vitamin D absorption because CCK stimulates gallbladder emptying. In a different experiment in healthy young volunteers, treatment with the PPI, omeprazole, 40 mg in the morning for 10 days, significantly decreased plasma secretin levels during and in the 3-h period after a high-fat meal [27]. Secretin has a physiologic role in the regulation of pancreatic bicarbonate secretion, and it also stimulates bile secretion. Decreased bile secretion is potentially important for vitamin D absorption because bile acids are essential for micelle formation [30] and thus for the transport of vitamin D to the gastric mucosal absorptive surface. Older men and women have both an increased prevalence of achlorhydria and an increased use of medications that block gastric acid production. It will be important to define the impact of gastric acid secretion on vitamin D absorption.

Dietary Phytosterols

In an extension of the work of Reboul and colleagues on the role of cholesterol transporters in vitamin D absorption cited above [25], Goncalves and colleagues investigated whether phytosterols affect vitamin D absorption [31]. This question is clinically relevant because many functional foods now contain phytosterols for the purpose of lowering cholesterol levels. It is scientifically plausible that phytosterols affect vitamin D absorption because of the structural similarity of phytosterols to cholesterol. The first experiment demonstrated that the phytosterol, ß-sitosterol, greatly impaired the absorption of vitamin D in mice [31]. Subsequent experiments revealed two mechanisms by which absorption was impaired: (1) by reducing vitamin D solubility in micelles and (2) by inhibiting the mucosal uptake of vitamin D, the latter based on an investigation in mouse intestinal fragments and in Caco-2 cells [31]. No effect of phytosterols was observed on the movement of vitamin D out of the mucosal cell onto the basolateral side of the Caco-2 cells. These findings suggest that taking vitamin D with meals high in phytosterols (or cholesterol) may impair its absorption, but this has not been directly tested in humans.

Effects of a Meal and the Fat Content of the Meal on the 25OHD Response to Supplemental Vitamin D

Contrary to the popular belief that dietary fat is required for vitamin D absorption, the impact of dietary fat on D_3 absorption is far from clear. Absorption of D_3 appears to be enhanced by the presence of small amounts of dietary fat, presumably because fat ingestion stimulates bile acid secretion [24, 32]; however, larger amounts of fat may have a null effect [33] or even be detrimental [24]. The effect of dietary fat in amounts usually consumed, i.e., 30 % of energy, on D_3 absorption in humans is unknown. Absorption of other fat-soluble vitamins, E and K, is enhanced by the presence of small amounts of dietary fat, presumably because fat ingestion stimulates bile acid secretion.

A recent clinical report indicated that serum 25OHD levels increased by an average of 57 % over a 2–3-month period in 17 patients when they were instructed to take their usual doses of vitamin D with the largest meal of the day as opposed to receiving no advice on when to take their supplements [34]. These patients had been prescribed different doses, ranging from 1,000 IU per day to 50,000 IU per week. At least five of the patients had known malabsorption and many were osteoporotic. There was no control group in this study, and the impact of season, weight, and other factors known to affect 25OHD levels was not taken into account in the analyses. Nonetheless, the findings are consistent with increased absorption of vitamin D when the vitamin is taken with a meal. No information is available on the composition of the meals.

Tangpricha and colleagues compared serum 25OHD responses in 18 adults treated with 25,000 IU of vitamin D_2 three times each, with different vehicles, 0.1 ml of corn oil on toast, 240 ml of

skim milk, and 240 ml of whole milk [33]. Serum 25OHD increments did not differ significantly with the three vehicles [33]. In 26 adults, they assessed whether orange juice was an appropriate vehicle for vitamin D fortification by examining the 25OHD response to 1,000 IU of vitamin D_3 given in 240 ml of orange juice. The control group received orange juice alone. They observed that 25OHD increased by 150 % at 12 weeks in the supplemented group and by 45 % in the control group, the latter attributed to season [33]. They concluded that fat is not necessary for vitamin D absorption and that orange juice is an effective vehicle. Grossman and Tangpricha later assessed the effect of vehicle on the utilization of supplemental vitamin D_3 in a systematic review [32] and reported that vitamin D dissolved in small amounts of fish oil, either in a capsule or liquid, produced a greater increment in 25OHD than the same amount of vitamin D_3 given as a powder or dissolved in ethanol. The authors acknowledged that available evidence is inconclusive because the experimental conditions varied greatly in the different studies. Specifically, the starting 25OHD levels, study durations, and dosing schedules in the available studies differed substantially. Moreover, the increment in 25OHD but not in parent vitamin D was measured. As discussed above, absorption is only one of many determinants of the serum 25OHD concentration. Of the two studies that compared the same dose of vitamin D administered as a powder and in fish oil, one found no difference in 25OHD increment [35] and the other found the 25OHD increment to be greater with the oil vehicle [36]. More recently, Raimundo and colleagues compared the effects of a single dose of 50,000 IU of vitamin D_3 given with a high (25.6 g)- or a low (1.7 g)-fat meal on serum 25OHD levels 7 and 14 days after dosing [37]. The starting mean 25OHD levels in the two groups were 42.7 and 36.4 nmol/L, respectively. The responses at 7 days in the two groups did not differ significantly. By day 14, however, the 25OHD level in the high-fat group was significantly greater than that in the low-fat group. Specifically, in the high-fat meal group, an increase of 4 nmol/L was seen on day 7 and a larger increase of 12 nmol/L was present on day 14. In the low-fat meal group, serum 25OHD was about 3 nmol/L lower than baseline at both 7 and 14 days. This experiment should be interpreted with caution because of the different starting 25OHD levels of the two groups and because of the unusual (delayed) timing of the 25OHD increase in the high-fat meal group. In a similar single 50,000 IU vitamin D_3 dose study, Armas and colleagues found that almost all of the observed increase in 25OHD occurred in the first week after dosing [38]. In her study, when compared with baseline, serum 25OHD had increased by 16 nmol/L on day 7 and by 17 nmol/L on day 15 [38]. Thus, the true role of dietary fat in the absorption of vitamin D remains unclear.

The Impact of Type of Dietary Fat on the 25OHD Response to Supplemental Vitamin D

The type of fat in the diet may impact D_3 absorption. In in vivo studies in the rat, fatty acids with greater chain length and degree of unsaturation slowed the rate of D_3 absorption [24]. Hollander and colleagues postulated that polyunsaturated fatty acids (PUFAs) impair D_3 absorption by increasing the solubility of D_3 in the micelles and changing the partition coefficient such that the D_3 stays in the micelle and/or by increasing the size of the micelle, thereby increasing its difficulty in crossing the unstirred water layer lining the intestinal mucosa [24]. The PUFAs, linoleic and linolenic acids, were particularly effective in *decreasing* vitamin D absorption [39].

We recently undertook an investigation to determine whether intakes of different dietary fats influenced the increment in serum 25OHD following supplementation with vitamin D_3.This secondary analysis was conducted with use of data from the active treatment arm of a randomized, double-blind, placebo-controlled trial of vitamin D_3 and calcium supplementation to prevent bone loss, STOP/IT, [40]. The analysis included 152 healthy men and women, age 65 years and older, who took 700 IU of vitamin D_3 and 500 mg of calcium daily, at bedtime, for 3 years [41]. Intakes of monounsaturated fatty acid (MUFA), PUFA, and saturated fatty acids (SFA) were estimated by food frequency questionnaire after 18 months of supplementation. Change in serum 25OHD was calculated as the

Table 24.1 Intake of macronutrients, fatty acids, and total energy by tertiles of the dietary monounsaturated to polyunsaturated fatty acid ratio (MUFA/PUFA)

	MUFA/PUFA			
	<1.77	1.77–2.10	>2.10	*P*
N	50	51	51	
MUFA/PUFA ratio	1.5 ± 0.2	2.0 ± 0.1	2.5 ± 0.4	<0.001
Fat intake (g/day)	48.2 ± 17.7	57.6 ± 27.5	60.5 ± 29.2	0.043
MUFA (g/day)	18.8 ± 7.2	23.9 ± 11.8	24.4 ± 10.7	0.010
PUFA (g/day)	12.7 ± 4.8	12.3 ± 6.1	9.8 ± 4.0	0.008
SFA (g/day)	16.7 ± 6.4	21.4 ± 10.0	26.2 ± 15.6	<0.001
Protein intake (g/day)	74.3 ± 20.7	84.1 ± 47.9	83.8 ± 26.3	0.255
Carbohydrate intake (g/day)	265.0 ± 97.5	262.7 ± 128.9	246.0 ± 84.6	0.614
Energy intake (kcal/day)	1834.2 ± 526.4	1996.0 ± 936.9	1943.5 ± 667.8	0.528
Fat % of energy intake	23.7 ± 6.3	25.9 ± 5.3	27.4 ± 5.6	0.006

value at the 2-year visit minus the baseline value. Initial analyses revealed associations of both MUFA (positive) and PUFA (inverse) intake with increment in serum 25OHD. Therefore, the subjects' clinical characteristics were examined across tertiles of MUFA/PUFA ratio. There were no significant differences in age (mean age 70 years) or BMI (mean 26.8 kg/m) across the tertiles; however, there tended to me more males in the highest tertile of MUFA/PUFA intake. Mean starting serum 25OHD levels across the tertiles were similar (77.4 ± 37.7, 76.1 ± 35.7, and 76.4 ± 36.2 nmol/L). The self-reported dietary intakes of fats and other nutrients across the tertiles are shown in Table 24.1. On average, the diets contained 24–27 % of energy intake as fat. Protein intake, carbohydrate intake, and total energy intake did not differ significantly across the tertiles. Mean compliance with vitamin D supplements over the 2-year study period was high at 96.7 ± 16.6 % and did not differ significantly across the tertiles. The mean MUFA/PUFA ratios in the tertiles were 1.5, 2.0, and 2.5, respectively. Total fat intake was not significantly associated with the change in 25OHD during supplementation. However, the change in 25OHD, nmol/L, during vitamin D supplementation was positively associated with MUFA intake, g ($\beta = 2.50$, $p = 0.016$); negatively associated with PUFA intake, g ($\beta = -2.32$, $p = 0.038$); and positively associated with the MUFA/PUFA ratio ($\beta = 16.12$, $p = 0.014$), after adjustment for baseline BMI and serum 25OHD and total energy and SFA intakes. Thus, based on the adjusted model, for every unit increase in the MUFA/PUFA ratio (i.e., from 1.5 to 2.5), the mean increment in 25OHD was 16.12 nmol/L greater. The MUFA/PUFA ratio of individual diets varies widely because the MUFA/PUFA ratio of commonly consumed oils is quite variable. For example, the MUFA/PUFA ratio of olive oil is 9.0 and that of corn oil is only 0.4. This preliminary investigation does not allow us to comment on the effect of timing of fat intake in relation to taking the vitamin D supplement; however, dinner, often the largest meal of the day, would have been the closest meal, as the subjects were asked to take their study pills at bedtime. The dietary intake data also do not allow us to assess the MUFA/PUFA ratio of any specific meal. We are aware of no other evidence for or against an impact of the MUFA/PUFA ratio of the diet on the utilization of supplemental vitamin D. This study does lend support to the hypothesis that a high MUFA/PUFA ratio in the diet promotes the absorption of vitamin D, but this remains to be tested prospectively.

Summary and Conclusion

Vitamin D absorption, once thought to occur solely by passive diffusion, is far more complex and appears to involve both active and passive transport mechanisms. The relative contributions of these two mechanisms to total vitamin D absorption are not clear. Several dietary factors

may influence vitamin D absorption, including cholesterol, phytosterols, and possibly also the fat composition of the diet, specifically, the MUFA/PUFA ratio. Genetic factors are involved in vitamin D absorption. To date, genetic variants in Gc, or vitamin D-binding protein, and CYP2R1, which regulates 25-hydroxylation of vitamin D in the liver, have been shown to influence 25OHD levels and the serum 25OHD response to supplemental vitamin D. It is important to better define the dietary circumstances under which vitamin D absorption is more consistent. This would help to alleviate the costly clinical problem of multiple 25OHD tests and dose adjustments in order to have individual patients achieve the desired 25OHD level through supplementation.

References

1. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988–1994 compared with 2000–2004. Am J Clin Nutr. 2008;88:1519–27.
2. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int. 2005;16:713–6.
3. Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt P, Fuleihan GE, Josse RG, Lips P, Morales-Torres J, Yoshimura N. IOF position statement: vitamin D recommendations for older adults. Osteoporos Int. 2010;21:1151–4.
4. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino Sr RB, Ordovas JM, O'Donnell CJ, Dawson-Hughes B, Vasan RS, Booth SL. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr. 2009;63:458–64.
5. Wjst M, Altmuller J, Braig C, Bahnweg M, Andre E. A genome-wide linkage scan for 25-OH-D(3) and 1,25-(OH)2-D3 serum levels in asthma families. J Steroid Biochem Mol Biol. 2007;103:799–802.
6. Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta. 2006;372:33–42.
7. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, Nexo E. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int. 2005;77:15–22.
8. Kawakami M, Blum CB, Ramakrishnan R, Dell RB, Goodman DS. Turnover of the plasma binding protein for vitamin D and its metabolites in normal human subjects. J Clin Endocrinol Metab. 1981;53:1110–6.
9. Bu FX, Armas L, Lappe J, Zhou Y, Gao G, Wang HW, Recker R, Zhao LJ. Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. Hum Genet. 2010;128:549–56.
10. Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxilase. J Biol Chem. 2003;278:38084–93.
11. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010;376:180–8.
12. Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. Clin Biochem. 2009;42:1174–7.
13. Kamboh MI, Ferrell RE. Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. Hum Genet. 1986;72:281–93.
14. Signorello LB, Shi J, Cai Q, Zheng W, Williams SM, Long J, Cohen SS, Li G, Hollis BW, Smith JR, Blot WJ. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D levels among African Americans. PLoS One. 2011;6:e28623.
15. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW, Norris JM. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. J Clin Endocrinol Metab. 2008;93:3381–8.
16. Barger-Lux MJ, Heaney RP, Dowell S, Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos Int. 1998;8:222–30.
17. Heaney RP. The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol. 2005;97:13–9.
18. Gallagher JC, Sai A, Templin 2nd T, Smith L. Dose response to vitamin d supplementation in postmenopausal women: a randomized trial. Ann Intern Med. 2012;156:425–37.
19. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3.
20. Blum M, Dallal GE, Dawson-Hughes B. Body size and serum 25 hydroxy vitamin D response to oral supplements in healthy older adults. J Am Coll Nutr. 2008;27:274–9.
21. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity. 2012. doi:10.1038/oby.2011.404.
22. Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, Saltzman E, Dawson-Hughes B. Vitamin D(3) in fat tissue. Endocrine. 2008;33:90–4.
23. Wagner D, Hanwell HE, Schnabl K, Yazdanpanah M, Kimball S, Fu L, Sidhom G, Rousseau D, Cole DE, Vieth R. The ratio of serum 24,25-dihydroxyvitamin D(3) to 25-hydroxyvitamin D(3) is predictive of 25-hydroxyvitamin D(3) response to vitamin D(3) supplementation. J Steroid Biochem Mol Biol. 2011;126:72–7.

24. Hollander D, Muralidhara KS, Zimmerman A. Vitamin D-3 intestinal absorption in vivo: influence of fatty acids, bile salts, and perfusate pH on absorption. Gut. 1978;19:267–72.
25. Reboul E, Goncalves A, Comera C, Bott R, Nowicki M, Landrier JF, Jourdheuil-Rahmani D, Dufour C, Collet X, Borel P. Vitamin D intestinal absorption is not a simple passive diffusion: evidences for involvement of cholesterol transporters. Mol Nutr Food Res. 2011;55:691–702.
26. Lo CW, Paris PW, Clemens TL, Nolan J, Holick MF. Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. Am J Clin Nutr. 1985;42:644–9.
27. Rasmussen L, Oster-Jorgensen E, Qvist N, Hovendal CP, Kraglund K, Olsen O, de Muckadell OB S, Rehfeld J, Pedersen SA. The effects of omeprazole on interdigestive motility and early postprandial levels of gastrin and secretin. Scand J Gastroenterol. 1992;27:119–23.
28. Rasmussen L, Qvist N, Oster-Jorgensen E, Rehfeld JF, Holst JJ, Pedersen SA. A double-blind placebo-controlled study on the effects of omeprazole on gut hormone secretion and gastric emptying rate. Scand J Gastroenterol. 1997;32:900–5.
29. Piper DW, Fenton BH. pH stability and activity curves of pepsin with special reference to their clinical importance. Gut. 1965;6:506–8.
30. Masclee AA, Jansen JB, Driessen WM, Geuskens LM, Lamers CB. Effect of truncal vagotomy on cholecystokinin release, gallbladder contraction, and gallbladder sensitivity to cholecystokinin in humans. Gastroenterology. 1990;98:1338–44.
31. Goncalves A, Gleize B, Bott R, Nowicki M, Amiot MJ, Lairon D, Borel P, Reboul E. Phytosterols can impair vitamin D intestinal absorption in vitro and in mice. Mol Nutr Food Res. 2011;55 Suppl 2:S303–11.
32. Grossmann RE, Tangpricha V. Evaluation of vehicle substances on vitamin D bioavailability: a systematic review. Mol Nutr Food Res. 2010;54:1055–61.
33. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr. 2003;77:1478–83.
34. Mulligan GB, Licata A. Taking vitamin D with the largest meal improves absorption and results in higher serum levels of 25-hydroxyvitamin D. J Bone Miner Res. 2010;25:928–30.
35. Holvik K, Madar AA, Meyer HE, Lofthus CM, Stene LC. A randomised comparison of increase in serum 25-hydroxyvitamin D concentration after 4 weeks of daily oral intake of 10 microg cholecalciferol from multivitamin tablets or fish oil capsules in healthy young adults. Br J Nutr. 2007;98:620–5.
36. Maalouf J, Nabulsi M, Vieth R, Kimball S, El-Rassi R, Mahfoud Z, El-Hajj Fuleihan G. Short- and long-term safety of weekly high-dose vitamin D3 supplementation in school children. J Clin Endocrinol Metab. 2008;93:2693–701.
37. Raimundo FV, Faulhaber GA, Menegatti PK, Marques Lda S, Furlanetto TW. Effect of high- versus Low-Fat meal on serum 25-hydroxyvitamin D levels after a single oral dose of vitamin D: a single-blind, parallel, randomized trial. Int J Endocrinol. 2011;2011:809069.
38. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab. 2004;89:5387–91.
39. Hollander D. Intestinal absorption of vitamins A, E, D, and K. J Lab Clin Med. 1981;97:449–62.
40. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med. 1997;337:670–6.
41. Niramitmahapanya S, Harris SS, Dawson-Hughes B. Type of dietary fat is associated with the 25-hydroxyvitamin D3 increment in response to vitamin D supplementation. J Clin Endocrinol Metab. 2011;96:3170–4.

Strategies for Improving Vitamin D Status: Focus on Fortification

25

Timothy J. Green, Wangyang Li,
and Susan J. Whiting

Abstract

A lack of vitamin D leads to a number of adverse health outcomes, and the most widely accepted is an increased risk of osteoporotic fracture. A number of national surveys have shown that 25 hydroxyvitamin D (25OHD) concentrations, the best indicator of vitamin D status, are below even conservative cutoffs for sufficiency (<50 nmol/L). Strategies are needed to improve vitamin D status. Sunlight exposure is an important contributor to vitamin D status; however, at higher latitudes during the winter and in populations that do not receive adequate sunlight exposure for various reasons, an exogenous source of vitamin D is required. Vitamin D intake from natural food sources is low in most populations. Furthermore, supplementation, although effective and very important as method to combat vitamin D insufficiency, will not work for the entire population. Vitamin D fortification does improve vitamin D intakes and status of populations, but a greater range of food vehicles and/or greater amounts of vitamin D may be necessary to improve vitamin d status.

Keywords

Vitamin D • 25 hydroxyvitamin D • Vitamin D fortification • Vitamin D supplementation • Vitamin D intakes

Introduction

Although the prevalence of rickets and osteomalacia, the most severe consequences of vitamin D insufficiency, are reportedly on the rise, this is most likely only within certain high-risk populations [1–3]. Of greater public health concern is vitamin D insufficiency not severe enough to cause rickets or osteomalacia but could increase the risk of other adverse health outcomes. Specifically, low vitamin D status has been associated with increased falls, poor

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dental health, several autoimmune conditions, and increased risk of type 1 diabetes, as well as certain types of cancer [4]. However, the most convincing evidence links low vitamin D status with poor bone outcomes. In several clinical trials, vitamin D supplementation, with or without calcium, reduced osteoporotic fracture risk and/or improved bone mineral density [5]. While much of the evidence comes from studies in older populations, maintaining optimal vitamin D status over the entire lifetime or during critical life periods, such as childhood or adolescence, is required for optimal bone health. Furthermore, there is some evidence that even in utero vitamin D exposure influences bone mineral density later on in life [6].

A high prevalence of vitamin D insufficiency has been documented in population-based surveys in many countries across diverse geographical conditions [7–11]. Strategies are required to improve the vitamin D status of these populations, such as increasing UV light exposure, providing supplementation, and improving dietary intake through food fortification or vitamin D-rich foods. In this chapter, the advantages and limitations of the various strategies will be discussed with emphasis on food fortification, following a brief review of current vitamin D intake recommendations and vitamin D status in select populations.

Vitamin D Status Is Suboptimal

Circulating 25 hydroxyvitamin D (25OHD) is the best indicator of vitamin D status as it represents vitamin D obtained from both UV skin synthesis and exogenous sources (diet and supplements) over a prolonged period of time. A circulating 25OHD of at least 30 nmol/L is needed to prevent risk of rickets and osteomalacia in the population [12]. However, there is a lack of consensus on the concentration of 25OHD required for optimal health [13].

The Institute of Medicine (IOM), which sets dietary reference intakes in Canada and the United States, has recently recommended that maintaining a serum concentration of 25OHD of approximately 50 nmol/L is desirable in all life-stage groups [12]. In setting this recommendation, the IOM only considered bone health because the evidence linking vitamin D to other outcomes was deemed insufficient. A number of individuals and groups felt that the IOM should have considered non-bone outcomes and set the desirable serum 25OHD concentration much higher [13]. Nevertheless, what is clear is that even using a "conservative" cutoff of 50 nmol/L, a substantial proportion of populations in many countries are vitamin D insufficient.

Serum concentrations of less than 50 nmol/L, often much less, have been documented in surveys using convenience samples from high-risk groups such as institutionalized elderly [14], veiled women [15], and dark-skinned immigrants living in northern countries [16]. Perhaps more surprising is the high levels of vitamin D insufficiency described in populations living near the equator such as Malaysia, Indonesia, and Southern India [17]. However, the most convincing evidence of widespread vitamin D insufficiency comes from large national population representative surveys in Western countries (Table 25.1). The prevalence of vitamin D insufficiency (25OHD <50 nmol/L) ranged from 26 % in Canadian Health Measures Survey (2007–2009; 6–79 years) [8] to ~55 % (19–64 years) in the United Kingdom National Diet and Nutrition Survey (2001–2002) [11]. Nearly a quarter of Britons had a 25OHD less than 30 nmol/L, indicating that national averages of vitamin D insufficiency obscure disparities that exist within populations.

Darker-skinned ethnic groups tend to have lower 25OHD concentration than lighter-skinned groups. In the US National Health and Nutrition Examination Surveys (2001–2006), 73 and 21 % of African Americans and Whites (≥1 year), respectively, had 25OHD less than 50 nmol/L [7]. Similarly, in the New Zealand 1997 National Nutrition Survey (>15 years), nearly 80 % of Pacific People but only 40 % of Whites had a serum 25OHD less than 50 nmol/L [10].

There are also large seasonal differences in 25OHD in some countries. In the New Zealand survey, a much greater percentage of the population had a serum 25OHD less than

Table 25.1 Vitamin D status reported in national surveys in Canada, New Zealand, United Kingdom, and the United States

	Canadian Health Measures Survey [8]	National Nutrition Survey, New Zealand [10]	National Diet and Nutrition Survey Adults, UK [11]	National Adult Nutrition Survey, Ireland [9]	National Health and Nutrition Examination Surveys, US [7]
Year of survey	2007–2009	1997	2001–2002	2008–2010	2001–2006
Method of analysis	Diasorin Chemiluminescence	Diasorin RIA	Diasorin RIA	Immunodiagnostic Systems Limited ELISA	Diasorin RIA
Age range (year)	6–79	≥15	19–64	18–84	≥1
Mean (nmol/L)	67	50	~50	60	60
<30 nmol/L (%)	5	12	~23	6.7	8
<50 nmol/L (%)	26	48	~55	40.1	32

50 nmol/L in winter than summer. The mean difference in 25OHD, in women during the winter compared with the summer months was over 30 nmol/L (Fig. 25.1). However, vitamin D insufficiency exists in all age and gender groups. Remarkably, the highest prevalence of vitamin D insufficiency in the Canadian Health Measures Survey (2007–2009) was males 20–39 years at 35 % (Fig. 25.2). Only 16 % of females 60–79 years were vitamin D insufficient; a group often thought to be at highest risk [8].

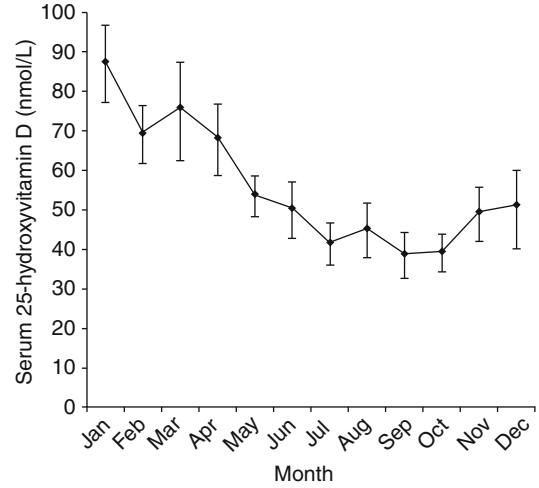

Fig. 25.1 Mean (99 % CI) serum 25-hydroxyvitamin D concentrations by month of New Zealand women ($n = 1{,}604$) who participated in the 1997 National Nutrition Survey (Reprinted from Rockell et al. [10]. With permission from Springer Science + Business Media)

Sunlight Exposure

An approach to improve the vitamin D status of the population is to recommend daily UV exposure to sunlight. It is well established that exposing people to UV from sunlight is an effective means of increasing 25OHD concentrations [18]. However, this would be a confusing message for the public as it is at odds with recommendations from public health agencies in most countries to restrict sunlight exposure to reduce the risk of melanoma [19, 20]. Furthermore, recommendations for duration of sun exposure would need to vary by the amount of skin exposed, season, latitude, and skin color; therefore, guidelines would need to be highly individualized.

The complexity of this message is illustrated by a position statement on vitamin D and adult bone health that recommends sunlight exposure times (hands, face, and arms) to synthesize the equivalent of a 1,000 IU of vitamin D in fair-skinned people (Table 25.2) in various parts of Australia and New Zealand in the summer and winter. In the summer

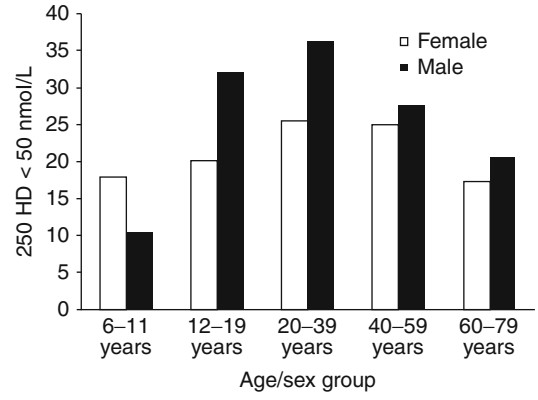

Fig. 25.2 Percentage of population with 25 hydroxyvitamin D less than 50 nmol/L by age and sex in the Canadian Health measures Survey (2007–2009) (Based on data from [8])

Table 25.2 Recommended sun exposure times (minutes) for Australians and New Zealanders with moderately fair skin[a] at different times of day

	July–August		December–January
Region, latitude	10:00 or 14:00	At 12:00	10:00 or 14:00
New Zealand			
Christchurch, 43ºS	49–97	40	6–9
Auckland, 36ºS	30–47	24	6–8
South Australia			
Hobart, 42ºS	40–47	29	7–9
Melbourne, 37ºS	32–52	25	6–8
Adelaide, 34ºS	25–38	19	5–7
Sydney, 33ºS	26–28	16	6–8
Central Australia			
Perth, 31ºS	20–28	15	5–6
Brisbane, 27ºS	15–19	11	6–7
Northern Australia			
Townsville, 19ºS	9–13	7	5–7
Cairns, 16ºS	9–12	7	6–7

Based on data from the Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis New Zealand Diamond et al. [21]
[a]Exposure times for people with highly pigmented skin would be three to four times greater

(Dec to Jan), only 5–10 min of UV exposure was required in all regions at 10 a.m. or 2 p.m., whereas in the winter (July–Aug) at 12 p.m., times ranged from 7 min in Cairns to 40 min in Christchurch. The authors of the position statement also indicate that exposure times would be three to four times greater in darker pigmented people [21].

Moreover, in countries at higher latitudes (i.e., Canada, United Kingdom, and parts of the United States), no amount of time in the sun would be sufficient for adequate vitamin D synthesis in the winter. Covering up for religious or cultural beliefs, sun avoidance for cosmetic reasons, as well as indoor lifestyles may explain poor vitamin D status in countries with abundant sunlight [17]. Exogenous sources of vitamin D are required if adequate vitamin D status is to be achieved at the population level in countries with climates, geography, religions, cultures, and lifestyles not conducive to increased sun exposure.

Recommended Vitamin D Intakes

A number of governmental and professional organizations have issued recommendations for vitamin D intake, and there is considerable controversy as to the most appropriate recommendations [22–25]. The amounts recommended have varied considerably, in part, because of uncertainties as to the optimal 25OHD they were intended to achieve. In setting the recommended dietary intake for Canadians and Americans, the IOM estimated the amount of vitamin D to achieve a 25OHD of 50 nmol/L in almost all individuals in the population. This level of intake known as the Recommended Dietary Allowance (RDA) was set at 600 IU/day for almost all life-stage groups. Two exceptions are as follows: the RDA for older adults (>70 years) was set at 800 IU and an Adequate Intake (AI)[1] for infants up to 12 months of age was set at 400 IU/day.

Vitamin D Supplementation

Many clinical trials have confirmed the efficacy of vitamin D supplementation at increasing 25OHD concentrations [26]. Vitamin D

[1]AI is used when an RDA cannot be developed due to insufficient data and is usually based on average intake level based on observed or experimental intakes. In the case of infants, the AI is normally set as the amount provided by exclusive breastfeeding.

supplement use is also associated with higher 25OHD concentrations at the population level. In the CHMS (2007–2009), Canadians (6–79 years; $n = 1{,}274$) who took supplements had a much higher mean (95 % CI) 25OHD than those who did not, especially in the winter months [74 (66, 83) versus 59 (53, 64) nmol/L] [8]. In the NHANES 2003–2006, 37 % of the US population reported taking a vitamin D containing supplements, and those who did were more likely to meet their vitamin D requirements [27].

Vitamin D supplementation is likely the only means to achieve recommended intakes in some groups. The IOM recommendations are intended for healthy people; therefore, they do not apply to those with certain diseases, such as Crohn's disease, cystic fibrosis, or morbid obesity, who have higher requirements [4]. Also, public health authorities worldwide recommend exclusive breastfeeding for the first 6 months of life for healthy term infants, and while breast milk is the best food for an infant, it does not generally supply adequate amounts of vitamin D [28]. As such, the American Academy of Pediatrics (AAP) [29] and Health Canada [28], as well as several European countries (Norwegian Food Safety Authority: Matportalen (The Food Portal), 2006, personal communication; Swedish National Food Administration, 2006, personal communication), recommend that breastfed infants receive a daily vitamin D supplement, usually 400 IU.

Data from the Infant Feeding Practices Study II (2005–2007) [30] suggest that US vitamin D supplementation rates are low; 43 % of infants were breastfed at 2 months and of these only 10 % were receiving a vitamin D supplement. In Canada, rates of vitamin D supplementation of breastfed babies appear to be much higher. In a survey conducted of infants in Vancouver, British Columbia who were being breastfed at 2 months, greater than 90 % were being supplemented with vitamin D [31]. It should be noted that the AAP only began recommending infant supplementation in November 2008, whereas the Canadian recommendation has been present in some form since 1967. The AAP also recommends that children and adolescents, who do not obtain 400 IU/day from diet, should take a 400 IU/day vitamin D supplement [32]. Some countries and organizations recommend vitamin D supplementation during pregnancy [22, 33]. It is not clear to what extent this advice is being followed.

Canada's Food Guide (2007) recommends that Canadians over the age of 50 years take a daily vitamin supplement providing 400 IU of vitamin D [34]. The reason for this is that the eating pattern recommended for this age group provides around 240, 400–600 IU/day short of the recommended intake for this age group. The uptake of this message has been mixed. In the CHMS (2007–2009) study, about 50 % of Canadians 60–79 years reported taking vitamin D supplements. It was recently reported that almost 60 % of people 50 years and over living in British Columbia had taken a supplement containing vitamin D in the month prior to the survey (Fall 2008). The median dose was 400 IU/day and the majority of people were receiving vitamin D as part of a multivitamin supplement [8]. It is noteworthy, that Health Canada issued this recommendation for adults >50 years only as the IOM's older 1997 recommendations for those under 50 years was only 200 IU/day, achievable through diet [34]. Given that the RDA is now 600 IU/day for those less than 50 years and that few Canadians meet this recommended intake from diet alone, it will be interesting to observe whether Health Canada recommends all Canadians take a supplement.

So is universal vitamin D supplementation by the population the answer to achieving adequacy in vitamin D? Currently, supplement users tend to be female, white, older, better educated, and have a higher income than nonusers [35]. Greater supplement use is certainly the reason that the prevalence of vitamin D insufficiency is lower in older women than middle-aged and younger men in Canada [36]. But several barriers to widespread usage of vitamin D supplements hinder universal supplementation. These barriers include the cost of supplements and the need for public health education, as well as long-term compliance [37].

Although vitamin D supplements are relatively inexpensive, cost will be a barrier for some individuals and families [37]. Extensive public

health education about the benefits of vitamin D supplements, particularly targeting minorities and the poor, would be required. Incidentally, the general failure of public health campaigns to increase folic acid supplement use in women of childbearing age in North America in order to prevent birth defects casts doubt on the effectiveness of such an approach [38]. Finally, the safety concerns related to overdose possibilities, especially in children, need to be considered. As a result of reports of infant overdosing with vitamin D in the USA, the Food and Drug Administration issued a warning of the potential risk of liquid vitamin D overdose in infants [39].

So although supplementation remains an important means of improving vitamin D status, it is not practical or feasible for the general public as a whole. Other alternatives need to be considered.

Vitamin D Found Naturally in Food

Vitamin D is found naturally in some foods but is also added as a fortificant. The food composition databases for vitamin D are incomplete and dissimilar analytical approaches yield very different estimates of vitamin D content. Vitamin D in foods is found as vitamin D_2 (ergocalciferol), D_3 (cholecalciferol), and 25OHD. Vitamin D_2 is found in UVB-irradiated mushrooms and yeast, whereas vitamin D_3 comes from animal sources. Vitamin D_2 is thought to be less effective than D_3 at increasing 25OHD concentrations. Vitamin D_3 is the form of the vitamin usually added to foods, but vitamin D_2 can also be used [26]. Animal products can contain significant amounts of 25OHD, which has five times the activity of vitamin D_3, but is usually ignored in food composition tables [40]. Health Canada has started to update its food composition to reflect this. For example, pork contains just 24 IU of vitamin D_3/100 g but also contains 30 IU of 25OHD ($30 \times 5 = 150$ IU), giving a total vitamin D activity of 174 IU/100 g [41].

Fatty fish such as salmon and sardines are high sources of naturally occurring vitamin D. Interestingly, within a species of fish, considerable variation appears to exist in vitamin D content. Lu et al. [42] reported that farmed salmon contains only 860 IU per 100 g, whereas wild salmon contains 3,500 IU/per 100 g. This difference is likely due to differing diets of the fish. Aside from fish, eggs are another source of natural vitamin D at 40 IU per large egg, but this increases to 115 IU when its 25OHD content is considered [41]. Strategies are being developed, sometimes termed bio-addition, to enhance the natural vitamin D content of foods such as supplementing hens with vitamin D to increase the vitamin D content of their eggs as well as exposing mushrooms to UV light postharvest [43, 44].

Can sufficient vitamin D be obtained from natural food sources? Certainly, indigenous people in the North, eating traditional diets, can achieve vitamin D intakes from natural sources that exceed IOM recommendations. Inuit, Denis/Métis, and Yukon, indigenous [29] people in Canada's North (>60° N), had significantly higher vitamin D intakes on days they ate traditional foods compared with days they did not (Table 25.3) [45]. Vitamin D-rich traditional foods varied by group but included salmon, trout, arctic char, seal, and whale meat. In Greenland (>60°N), individuals who ate mostly traditional diets were more likely to have a 25OHD greater than 50 nmol/L than those who ate mostly Western diets (~80 % versus 22 %) [46]. In areas where fish consumption is high, vitamin D found naturally in food also contributes significantly to vitamin D intake. In Norway, fish provides 60–70 IU/day of vitamin D [47], and in a study of older Japanese women, fish provided 256 IU/day [48]; however, both are well short of the 600–800 IU/day recommended by the IOM.

Current vitamin D intake from natural food sources in the USA and UK, as well as most other Western countries, is well below the IOM's recommended intakes. In the US NHANES 1999–2000 [49], vitamin D intake from natural sources did not exceed 75 IU/day for any age group or gender. Similarly, in the UK NDNS (2000–2001) [50], median vitamin D intake from natural sources did not exceed 80 IU/day for any age group surveyed (19–64 years). These estimates do not include the contribution of 25OHD from

Table 25.3 Vitamin D intake on days with and without traditional food for Yukon, Dene/Métis, and Inuit adults and Yukon and Dene children (>60°N)

	Days with TF		Days without TF	
	N	Vitamin D, IU	*N*	Vitamin D, IU
Adults[a]				
Yukon	410	292 ± 24[b]	387	84 ± 28[c]
Dene/Métis	661	316 ± 36[b]	346	140 ± 52[c]
Inuit	968	1,004 ± 52[b]	632	344 ± 68[c]
Children[a]				
Yukon and Dene	58	128 ± 16	40	100 ± 20

Based on data from [45]
TF traditional food
[a]Significant differences in nutrient intakes with TF and without TF are represented by the presence of different superscript in the same row (b, c). Absence of superscripts means results are not significantly different. Adults, $P < 0.01$; children $P < 0.05$

animal sources, but even if they did, vitamin D intakes would remain low. Bio-addition holds some promise for increasing vitamin D levels; however, shifting eating patterns to greater fish and egg consumption to meet requirements is not practical.

Vitamin D Fortification of Food

Food fortification has been used as a method of improving population micronutrient intakes for many years, especially in Canada and the United States. Food fortification can be mandatory or discretionary. With mandatory fortification, the government legislates the addition of nutrients to a food or food group; an example is the folic acid added to enriched grains in Canada and the United States. Mandatory fortification is the best method of improving intakes at the population level because the nutrient is supplied in a continuous and passive manner.

With discretionary fortification, the government legislates what foods may be fortified and to what level, but the decision to fortify it is left with the food manufacturers. Often there is variable uptake by manufacturers; moreover, the foods that are fortified are usually the more expensive premium brands. On the other hand, discretionary fortification preserves consumer choice. This is an important consideration, especially outside Canada and the United States, where mandatory fortification is sometimes described as "mass medication" of the population [51, 52].

In Canada, milk and milk alternatives (40 IU/100 ml), as well as margarine (530 IU/100 g), must be fortified with vitamin D. In the United States, only milk in which the label claims the milk is fortified needs to be fortified with vitamin D (40 IU/100 g); in practice, this represents over 90 % of the milk sold [53]. In the United Kingdom [54] and Australia [55], margarine (~320 IU/100 g) must be fortified with vitamin D. Vitamin D fortification of margarine was introduced in the UK in 1945. This was done because margarine replaced butter in the diet, but fortified margarine contains significantly more vitamin D than butter (25–50 IU/100 g). The number of foods that may be fortified on a voluntary basis varies by country and is extensive, especially in the United States (Table 25.4).

Fortified foods make up the majority of vitamin D intake in Canada and the United States. In the US NHANES 1999–2000 [49], vitamin D intake from fortified food makes up greater than 60 % of vitamin D intake for each age group and gender. In fact, fortified foods make up greater than 75 % of vitamin D intake in children and adolescent in the United States (Fig. 25.3). In the 2004 Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) [56], fortified dairy contributed to nearly all the vitamin D intake of children 1–8 years. In Canadians greater than 8 years, fortified dairy and other fortified foods make up greater than 50 % of vitamin D intake (Fig. 25.4).

The efficacy of vitamin D fortification at increasing 25OHD concentrations has been

Table 25.4 Foods that may be fortified with vitamin D on a discretionary basis in select countries

United States [72]	Canada [53, 73]
Enriched farina	Goat's milk
Ready-to-eat breakfast cereals	Fortified plant-based beverages
Enriched rice	Fruit-flavored drinks
Enriched cornmeal products	Cheese (using fortified milk)
Enriched noodle products	Yogurt (using fortified milk)
Enriched macaroni products	Meal replacements
Fluid milk	Formulated liquid diets
Acidified milk	Some egg products
Cultured milk	*Australia/New Zealand* [55]
Concentrate milk	Dried milk
Nonfat dry milk, A and D fortified	Modified milks and skim milk
Evaporated milk, fortified	Cheese and cheese products
Dry whole milk	Yogurt (with or without other foods)
Yogurt	Dairy desserts containing no less than 3.1 % m/m milk protein
Low-fat yogurt	Butter
Nonfat yogurt	Edible oil spreads
Cheese	Analogues of yogurt and dairy desserts containing no less than 3.1 % m/m protein derived from legumes
Margarine	Analogues of cheese containing no less than 15 % m/m protein derived from legumes
Calcium-fortified 100 % fruit juice	Beverages containing no less than 0.3 % m/m protein derived from cereals
Calcium-fortified fruit juice drinks	Formulated Beverages
Soy-protein meal-replacement beverage	
Meal-replacement and other-type bars	
Cheese and cheese products	

demonstrated for a number of foods including milk [57, 58], orange juice [59], cheese [60], and bread [61–63]. In a recent systematic review of 16 randomized trials of fortified foods (mainly milk), all but two showed a significant impact on 25OHD [64]. Higher dose and latitude, as well as lower baseline 25OHD, resulted in a greater increase in 25OHD. The authors estimated that for each 40 IU of additional vitamin D from a fortified food corresponded to a 1.2 nmol/L increase in 25OHD.

Consumption of fortified foods leads to greater 25OHD concentrations at the population level. Despite being at a higher latitude and much colder in the winter, the mean difference in 25OHD between winter and summer months was 9 nmol/L in the Canadian Health Measures Survey (excluding supplement users) [8] compared with 18 nmol/L in the 1997 New Zealand National Nutrition Survey [10]. The most likely explanation for this is that Canada's greater food fortification compared to that of New Zealand (virtually none in 1997) is protecting Canadians against low 25OHD, especially in the winter months.

Within Canada, individuals in the CHMS (6–79 years) who consumed >1 serving of milk per day had a higher mean (95 % CI) 25OHD [75.0 (72.5, 77.5)] than those who consumed one serving a day [68.1 (65.3, 71.0)], who in turn had a higher 25OHD than those who consumed less than one serving per day [62.7 (60.5, 64.9)]. In the wintertime, 25OHD concentrations were 66.0, 59.8, and 53.7 in these groups, respectively, excluding supplement users who are more likely to be milk consumers [8] (Whiting, unpublished).

The Need for Greater Vitamin D Fortification

Despite fortification in Canada and the United States, mean total vitamin D intakes still fall short of IOM recommendations of 600 IU/day. In the US NHANES [49], the group with the highest mean vitamin D intake from food was non-Hispanic white males (9–18 years) at 260 IU/day. Even with supplements included, the highest mean intake achieved was ~400 IU/day in older non-Hispanic white females (51+ years).

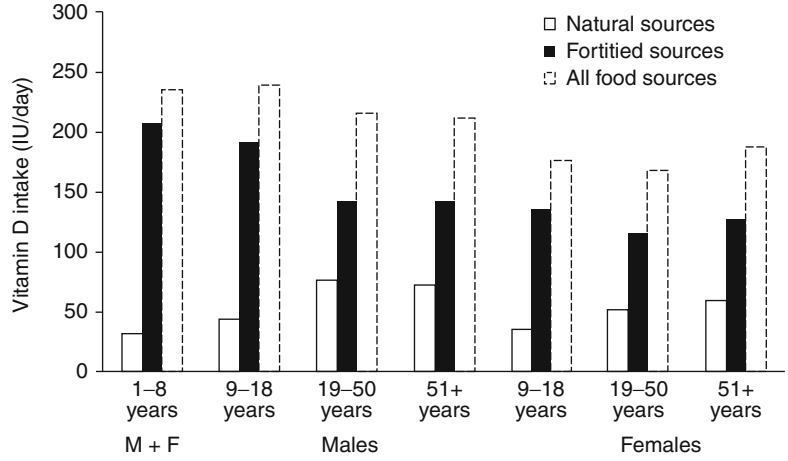

Fig. 25.3 Vitamin D intake natural sources of vitamin D, fortified sources, and total vitamin D intakes in the National Health by age and sex in the US National Health and Nutrition Examination Survey (1999–2000) (Based on data from [49])

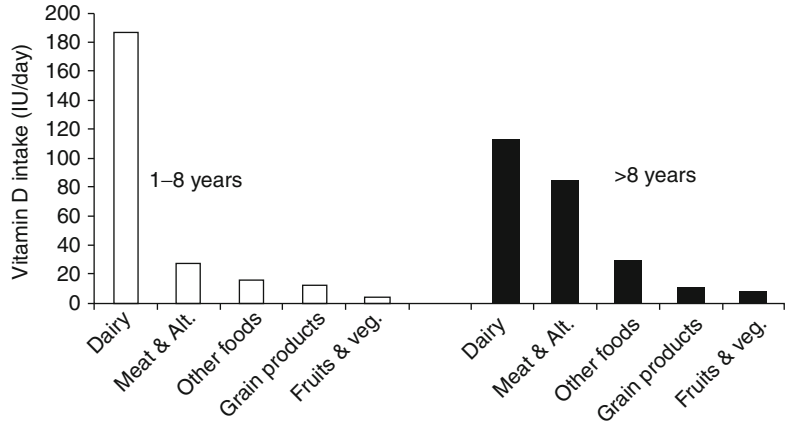

Fig. 25.4 Vitamin D intake by food group in the Canadian Community Health Survey (Based on data from [56])

Data from the 2004 CHMS [8] indicates that Canadian total vitamin D intakes from food are higher than those of Americans, although differences in the food composition database may partially explain the difference. Nevertheless, the mean intake was only 250 IU/day, with males 9–18 years having the highest mean intakes (300 IU/day) and females 51–70 years having the lowest intakes (200 IU/day). In the UK, where there is less fortification, mean intake from food sources was 170 IU/day for men and 150 IU/day for women. Almost the entire population was receiving less than 360 IU/day even when vitamin D supplements were considered [50]. Clearly, vitamin D intakes need to increase substantially in all countries if most of the population is to achieve intakes of 600 IU, the amount recommended by the IOM.

Based on the data presented, we contend that greater fortification is the best way to improve vitamin D intakes at the population level. This could be done by either increasing the amount of vitamin D added to foods or increasing the range of foods fortified with vitamin D. The best fortification strategy would maximize the number of individuals exceeding 600 IU/day while limiting the number of individuals reaching an undesirable intake. The IOM sets an upper limit (UL) of 4,000 IU/day for vitamin D [12]. The UL is the highest average daily intake of nutrient that is likely to pose no risk of adverse effects to almost all individuals in the general population [12]. Fortification tends to increase the intake of those who eat more (i.e., young males), rather than those who eat less (i.e., older women). Fortunately, there is a greater than sixfold

Table 25.5 Theoretical mean (95 % CI) vitamin D intakes of Canadians resulting from the mandatory fortification of white wheat flour[a]

	Predicted mean vitamin D intake IU/day (95 % CI)	
Age (year)	Male	Female
14–18	889 (321, 1,456)	658 (168, 1,147)
19–30	836 (276, 1,395)	542 (155, 930)
31–50	738 (209, 1,266)	516 (125, 906)
51–70	587 (47, 1,127)	453 (68, 838)
>70	507 (80, 934)	400 (38, 762)

[a]Based on data from Canadian Community Health Survey (CCHS 2.2) [56] and Shakur et al. [69] Women >70 years assigned an intake of 400 IU/day

difference between the recommended intake and the UL that can accommodate this disparity.

Mandatory fortification is favored because if the food vehicle and the amount of vitamin D added are chosen correctly, it does not discriminate against the disadvantaged in the population. How does one choose the best food vehicle for vitamin D fortification? It is best done for each country by modeling vitamin D fortification with population-representative food intakes. By doing this, it is possible to determine what percentage of the population will achieve 600 IU/day and who is at risk for exceeding the UL. Therefore, the best vehicle will be a food that is eaten daily by most of the population and in sufficient quantity.

In New Zealand, we used the 1997 National Nutrition Survey to model the best fortification vehicle for a range of nutrients [65]. Although milk was consumed by 92 % of the population on any given day, adults consumed only small amounts (most likely in coffee and tea), while children and adolescents consumed much greater amounts. Greater coverage could be achieved by fortifying other dairy foods such as yogurt and cheese in addition to milk. Ready-to-eat breakfast cereals were consumed by only 26 % of the New Zealand population with young people consuming greater amounts. Bread, and to an even greater extent wheat flour, was the best vehicle to reach the entire population and in sufficient quantity. Adding vitamin D to flour during milling would result in vitamin D ending up in the many foods that contain flour, not just bread. A similar approach has proposed by others [66]. There are technical issues that would need to be overcome before flour could be fortified.

However, such an approach was used in Canada and the United States to reduce birth defects, where the government mandated the addition of folic acid to white wheat flour and as a result substantially increased folic acid intakes of the population [67, 68]. The impact of fortification of white wheat flour on folic acid intakes of the Canadian population was recently estimated using data from the 2004 Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) [69]. From that we estimated the theoretical intakes of vitamin D if Canada fortified white flour in the various age and gender groups. This was done by setting the desired intake of vitamin from enriched grains at 400 IU/day for women 50–70 years. Women in this age group receive the least amount of vitamin D from food and this is the amount required to bring them up to a mean intake of 600 IU/day. As shown in Table 25.5, if women (>70 years) were to receive on average 400 IU/day from white wheat flour, we would predict that males 14–18 years (the highest group) would receive 899 IU/day with only the top 5 % consuming more than 1,456 IU/day, well below the UL. Unfortunately, the lowest 5 % of women (>70 years) would be receiving 80 IU, demonstrating the limitation of fortification. White flour is just an example of a potential fortification vehicle for vitamin D and given that government policy in most countries is to encourage whole grain consumption over refined grains other vehicles should be considered [34, 70].

Conclusion

Vitamin D insufficiency is associated with a number of adverse health outcomes, most widely accepted is the increased risk of

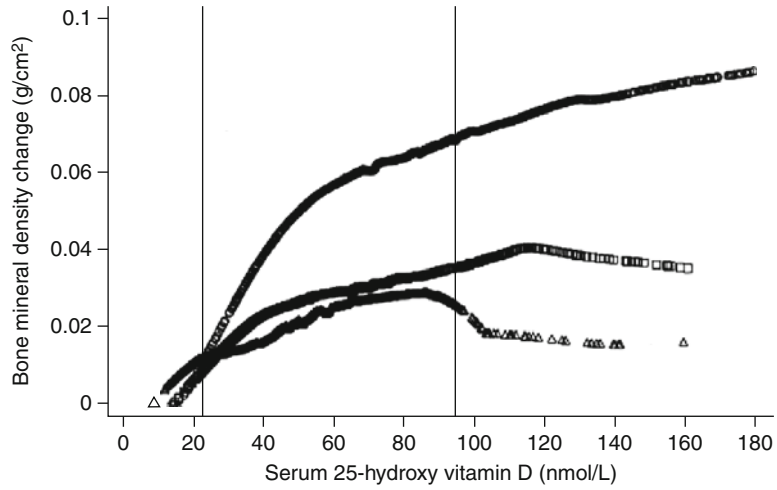

Fig. 25.5 Regression plot of bone mineral density by 25 hydroxyvitamin D concentration in younger adults (20–49 years) in the National Health and Nutrition Examination Survey III. *Circles* represent whites, *squares* represent Mexican Americans, and *triangles* represent blacks (Reprinted from Bischoff-Ferrari et al. [71]. With permission from Elsevier)

osteoporotic fracture. A number of national surveys have shown that 25OHD concentrations are below even the most conservative cutoffs for sufficiency in a substantial proportion of populations. Sunlight exposure is an important contributor to vitamin D status; however, at higher latitudes during winter and in populations that do not receive adequate sunlight exposure for various reasons, an exogenous source of vitamin D is required. Vitamin D intake from natural food sources is not sufficient; furthermore, supplementation, although effective and very important as a method to combat vitamin D insufficiency, will not work for everyone in the population.

Vitamin D fortification does improve vitamin D intakes and status of populations, but a greater range of food vehicles and/or greater amounts of vitamin D are necessary to make this option viable. However, no amount of fortification that would be acceptable to government regulators, and perhaps the public, would ensure that the entire population achieves the recommended vitamin D intakes of the IOM. Achieving 25OHD concentrations of 70–100 nmol/L, recommended some vitamin D proponents, through fortification is impossible. It must be remembered that cutoffs do not define who is healthy and who is not, but rather there is a continuum of risk between vitamin D intake, 25OHD, and adverse outcomes. Bischoff-Ferrari et al. [71]. examined the association between 25OHD and bone mineral density in younger adults (20–49 years) in the National Health and Nutrition Examination Survey III. Although there was an association between bone mineral densities up to 93 nmol/L, the steepest part of the slope was between 10 and 50 nmol/L suggesting that even small increases in 25OHD concentration will improve bone outcomes. (Fig. 25.5)

References

1. Primary vitamin D deficiency in adults. Drug Ther Bull. 2006;44:25–9.
2. Ward LM, Gaboury I, Ladhani M, Zlotkin S. Vitamin D–deficiency rickets among children in Canada. Can Med Assoc J. 2007;177:161–6.
3. Tserendolgor U, Mawson JT, MacDonald AC, Oyunbileg M. Prevalence of rickets in Mongolia. Asia Pac J Clin Nutr. 1998;7:325–8.
4. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.
5. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ. 2009;339:1–11.
6. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet. 2006;367:36–43.
7. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS Data Brief. 2011; 59:1–8.

8. Langlois K, Greene-Finestone L, Little J, Hidiroglou N, Whiting S. Vitamin D status of Canadians as measured in the 2007 to 2009 Canadian Health Measures Survey. Health Rep. 2010;21:47–55.
9. Cashman KD, Muldowney S, Breige M, Anne N, FitzGerald AP, Kiely M, et al. Vitamin D status of Irish adults: findings from the National Adult Nutrition Survey. Br J Nutr. 2012. doi:10.1017/S0007114512003212 [Epub ahead of print].
10. Rockell JE, Skeaff CM, Williams SM, Green TJ. Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older. Osteoporos Int. 2006;17:1382–9.
11. Ruston D, Hoare J, Henderson L, Gregory J, Bates CJ, Prentice A, et al. The National Diet & Nutrition Survey: adults aged 19 to 64 years (nutritional status, blood pressure and physical activity). National Diet and Nutrition Survey. 2004;4:1–156.
12. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, D.C.: National Academy Press; 2010.
13. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006; 84:18–28.
14. Liu BA, Gordon M, Labranche JM, Murray TM, Vieth R, Shear NH. Seasonal prevalence of vitamin D deficiency in institutionalized older adults. J Am Geriatr Soc. 1997;45:598–603.
15. Serenius F, Elidrissy AT, Dandona P. Vitamin D nutrition in pregnant women at term and in newly born babies in Saudi Arabia. J Clin Pathol. 1984;37:444–7.
16. Henriksen C, Brunvand L, Stoltenberg C, Trygg K, Haug E, Pedersen JI. Diet and vitamin D status among pregnant Pakistani women in Oslo. Eur J Clin Nutr. 1995;49:211–8.
17. Green TJ, Venn BJ. Vitamin D in Asia. In: Holick MF, editor. Vitamin D physiology, molecular biology, and clinical applications. 2nd ed. New York: Springer; 2010. p. 563–87.
18. Holick MF. Vitamin D, deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. Nat Rev Endocrinol. 2011;7:73–5.
19. SunSmart [Internet]. Victoria: Cancer Council Victoria. 2012. Available from: http://www.sunsmart.com.au/default.asp. Updated 5 Sept 2012; cited 5 Sept 2012.
20. Lucas R, McMichael T, Smith W, Armstrong B. Solar ultraviolet radiation: global burden of disease from solar ultraviolet radiation, vol. Environmental Burden of Disease Series, No. 13. Switzerland: World Health Organization; 2006.
21. Diamond TH, Eisman JA, Mason RS, Nowson CA, Pasco JA, Sambrook PN, et al. Vitamin D and adult bone health in Australia and New Zealand: a position statement. Med J Aust. 2005;182:281–5.
22. Canadian Pediatric Society. Vitamin D supplementation: recommendations for Canadian mothers and infants. Paediatr Child Health. 2007;12:583–9.
23. Osteoporosis Society of Canada [Internet]. Toronto: Osteoporosis Canada. 2011. Available from: http://www.osteoporosis.ca/. Updated 2002; cited 6 Sept 2012.
24. Canadian Cancer Society [Internet]. Toronto: Canadian Cancer Society. 2012. Available from: http://www.osteoporosis.ca/. Updated 2007; cited 6 Sept 2012.
25. National Osteoporosis Foundation [Internet]. Washington, D.C.: National Osteoporosis Foundation; 2012. Available from: http://www.nof.org. Updated 2008; cited 6 Sept 2012.
26. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;95:1357–64.
27. Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, et al. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr. 2010;140:817–22.
28. Canada H. Vitamin D supplementation for breastfed infants: 2004 Health Canada recommendation. Ottawa: Health Canada; 2004.
29. American Academy of Pediatrics Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics. 2005;115:496–506.
30. Perrine CG, Sharma AJ, Jefferds MED, Serdula MK, Scanlon KS. Adherence to vitamin D recommendations among US infants. Pediatrics. 2010;125:627–32.
31. Crocker B, Green TJ, Barr SI, Beckingham B, Bhagat R, Dabrowska B, et al. Very high vitamin D supplementation rates among infants aged 2 months in Vancouver and Richmond, British Columbia, Canada. BMC Public Health. 2011;11:905–12.
32. Gartner LM, Greer FR. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics. 2003;111:908–10.
33. Department of Health. Dietary reference values for food energy and nutrients for the United Kingdom. Report on health and social subjects 41. London: HMSO; 1991.
34. Canada's Food Guide [Internet]. Ottawa: Health Canada; 2012. Available from: http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php. Updated 9 July 2012; cited 6 Sept 2012.
35. Greger JL. Dietary supplement use: consumer characteristics and interests. J Nutr. 2001;131:1339S–43.
36. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. The vitamin D status of Canadians relative to the 2011 dietary reference intakes: an examination in children and adults with and without supplement use. Am J Clin Nutr. 2011;94:128–35.
37. Whiting SJ, Vatanparast H, Taylor JG, Adolphe JL. Barriers to healthful eating and supplement use in lower-income adults. Can J Diet Pract Res. 2010; 71:70–6.
38. Rofail D, Colligs A, Abetz L, Lindemann M, Maguire L. Factors contributing to the success of folic acid public health campaigns. J Public Health. 2012; 34:90–9.

39. Food and Drug Administration Consumer Health Information [Internet]. Infant overdose risk with liquid vitamin D. Silver Spring: Food and Drug Administration; 2012. Available from: http://www.fda.gov/consumer. Updated 2012; cited 10 June 2011.
40. Cashman KD, Seamans KM, Lucey AJ, Stöcklin E, Weber P, Kiely M, et al. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. Am J Clin Nutr. 2012;95:1350–6.
41. Health Canada. Nutrient intakes from food, provincial, regional and national summary data tables. Ottawa: Health Canada Publications; 2008.
42. Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, Berkowitz R, et al. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J Steroid Biochem Mol Biol. 2007;103:642–4.
43. O'Mahony L, Stepien M, Gibney MJ, Nugent AP, Brennan L. The potential role of vitamin D enhanced foods in improving vitamin D status. Nutrients. 2011; 3:1023–41. doi:10.3390/nu3121023.
44. Kristensen HL, Rosenqvist E, Jakobsen J. Increase of vitamin D2 by UV-B exposure during the growth phase of white button mushroom (Agaricus bisporus). Food Nutr Res. 2012;56. doi:10.3402/fnr.v56i0.7114.
45. Kuhnlein HV, Receveur O. Local cultural animal food contributes high levels of nutrients for Arctic Canadian indigenous adults and children. J Nutr. 2007;137: 1110–4.
46. Andersen S, Laurberg P, Hvingel B, Kleinschmidt K, Heickendorff L, Mosekilde L. Vitamin D status in Greenland is influenced by diet and ethnicity: a population-based survey in an Arctic society in transition. Br J Nutr. 2012. doi:10.1017/S0007114512002097 [Epub ahead of print].
47. Jorde R, Bønaa KH. Calcium from dairy products, vitamin D intake, and blood pressure: the Tromsø study. Am J Clin Nutr. 2000;71:1530–5.
48. Nakamura K, Nashimoto M, Okuda Y, Ota T, Yamamoto M. Fish as a major source of vitamin D in the Japanese diet. Nutrition. 2002;18:415–6.
49. Moore CE, Murphy MM, Holick MF. Vitamin D intakes by children and adults in the United States differ among ethnic groups. J Nutr. 2005;135:2478–85.
50. Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, et al. The National Diet & Nutrition Survey: adults aged 19 to 64 years (vitamin and mineral intake and urinary analytes). National Diet and Nutrition Survey. 2003;3:1–160.
51. Begley A, Coveney J. Wonder vitamin or mass medication? Media and academic representation of folate fortification as a policy problem in Australia and New Zealand. Aust N Z J Public Health. 2010;34: 466–71.
52. Boseley S. Add vitamin D to Scotland's food: experts. The guardian. 2011. Available from: http://www.guardian.co.uk/uk/2011/dec/23/vitamin-d-scotland-food-multiple-sclerosis. Last accessed 18 Sept 2012.
53. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004;80: 1710S–6.
54. Statutory instrument 1999 No.2457 spreadable fats (marketing standards). Mandatory fortification for margarine and voluntary fortification for fat spreads. 1999.
55. Australia Government. Australia New Zealand food standards code – Standard 1.3.2 – Vitamins and Minerals. Canberra: 2011 epub ahead of print.
56. Vatanparast H, Calvo MS, Green TJ, Whiting SJ. Despite mandatory fortification of staple foods, vitamin D intakes of Canadian children and adults are inadequate. J Steroid Biochem. 2010;121:301–3.
57. Daly RM, Brown M, Bass S, Kukuljan S, Nowson C. Calcium- and vitamin D3-fortified milk reduces bone loss at clinically relevant skeletal sites in older men: a 2-year randomized controlled trial. J Bone Miner Res. 2006;21:397–405.
58. Daly RM, Petrass N, Bass S, Nowson CA. The skeletal benefits of calcium- and vitamin D3–fortified milk are sustained in older men after withdrawal of supplementation: an 18-mo follow-up study. Am J Clin Nutr. 2008;87:771–7.
59. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr. 2003;77:1478–83.
60. Wagner D, Sidhom G, Whiting SJ, Rousseau D, Vieth R. The bioavailability of vitamin D from fortified cheeses and supplements is equivalent in adults. J Nutr. 2008;138:1365–71.
61. Tylavsky FA, Cheng S, Lyytikäinen A, Viljakainen H, Lamberg-Allardt C. Strategies to improve vitamin D status in northern European children: exploring the merits of vitamin D fortification and supplementation. J Nutr. 2006;136:1130–4.
62. Mocanu V, Stitt PA, Costan AR, Voroniuc O, Zbranca E, Luca V, et al. Long-term effects of giving nursing home residents bread fortified with 125 μg (5000 IU) vitamin D3 per daily serving. Am J Clin Nutr. 2009;89:1132–7.
63. Natri A-M, Salo P, Vikstedt T, Palssa A, Huttunen M, Kärkkäinen MUM, et al. Bread fortified with cholecalciferol increases the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplement. J Nutr. 2006;136:123–7.
64. Black LJ, Seamans KM, Cashman KD, Kiely M. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. J Nutr. 2012;142:1102–8.
65. Green TJ, Newton R, Bourn D. Estimated folic acid intakes from simulated fortification of the New Zealand food supply. N Z Med J. 2003;116:U294.
66. Newmark HL, Heaney RP, Lachance PA. Should calcium and vitamin D be added to the current enrichment program for cereal-grain products? Am J Clin Nutr. 2004;80:264–70.
67. Health Canada. Food and drug regulations, Amendment Schedule no. 1066. Ottawa: Health Canada; 1997.

68. U.S. Food and Drug Administration. Food standards. Amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Regist. 1996;61:8781–97.
69. Shakur YA, Garriguet D, Corey P, O'Connor DL. Folic acid fortification above mandated levels results in a low prevalence of folate inadequacy among Canadians. Am J Clin Nutr. 2010;92:818–25.
70. Choose my plate.gov [Internet]. Alexandria: United States Department of Agriculture; 2012. Available from: http://www.choosemyplate.gov/. Updated 2012; cited 6 Sept 2012.
71. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin d levels and bone mineral density: a population-based study of younger and older adults. Am J Med. 2004;116:634–9.
72. Calvo MS, Whiting SJ. Determinations of vitamin D intake. In: Holick MF, editor. Vitamin D physiology, molecular biology, and clinical applications. 2nd ed. New York: Springer; 2010. p. 374–5.
73. Health Canada. Addition of vitamins and minerals to foods. Ottawa: Health Canada; 2005.

Vitamin D and Calcium Absorption: Toward a New Model

26

Robert P. Heaney

Abstract

The standard model for regulation of calcium absorption identifies calcitriol as the vitamin D metabolite responsible for active transport of calcium across the intestinal mucosa, with 25-hydroxy vitamin D [25(OH)D] functioning as the substrate for renal synthesis of calcitriol. However, as experience with measurement of calcium absorption has accumulated, it has become evident that (1) calcitriol is sometimes ineffective in elevating calcium absorption and (2) 25(OH)D is sometimes effective, apparently in its own right. Additionally, supplemental administration of vitamin D or 25(OH)D sometimes increases calcium absorption, and sometimes does not. A new model that integrates the growing number of seemingly contradictory observations is needed. Missing in the current model is the fact of calcium need, which helps to explain many of the discrepancies. Finally, the apparent cooperation of 25(OH)D and 1,25(OH)2D may be explainable by sequential binding of both metabolites to different pockets of the vitamin D receptor.

Keywords

Vitamin D • Calcitriol • 25(OH)D • PTH • Vitamin D receptor • Calcium absorption

Introduction

There are probably few facts in the field of nutrition better established than the relationship between vitamin D and intestinal calcium absorption. The general scheme for such regulation, accepted for the past 30-plus years, is set forth in Fig. 26.1. Surprisingly, little is known about quantitative regulation of this control loop at the level of the intact organism. Further, as more and more data from studies measuring absorption in humans have been published, it has become clear that the system does not always function as the figure indicates. There is need, therefore, for a model that accommodates, insofar as possible, the apparent exceptions to the rule as well as its canonical operation.

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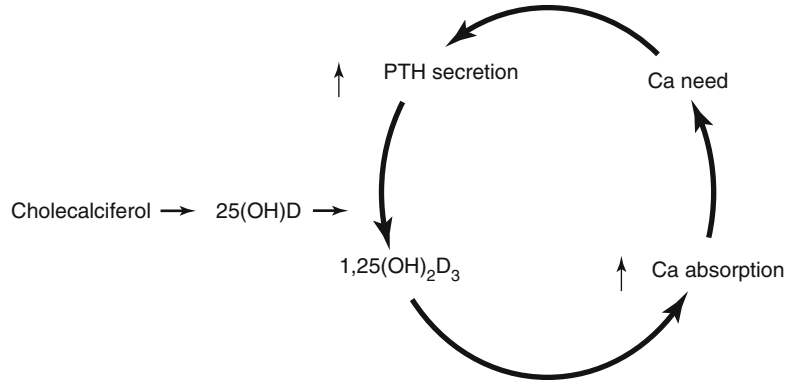

Fig. 26.1 *Schematic diagram* depicting the canonical relationship of vitamin D_3 (*cholecalciferol*) to the control loop regulating active intestinal calcium absorption. Vitamin D and its 25-hydroxyl derivative function as precursors for the active form of the vitamin, $1,25(OH)_2D$ (calcitriol), which is synthesized in the kidney in response to stimuli evoked by sensed calcium need (Copyright Robert P. Heaney 2011. Used with permission)

In the absence of vitamin D, transfer of calcium from gut lumen into blood in nonpregnant organisms occurs mainly by passive, paracellular diffusion, producing *gross* absorption in humans of roughly 15 % of the ingested calcium. However, since appreciable endogenous calcium is transferred from the blood into the gut [1, 2], this passive absorption is effectively countered by secretion, and thus little or no *net* absorption occurs. Vitamin D changes that. It leads to the expression in the enterocyte of membrane calcium transporters and calcium-binding proteins which complex with calcium entering the mucosal cell and shuttle it to the basolateral surface where it is released into the extracellular fluid (ECF) [3].

The quantitative relationships between calcium intake, net calcium absorption, and active transport are shown in Fig. 26.2, which makes explicit the fact that net absorption rises as a function of both calcium intake and active (i.e., vitamin D-mediated) absorption. The diagonal lines in the figure plot, for any degree of active absorption, the relation of net absorption to oral calcium intake after factoring in the reverse flux of calcium across the mucosa. For example, for an active absorption fraction of 14–16 % (typical for healthy adults), net absorption equals 200 mg/day at a total calcium intake of about 1,200 mg/day, the current RDA in the USA for postmenopausal women [4]. As active absorption falls (see the right-hand axis of Fig. 26.2), net absorption for any given intake falls as well, and at zero active transport, net absorption is zero or negative for all intakes below 1,100 mg/day.[1] This quantification (and Fig. 26.2) simply makes concrete the generalization that vitamin D affects calcium absorption.

As Fig. 26.1 notes, the metabolite of vitamin D responsible for stimulating active calcium absorption is $1,25(OH)_2D$ (calcitriol), delivered to the intestinal mucosa in the blood, and produced in the kidney under a complex set of controls, but mainly regulated by secretion of parathyroid hormone (PTH). In the enterocyte, calcitriol complexes with the vitamin D receptor (VDR) which, together with other proteins, forms a complex that binds to the vitamin D response elements of the genes encoding the component proteins of the transport apparatus, leading to their expression and ultimately to augmented active transport of calcium across the mucosal cell [3].

In this action calcitriol acts as one arm of a classical negative feedback loop typical of endocrine

[1]The specific contours in the figure were developed for midlife women and may differ in degree for men or for adolescents, depends on the value assigned for entry of endogenous calcium into the gut. Nevertheless, the basic relationships depicted remain true for both sexes and all ages.

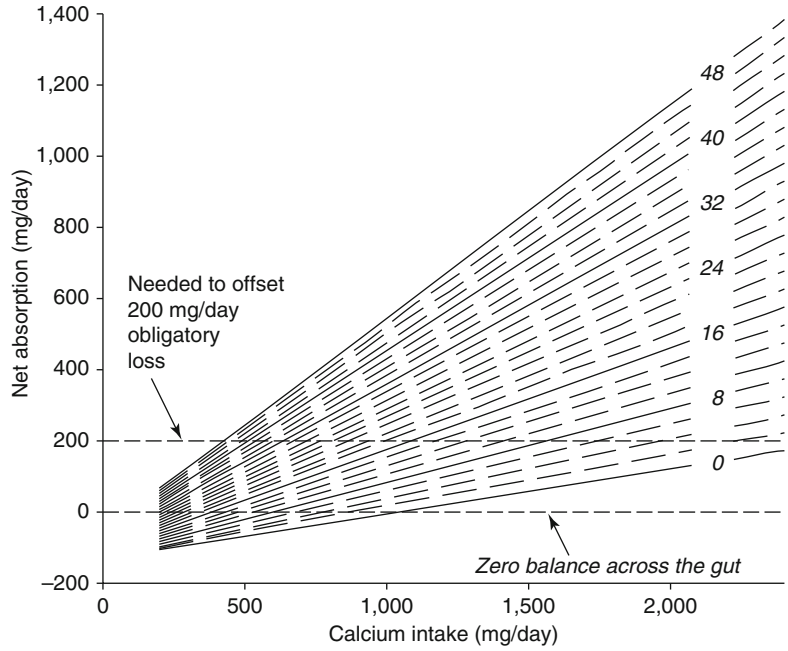

Fig. 26.2 Nomogram characterizing net intestinal calcium absorption as a function (1) of oral calcium intake and (2) percent active (i.e., vitamin D-mediated) absorption. Each *diagonal line* represents the relationship between intake and net absorption for a specified level of active calcium absorption. The *dashed horizontal lines* denote (1) zero net absorption and (2) the net absorption needed to offset obligatory loss through cutaneous and excretory routes (i.e., ~200 mg/day) [11] (Copyright Robert P. Heaney 1999. Used with permission)

controls. Briefly, when the parathyroid glands sense calcium need, they increase the release of PTH which, among other effects, leads to increased renal 1-α-hydroxylation of 25-hydroxy vitamin D [25(OH)D], with secretion of calcitriol into the blood and, ultimately, to increased calcium entry from the gut, thereby partially counteracting the stimulus that had led to increased PTH secretion.

Obviously this mechanism increases calcium input only when the gut lumen contains unabsorbed calcium, and because of this limitation the body does not depend solely upon control of calcium absorption for its defense of ECF [Ca^{++}]. In addition to stimulating calcitriol synthesis, PTH both raises the renal excretory threshold (reducing urinary loss of calcium) and augments calcium release from bone. With these features in mind, consider the response that occurs with a reduction in calcium intake from 1,200 to 600 mg/day in an individual who had established a homeostatic equilibrium at the higher intake. PTH secretion rises and all three of the PTH effects are upregulated, even though the reduced calcium input in this instance is located to a single one of the effector organs. Thus, a key feature of this three-effector response is that the three arms of this feedback loop *jointly* offset the stress on ECF [Ca^{++}] that had been produced by reduced input from one of them, and the offending input (i.e., net absorbed calcium), while improved, is not itself restored to its prior level. In other words, the feedback loop of Fig. 26.1 only partially offsets the absorptive deficit.

Cholecalciferol, Parathyroid Hormone, and Calcium Absorption

Concrete expression of this incomplete response is seen in the well-attested, inverse relationship between serum PTH and 25(OH)D concentrations, a recent instance of which is depicted in Fig. 26.3, which plots data derived from NHANES 2003–2006 [5]. The relationship is usually found to be curvilinear (as in Fig. 26.3), with PTH approaching an apparently asymptotic low value at serum 25(OH)D concentrations above 75 nmol/L (30 ng/mL). It is sometimes said that many studies fail to show

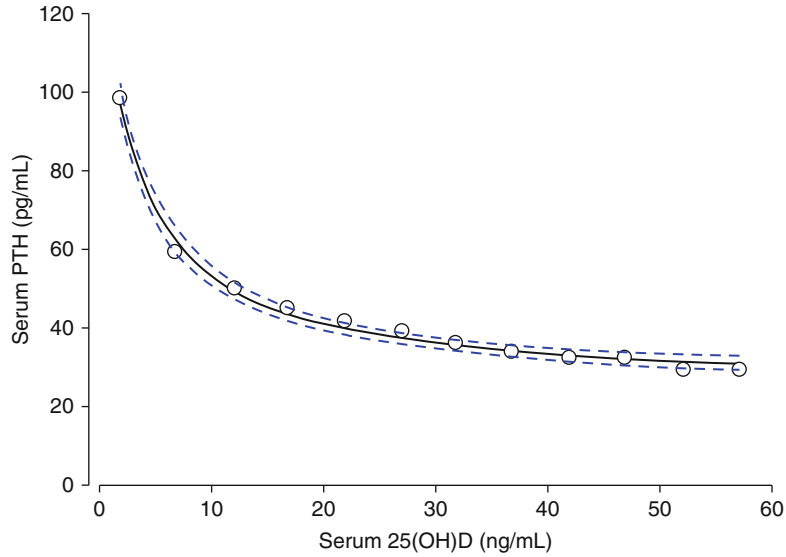

Fig. 26.3 Plot of serum PTH concentration as a function of serum 25(OH)D concentration, derived from NHANES 2003–2006. Points represent means for the various groupings of serum 25(OH)D concentration, and the *dashed lines* represent the 95 % confidence interval for the regression line through those means. That regression equation is as follows: $PTH = 25.35\ e^{(13.45/(25D+8.09))}$. The 95 % confidence range for the coefficient is ± 2.66 pg/mL (Based on data from [5]. Copyright Robert P. Heaney 2011. Used with permission)

an "inflection point" [6]. By that is meant a point at which the generally downward trend flattens out and PTH concentration becomes invariant with respect to 25(OH)D. That may be factually accurate for a particular data set, but it cannot be an accurate reflection of the underlying physiology, as PTH cannot go negative (as is predicted by a linear model). Nor is there likely to be a sharp point where the downward trend suddenly becomes flat. As in other negative feedback control systems, the control signal is proportional to the "error" – i.e., the deviation from a set point. That means the curve is best represented by a decreasing exponential (as in the equation generating Fig. 26.3). Finally, although the many studies documenting this relationship are observational in character, there can be little doubt that the basic relationship is causal, as elevating serum 25(OH)D with vitamin D usually lowers serum PTH.

While the fact of this inverse relationship seems incontrovertible, its use in estimating the vitamin D requirement has not elicited a consensus. Seemingly ignored in the associated controversy is what the relationship itself tells us about vitamin D and calcium absorption. As noted above, PTH rises in response to calcium need. Hence, the most straightforward interpretation of the higher PTH values observed at low vitamin D status is that calcium transfer from gut to blood is inadequate for current needs, i.e., that not enough calcium is entering the body from food, thus requiring the calcium homeostatic system to compensate. Similarly, the most plausible explanation for the lower PTH concentration at higher 25(OH)D values is greater calcium input from the gut. In brief, any elevation of PTH above its set point value indicates a level of calcium input that would be insufficient in the absence of PTH.

Moreover, the continuous character of the relationship depicted in Fig. 26.3 means that calcium absorptive efficiency must itself be a continuum. In other words, the lower PTH values at higher 25(OH)D concentrations must mean that calcium need is less, i.e., that, other things being equal, calcium absorption is greater at a 25(OH)D of 30 nmol/L than at 20 and greater at 40 nmol/L than at 30, up to the point where the curve reaches its apparent asymptote. Then, no further increase in absorption occurs (and PTH ceases to fall). It is exactly this continuum of response to cholecalciferol that is represented in the active absorption isocontours along the right-hand vertical axis of Fig. 26.2.

Quantifying the Relationship Between Vitamin D Status and Calcium Absorption

The first study formally addressing the relationship of absorption to vitamin D status [7] used a pharmacokinetic method, reflecting *net* calcium transfer out of the gut (i.e., gross absorption from

lumen to blood, less offsetting calcium secretion into the gut). The same women were tested twice, once at mean 25(OH)D values of 50.2 and again at 86.5 nmol/L (20.1 and 34.6 ng/mL). Area under the curve for the increment in serum calcium (AUC) was found to be 65 % greater at the higher of the two 25(OH)D values ($P<0.001$).[2]

More recently Shapses et al. [8], using a double-tracer method, reported an increase in fractional absorption of about 23 % for a difference in serum 25(OH)D of 23 nmol/L (9.3 ng/mL). This percentage change, per unit 25(OH)D increment, was almost identical to the proportional change reported for the pharmacokinetic method [7]. Shapses et al. downplay the role of the administered vitamin D in the rise in absorption which they found, because the subjects concerned received alendronate as well, an agent that reduces calcium release from bone and thereby elicits a rise in PTH in its own right. However, the increase in absorption that they report illustrates a key feature of calcium absorption, i.e., that it is always in response to calcium need – a need augmented in this case by reduced bone resorptive response to PTH. Arguably, therefore, the extra vitamin D given to the alendronate-treated subjects was critically important, as it enabled the observed increase in calcium absorption efficiency.

This mechanism is perhaps most dramatically illustrated in the fact that patients with undetected but real vitamin D deficiency develop severe hypocalcemia when treated with potent antiresorptive agents [9]. At low vitamin D status, they are unable to increase absorption sufficiently to offset the treatment-induced inaccessibility of skeletal calcium reserves, no matter how much PTH they produce.

These two absorption studies comprise the bulk of the world's literature directly testing responses of calcium absorption to altered vitamin D status. They complement and confirm the interpretation of the inverse relationship between serum PTH and 25(OH)D concentrations shown in Fig. 26.3. However, there is one other study, also published just recently, that bears indirectly on this issue. Kimball et al. [10], evaluating indicators of possible toxicity from high doses of vitamin D, described the relationship between serum 25(OH)D and urine calcium-to-creatinine ratio (UCa:cr). The latter is a reflection of net calcium absorption precisely because the small absorptive rise in serum calcium increases the renal filtered load of calcium and leads to a rise in urine calcium. Teasing out that absorptive effect, Kimball et al. present a scatterplot of UCa:cr against serum 25(OH)D and, using a LOESS method, show that the data themselves indicate a rise in UCa:cr as serum 25(OH)D values rises from 33 nmol/L (13.2 ng/mL) to about 80 nmol/L (32 ng/mL) [10].

Using the piecewise regression routine of SAS (PROC_NLIN) on their data (S. Kimball, personal communication), the break point seen in the LOESS plot was found to be 79.9 nmol/L (31 ng/mL), with a 95 % confidence interval of ± 31 nmol/L (12 ng/mL). While this result does not locate the transition point with sufficient precision to resolve controversy concerning optimal vitamin D status, it does reinforce the conclusion that, in its effect on calcium absorption, vitamin D, like nutrients such as iron and calcium, exhibits threshold behavior, i.e., below a certain vitamin D status, calcium absorption rises when vitamin D status is improved, and above that level, no further rise in calcium absorption occurs. The best fit regression lines through the data of Kimball et al. (and their confidence limits) are shown in Fig. 26.4, for serum 25(OH)D values extending from a clearly deficient 33 nmol/L (13 ng/mL) to 225 nmol/L (90 ng/mL), the upper end of the physiological range.

The plateau above 80 nmol/L seen in Fig. 26.4 had been described previously [7, 11] and is an important feature of vitamin D physiology. It is the range of 25(OH)D concentrations at which physiological adaptation is no longer limited by vitamin D status. What it also means (and what is little appreciated outside the vitamin D field) is

[2]In its report setting forth the DRIs for calcium and vitamin D [4], the IOM discounted this study, on the grounds that it used a method that was "nonstandard" and "indirect." This characterization was inaccurate for several reasons, not least of which is that (1) the method is, in fact, the gold standard in the European Union; (2) it is the only method that can be used for sources that cannot be intrinsically labeled (e.g., preformulated supplements and prepared foods); and (3) it does, in fact, directly measure the rise in serum calcium produced by net calcium absorption.

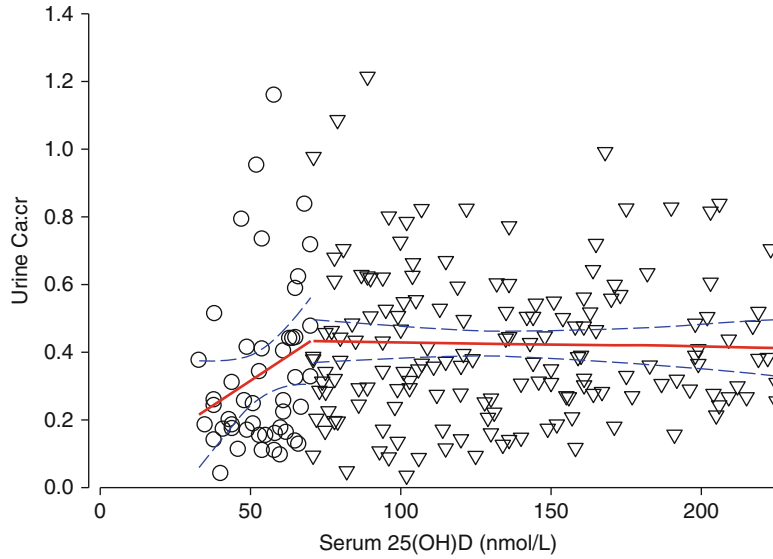

Fig. 26.4 Piecewise regression through the urine calcium:creatinine ratios (mmol:mmol) from the data supplied by Kimball and Vieth (personal communication). Both *above* and *below* the *breakpoint* the *solid lines* represent the least squares, linear regression lines through their data, and the *dashed lines*, the 95 % confidence interval for the two regression lines (Copyright Robert P. Heaney 2011. Used with permission)

that cholecalciferol does not itself cause calcium absorption. This is shown clearly on the long plateau region of Fig. 26.4 over which serum 25(OH)D concentration rises threefold but urine calcium does not change. This same conclusion is also supported by the fact that outdoor summer workers [who often have 25(OH)D values in the range of 150 nmol/L (60 ng/mL)] do not hyperabsorb calcium [12].

Rethinking the Model

A satisfactory model of absorption control must provide a way not only to quantify absorptive performance but must also make provision for what would seem to be exceptions to its currently presumed operation. Several such anomalies will be described as follows: (1) the multisystem functionality of 25-hydroxy vitamin D [25(OH)D] itself; (2) the fact that there are many reports of vitamin D administration in quantities sufficient to change serum 25(OH)D appreciably, which nevertheless do not show the change in calcium absorption efficiency that Figs. 26.1 and 26.2 predict; (3) the fact that in vitamin D deficiency osteomalacia, active calcium absorption is low or absent, despite frequently normal or even high serum concentrations of calcitriol; (4) the greater effectiveness of 25(OH)D vs. calcitriol in treatment of nutritional vitamin D deficiency; and (5) the vitamin D-independent rise in calcium absorption in pregnancy and in late adolescence.

25-Hydroxy-vitamin D

25(OH) D, the first of the derivatives of cholecalciferol to be discovered [13], was for a time thought to be *the* active form of the vitamin and was developed originally as a drug. However, such use was soon eclipsed when calcitriol, the true active form, was discovered [13]. In 1997, the Food and Nutrition Board, in the first of the Institute of Medicine's (IOM) Dietary Reference Intake volumes, designated serum 25(OH)D concentration as the functional indicator of vitamin D status [14]. Since then, quantitative studies have shown that circulating 25(OH)D also comprises an important portion of total body vitamin D stores: under prevailing inputs, it can account for perhaps one-third of total body vitamin D content [15].

But there is more to the importance of 25(OH)D than these somewhat passive roles might suggest. Prior to the discovery and deployment of calcitriol, 25(OH)D had demonstrated what, in hindsight, is a surprising functionality, increasing calcium absorption efficiency and improving bone status in patients with end-stage renal disease. Further, in side-by-side comparisons, it functioned as well or better than calcitriol (e.g., [16–19]). In a 1978

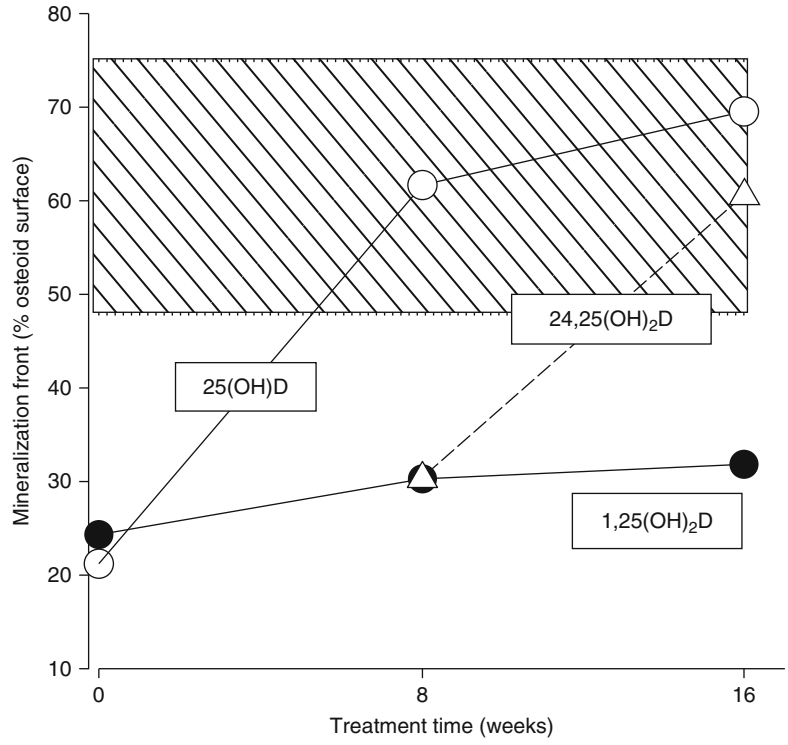

Fig. 26.5 Histomorphometric response of the mineralization front to treatment of nutritional vitamin D deficiency with 25(OH)D, calcitriol, or calcitriol augmented at week 8 with $24,25(OH)_2D$. The *shaded zone* demarcates the range of values found in healthy individuals (Based on data from [16]. Copyright Robert P. Heaney 2011. Used with permission)

paper describing response to various treatments of nutritional vitamin D deficiency [16], Bordier et al. showed that 25(OH)D completely normalized defective bone mineralization, while calcitriol did not (Fig. 26.5). This failure of calcitriol to correct the bone manifestations of nutritional vitamin D deficiency almost certainly reflects an underlying failure of calcitriol by itself to increase calcium absorption. This conclusion follows from the fact that calcium alone, without any vitamin D, will heal the bone lesions of osteomalacia. Any comprehensive model of vitamin D action will have to explain both the failure of calcitriol and the functional effects of 25(OH)D which, in standard theory, serves mainly as the substrate for renal and peripheral 1-α-hydroxylases (as in Fig. 26.1).

Failure of Calcium Absorption to Respond to Vitamin D

In several vitamin D trials, calcium absorption did not increase as predicted. Several of these were also calcium supplement trials, in which calcium intake was augmented by 1,000–1,500 mg/day. In one, *fractional* calcium absorption actually decreased in the face of an appreciable rise in serum 25(OH)D [20]. [However, with the augmented calcium intake, *net* calcium absorption had to have increased (see, e.g., Fig. 26.2)]. In another study, Park et al. gave vitamin D to growing adolescent girls with suboptimal serum 25(OH)D concentrations [21]. Despite improved vitamin D status, calcium absorption fraction actually fell. [It is important to note, however, that the pretreatment net absorption in these girls averaged over 400 mg/day, more than enough to support bone accretion at the adolescent growth spurt. Whatever may be the mechanism responsible for that absorptive performance, it seems clear that the calcium need of these girls was being met without extra vitamin D.]

More puzzling than the failure to respond to additional cholecalciferol is the high absorption fraction observed in adolescents. Earlier, O'Brien et al. [22] had shown, across a broad range of calcium intakes and altogether apart from vitamin D status, that adolescent girls exhibit absorption fractions about 45 % higher than adult women for the same calcium intakes. Assuming nominal values for entry into the gut of endogenous calcium, an

increase in absorption fraction of this magnitude is fully sufficient to explain the retention values reported by Park et al. [21]. Clearly, however, this absorptive performance is either independent of vitamin D or is a result of greatly increased responsiveness of the renal 1-α-hydroxylase to ambient vitamin D status. Some degree of vitamin D independence is clearly shown in patients with vitamin D-resistant rickets who have low absorption efficiency during childhood but above expected normal efficiency in late adolescence [23].

These studies illustrate the importance of calcium need as the initiating and sustaining stimulus in the endocrine control loop that regulates calcium absorption. Substantially augmenting calcium intake is one way to counteract calcium need (see Figs. 26.1 and 26.2), and when that occurs, calcium absorption *fraction* appropriately falls, even in the face of increased cholecalciferol intake and rising serum 25(OH)D (e.g., [21]).

Calcium need, which I address further below, is the one constant running through the foregoing discussion. The ability to respond to calcium need is nominally dependent upon vitamin D status, but how the prevailing model (Fig. 26.1) incorporates that dependence has been unclear.

The Paradox of High (or Normal) Calcitriol Concentrations and Low Calcium Absorption in Nutritional Osteomalacia

It has proved difficult to explain why the endocrine feedback loop fails to operate as expected in the face of outspoken vitamin D deficiency, when calcium is poorly absorbed despite calcitriol levels that are sometimes normal or even high. Figure 26.5 illustrates a further instance of this failure of calcitriol to elevate absorption when vitamin D status is low. Although the measure used in Fig. 26.5 was bone mineralization, the poor performance of calcitriol, as noted earlier, has to mean that calcium absorption was not increased appreciably, since calcium alone, if given in sufficient quantity, will heal the mineralization defect in osteomalacic bone. It has been suggested in this instance that the enterocyte, in addition to accessing administered calcitriol circulating in the blood, expresses its own 1-α-hydroxylase and synthesizes additional calcitriol for itself from circulating 25(OH)D, a process which would be impeded if serum 25(OH)D values were low. This seems an unsatisfactory explanation for two reasons: (1) it fails to address why additional calcitriol would be necessary if serum calcitriol is already "normal" and (2) it ignores the controls built into the system to prevent hyperabsorption when vitamin D status is normal and calcium absorption is adequate.

On a related issue (as Fig. 26.5 also shows), calcitriol's efficacy in correcting the mineralization defect of osteomalacic bone was rescued by coadministration of $24,25(OH)_2D$. A parallel study illustrating this same failure of calcitriol used a fracture healing model, and showed, as had Bordier et al. [16], that the combination of calcitriol with $24,25(OH)_2D$ supported fracture healing, while calcitriol alone did not [24].

Another instance of the same failure is the inability of calcitriol to elevate calcium absorption appreciably in patients with end-stage renal disease [25], at least so long as serum 25(OH)D is low. The serum concentration of $24,25(OH)_2D$ was not measured in these studies, nor was it used as co-therapy. Nevertheless, as $24,25(OH)_2D$ is synthesized in the kidney [26], just as is calcitriol, it is worth noting that supplying calcitriol alone provides only one of the two metabolites missing in end-stage renal disease.

The same basic failure of increased serum calcitriol fully to reverse calcium need is implicit in the relationship of PTH to 25(OH)D concentrations (Fig. 26.3). The high PTH values at low vitamin D status elicits a rise in renal synthesis of calcitriol – as shown explicitly, e.g., in the study of Shapses et al. [8] (among many others) – and should thus have led to a reduction in PTH. But PTH remains elevated. This failure to return to a prior equilibrium at the level of the whole organism can only mean that calcitriol, while necessary for absorption, is not itself fully sufficient and that the lower the vitamin D status, the greater the insufficiency. In other words, absorptive response

to a given level of circulating calcitriol falls as serum 25(OH)D concentration falls. Although studies directly addressing this question are virtually nonexistent, the available data suggest that, for the calcium absorptive response to occur, both calcitriol and 25(OH)D must be present in adequate concentration, presumably at the level of the mucosal cell, but somewhere in the system at least, a point that I shall develop below.

Pregnancy

A different kind of problem arises in the context of pregnancy [27]. While serum calcitriol concentrations rise dramatically throughout pregnancy, *free* calcitriol does not rise till the third trimester. However, calcium absorption efficiency rises by the second trimester [27, 28], suggesting involvement of a non-vitamin D mechanism for absorption. That this may indeed be the case is indicated by the effect of pregnancy in the VDR-null mouse which, as would be predicted, has poor calcium absorption in the basal state. However, the mouse, when pregnant, increases calcium absorption just as do wild-type mice (and humans) [29]. Absorption becomes so efficient that, by term, the mouse emerges from pregnancy with a normal skeletal phenotype (whereas the unbred VDR-null mouse has an osteoporotic phenotype). Brommage et al. [30] had earlier shown the same effect of pregnancy on calcium absorption in vitamin D deficient rats. The mechanism of this pregnancy effect is unknown. It must involve either an alternative mechanism for transcellular transport of calcium ions (active absorption) or greatly enhanced paracellular diffusion (passive absorption). Either way, some unknown pregnancy-related stimulus triggers this surprising effect.

Calcium Need

A key feature of the forgoing discussion is the notion of "calcium need," which is a concept, not a measured variate. Fortunately free serum calcitriol serves as biomarker for this need, if not a sufficient corrective thereof. When the organism senses calcium need, it upregulates calcitriol production, and when calcium requirements are satisfied, downregulates it (Fig. 26.1). If adequate calcium is somehow being made available, further vitamin D will have no effect on calcium absorption; there simply is no calcium need.

However, most adults today need additional calcium at prevailing calcium intakes [31] and prevailing vitamin D levels. (The fact that, in the populations often studied, intakes of *both* nutrients are commonly inadequate confounds the interpretation of single-nutrient supplementation trials.) Even at relatively low calcium intakes, there is plenty of unabsorbed calcium in the gut lumen, as net absorption averages only about 10–12 % of intake. The fact that individuals are not extracting more of the calcium present in their diets in the face of manifest need indicates a failure adequately to elevate active calcium transport, i.e., a relative vitamin D inadequacy. Had their active absorption been as high as, e.g., 32 % (as would be the case in primary hyperparathyroidism and thus physiologically plausible), a calcium intake as low as 500 mg/day would have been sufficient to meet typical systemic calcium needs (see Fig. 26.2).

Increasing either calcium intake or vitamin D status (or both) inevitably augments *net* calcium absorption so long as there is calcium need. This explains both the rise in calcium absorption in otherwise untreated postmenopausal women receiving cholecalciferol (but no extra calcium) [7] and at the same time the absence of a rise in absorption fraction in postmenopausal women given large calcium supplements (plus cholecalciferol) [21]. It also explains why outdoor workers with high vitamin D status levels do not hyperabsorb [12].

The Two Binding Site Mechanisms

As noted above, existing data strongly suggest that both calcitriol and 25(OH)D [and/or $24,25(OH)_2D$] are required for optimal control of

calcium absorption. How can 25(OH)D be acting in this context? A possible answer is suggested by recent work of Norman's group [32–35] demonstrating that the VDR exhibits two ligand pockets, one needed for genomic action (the genomic pocket – GP – and exhibiting 1,000-fold greater affinity for calcitriol than for 25(OH)D) and the other (the AP or "alternate pocket") able to bind 25(OH)D as readily as calcitriol. In at least one model system tested to date, function of both pockets is required for the full effect of the VDR ligand complex to produce the vitamin D-specific effect [35]. If, instead of being simply a calcitriol precursor, 25(OH)D [or 24,25$(OH)_2$D] is serving as an essential cofactor in the vitamin D signaling sequence that leads to calcium absorption, then we have both a richer model with which to work *and* an explanation for some of the anomalies discussed above.

Given the large serum concentration difference between calcitriol and 25(OH)D in the intact organism, the VDR-AP would be expected to have 25(OH)D as its regular ligand, at least so long as 25(OH)D concentrations are adequate. This model proposes that, without such occupancy, calcitriol, despite being present in "normal" concentrations (and accordingly available for binding to the VDR), would not by itself be able to cause full expression of the genes necessary to activate the intestinal calcium absorptive mechanism. Evaluation of this possible mechanism would be aided by the elucidation of the dissociation constant of the VDR-AP for 25(OH)D, i.e., determining whether occupancy of the VDR-AP is likely to vary appreciably in the range of physiological concentrations of 25(OH)D.

Summary

While the details of the mechanism by which 25(OH)D and calcitriol jointly interact to regulate absorption are still to be worked out in detail, several other features of the homeostatic context, of which calcium absorption is one component, can be asserted with some confidence:

- Calcitriol is the principal factor that directly stimulates active intestinal calcium absorption through established genomic mechanisms.
- The organism responds to calcium need by upregulating renal synthesis of calcitriol.
- This function is observed in nonpregnant individuals with normal vitamin D status and renal function, but may not be so straightforward otherwise (see below).
- With the exception of the vitamin D-independent calcium absorption of pregnancy and possibly adolescence, *active* calcium transport usually requires calcitriol bound to the VDR, i.e., calcitriol is a necessary, but probably not sufficient, condition for active calcium absorption to occur.
- Calcitriol's effect on absorption efficiency is dependent upon vitamin D status, i.e., it needs 25(OH)D to be present in the system in order to increase calcium absorption adequately. That dependence on 25(OH)D is not just as a precursor of calcitriol but as a cofactor *with* calcitriol, possibly involving a prior step in the signaling sequence leading up to calcitriol's genomic effect.
- Cholecalciferol and 25(OH)D have no independent effect on calcium absorption in the absence of calcium need. The many studies showing an absorptive response to increases in 25(OH)D concentration reflect the presence of an underlying calcium need which calcitriol, alone, had not been able to satisfy, i.e., 25(OH)D enables response to calcitriol.
- Thus, raising vitamin D status (short of intoxication), even from nominally low levels, will not increase calcium absorption unless there is calcium need, as reflected, e.g., by elevated free serum calcitriol concentrations (the biomarker for calcium need).

References

1. Heaney RP. The calcium economy. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totowa: Humana; 2006. p. 145–62.
2. Heaney RP, Abrams SA. Improved estimation of the calcium content of total digestive secretions. J Clin Endocrinol Metab. 2004;89:1193–5.

3. Fleet J. Molecular regulation of calcium metabolism. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totowa: Humana; 2006. p. 163–89.
4. IOM (Institute of Medicine). Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.
5. Schwartz RS, Camargo Jr CA, Wolfe P, Wolfe P, Ginde AA. Defining vitamin D status by secondary hyperparathyroidism in the U.S. Population. J Endocrinol Invest. 2012;35:42–8.
6. Aloia JF, Talwar SA, Pollack S, Feuerman M, Yeh JK. Optimal vitamin D status and serum parathyroid hormone concentrations in African American women. Am J Clin Nutr. 2006;84:602–9.
7. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr. 2003;22:142–6.
8. Shapses SA, Kendler DL, Robson R, Hansen KE, Sherrell RM, Field MP, et al. Effect of alendronate and vitamin D_3 on fractional calcium absorption in a double-blind, randomized, placebo-controlled trial in postmenopausal osteoporotic women. J Bone Miner Res. 2011;26:1836–44.
9. Rosen CJ, Brown S. Severe hypocalcemia after intravenous bisphosphonate therapy in occult vitamin D deficiency. N Engl J Med. 2003;348:1503–4.
10. Kimball S, Burton JM, O'Connor PG, Vieth R. Urinary calcium response to high dose vitamin D_3 with calcium supplementation in patients with multiple sclerosis. Clin Biochem. 2011;44:930–2.
11. Heaney RP. Serum 25-hydroxy-vitamin D and the health of the calcium economy. In: Burckhardt P, Dawson-Hughes B, Heaney RP, editors. Nutritional aspects of osteoporosis. 2nd ed. San Diego: Elsevier; 2004. p. 227–44.
12. Barger-Lux MJ, Heaney RP. Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. J Clin Endocrinol Metab. 2002;87:4952–6.
13. DeLuca HF. Historical overview of vitamin D. In: Feldman D, Pike JW, Adams JS, editors. Vitamin D. 3rd ed. San Diego: Elsevier; 2011. p. 3–12.
14. IOM (Institute of Medicine). Dietary reference intakes for calcium, magnesium, phosphorus, vitamin D, and fluoride. Washington, DC: The National Academies Press; 1997.
15. Heaney RP, Horst RG, Cullen DR, Armas LAG. Vitamin D_3 distribution and status in the body. J Am Coll Nutr. 2009;28:252–6.
16. Bordier P, Rasmussen H, Marie P, Miravet L, Gueris J, Ryckwaert A. Vitamin D metabolites and bone mineralization in man. J Clin Endocrinol Metab. 1978;46:284–94.
17. Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. J Clin Endocrinol Metab. 1997;82:4111–6.
18. Colodro IH, Brickman AS, Coburn JW, Osborn TW, Norman AW. Effect of 25-hydroxy-vitamin D_3 on intestinal absorption of calcium in normal man and patients with renal failure. Metabolism. 1978;27:745–53.
19. Recker R, Schoenfeld P, Letteri J, Slatopolsky E, Goldsmith R, Brockman S. The efficacy of calcifediol in renal osteodystrophy. Arch Intern Med. 1978; 138:857–63.
20. Zhu K, Devine A, Dick IM, Wilson SG, Prince RL. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory Australian women: a five-year randomized controlled trial. J Clin Endocrinol Metab. 2008;93:743–9.
21. Park CY, Hill KM, Elble AE, Martin BR, DiMeglio LA, Peacock M, et al. Daily supplementation with 25 μg cholecalciferol does not increase calcium absorption or skeletal retention in adolescent girls with low serum 25-hydroxyvitamin D. J Nutr. 2010; 140:2139–44.
22. O'Brien KO, Abrams SA, Liang LK, Ellis KJ, Gagel RF. Increased efficiency of calcium absorption during short periods of inadequate calcium intake in girls. Am J Clin Nutr. 1996;63:579–83.
23. Tiosano D, Hadad S, Chen Z, Nemirovsky A, Gepstein V, Militianu D, Weisman Y, Abrams SA. Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. J Clin Endocrinol Metab. 2011;96:3701–9.
24. Seo E-G, Einhorn TA, Norman AW. 24R,25-dihydroxyvitamin D_3: an essential vitamin D_3 metabolite for both normal bone integrity and healing of tibial fracture in chicks. Endocrinology. 1997;138:3864–72.
25. Lund RJ, Andress DL, Amdahl M, Williams LA, Heaney RP. Differential effects of paricalcitol and calcitriol on intestinal calcium absorption in hemodialysis patients. Am J Nephrol. 2010;31:165–70.
26. Holick MF, Schnoes HK, DeLuca HF, Gray RW, Boyle IT, Suda T. Isolation and identification of 24,25-dihydroxycholecalciferol, a metabolite of vitamin D_3 made in the kidney. Biochemistry. 1972;11:4251–5.
27. Heaney RP, Skillman TG. Calcium metabolism in normal human pregnancy. J Clin Endocrinol Metab. 1971;33:661–70.
28. Kovacs CS. Vitamin D, in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr. 2008;88(suppl): 520S–8.
29. Brommage R, Baxter DC, Gierke LW. Vitamin D-independent intestinal calcium and phosphorus absorption during reproduction. Am J Physiol. 1990; 259:G631–8.
30. Fudge NJ, Kovacs CS. Pregnancy up-regulates intestinal calcium absorption and skeletal mineralization independently of the vitamin D receptor. Endocrinology. 2010;151:886–95.
31. Bone Health and Osteoporosis: A Report of the Surgeon General. Rockville: U.S. Department of Health and Human Services, Office of the Surgeon General; 2004.
32. Norman AW, Mizwicki MT, Norman DPG. Steroid hormone rapid actions, membrane receptors and a conformational ensemble model. Nat Rev Drug Discov. 2004;3:27–41.

33. Mizwicki MT, Norman AW. Vitamin D sterol/VDR conformational dynamics and nongenomic actions. In: Feldman D, Pike JW, Adams JS, editors. Vitamin D. 3rd ed. San Diego: Elsevier; 2011. p. 271–97.
34. Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds $1\alpha,25(OH)_2$-vitamin D_3 in vivo and in vitro. Mol Endocrinol. 2004;18:2660–71.
35. Menegaz D, Mizwicki MT, Barrientos-Duran A, Chen N, Henry HL, Norman AW. Vitamin D receptor (VDR) regulation of voltage-gated chloride channels by ligands preferring a VDR-alternative pocket (VDR-AP). Mol Endocrinol. 2011;25:1289–300.

Do Desirable Vitamin D Levels Vary Globally?

27

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Abstract

Vitamin D insufficiency is a common problem worldwide, with a varying prevalence depending on the population of interest and cutoff used to define insufficiency. The medical literature has witnessed an explosion in the number of vitamin D publications over the last three decades, most convincingly supporting a beneficial effect of vitamin D on musculoskeletal parameters. This led the Institute of Medicine (IOM) to issue an update in 2011 with an increase in the recommended vitamin D intake across all age groups and to set the desirable level at 50 nmol/L. This compares modestly to the desirable level recommended by the Endocrine Society (ES) of 75 nmol/L, which is similar to that recommended by the International Osteoporosis Foundation for older individuals. While the IOM Committee focused on the population needs in North America, the Endocrine Society tried to target high-risk populations. Some of the lowest vitamin D levels are recorded in black subjects and in non-western populations, populations in whom data on fractures and falls are scarce. Information using surrogate markers for the beneficial effect of vitamin D action on musculoskeletal health has many limitations, even in Caucasian subjects where it is the most available. The calcium–vitamin D economy in blacks seems different, and the desirable vitamin D level to optimize musculoskeletal health may be lower than that of Caucasians. Furthermore, some evidence from association studies suggests an increase in the risk of fractures in blacks, and possibly Asians, at 25(OH)D levels exceeding the desirable level for Caucasians. In view of this apparent divergence, the lack of solid outcome data in other ethnic and racial groups, and the multitude of modulators that affect vitamin D metabolism and action, the notion of a global desirable vitamin D level to date is not tenable.

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25(OH)D • Desirable • Falls • Fractures • Surrogate markers • Ethnicity • PTH • Inflection point • Plateau • Pleiotropic effect of 25(OH)D

Abbreviations

$1.25(OH)_2$ D	1,25-Dihydroxyvitamin D
25(OH)D	Hydroxyvitamin D
AIDS	Acquired immune deficiency syndrome
BMI	Body mass index
CI	Confidence interval
ER	Estrogen receptor
ES	Endocrine Society
HIV	Human Immunodeficiency virus
HR	Hazard risk
IOF	International Osteoporosis Foundation
IOM	Institute of Medicine
LASA	Longitudinal Aging Study Amsterdam
NHANES	National Health and Nutrition Examination
OC	Osteocalcin
OR	Odds ratio
PM	Postmenopausal
PTH	Parathyroid hormone
RIA	Radioimmunoassay
RR	Relative risk or risk ratio
SNP	Single-nucleotide polymorphism
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TB	Tuberculosis
USA	United States of America
VDR	Vitamin D receptor
WHI	Women's Health Initiative

Introduction

Vitamin D is a steroid hormone that controls more than 200 genes and thus impacts a wide range of molecular and cellular functions. While the impact of vitamin D on musculoskeletal health and bone and mineral metabolism are well established [1–3], an increasing body of evidence over the last three decades supports associations with decreased mortality [4, 5], in addition to pleiotropic effects [6, 7]. In a systematic review of 50 randomized trials, including 94,148 participants, vitamin D3 was associated with decreased mortality, with a relative risk (RR) of 0.94, 95 % CI 0.91–0.98 [8]. Nonclassical proposed beneficial effects are on cardiovascular diseases [6–10], type 2 diabetes mellitus (T2DM) and the metabolic syndrome [11, 12], type 1 diabetes mellitus (T1DM) [13–15], innate immune responses and self-tolerance [16–18], multiple sclerosis [19–21], and selected cancers [22–25].

Vitamin D insufficiency is a common problem worldwide, with a prevalence of 50–90 %, depending on the cutoff used to define insufficiency and on the population of interest. Some of the highest prevalences are described in India, China, the Middle East, and Southern Europe, as opposed to Northern America and Europe [1, 26–29]. Vitamin D status depends on the production of vitamin D3 in the skin, under the influence of ultraviolet light, and on vitamin D intake, from dietary or supplemental sources. The serum 25-hydroxyvitamin D [25(OH)D] concentration is the preferred indicator of vitamin D status, skin being the source for 50–90 % of circulating levels. Consistent predictors of low vitamin D levels are latitude, race–ethnicity skin color, clothing style, body mass index (BMI), and socioeconomic status. At particular risk are young children, pregnant women, and the elderly [1, 26–28]. Ganji et al. recently described secular trends in vitamin D status in the NHANES study, with a decrease in mean levels and an increase in prevalence estimates. Indeed, the overall prevalence of hypovitaminosis D, defined as serum 25(OH)D <25 nmol/L, increased by approximately 100 % from 1988–1994 to 2001–2006 (from 2.4 to 4.7 %; $P < 0.001$)

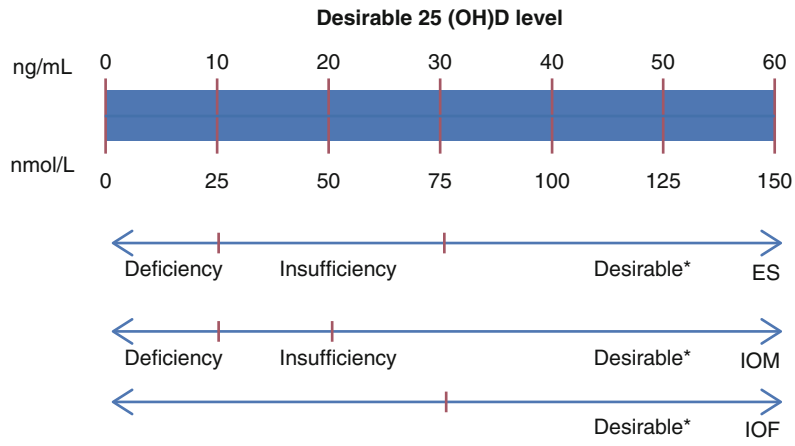

Fig. 27.1 Recommended desirable 25(OH)D levels. Desirable 25(OH)D levels as recommended by the Endocrine Society (ES) 2011 Guidelines, the Institute of Medicine (IOM) 2011 Report, and the IOF 2010 Position statement. *Desirable 25(OH)D level: the IOM Report states that levels above 125 nmol/L should raise concerns about potential adverse effects, whereas no clear statement was made by ES. The IOF recommendation was specifically targeting the elderly

in the overall group. The overall geometric mean serum 25(OH)D concentrations decreased, after adjusting for assay variation, by 9 % from 61 nmol/L in 1988–1994 to 55 nmol/L in 2001–2006 ($P<0.001$) in the overall group, a decline that was even greater, by 16 %, that is from 64 to 54 nmol/L, $P<0.001$, in adolescents [30]. The wide variation in the prevalence of hypovitaminosis D globally is in part explained by the different vitamin D assays and variable cutoffs used in the various studies.

Two recent sets of recommendations both issued in 2011 by the Institute of Medicine (IOM) and the Endocrine Society (ES) defined differing desirable 25(OH)D levels, see Fig. 27.1 [31, 32]. The charge of the IOM Committee was to determine the population needs for calcium and vitamin D in North America, and the objective of the ES task force was to provide guidelines to clinicians for the evaluation, treatment, and prevention of vitamin D deficiency, with an emphasis on the care of patients who are at risk for deficiency. Although the authors of both guidelines conducted extensive and thorough reviews of the literature; used for the most part the same studies, identical predefined musculoskeletal outcomes, and surrogate measures for such outcomes; and established criteria to grade the quality and strength of the evidence, they reached different recommendations. Furthermore, their recommendations were most relevant to western populations.

Mean circulating 25(OH)D levels vary between ethnic groups in the USA, being lower in blacks and Hispanics, than non-Hispanic whites [33–35], and also in many other populations worldwide [1, 26, 27]. In this chapter, we address the question whether desirable 25(OH)D levels vary globally. To define desirable levels, the beneficial effects of vitamin D on musculoskeletal outcomes, that is, fractures and falls, and on surrogate measures and markers of mineral metabolism are considered as primary endpoints. Pertinent studies relevant to pleiotropic effect of vitamin D on nonclassical outcomes are also briefly overviewed. The term globally was used in its literary meaning (dictionary definition), and thus the purpose of this chapter is to determine whether desirable vitamin D levels vary worldwide, between different populations and differing ethnicities, or whether a common universal desirable level can be defined.

Methods

A PubMed search covering the period between January 1, 1966, and May 10, 2012, was conducted. It was updated up to Aug 15, 2012, for the primary outcomes of falls and fractures. The search was limited to English language. For the primary outcomes of falls and fractures, the key terms vitamin D, falls, fractures, and meta-analysis were used. For the surrogate markers of bone and mineral metabolism, that is, vitamin D–PTH relationship, calcium absorption, and bone mineral density, these keywords were used each separately, combined with the key terms ethnicity and vitamin D.

All articles were scanned by title to select relevant publications; abstracts for these were reviewed, leading to the ultimate publications that were reviewed in full for relevant information. Additional pertinent papers not identified through the above searches, or in press, identified by authors were also taken into consideration. For surrogate markers of bone and mineral metabolism population-based studies were favored, but if unavailable, as was the case for most ethnic groups excluding blacks, smaller studies were reviewed.

Beneficial Effect of Vitamin D on Classical Outcomes

Direct Evidence from Randomized Controlled Trials on Fractures and Falls

A PubMed search at the time of the symposium, updated to Aug 2012, revealed a total of 11 relevant meta-analyses on fractures, summarized in Table 27.1 [25, 36–45]. Similarly, a total of nine meta-analyses on falls were retrieved, summarized in Table 27.2 [39, 46–53].

For fractures, a total of 60 publications were originally identified by title, of which 10 studies were relevant and had their abstract screened, in addition to one study retrieved from our database [38] for a total of 11 publications. A protective effect of vitamin D supplementation on the incidence of fractures including hip and non-hip fractures was observed in some, but not all studies, with odds ratios (ORs) or relative risks (RRs) varying between 0.70 and 0.92 for positive studies (Table 27.1). The most consistent effect was on hip fractures, where out of 13 positive estimates, 7 were on hip fractures, and more likely to be in trials on vitamin D plus calcium or in trials using high doses of vitamin D (Table 27.1). Conversely, three positive estimates were on non-vertebral fractures (four if subgroup analyses included) and two on overall/total fractures. The differences between the meta-analyses could in part be explained, by differences in the criteria for trial selection, vitamin D formulation used (oral vs. injectable), varying adherence with study drugs, and/or methodologies used to implement analyses. In an attempt to resolve the controversy above, Bischoff-Ferrari et al. conducted a pooled analysis from 11 double-blind, randomized, controlled trials of oral vitamin D supplementation (daily, weekly, or every 4 months), with or without calcium, as compared to placebo or calcium alone, in persons 65 years of age or older [45]. At doses greater than 800 IU/day, the authors demonstrated a 30 % reduction in the risk of hip fracture hazard ratio (HR) = 0.70 [0.58–0.86] and a 14 % reduction in the risk of any non-vertebral fracture (HR) = 0.86 [0.76–0.96], in these elderly subjects. The advantage of the latest analyses is the fact that the investigators considered participant-level pooled analysis and the actual dose of vitamin D consumed by study subjects [45]. Threshold assessment of hip fracture risk according to 25(OH)D cutoffs revealed a significantly protective effect of vitamin D at levels >43–60 nmol/L for non-hip fractures and ≥61 nmol/L for hip fractures. Similarly, Dawson-Hughes et al. suggested that a mean 25(OH)D level of 65 nmol/L would be needed to reduce non-vertebral fracture risk and of 75 nmol/L to reduce hip fracture risk [54]. The overwhelming majority of randomized trials were conducted in western countries, with an anticipated predominance of Caucasian subjects. Indeed, ethnicity was specified in only two US studies, both of which were included in six of the meta-analyses, whereas the meta-analyses of Bischoff-Ferrari et al. 2005 and DIPART 2010 only used one.

Table 27.1 Meta-analysis of randomized controlled trials on vitamin D by fracture type

Author (year) [reference]	Fracture type	Age/*N* study subjects	Country of origin (ethnicity)	Treatment	OR or RR [CI]	Outcome
Bischoff-Ferrari et al. (2005) [36]	*Hip*	Age ≥ 60 years	France, Germany, the Netherlands, Norway, the UK, the USA (*1 with mixed ethnicity*)			
	3 RCTs	5,572 ♀♂		Vit D 700–800 IU/day vs. Ca/Pbo	RR 0.74 [0.61–0.88]	Positive
	2 RCTs	3,722 ♀♂		Vit D 400 IU/day vs. Ca/Pbo	RR 1.15 [0.88–1.50]	
	Non-vertebral	Mean age 79 years				
	5RCTs	6,098 ♀♂		Vit D 700–800 IU/day vs. Ca/Pbo	RR 0.77 [0.68–0.87]	Positive
	2 RCTs	3,722 ♀♂		Vit D 400 IU/day vs. Ca/Pbo	RR 1.03 [0.86–1.24]	
Boonen et al. (2007) [37]	*Hip*	≥50 years	France, the Netherlands, Norway, the UK, the USA (*2 with mixed ethnicities*)			
	4 RCTs	9,083 PM ♀, ♂		Vit D vs. Pbo	RR 1.10 [0.89–1.36]	
	6 RCTs	45,509 PM ♀, ♂		Vit D ± Ca vs. Pbo/no treatment	RR 0.82 [0.71–0.94]	Positive
Cranney et al. (2007) [38]	*Hip*		Finland, France, Germany, the Netherlands, the UK, the USA (*2 with mixed ethnicities*)			
	3 RCTs	7,939♀♂		Vit D3 vs. Pbo	OR 1.11 [0.86–1.44]	
	3 RCTs	2,997♀ and ♀♂		Vit D3 + Ca vs. Ca	OR 0.91 [0.61–1.36]	
	7 RCTs	46,072 ♀ and ♀♂		Vit D3 + Ca vs. Pbo	OR 0.83 [0.68–1.00]	
	Non-vertebral					
	7 RCTs	46,074♀ and ♀♂		Vit D3 + Ca vs. Pbo	OR 0.87 [0.75–1.00]	
	3 RCTs	7,939♀♂		Vit D3 vs. Pbo	OR 0.99 [0.83–1.17]	
	Vertebral					
	3 RCTs	44,260♀♂		Vit D2/D3 ± Ca vs. Pbo/Ca	OR 0.88 [0.73–1.07]	
Jackson et al. (2007) [39]	*Non-vertebral*	≥65 years ♀♂	Finland, Germany, the Netherlands, Norway, the UK, the USA			
	6 RCTs	8,524		Vit D3 vs. no Vit D3	RR 0.96 [0.84–1.09]	
	Vertebral	Only in PM ♀				
	2 RCTs	902			RR 1.22 [0.64–2.31]	

(continued)

Table 27.1 (continued)

Author (year) [reference]	Fracture type	Age/*N* study subjects	Country of origin (ethnicity)	Treatment	OR or RR [CI]	Outcome
Bischoff-Ferrari et al. (2009) [40]	*Hip*	Age ≥65 years	Australia, France, Germany, the Netherlands, Norway, the UK, the USA (*2 with mixed ethnicities*)	Vit D ± Ca, Ca/Pbo		
	8 RCTs	40,886♀♂		Any dose of Vit D	RR 0.91 [0.78–1.05]	
	3 RCTs	9,014		Low dose Vit D up to 400 IU/day	RR 1.09 [0.90–1.32]	
	5 RCTs	31,872		Vit D >400 IU/day	RR 0.82 [0.69–0.97]	Positive
	Non-vertebral					
	12 RCTs	42,279 ♀♂		Any dose of Vit D	RR 0.86 [0.77–0.96]	Positive
	3 RCTs	9,014		Low dose Vit D up to 400 IU/day	RR 1.02 [0.92–1.15]	
	9 RCTs	33,265		Vit D >400 IU/day	RR 0.80 [0.72–0.89]	Positive
Avenell et al. (2009) [41]		Age >65 years	Australia, Finland, France, Germany, the Netherlands, Norway, Switzerland, the UK, the USA (*2 with mixed ethnicities*)			
	Hip	♂/PM♀				
	9 RCTs	24,749		Vit D vs. Pbo/no treatment	RR 1.15 [0.99–1.33]	
	4 RCTs	6,988		Vit D + Ca vs. Ca	RR 0.83 [0.61–1.12]	
	2 RCTs	2,718		Vit D vs. Ca	RR 0.90 [0.61–1.32]	Positive
	8 RCTs	46,658		Vit D + Ca vs. Pbo /no treatment	RR 0.84 [0.73–0.96]	
	Non-vertebral	♂/PM♀				
	1 RCT	3,440		Vit D vs. Pbo /no treatment	RR 0.96 [0.80–1.15]	
	4 RCTs	3,061		Vit D + Ca vs. Ca	RR 0.96 [0.79–1.16]	
	3 RCTs	2,976		Vit D vs. Ca	RR 1.08 [0.90–1.31]	
	9 RCTs	46,781		Vit D + Ca vs. Pbo/ no treatment	RR 0.95 [0.90–1.00]	
	Vertebral	♂/PM♀				
	5 RCTs	9.138		Vit D vs. Pbo/no treatment	RR 0.90 [0.42–1.92]	
	2 RCTs	2,681		Vit D + Ca vs. Ca	RR 0.14 [0.01–2.77]	
	3 RCTs	2,976		Vit D vs. Ca	RR 2.21 [1.08–4.53]	
	3 RCTs	38,990		Vit D + Ca vs. Pbo/ no treatment	RR 0.91 [0.75–1.11]	
	Any new fracture	♂/PM♀				
	10 RCTs	25,016		Vit D vs. Pbo/no treatment	RR 1.01 [0.93–1.09]	
	2 RCTs	927		Vit D + Ca vs. Ca	RR 0.76 [0.48–1.21]	

Bergman et al. (2010) [42]	*Hip*	≥50 years ♀	France, Germany, the UK			
	3 RCTs	7,473		Vit D3 + Ca vs. Pbo	OR fixed effect 0.70 [0.53–0.90] OR random effects 0.72 [0.32–1.40]	Positive
	2 RCTs			Vit D3+ Ca vs. Ca	OR fixed effects 1.03 [0.39–2.25] OR random effects 1.12 [0.23–3.04]	
	Non-vertebral	3,510				
	2 RCTs			Vit D3 + Ca vs. Pbo	OR fixed effects 0.77 [0.63–0.93] OR random effects 0.96 [0.28–2.54]	Positive
	2 RCTS			Vit D3+ Ca vs. Ca	OR fixed effects 0.68 [0.43–1.01] OR random effects 0.73 [0.17–1.9]	
DIPART Study (2010) [43]	7 RCTs	Mean age 70 years ♀♂	Denmark, Norway, the UK, the USA (*1 with mixed ethnicity*)			
	Overall fracture	68,517		Vit D + Ca	HR 0.92 [0.86–0.99]	Positive
	Hip			All studies	HR 0.84 [0.70–1.01]	
				Vit D 400 IU + Ca	HR 0.74 [0.60–0.91]	Positive
				Vit D alone 400–800 IU	HR 1.09 [0.92–1.29]	
	Vertebral			Vit D 400 IU + Ca	HR 0.85 [0.66–1.11]	
				Vit D alone 400–800 IU	HR 1.12 [0.70–1.79]	
Lai et al. (2010) [44]	7 RCTs	Mean age range for all RCTs 75–85 years ♀♂	the Netherlands, Norway, the UK			
	Hip	29,125		6 RCTS with Vit D3 vs. Pbo 1 Factorial design with Vit D vs. Pbo + Vit D + Ca vs. Ca.	RR 1.13 [0.98–1.29]	

(continued)

Table 27.1 (continued)

Author (year) [reference]	Fracture type	Age/*N* study subjects	Country of origin (ethnicity)	Treatment	OR or RR [CI]	Outcome
Chung et al. (2011) [25]	*Total fracture risk*	Age ≥65 years	Australia, France, Finland, Germany, the Netherlands, the UK, the USA (*2 with mixed ethnicities*)			Positive
	5 RCTs	14,583 ♀♂		VIT D 400–1,370 IU/day vs. Pbo	RR 1.03 [0.84–1.26]	
				Total fracture risk		
	11 RCTs	52,915 (69 % PM ♀)		Vit D 300–1,000 IU/day + Ca	RR 0.88 [0.78–0.99]	
				Total fracture risk		
Bischoff-Ferrari et al. (2012) [45]	11 RCTs	Mean age 76 years 31,022	Australia, France, Germany, the Netherlands, Norway, the UK, Switzerland, the USA (*2 with mixed ethnicities*)			Positive
	Hip	♀♂		Vit D (792–2000 IU/day)	RR 0.72 [0.59–0.89]	
				Vit D (638–791 IU/day)	RR 1.01 [0.82–1.23]	
				Vit D (361–637 IU/day)	RR 1.16 [0.76–1.77]	
				Vit D (0–360 IU/day)	RR 1.13 [0.77–1.67]	
	Non-vertebral	♀♂		Vit D (792–2000 IU/day)	RR 0.88 [0.74–1.04]	
				Vit D (638–791 IU/day)	RR 0.89 [0.80–1.01]	
				Vit D (361–637 IU/day)	RR 1.12 [0.90–1.40]	
				Vit D (0–360 IU/day)	RR 0.99 [0.89–1.11]	

Meta-analysis sorted by country of origin/ethnicity, with the specified ethnicity being presumably predominantly Caucasian, unless otherwise specified. Two fracture studies specified mixed ethnicity and are included in 8/11 fracture meta-analyses summarized in the table. Only studies with positive outcome are specified in the last column. *PM* postmenopausal women

Table 27.2 Meta-analysis of randomized controlled trials on vitamin D and falls

Author (year) [reference]	*N* studies	Age/*N* study subjects	Country of origin (ethnicity)	Treatment	OR or RR [CI]	Outcome
Bischoff-Ferrari et al. (2004) [46]		Mean age 70 years	Germany, the Netherlands, Switzerland, the USA (*1 with mixed ethnicity*)	Vit D vs. Ca/Pbo		
	5 RCTs	1,237 (81 %♀)			OR 0.78 [0.64–0.92]	Positive
	+5 additional RCTs	11,238 (1,237 + 10,001)♀♂			OR 0.87 [0.80–0.96]	Positive
Jackson et al. (2007) [39]		Age ≥65 years	Finland, Germany, the UK, the Netherlands, Norway, the USA			
	4 RCTs +1 prospective	3,776 PM♀, ♂		Vit D3 ± Ca vs. Pbo	RR 0.88 [0.78–1.00]	
	4 RCTs	784 PM ♀		Vit D3 ± Ca	RR 0.92 [0.75–1.12]	
Richy et al. (2008) [47]		Mean age 78 years	Australia, Denmark, France, Germany, the Netherlands, New Zealand, Switzerland, the UK, the USA (*1 with mixed ethnicity*)			
	14 RCTs	21,268 ♀/♀♂		Vit D analogs vs. Pbo, blinded data	RR 0.79 [0.64–0.96]	Positive
				Vit D vs. Pbo, all data	RR 0.94 [0.90–0.99]	Positive
Bischoff-Ferrari et al. (2009) [48]		Age ≥65 years	Australia, Germany, the Netherlands, Switzerland, the USA			
	7 RCTs	1,921 ♀♂		High-dose Vit D (700–1000 IU/day)	RR 0.81 [0.71–0.92]	Positive
Gillepsie et al. (2009) [49]		Age ≥65 years	Australia, Germany, Japan, New Zealand, Switzerland, the UK, the USA (*1 with mixed ethnicity*)	Vit D ± Ca vs. control/ Pbo/Ca		
	5 RCTs	3,929♀♂		Rate of falls	RR 0.95 [0.80–1.14]	
	10 RCTs	21,110 ♀♂		Number of fallers	RR 0.96 [0.92–1.01]	
Cameron et al. (2010) [50]		Mean age 83 years	Australia, France, Switzerland, the UK, the USA	Vit D3 + Ca vs. Ca		
				Vit D2 vs. no intervention		
	4 RCTs	4,512 ♀/♀♂		Rate of falls		
	5 RCTs	5,095 ♀/♀♂		Risk of falls	RR 0.72 [0.55–0.95]	Positive
					RR 0.98 [0.89–1.09]	
Kalyani et al. (2010) [51]		Mean age range 71–92 years	Australia, Germany, the Netherlands, New Zealand, Switzerland, the UK, the USA (*1 with mixed ethnicity*)	Vit D 200–1,000 IU/ day vs.Ca/Pbo		
				Number of falls		
	10 RCTs	2,932♀♂		Primary analysis	RR 0.86 [0.79–0.93]	Positive
	+7 additional RCTs	18,068 ♀♂		Post hoc analysis	RR 0.92 [0.87–0.98]	Positive

(continued)

Table 27.2 (continued)

Author (year) [reference]	*N* studies	Age/*N* study subjects	Country of origin (ethnicity)	Treatment	OR or RR [CI]	Outcome
Micheal et al. (2010) [52]	9 RCTs	Age ≥65 years 5,780 (♀/♀♂)	Australia, Finland, Germany, Switzerland, the UK, the USA (*1 with mixed ethnicity*)	Vit D Risk of falls	RR 0.83 [0.77–0.89]	Positive
Murad et al. (2011) [53]	26 RCTs	Mean age 83 years 45,782; 78 % ♀	Amsterdam, Austria, Australia, Denmark, Finland, France, Germany, Japan, the Netherlands, New Zealand, Sweden, Switzerland, the UK, the USA	Vit D or no intervention Risk of falls	OR 0.86 [0.77–0.96]	Positive

Meta-analysis sorted by country of origin/ethnicity, with the specified ethnicity being presumably predominantly Caucasian, unless otherwise specified. One fall study specified mixed ethnicity and is included in 5/9 fall meta-analyses summarized in the table. Only studies with positive outcome are specified in the last column. *PM* postmenopausal women

Regarding falls, the search identified 16 titles, a total of 8 were used, in addition to one that was identified from our database, for a total count of 9 publications. The results for falls as shown in Table 27.2 were more consistently positive, with ORs/RRs ratios varying between 0.72 and 0.94, with the exception of the study by Gillespie et al. [49] and Jackson et al. [39]. However, when assessing efficacy of vitamin D in reducing number of fallers by vitamin D level at study entry, the risk ratio in 563 subjects partaking in three studies was significantly reduced, RR = 0.65 [0.46–0.91] [49]. The overall positive results for falls are also consistent with the sensitivity analyses of six trials with high-quality fall assessment, the IOM implemented showing a 21 % reduction in the odds of falling (OR = 0.79; 0.65–0.96; heterogeneity $I^2 = 0$ %). Again, the overwhelming majority of randomized trials were conducted in western countries, with an anticipated predominance of Caucasian subjects; however, ethnicity was specified in only one study. The study was conducted in the USA, stated that ethnicity was mixed, and was included in five meta-analyses (Table 27.2). Therefore, based on the randomized controlled trials available to date, there is no good evidence for a beneficial effect, or the absence thereof, of vitamin D supplementation on falls or fractures in non-Caucasian subjects.

Indirect Evidence: Surrogate Markers for the Effect of Vitamin D on Mineral Metabolism

Low levels of 25(OH)D are associated with calcium malabsorption, secondary hyperparathyroidism, increased bone resorption, bone loss, and fractures. Very low levels cause osteomalacia in adults. Desirable 25(OH)D levels have thus also been defined by examining the relationship between this metabolite and surrogate markers of its impact on mineral metabolism, namely, PTH levels, calcium absorption, bone remodeling, and density. The challenges with such approach for all such surrogates are numerous and include the fact that such relationships are modulated by many other variables that are not captured in these two-dimensional models, including age, gender, race, calcium intake, renal function, body mass index (BMI), and genetic polymorphisms (VDR, ER, etc.). This is to be added to the scarce head-to-head comparisons between racial and ethnic groups and the inherent variations between the 25(OH)D assays used (see below). This section will examine the evidence stemming from such relationships in general and how it pertains to ethic and racial groups in particular.

Vitamin D and PTH

25(OH)D insufficiency thresholds have been suggested based on the inverse association between 25(OH)D and PTH, because low vitamin D causes hyperparathyroidism and high PTH levels are associated with increased bone turnover, leading to greater bone loss. Thus, maintenance of sufficient 25(OH)D levels would be anticipated to enhance bone health. In his review of the topic, Willet underscored the wide range of proposed 25(OH)D thresholds in the various studies available at the time, ranging from 25 to 90 nmol/L, depending on the study, and concluded that such approach may not be the best mean to identify vitamin D insufficiency in various populations [55]. Similarly, in their review of the literature on this topic from 1988 to June 2010, Sai et al. reviewed a total of 70 studies showing an inverse relationship between serum 25(OH)D and PTH and again outlined the wide variability in 25(OH)D levels at which PTH plateaus, where levels ranged from 25 to 125 nmol/L. Similar wide variations were presented when the data was analyzed by age, gender, or world region (the USA, Europe, Asia, Australia, and the Middle East) [56]. Three studies had differing plateaus based on age, two based on gender and one by ethnicity. The wide variations detailed are explained by the numerous modulators detailed above, data from the most established ones are discussed below. Finally, no plateau could be defined in eight studies, and no relationship between 25(OH)D and PTH could be described in three studies [56].

The PubMed search we conducted included ethnicity as a keyword and revealed a total of 121

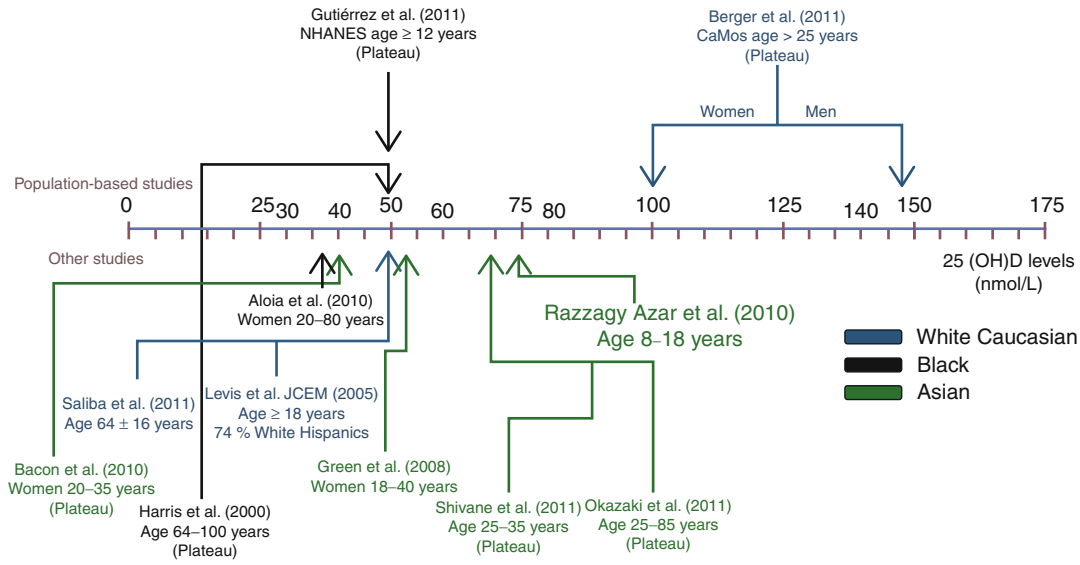

Fig. 27.2 Inflection point or plateau determined from the curves relating PTH to 25(OH)D levels. Data points represent the inflection point, 25(OH)D level below which PTH levels rise sharply, or a plateau, the 25(OH)D level above which PTH levels did not suppress any further. Information provided from population-based studies are illustrated in *upper panel*, and in other studies on *lower panel*, by ethnicity (*blue* for Caucasians, *green* for Asians, and *black* for blacks). The data points include both genders unless otherwise specified. The study by Saliba et al. also included information on the plateau that was reached at a 25(OH)D level between 75 and 85 nmol/L. To convert from nmol/L to ng/mL, divide by 2.496

publications, and although many included information on 25(OH)D and or PTH in different ethnic groups, most did not analyze the data in terms of evaluating the 25(OH)D–PTH relationship nor defined an inflection point, that is, 25(OH)D level below which PTH levels rise sharply, or a plateau, the 25(OH)D level above which PTH levels did not suppress any further. Therefore, a total of 41 relevant publications were reviewed in full, of which 35 are detailed in this manuscript.

For studies conducted in white (Caucasian) subjects, we elected to focus on population-based studies, as the most powerful and representative. These include the two recent population-based studies, NHANES in the United States and CaMOS in Canada, that provided differing conclusions [57, 58]. In the former, a total of 8,415 individuals, mean age between 37 and 45 years, including both genders, recruited in 2003–2004 and 2005–2006, were included: 51 % were white, 25 % blacks, and 25 % Mexican Americans. Whereas a continuous inverse association between 25(OH)D and PTH was observed across 25(OH)D levels in white and in Mexican American subjects, with no plateau, the curve became flat at 25(OH)D levels above 50 nmol/L in blacks, using the DiaSorin radioimmunoassay (RIA) ([57], Fig. 27.2). The lack of an inflection point in white subjects is consistent with findings from the LASA (Longitudinal Amsterdam Study of Aging, 59) and the British Diet and Nutritional Survey, conducted in elderly individuals between 1994 and 1995 [59]. Conversely, in the CaMos study of 3,896 individuals recruited in 1995–1997 and 2005–2007, the investigators showed differing plateau points by gender, defined at 147 nmol/L in men and 104 nmol/Lin women, using the DiaSorin LIAISON rapid platform assay ([58], Fig. 27.2). This gender difference is consistent, albeit with very different values for plateau points, with an opposite gender pattern described in an earlier study conducted in the national FINRISK survey, conducted in Finland on 328 young adults, age 30–42 years. PTH concentration started to increase at 25(OH)D concentrations lower than

40 nmol/L in men and 80 nmol/L in women, using the Incstar/DiaSorin 25(OH)D RIA [60]. In the same study, the PTH plateau was reached at 65 nmol/L in women and 57 nmol/L in men.

Several publications have illustrated the impact of other modulators of the 25(OH)D–PTH relationship including age [61, 62], renal function [63], and calcium intake to name a few [64]. Indeed, Arabi et al. evaluated this relationship in 340 Lebanese adolescents and 443 elderly and showed PTH levels to be higher in the elderly, in the overall population, and by subgroup analysis by gender. For the same 25(OH)D level measured by DiaSorin RIA, PTH levels were comparable across genders but were 1.5- to 2-folds higher in the elderly compared to adolescents. PTH correlated positively with age and body fat and negatively with calcium intake and 25-OHD; on multivariate analyses, age, 25(OH)D, and fat mass were independent predictors for PTH [62]. The impact of age may in part be confounded by a rise in serum creatinine. Indeed, in his evaluation of over 19,000 individuals partaking in a health maintenance organization, mean age 64 years, Saliba et al. demonstrated PTH levels to rise sharply at 25(OH)D levels below 50 nmol/L and to plateau at levels between 75 and 85 nmol/L, using the DiaSorin LIAISON rapid platform assay. The plateau was reached at a lower 25(OH)D level, of 46 nmol/L, when subjects with renal insufficiency were excluded. Finally, the impact of calcium intake on the 25(OH)D–PTH relationship was demonstrated in a cross-sectional study of 977 individuals, where serum PTH was lowest in subjects with a serum 25(OH)D of more than 45 nmol/L and highest in the group with a serum 25(OH)D level of less than 25 nmol/L, using a DiaSorin RIA [64]. At a level below 25 nmol/L, a calcium intake of less than 800 mg/day vs. more than 1,200 mg/day was significantly associated with higher serum PTH; at a calcium intake of more than 1,200 mg/day, there was a significant difference in PTH levels between the lowest and highest vitamin D groups [64].

Few are the studies evaluating inflection points or plateaus in other ethnic groups. Tables 27.3, 27.4, and 27.5 overview studies that have investigated the PTH 25(OH)D relationship in African American (Table 27.3, blacks) and Asian individuals (Tables 27.4 and 27.5). Studies on ethnic subgroups were mostly conducted on blacks, in the USA. A plateau point of 50 nmol/L was defined in black adults in the NHANES study and similarly in the study by Harris et al. [57, 65]. The latter was driven by only 12 subjects who had 25(OH)D level above 75 nmol/L. Conversely, Aloia et al. defined an inflection point of 37 nmol/L in black adults in one study [68], but the same author did not define it clearly in another [66]. Weaver et al. described an inverse slope relationship and no inflection point could be defined in black adolescents ([67], Table 27.3). As mentioned above, an inflection point could not be defined in Mexican Americans similarly to white subjects, in NHANES, whereas a study conducted in Florida on 212 subjects, age > 18 years, that included 74 % white Hispanics demonstrated an inflection point at 50 nmol/L [81]. Therefore, there was no agreement on the inflection point in black individuals, whereas 2 studies concurred on a plateau of 50 nmol/L (Table 27.3).

The 25(OH)D–PTH relationship was evaluated in few studies in other ethnic groups, including in adults from China, Japan, Lebanon, and Bangladeshi and Somali immigrants in Finland (Table 27.4, [62, 69–75]), as well as in children from Lebanon, India, and Iran (Table 27.5, [76–80]), and see Fig. 27.2. A plateau of 40 nmol/L was described in women from Hong Kong [73], and of 70 nmol/L was described both in 1,137 women from India [75] and in 107 women from Japan [74]. Conversely, an inflection point of 52 nmol/L was described in 504 women from Malaysia [72], and of 75 nmol/L was described in 313 Iranian children (Table 27.5, [80], see Fig. 27.2). Finally, few are the studies that evaluated inflection points in different ethnic groups, concomitantly. Consistent with observations from the NHANES study [57], the apparent plateau in blacks, based on only 12 subjects with 25(OH)D concentrations above 50 nmol/L, was not observed in whites, in the study by Harris et al. [65]. Conversely, no real inflection point could be identified by evaluating data in whites and blacks in two studies [66, 82]. Finally, many

Table 27.3 25(OH)D–PTH relationship in black subjects

Author (year) [reference], country	*N*	Gender (*N*) Age (years)	25(OH)D inflection point or plateau[a] (nmol/L)	25(OH)D assay used
Harris et al. (2000) [65], USA	136	Men (52) Women (84) 64–100	Plateau: 50 nmol/L	Competitive protein-binding assay (in-house)
Aloia et al. (2006) [66], USA	235	Pre-menopausal (148) Postmenopausal (87)	NA	Radioreceptor Assay, Incstar
Weaver et al. (2008) [67], USA	52	Girls 11–15	No inflection point Opposite slope	RIA, DiaSorin
Aloia et al. (2010) [68], USA	235	Women 20–80	Inflection point: 37 nmol/L	RIA, DiaSorin
Gutiérrez et al. (2011)[b] [57], USA NHANES 2003–2004, 2005–2006	2, 081	Men (957) Women (1,124) ≥12	Plateau: 50 nmol/L	RIA, DiaSorin

25(OH)D inflection point/plateau (nmol/L) determined from the PTH–25(OH)D relationship in black subjects. Data points represent the inflection point, 25(OH)D level below which PTH levels rise sharply, or plateau, the 25(OH)D level above which PTH levels did not suppress any further. All studies are from the USA

[a]Either inflection point or plateau detailed, as defined in original paper

[b]The only population-based study defined in blacks

are the studies that could not identify an inflection point or plateau, in several ethnic groups, few of which are detailed herein [56, 57, 59, 65, 67, 69–71, 82]. Thus, there was no convergence on either inflection point or plateau in the Asian subjects (Tables 27.4 and 27.5 and Fig. 27.2).

The challenge in deriving an inflection point and/or a plateau from the 25(OH)D–PTH relationship lies in the model chosen for curve fitting and its assumptions, be it based on visual examination of the curves, or through the application of mathematical curve fitting equations [83, 84]. Indeed, the PTH 25(OH)D relationship has been submitted to various models including exponential, log transformed, quadratic, fractional polynomials, cubic splines, two-phase, and three-phase models. For example, a systematic evaluation of the impact the choice of statistical approach has on the threshold point revealed that the three-phase model was superior to the two-phase model and identified two thresholds at 30 and 70 nmol/L, in white subjects, using the same dataset [85]. These findings illustrate the substantial impact the statistical approach has on threshold and plateau determination and the wide variability observed, within and between ethnic groups [85].

In light of considerations presented above including the confounding effect of age, renal function, gender, mathematical model used, and assay variation (see section below), on the 25(OH)D–PTH relationship, the determination of whether a desirable vitamin D level is the same or vary worldwide using such relationship is deeply flawed. Furthermore, very few if any are the studies that have attempted to do so by including several ethnic groups within the same study [57, 65, 66, 82], and only one was population based [57]. Therefore, such approach to define desirable 25(OH)D levels is therefore inadequate and should clearly be abandoned.

Vitamin D and Intestinal Calcium Absorption

Intestinal calcium absorption is the sum of a saturable (active) and a non-saturable (passive) pathways, the latter being dependent on Ca concentration in the lumen. In general 35 % of a dietary load is absorbed and calcitriol, also known as 1,25 dihydroxyvitamin D [1,25$(OH)_2$D], is the primary regulator of the saturable pathway and thus of intestinal Ca absorption efficiency [3, 86].

Table 27.4 25(OH)D–PTH relationship in Asian adult subjects

Author (year) [reference], country	*N*	Gender (*N*) Age (years)	25(OH)D inflection point or plateau[a] (nmol/L)	25(OH)D assay used
G-Yared et al. (2000) [69], Lebanon	316	Men (99) Pre-menopausal women (217) 30–50	Inflection point: logarithmic relationship with steep increase in PTH level at 25OHD	RIA, Incstar
Yan et al. (2003) [70], China vs. the UK	352	Men (175) Women (177) 60–83	Linear continuous	RIA, DiaSorin
Ono et al. (2005) [71], Japan	197	Men (95) Women (102) 43	Linear continuous	RIA, DiaSorin
Green et al. (2008) [72], Indonesia and Malaysia	504	Women 18–40	Inflection point: 52 nmol/L	RIA, DiaSorin
Arabi et al. (2010) [62], Lebanon	460	Men (150) Women (300) >65	NA	Competitive protein-binding assay, DiaSorin, Incstar
Bacon et al. (2010) [73], China	221	Women 20–35	Plateau: 40 nmol/L	RIA, DiaSorin
Okazaki et al. (2011) [74], Japan	107	Men (25) Women (82) 25–85	Plateau: 62–75 nmol/L	Chemiluminescence assay LIAISON, DiaSorin
Shivane et al. (2011) [75], India	1,137	Men (579) Women (558) 25–35	Plateau: 62–75 nmol/L	RIA, DiaSorin

25(OH)D inflection point/plateau (nmol/L) determined from the PTH–25(OH)D relationship in Asian adult subjects. Data points represent the inflection point, 25(OH)D level below which PTH levels rise sharply, or plateau, the 25(OH)D level above which PTH levels did not suppress any further

[a]Either inflection point or plateau detailed, as defined in original paper

Although it is clear that signaling through the VDR is needed for vitamin D-mediated intestinal Ca absorption, the exact mechanism(s) for calcium absorption is still being elucidated [86]. For example, VDR Fok1 polymorphisms can affect Ca absorption as shown with Chinese youth [87], but not in African American [88]. In addition, several other factors and hormones modulate calcium absorption including PTH, thyroid hormone, sex steroids, and IGF1 [86].

Whether 25(OH)D directly controls Ca absorption is under debate [56, 89–93]. Using a stable isotope technique in 93 young Caucasian adolescents, mean age 12.7 (±1.0) years, receiving diets averaging approximately 900 mg/day calcium, serum $1,25(OH)_2D$ but not 25(OH)D level, was significantly correlated to calcium absorption [92]. These finding are consistent with the results of studies conducted in 488 elderly Caucasian subjects, mean age 71 years [56]. In this study, calcium absorption was measured as percentage absorbed 2 h after the ingestion of 5 μCi of Ca in 200 mL distilled water, and while it was significantly and positively correlated with serum $1,25(OH)_2D$ after adjustment for age, body weight, calcium intake, serum 1,25(OH)2D, and serum creatinine ($r=0.224$, $P=0.0005$), it was not associated with serum 25(OH)D ($r=0.05$, $P=0.247$) after similar adjustments [56].

Two frequently quoted studies, which were extensively detailed in the Endocrine Society 2011 Guidelines and the IOM 2011 Report, have reached strikingly differing desirable 25(OH)D levels with regard to intestinal calcium absorption. The Endocrine Society relied on a study by Heaney et al., a study criticized for the fact that

Table 27.5 25(OH)D–PTH relationship in Asian children

Author (year) [reference], country	*N*	Gender (*N*) Age (years)	25(OH)D inflection point or plateau[a] (ng/mL)	25(OH)D assay used
El-Hajj Fuleihan et al. (2001) [76], Lebanon	346	Males (164) Females (182) 10–17	NA	Competitive protein-binding assay, DiaSorin, Incstar
Marwaha et al. (2005) [77], India	5,137	Males (2,047) Females (3,090) 10–18	NA	RIA, DiaSorin
El-Hajj Fuleihan et al. (2006) [78], Lebanon	179	Females 10–17	NA	Competitive protein-binding assay, DiaSorin, Incstar
Ghazi et al. (2010) [79], Iran	207	Males (103) Females (104) 14–20	NA	ELISA, IDS
Razzagy Azar et al. (2010) [80], Iran	313	Males (121) Females (192) 8–18	Inflection point: 75 nmol/L	RIA, IDS

25(OH)D inflection point/plateau (nmol/L) determined from the PTH–25(OH)D relationship in Asian children. Data points represent the inflection point, 25(OH)D level below which PTH levels rise sharply, or a plateau, the 25(OH)D level above which PTH levels did not suppress any further

[a]Either inflection point or plateau detailed, as defined in original paper

40 % of subjects were on hormone therapy and half did not have formal calcium absorption studies [94]. The same authors have compiled these data with two additional studies and concluded that absorption efficiency rises and reaches a plateau at a serum 25(OH)D level of 80 nmol/L [91]. Conversely, the IOM Report heavily relied on a key study demonstrating that calcium absorption reaches a near maximum at a 25(OH)D level of 20 nmol/L [95].

Thacher et al. showed that calcium absorption in Nigerian adolescents with calcium deficiency rickets was not closely related to baseline 25(OH)D ($r=0.01$, $P=0.93$) or 1,25(OH)2D concentrations ($r=0.21$, $P=0.24$) nor to changes in these metabolites in response to supplementation to a single oral dose of 50,000 IU of vitamin D [96]. Using results from pooled studies, the same authors showed that while the relationship between fractional calcium and serum 25(OH)D concentrations was absent in control children, it was marginally inverse in calcium-deficient rachitic children ($r=-0.26$, $P=0.06$) [93, 96]. We are unaware of any other absorption studies conducted in non-Caucasian populations. However, it was recently suggested that single-nucleotide polymorphisms (SNPs) of TRPV6 and TRPV5, highly selective transcellular calcium transporters expressed at the apical site of intestinal epithelia, exhibit unusually high levels of single-nucleotide polymorphisms in African populations compared to other populations and are likely contributed to the calcium conservation mechanisms in these populations [97].

In conclusion, the evidence to support a key role for 25(OH)D in calcium absorption is debatable, and for a desirable 25(OH)D level rather limited in Caucasian populations, let alone other ethnic groups. Furthermore, none take into consideration modulators of absorption such as gender, calcium intake, hormonal status, and polymorphisms for receptors involved in signaling by known key modulators, such as ER, VDR, CaSR, TRPV5, and TRPV6 polymorphisms. In light of all of the above, a conclusive statement regarding desirable 25(OH)D levels in general, and in ethnic subgroups in particular, using calcium absorption cannot be drawn.

Vitamin D, Biochemical Markers of Bone Remodeling, and Bone Mineral Density

Adults

The relationship between 25(OH)D, bone remodeling markers, bone mineral density (BMD), and bone loss has also been used to define optimal vitamin D levels. In the Longitudinal Amsterdam Study of Aging (LASA), there was an inverse relationship between 25(OH)D and serum osteocalcin (OC) and urinary deoxypyridinoline across four categories of the metabolite: <25, 25–50, 50–75, and >75 nmol/L [98]. In adjusted analyses, on weighted regression smoothing (LOESS plots), there was a decrease in both markers up to a 25(OH)D of 40 nmol/L, followed by a plateau, and both hip and trochanteric BMD increased up to a 25(OH)D level of 50 nmol/L. In post hoc analyses in a subgroup of elderly women partaking in the STOP-IT trial, nonlinear and piecewise regression analyses revealed increments in serum osteocalcin and urine N-telopeptides only at a 25(OH)D below 45 nmol/L [56]. In the NHANES study, hip BMD increased with increasing 25(OH)D levels in all ethnic groups, the curve being steepest in white subjects, up to a level of 80 nmol/L, and above which it increased less steeply [99]. In subsequent analyses, hip BMD significantly decreased as 25(OH)D levels declined, by approximately 0.6 SD among white and 0.33 SD among Mexican Americans, but it did not change among black subjects [57]. The findings in NHANES are consistent with those described in adult white and black men residing in Boston [82]. Furthermore, the BMD–vitamin D relationship in the NHANES study did not change after adjustments for gender, BMI, and serum creatinine, but whereas age did not alter this relationship in blacks and Mexican Americans, it did only among white subjects ≥50 years [57].

This divergent relationship between vitamin D and skeletal parameters between black and white subjects is further illustrated in the nested case–control study within the prospective Women's Health Initiative (WHI) observational cohort [100]. During the 8-year follow-up, 400 incident fractures were identified in white, 381 in black, 192 in Hispanic, 113 in Asian, and 46 in Native American subjects. In the multivariate analyses, 25(OH)D levels above 75 nmol/L were associated with reduced risk of fractures in white individuals, whereas a trend for an increased risk was observed in black and Asian individuals and no association could be detected in Hispanics and Native Americans, possibly due to the lower power in the latter ethnic groups [100]. Spline analyses revealed a significant harmful effect of 25(OH)D levels above 42 nmol/L in black subjects, justifiably leading the authors to conclude that optimal levels vary across race and ethnicities. Similarly, in a randomized vitamin D trial conducted in 208 postmenopausal black women, vitamin D at doses of 800 IU/day during the first 2 years and 2000 IU/day during the third year raised 25(OH)D from a mean of 47 to 71 nmol/L, but did not affect bone turnover markers nor bone loss prospectively [101]. This is in sharp contrast to the protective effect of vitamin D, on bone loss rates in white subjects, as shown in 1,279 community dwelling elderly white men (age 73 years), partaking in the MrOs study [102], and in white postmenopausal women (age 62 years) in the OFELY study [103]. A 25(OH)D greater than 50 nmol/L was associated with decreased bone loss in men [102] and decreased fracture risk in women [103]. The protective effect of 25(OH)D on fractures in white women is consistent with those made in a cohort of 1,194 white elderly Swedish men, mean age 71 years, followed for a median of 11 years, 309 of whom sustained a fracture. The multivariate-adjusted hazard ratio for fracture was 1.65 [1.09–2.49] in those with a 25(OH)D below 40 nmol/L [104].

Very few are the studies that investigated other ethnic groups outside the United States. The prevalence of vitamin D deficiency, secondary hyperparathyroidism, and associations with forearm bone mineral density were studied in 48 premenopausal Somali and 34 Bangladeshi immigrants, compared to 61 ethnic Finnish women, during the winter [105]. The prevalence of serious vitamin D deficiency, defined by a PTH level above 7.2 pmol/L, was five times higher

in Somali and two times higher in Bangladeshi women, confirming the well-established high prevalence of vitamin D insufficiency in Asian immigrants in Europe. There was a significant correlation between 25(OH)D level and both proximal and distal radius BMD, $r = 0.25–0.27$, an observation that has been made in several other smaller studies in subjects from the Middle East and Asia [106–116]. Resistance to the bone resorbing effect of high PTH levels, in response to low 25(OH)D, had been suggested in African American subjects, but was not consistently demonstrated [117–119]. Ethnic differences in bone metabolism in response to a phosphate load were examined in healthy older individuals from the UK, China, and the Gambia [119]. Although ethnic differences in changes in bone markers were detected, the relationship with changes in PTH was comparable between groups, and there was no evidence for skeletal resistance to PTH [119].

Finally, in a population-based study of elderly Lebanese, mean age 72 years, 25(OH)D of 50 nmol/L by itself was not a predictor of low bone mass nor of prospective bone loss over 4 years, unless coupled with elevated PTH levels above 8.4 pmol/L [120, 121], thus again reflecting the multidimensional nature of the relationship between vitamin D and skeletal health [122]. Furthermore, few are the studies evaluating the relationship between 25(OH)D and BMD taking into consideration other modulators of this association, such as VDR polymorphisms [114, 115, 123].

Children and Adolescents

Studies evaluating the relationship between 25(OH)D, PTH, and bone remodeling markers in this age group are limited by the powerful confounding effect of growth on bone modeling and remodeling. While many are the cross-sectional studies illustrating the direct correlation between vitamin D and BMD in the pediatric age groups, including studies from Asia [77], few are the randomized trials investigating the beneficial effect of supplementation on bone mass. In a recent meta-analysis, information from six studies, totaling 343 participants receiving placebo and 541 receiving vitamin D, was analyzed [124]. Vitamin D supplementation had no statistically significant effects on total body bone mineral content or on bone mineral density of the hip or forearm, and there was a trend to a small effect on lumbar spine bone mineral density. In preplanned subgroup analyses, the authors concluded that supplementation of deficient children and adolescents could result in clinically useful improvements. These analyses were largely driven by findings in a randomized trial conducted in Lebanese adolescent girls, who were vitamin D insufficient at study entry [78].

Optimal levels of vitamin D may vary by ethnicity, reflecting differences in the calcium economy. While there is some converging evidence to support this hypothesis in blacks, data in other ethnic groups is scarce to nonexistent. Indeed, it appears that the calcium economy for bone health is quite different in blacks [117, 118, 125, 126], with a suggestion that desirable 25(OH)D levels for Caucasians may be harmful to black subjects [100]. The notion of a universal global desirable level for musculoskeletal health, at this point, therefore seems non-tenable.

Pleiotropic (Nonclassical) Effects of Vitamin D

Association studies, many of which are population based, have implicated vitamin D as a potential risk factor for a number of major chronic illnesses [4–25]. These studies are however limited by their cross-sectional nature. Both the IOM Committee and the ES task force members examined the data available and agree on the lack of convincing evidence for a beneficial effect of vitamin D on nonskeletal outcomes [31, 32, 127]. Cardiovascular diseases, infections, and maternal neonatal health are on the top of the health agenda of the WHO and health authorities in many countries worldwide, most of which are from Asia and Africa. In this section, pertinent available data specific and/or of particular relevance to non-western populations will be reviewed.

Cardiovascular Diseases and Infections

Cardiovascular diseases in particular are among leading causes of death worldwide, non-western populations included [128, 129]. In the Korean Longitudinal Study of Health, 439 men and 561 women aged 65 years or older were recruited by random stratified sampling and had their coronary artery calcium score, intima–media thickness, and pulse wave velocity measured [130]. Compared to subjects with 25(OH)D levels above 75 nmol/L, those with levels between 37.5 and 75 nmol, and below 37.5 nmol/L, had a two-fold and three-fold higher risk of stenosis, respectively [130].

Acute respiratory infections cause 4.5 million deaths among children per year in developing countries and are the leading cause of morbidity and mortality in preschool children in these countries [27]. Strong associations between hypovitaminosis D and tuberculosis (TB) infection and acute lower respiratory tract infections have been reported in Indian, Turkish, and sub-Saharan African populations [27]. TB, HIV, and AIDS are rife and still responsible for the most deaths in Africa and many other developing regions. TB has plagued human kind for thousands of years, and although the first antituberculosis drugs were discovered over 60 years ago, it still kills 1.7 million persons per year [131]. Because of the associations between vitamin D deficiency with susceptibility to TB in HIV-uninfected people in Europe and the high prevalence of vitamin D deficiency in Africa, a cross-sectional study was conducted to determine whether vitamin D deficiency was associated with susceptibility to active TB in HIV-uninfected ($n = 196$) and HIV-infected ($n = 174$) black Africans in Cape Town, South Africa [132]. Vitamin D deficiency defined as 25(OH)D <50 nmol/L was present in 63 % of participants and was associated with active TB both in HIV-uninfected and HIV-infected individuals, OR = 5.2 [2.8–9.7] and 5.6 [2.7–11.6], respectively. Reciprocal seasonal variation in serum 25(OH)D concentration and TB notifications over an 8-year period were noted, suggesting that vitamin D status and TB incidence are causally related [132]. However, vitamin D supplementation failed to improve clinical outcome or mortality in a randomized placebo-controlled trial of 367 adult patients with TB from Guinea-Bissau [27].

Maternal–Neonatal Health

Maternal, infant, and under-five child mortality rates in developing countries have declined significantly in the past two to three decades, but newborn mortality rates have reduced much more slowly. Vitamin D insufficiency may be associated with poorer maternal obstetrical outcomes and adverse outcomes in the offspring [133]. Pregnant women from Pittsburgh with 25(OH)D levels below 37.5 nmol/L during their first trimester had a five-fold higher risk of developing preeclampsia during pregnancy [134], and in a study from Boston using the same 25(OH)D cut-off, women were 2.5 times as likely to have a Cesarian section [135]. The association with preeclampsia could not be confirmed in a subsequent study [136], findings that may potentially be explained by the timing of the vitamin D measurement during pregnancy. Indeed, in a study of 700 women, mean age 30 years, 25(OH)D level below 50 nmol/L during the second, but not first trimester, conferred a three-fold higher risk of developing preeclampsia [137]. First trimester maternal 25(OH)D levels were also associated with an increased risk of small for gestational age in the offspring. In the USA, white women with 25(OH)D level less than 37.5 nmol/L had an increased risk of having a neonate who is small for gestational age (less than 10th percentile). The OR was 7.5 [1.8–31.9], but the risk was not increased in black women OR = 1.5 [0.6–3.5], [138]. Similarly, white Finnish women, with 25(OH)D less than 30 nmol/L during the first trimester, had an increased risk of small for gestational age (less than 10th percentile) OR = 1.9 [2.4–2.7], [139]. Finally, in a study of 374 Australian women, mean age 29 years, there was a significant association between 25(OH)D at 29–32 weeks and infant size at birth: birth weight, head circumference, knee–heel length, and crown–heel length [140]. In a longitudinal birth

cohort of 98 children born in Southampton [141], maternal 25(OH)D during the third trimester correlated with offspring bone mass at age 9 years [141]. Vitamin D supplementation during the first year of life was associated with increased bone mass at the hip 8 years later [142]. However, in a systematic review of trials using vitamin D supplementation during pregnancy, the authors could not find any vitamin D trials investigating the impact of intervention on gestational diabetes, preeclampsia, or preterm birth and found low-quality evidence for a positive effect on birth weight [143]. Considering trials that included calcium with vitamin D supplementation, the authors found low-grade evidence for a favorable effect on preeclampsia, but there were no trials to evaluate an impact on other outcomes listed above [143]. It is very important to underscore again the fact that many of the studies above were conducted in Caucasians subjects, where levels of 25(OH)D are anticipated to be higher than in non- Caucasians. Indeed, a systematic review of first trimester normative 25(OH)D levels that included 18 studies across the world revealed mean levels ranging between 29 and 73 nmol/L in white Caucasian women from the USA, Canada, Finland, Ireland, and Australia and lower levels ranging between 15 and 43 nmol/L in non-western women from the Netherlands, including Turkish, Moroccan, and black women from the USA and women from Japan [133]. Scarce are the studies conducted in such populations.

Investigations in Saudi Arabia, Kuwait, the United Arab Emirates, and Iran reveal that 10–60 % of mothers and 40–80 % of their neonates had undetectable or low vitamin D levels (0–25 nmol/L) at delivery [144–147]. Neonates born to mothers with low D levels have lower cord vitamin D levels and may be at risk for rickets and other complications [148, 149]. While there was no effect of maternal vitamin D levels on neonatal birth weight in a sample of 50 mothers–neonates from Iran after adjusting for maternal height, age, and parity [145], the opposite was seen in a larger study. In a sample of 449 mom–offspring pairs from Tehran, neonates of mothers with adequate calcium and vitamin D intake were 0.9 cm taller and had a better Apgar score at birth [150]. The impact of supplementation of mother or infants in these high-risk populations is largely unknown. The only study we are aware of is a randomized controlled vitamin D trial conducted in 2,000 neonates in India in which vitamin D administration at 35 μg/day (1,400 IU/day) significantly increased standard deviation (*Z*) scores for weight, length, and arm circumference and decreased the proportion of children with stunted growth at 6 months, but had no effect on death, hospitalization, and in- or outpatient visits [151].

Assay Variations

Available data to define the prevalence of hypovitaminosis D in population-based studies, and desirable 25(OH)D levels, has been in large part driven by older RIAs to measure vitamin D levels and for many the older DiaSorin RIA (see Tables 27.3, 27.4, and 27.5). However, this has not been a universal practice in the past and much more so in recent studies. Indeed, updates on NHANES, CaMoS, and recent trials have shifted to newer assays with diverging results [152–156]. Furthermore, it has become an increasingly common practice for clinical laboratories to shift from the classical RIA to more rapid, high-output, automated assays, a practice that has profound implications on results obtained [152, 153, 155], possibly contributing to under- or overreplacement of patients in the clinic [155, 157]. This variability between assays confounds international efforts to develop evidence-based clinical guidelines.

This problem can only be resolved through the implementation of quality assurance programs for 25(OH)D assays, pushing forward the agenda for global standardization of assays, and the consideration of assay-specific cutoff points. It is in this spirit that the Vitamin D Standardization Program (VDSP) was established in 2010, an initiative led by the NIH Office of Dietary Supplements, in collaboration with the CDC, National Institute of Standards and Technology (NIST), and Ghent University [156]. Its objectives include, among others, the standardization

of 25(OH)D concentration measurements in national health surveys around the world; the extension of standardization efforts to assay manufacturers and to clinical, commercial, and research laboratories; and the ability to use standardized data in patient care and public health. This will also allow the provision of guidance for the implementation of current vitamin D recommendations (IOM, ES, IOF, the WHO), ideally providing assay-specific desirable values, and/or ranges for desirable 25(OH)D levels, adjusting for assay differences.

Variability in 25(OH)D assays is a major problem worldwide, has a significant implications to research and practice, and has a major impact on guidelines and treatment recommendations. Such variability needs to be addressed before desirable 25(OH)D levels can be recommended and implemented.

Summary and Recommendations

A desirable level is one that is efficacious, optimizes outcomes, and is safe, with a relatively wide therapeutic window. Hypovitaminosis D is prevalent worldwide, and vitamin D status may vary not only by ethnicity but also age, gender, hormonal status, specific outcome of interest, and assay used. The use of surrogate measures to assess vitamin D economy has many limitations, leads to contradicting and unreliable results, and should be abandoned. However, the beneficial effect of vitamin D on musculoskeletal outcomes, that is, falls and fractures, is undisputed. Thus, the recent recommendations for desirable levels are 50 nmol/L by IOM (2011) and by the WHO (2003) and 75 nmol/L by ES (2011) and by IOF (2010) for the elderly only. The applicability of such recommendations is somewhat hampered by the problem of assay variability and by the limited information on the impact of various modulators of vitamin D on its circulating level and on its action.

Furthermore, while the most abundant outcome data are from western populations, some of the lowest 25(OH)D levels are reported in African Americans and Asians in the USA and worldwide. The relatively limited data by ethnicity from the USA reveals that the lower 25(OH)D level may be sufficient to maintain skeletal health in blacks, despite their elevated PTH levels, and that higher levels may be harmful. Data for other ethnicities are scarce to nonexistent. Adequately designed randomized controlled trials that aim to establish the optimal level/intake on musculoskeletal outcomes in non-western populations are needed. Other health outcomes of particular relevance to non-Caucasian ethnic groups and populations from developing countries worldwide, with the lowest circulating levels, such as infections and maternal–neonatal health, cannot be ignored. To date, desirable levels cannot be unequivocally established, and the concept of a universal optimal level is thus non-tenable.

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References

1. Holick MF. Vitamin D, deficiency. N Engl J Med. 2007;357(3):266–81.
2. Rosen CJ. Clinical practice. Vitamin D insufficiency. N Engl J Med. 2011;364(3):248–54.
3. Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best Pract Res Clin Endocrinol Metab. 2011;4:585–91.
4. Zittermann A, Iodice S, Pilz S, Grant WB, Bagnardi V, Gandini S. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. Am J Clin Nutr. 2012;95(1):91–100.
5. Sempos C, Vesper H, Phinny K, Thienpont L, Coates P, VDSP. Is there a U- or J-shaped Association between 25-Hydroxyvitamin D and all-cause mortality? Results from the US Nationally Representative NHANES, Submitted 2012.
6. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med. 2008;29(6):361–8.

7. Makariou S, Liberopoulos EN, Elisaf M, Challa A. Novel roles of vitamin D in disease: what is new in 2011? Eur J Intern Med. 2011;22(4):355–62.
8. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2011;(7): CD007470.
9. Reddy Vanga S, Good M, Howard PA, Vacek JL. Role of vitamin D in cardiovascular health. Am J Cardiol. 2010;106:798–805.
10. Pilz S, Kienreich K, Tomaschitz A, Lerchbaum E, Meinitzer A, März W, et al. Vitamin D and cardiovascular disease: update and outlook. Scand J Clin Lab Invest Suppl. 2012;243:83–91.
11. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. Eur J Clin Nutr. 2011;65(9):1005–15.
12. El-Hajj Fuleihan G. Can the sunshine vitamin melt the fat? Metabolism. 2012;61(5):603–10.
13. Pozzilli P, Manfrini S, Crinò A, Picardi A, Leomanni C, Cherubini V, et al. Low levels of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. Horm Metab Res. 2005;37(11):680–3.
14. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001;358(9292): 1500–3.
15. EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. Diabetologia. 1999;42(1):51–4.
16. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770–3.
17. Cantorna MT, Mahon BD. D-hormone and the immune system. J Rheumatol Suppl. 2005;76:11–20.
18. Hewison M. Vitamin D, and immune function: autocrine, paracrine or endocrine? Scand J Clin Lab Invest Suppl. 2012;243:92–102.
19. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296(23): 2832–8.
20. Mirzaei F, Michels KB, Munger K, O'Reilly E, Chitnis T, Forman MR, et al. Gestational vitamin D and the risk of multiple sclerosis in offspring. Ann Neurol. 2011;70(1):30–40.
21. Faridar A, Eskandari G, Sahraian MA, Minagar A, Azimi A. Vitamin D and multiple sclerosis: a critical review and recommendations on treatment. Acta Neurol Belg. 2012;112:327–333.
22. Sinha A, Avery P, Turner S, Bailey S, Cheetham T. Vitamin D status in pediatric patients with cancer. Pediatr Blood Cancer. 2011;57(4):594–8.
23. Buttigliero C, Monagheddu C, Petroni P, Saini A, Dogliotti L, Ciccone G, et al. Prognostic role of vitamin d status and efficacy of vitamin D supplementation in cancer patients: a systematic review. Oncologist. 2011;16(9):1215–27.
24. Ma Y, Zhang P, Wang F, Yang J, Liu Z, Qin H. Association between vitamin D and risk of colorectal cancer: a systematic review of prospective studies. J Clin Oncol. 2011;29(28):3775–82.
25. Chung M, Lee J, Terasawa T, Lau J, Trikalinos TA. Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force. Ann Intern Med. 2011;155(12):827–38.
26. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol. 2010;121(1–2):297–300.
27. Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nat Rev Endocrinol. 2010;6(10):550–61.
28. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20(11):1807–20.
29. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS Data Brief. 2011;59:1–8.
30. Ganji V, Zhang X, Tangpricha V. Serum 25-hydroxyvitamin D concentrations and prevalence estimates of hypovitaminosis D in the U.S. population based on assay-adjusted data. J Nutr. 2012;142(3): 498–507.
31. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96(1):53–8.
32. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.
33. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr. 1998;67:1232–6.
34. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone. 2002;30:771–7.
35. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2002;76:187–92.
36. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA. 2005;293(18):2257–64.
37. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional

calcium to reduce the risk of hip fracture with vitamin d supplementation: evidence from a comparative metaanalysis of randomized controlled trials. J Clin Endocrinol Metab. 2007;92(4):1415–23.
38. Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, et al. Effectiveness and safety of vitamin D in relation to bone health. Evid Rep Technol Assess (Full Rep). 2007;158:1–235.
39. Jackson C, Gaugris S, Sen SS, Hosking D. The effect of cholecalciferol (vitamin D3) on the risk of fall and fracture: a meta-analysis. QJM. 2007;100(4):185–92.
40. Bischoff-Ferrari HA, Willett WC, Wong JB, Stuck AE, Staehelin HB, Orav EJ, et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials. Arch Intern Med. 2009;169(6):551–61.
41. Avenell A, Gillespie WJ, Gillespie LD, O'Connell D. Vitamin D and vitamin D analogues for preventing fractures associated with involutional and postmenopausal osteoporosis. Cochrane Database Syst Rev. 2009;(2):CD000227.
42. Bergman GJ, Fan T, McFetridge JT, Sen SS. Efficacy of vitamin D3 supplementation in preventing fractures in elderly women: a meta-analysis. Curr Med Res Opin. 2010;26(5):1193–201.
43. DIPART (Vitamin D Individual Patient Analysis of Randomized Trials) Group. Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. BMJ. 2010;340:b5463.
44. Lai JK, Lucas RM, Clements MS, Roddam AW, Banks E. Hip fracture risk in relation to vitamin D supplementation and serum 25-hydroxyvitamin D levels: a systematic review and meta-analysis of randomised controlled trials and observational studies. BMC Public Health. 2010;10:331.
45. Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367(1):40–9.
46. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004;291(16):1999–2006.
47. Richy F, Dukas L, Schacht E. Differential effects of D-hormone analogs and native vitamin D on the risk of falls: a comparative meta-analysis. Calcif Tissue Int. 2008;82:102–7.
48. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ. 2009;339:b3692.
49. Gillespie LD, Robertson MC, Gillespie WJ, Lamb SE, Gates S, Cumming RG, et al. Interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev. 2009;(2):CD007146.
50. Cameron ID, Murray GR, Gillespie LD, Robertson MC, Hill KD, Cumming RG, et al. Interventions for preventing falls in older people in nursing care facilities and hospitals. Cochrane Database Syst Rev. 2010;(1):CD005465.
51. Kalyani RR, Stein B, Valiyil R, Manno R, Maynard JW, Crews DC. Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. J Am Geriatr Soc. 2010;58(7):1299–310.
52. Michael YL, Whitlock EP, Lin JS, Fu R, O'Connor EA, Gold R, et al. Primary care-relevant interventions to prevent falling in older adults: a systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med. 2010;153(12):815–25.
53. Murad MH, Elamin KB, Abu Elnour NO, Elamin MB, Alkatib AA, Fatourechi MM, et al. Clinical review: the effect of vitamin D on falls: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2011;96(10):2997–3006.
54. Dawson-Hughes B. What is the optimal dietary intake of vitamin D for reducing fracture risk? Calcif Tissue Int. 2012. doi:10.1007/S00223-012-9606-5.
55. Willett AM. Vitamin D, status and its relationship with parathyroid hormone and bone mineral status in older adolescents. Proc Nutr Soc. 2005;64(2):193–203.
56. Sai AJ, Walters RW, Fang X, Gallagher JC. Relationship between vitamin D, parathyroid hormone, and bone health. J Clin Endocrinol Metab. 2011;96(3):E436–46.
57. Gutiérrez OM, Farwell WR, Kermah D, Taylor EN. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int. 2011;22(6):1745–53.
58. Berger C, Greene-Finestone LS, Langsetmo L, Kreiger N, Joseph L, Kovacs CS. Temporal trends and determinants of longitudinal change in 25-hydroxyvitamin D and parathyroid hormone levels. J Bone Miner Res. 2012;27(6):1381–9.
59. Bates CJ, Carter GD, Mishra GD, O'Shea D, Jones J, Prentice A. In a population study, can parathyroid hormone aid the definition of adequate vitamin D status? A study of people aged 65 years and over from the British National Diet and Nutrition Survey. Osteoporos Int. 2003;14(2):152–9.
60. Lamberg-Allardt CJ, Outila TA, Kärkkainen MU, Rita HJ, Valsta LM. Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? J Bone Miner Res. 2001;16(11):2066–73.
61. Vieth R, Ladak Y, Walfish PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinol Metab. 2003;88(1):185–91.
62. Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G. Age but not gender modulates the relationship between PTH and vitamin D. Bone. 2010;47(2):408–12.
63. Saliba W, Barnett O, Rennert HS, Lavi I, Rennert G. The relationship between serum 25(OH)D and

parathyroid hormone levels. Am J Med. 2011;124(12): 1165–70.
64. Steingrimsdottir L, Gunnarsson O, Indridason OS, Franzson L, Sigurdsson G. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA. 2005;294(18): 2336–41.
65. Harris SS, Soteriades E, Coolidge JA, Mudgal S, Dawson-Hughes B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. J Clin Endocrinol Metab. 2000;85(11):4125–30.
66. Aloia JF, Feuerman M, Yeh JK. Reference range for serum parathyroid hormone. Endocr Pract. 2006;12(2): 137–44.
67. Weaver CM, McCabe LD, McCabe GP, Braun M, Martin BR, Dimeglio LA, et al. Vitamin D status and calcium metabolism in adolescent black and white girls on a range of controlled calcium intakes. J Clin Endocrinol Metab. 2008;93(10):3907–14.
68. Aloia JF, Chen DG, Chen H. The 25(OH)D/PTH threshold in black women. J Clin Endocrinol Metab. 2010;95(11):5069–73.
69. Gannagé-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res. 2000;15(9):1856–62.
70. Yan L, Zhou B, Wang X, D'Ath S, Laidlaw A, Laskey MA, et al. Older people in China and the United Kingdom differ in the relationships among parathyroid hormone, vitamin D and bone mineral status. Bone. 2003;33(4):620–7.
71. Ono Y, Suzuki A, Kotake M, Zhang X, Nishiwaki-Yasuda K, Ishiwata Y, et al. Seasonal changes of serum 25-hydroxyvitamin D and intact parathyroid hormone levels in a normal Japanese population. J Bone Miner Metab. 2005;23(2):147–51.
72. Green TJ, Skeaff CM, Rockell JE, Venn BJ, Lambert A, Todd J, et al. Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. Eur J Clin Nutr. 2008;62(3):373–8.
73. Bacon CJ, Woo J, Lau EM, Lam CW, Gamble GD, Reid IR. Effects of 25-hydroxyvitamin D level and its change on parathyroid hormone in premenopausal Chinese women. Osteoporos Int. 2010;21(11): 1935–41.
74. Okazaki R, Sugimoto T, Kaji H, Fujii Y, Shiraki M, Inoue D, et al. Vitamin D insufficiency defined by serum 25-hydroxyvitamin D and parathyroid hormone before and after oral vitamin D_3 load in Japanese subjects. J Bone Miner Metab. 2011;29(1):103–10.
75. Shivane VK, Sarathi V, Bandgar T, Menon P, Shah NS. High prevalence of hypovitaminosis D in young healthy adults from the western part of India. Postgrad Med J. 2011;87(1030):514–8.
76. El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A, et al. Hypovitaminosis D in healthy school children. Pediatrics. 2001;107(4):E53.
77. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr. 2005;82(2): 477–82.
78. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab. 2006;91(2):405–12.
79. Ghazi AA, Hosseinpanah F, M Ardakani E, Ghazi S, Hedayati M, Azizi F. Effects of different doses of oral cholecalciferol on serum 25(OH)D, PTH, calcium and bone markers during fall and winter in schoolchildren. Eur J Clin Nutr. 2010;64(12):1415–22.
80. Razzaghy-Azar M, Shakiba M. Assessment of vitamin D status in healthy children and adolescents living in Tehran and its relation to iPTH, gender, weight and height. Ann Hum Biol. 2010;37(5): 692–701.
81. Levis S, Gomez A, Jimenez C, Veras L, Ma F, Lai S, et al. Vitamin D deficiency and seasonal variation in an adult South Florida population. J Clin Endocrinol Metab. 2005;90(3):1557–62.
82. Hannan MT, Litman HJ, Araujo AB, McLennan CE, McLean RR, McKinlay JB, et al. Serum 25-hydroxyvitamin D and bone mineral density in a racially and ethnically diverse group of men. J Clin Endocrinol Metab. 2008;93(1):40–6.
83. Heaney RP. Serum 25-hydroxyvitamin D and parathyroid hormone exhibit threshold behavior. J Endocrinol Invest. 2005;28:180–2.
84. Vieth R, El-Hajj Fuleihan G. There is no lower threshold level for parathyroid hormone as 25-hydroxyvitamin D concentrations increase. J Endocrinol Invest. 2005;28:183–6.
85. Durazo-Arvizu RA, Dawson-Hughes B, Sempos CT, Yetley EA, Looker AC, Cao G, et al. Three-phase model harmonizes estimates of the maximal suppression of parathyroid hormone by 25-hydroxyvitamin D in persons 65 years of age and older. J Nutr. 2010;140(3):595–9.
86. Fleet JC, Schoch RD. Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors. Crit Rev Clin Lab Sci. 2010;47(4): 181–95.
87. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res. 1999;14(5): 740–6.
88. Zmuda JM, Cauley JA, Danielson ME, Theobald TM, Ferrell RE. Vitamin D receptor translation initiation codon polymorphism and markers of osteoporotic risk in older African-American women. Osteoporos Int. 1999;9:214–9.
89. Boyle IT, Gray RW, Omdahl JL, DeLuca HF, Schilling RF. The mechanism of adaptation of intestinal calcium absorption to low dietary calcium. J Lab Clin Med. 1971;78(5):813.

90. Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. J Clin Endocrinol Metab. 1997;82(12):4111–6.
91. Heaney RP. The Vitamin D requirement in health and disease. J Steroid Biochem Mol Biol. 2005;97(1–2):13–9.
92. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO. Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab. 2005;90(10):5576–81.
93. Thacher TD, Abrams SA. Relationship of calcium absorption with 25(OH)D and calcium intake in children with rickets. Nutr Rev. 2010;68(11):682–8.
94. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr. 2003;22(2):142–6.
95. Need AG, O'Loughlin PD, Morris HA, Coates PS, Horowitz M, Nordin BE. Vitamin D metabolites and calcium absorption in severe vitamin D deficiency. J Bone Miner Res. 2008;23(11):1859–63.
96. Thacher TD, Obadofin MO, O'Brien KO, Abrams SA. The effect of vitamin D2 and vitamin D3 on intestinal calcium absorption in Nigerian children with rickets. J Clin Endocrinol Metab. 2009;94(9):3314–21.
97. Peng JB. TRPV5 and TRPV6 in transcellular Ca(2+) transport: regulation, gene duplication, and polymorphisms in African populations. Adv Exp Med Biol. 2011;704:239–75.
98. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab. 2009;94(4):1244–50.
99. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med. 2004;116(9):634–9.
100. Cauley JA, Danielson ME, Boudreau R, Barbour KE, Horwitz MJ, Bauer DC, et al. Serum 25-hydroxyvitamin D and clinical fracture risk in a multiethnic cohort of women: the Women's Health Initiative (WHI). J Bone Miner Res. 2011;26(10):2378–88.
101. Aloia JF, Talwar SA, Pollack S, Yeh J. A randomized controlled trial of vitamin D3 supplementation in African American women. Arch Intern Med. 2005;165(14):1618–23.
102. Ensrud KE, Taylor BC, Paudel ML, Cauley JA, Cawthon PM, Cummings SR, et al. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. J Clin Endocrinol Metab. 2009;94(8):2773–80.
103. Garnero P, Munoz F, Sornay-Rendu E, Delmas PD. Associations of vitamin D status with bone mineral density, bone turnover, bone loss and fracture risk in healthy postmenopausal women. The OFELY study. Bone. 2007;40(3):716–22.
104. Melhus H, Snellman G, Gedeborg R, Byberg L, Berglund L, Mallmin H, et al. Plasma 25-hydroxyvitamin D levels and fracture risk in a community-based cohort of elderly men in Sweden. J Clin Endocrinol Metab. 2010;95(6):2637–45.
105. Islam MZ, Viljakainen HT, Kärkkäinen MU, Saarnio E, Laitinen K, Lamberg-Allardt C. Prevalence of vitamin D deficiency and secondary hyperparathyroidism during winter in pre-menopausal Bangladeshi and Somali immigrant and ethnic Finnish women: associations with forearm bone mineral density. Br J Nutr. 2012;107(2):277–83.
106. Scharla SH, Scheidt-Nave C, Leidig G, Woitge H, Wüster C, Seibel MJ, et al. Lower serum 25-hydroxyvitamin D is associated with increased bone resorption markers and lower bone density at the proximal femur in normal females: a population-based study. Exp Clin Endocrinol Diabetes. 1996;104(3):289–92.
107. Fradinger EE, Zanchetta JR. Vitamin D and bone mineral density in ambulatory women living in Buenos Aires, Argentina. Osteoporos Int. 2001;12(1):24–7.
108. Harinarayan CV, Sachan A, Reddy PA, Satish KM, Prasad UV, Srivani P. Vitamin D status and bone mineral density in women of reproductive and postmenopausal age groups: a cross-sectional study from south India. J Assoc Physicians India. 2011;59:698–704.
109. Khadgawat R, Brar KS, Gahlo M, Yadav CS, Malhotra R, Guptat N, et al. High prevalence of vitamin D deficiency in Asian-Indian patients with fragility hip fracture: a pilot study. J Assoc Physicians India. 2010;58:539–42.
110. Arya V, Bhambri R, Godbole MM, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporos Int. 2004;15(1):56–61.
111. Serhan E, Holland MR. Relationship of hypovitaminosis D and secondary hyperparathyroidism with bone mineral density among UK resident Indo-Asians. Ann Rheum Dis. 2002;61(5):456–8.
112. Hosseinpanah F, Rambod M, Hossein-nejad A, Larijani B, Azizi F. Association between vitamin D and bone mineral density in Iranian postmenopausal women. J Bone Miner Metab. 2008;26(1):86–92.
113. Rassouli A, Milanian I, Moslemi-Zadeh M. Determination of serum 25-hydroxyvitamin D(3) levels in early postmenopausal Iranian women: relationship with bone mineral density. Bone. 2001;29(5):428–30.
114. Vupputuri MR, Goswami R, Gupta N, Ray D, Tandon N, Kumar N. Prevalence and functional significance of 25-hydroxyvitamin D deficiency and vitamin D receptor gene polymorphisms in Asian Indians. Am J Clin Nutr. 2006;83(6):1411–9.

115. Mitra S, Desai M, Ikram Khatkhatay M. Vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Indian women. Maturitas. 2006;55(1):27–35.
116. El-Hajj Fuleihan G, Dib G. The Middle East & Africa Osteoporosis Regional Audit. Epidemiology, costs, burden of osteoporosis in 2011. http://staff.aub.edu.lb/~webcmop/downloads/ME_audit.pdf. Accessed 12 Sept 2012.
117. El-Hajj Fuleihan G, Gundberg CM, Gleason R, Brown EM, Stromski ME, Grant FD, et al. Racial differences in parathyroid hormone dynamics. J Clin Endocrinol Metab. 1994;79:1642–7.
118. Cosman F, Morgan DC, Nieves JW, Shen V, Luckey MM, Dempster DW, et al. Resistance to bone resorbing effects of PTH in black women. J Bone Miner Res. 1997;12:958–66.
119. Yan L, Schoenmakers I, Zhou B, Jarjou LM, Smith E, Nigdikar S, et al. Ethnic differences in parathyroid hormone secretion and mineral metabolism in response to oral phosphate administration. Bone. 2009;45(2):238–45.
120. Arabi A, Baddoura R, Awada H, Salamoun M, Ayoub G, El-Hajj Fuleihan G. Hypovitaminosis D osteopathy: is it mediated through PTH, lean mass, or is it a direct effect? Bone. 2006;39:268–75.
121. Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G. PTH level but not 25 (OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int. 2012;23(3):971–80.
122. Yamauchi M, Kaji H, Nawata K, Takaoka S, Yamaguchi T, Sugimoto T. Role of parathyroid hormone in bone fragility of postmenopausal women with vitamin D insufficiency. Calcif Tissue Int. 2011;88(5):362–9.
123. Arabi A, Mahfoud Z, Zahed L, El-Onsi L, El-Hajj Fuleihan G. Effect of age, gender and calciotropic hormones on the relationship between vitamin D receptor gene polymorphisms and bone mineral density. Eur J Clin Nutr. 2010;64(4):383–91.
124. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. BMJ. 2011;342:c7254.
125. Bell NH, Yergey AL, Vieira NE, Oexmann MJ, Shary JR. Demonstration of a difference in urinary calcium, not calcium absorption, in black and white adolescents. J Bone Miner Res. 1993;8:1111–5.
126. Bryant RJ, Wastney ME, Martin BR, Wood O, McCabe GP, Morshidi M, et al. Racial differences in bone turnover and calcium metabolism in adolescent females. J Clin Endocrinol Metab. 2003;88:1043–7.
127. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, et al. IOM committee members respond to Endocrine Society guidelines. J Clin Endocrinol Metab. 2012;97:1146–52.
128. Narayan VKM, Ali MK, Koplan J. Global non-communicable diseases – where worlds meet. N Engl J Med. 2010;363(13):1196–8.
129. WHO. Global status report on non-communicable diseases 2010 (Included as pdf: WHO_global_report). http://whqlibdoc.who.int/publications/2011/9789240686458_eng.pdf. Accessed 12 Sept 2012.
130. Lim S, Shin H, Kim MJ, Ahn HY, Kang SM, Yoon JW, et al. Vitamin D inadequacy is associated with significant coronary artery stenosis in a community-based elderly cohort: the Korean Longitudinal Study on Health and Aging. J Clin Endocrinol Metab. 2012;97(1):169–78.
131. Lawn SD, Zumla AL. Tuberculosis. Lancet. 2011;378:57–72.
132. Martineau AR, Nhamoyebonde S, Oni T, Rangaka MX, Marais S, Bangani N, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci USA. 2011;108(47):19013–7.
133. Nassar N, Halligan GH, Roberts CL, Morris JM, Ashton AW. Systematic review of first-trimester vitamin D normative levels and outcomes of pregnancy. Am J Obstet Gynecol. 2011;205(3):208.e1–7.
134. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. J Clin Endocrinol Metab. 2007;92(9):3517–22.
135. Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF. Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab. 2009;94(3):940–5.
136. Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA, et al. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension. 2010;56(4):758–63.
137. Wei SQ, Audibert F, Hidiroglou N, Sarafin K, Julien P, Wu Y, et al. Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. BJOG. 2012;119(7):832–9.
138. Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, et al. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. J Nutr. 2010;140(5):999–1006.
139. Leffelaar ER, Vrijkotte TG, van Eijsden M. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. Br J Nutr. 2010;104(1):108–17.
140. Morley R, Carlin JB, Pasco JA, Wark JD. Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size. J Clin Endocrinol Metab. 2006;91(3):906–12.
141. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, Arden NK, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet. 2006;367(9504):36–43.
142. Zamora SA, Rizzoli R, Belli DC, Slosman DO, Bonjour JP. Vitamin D supplementation during

infancy is associated with higher bone mineral mass in prepubertal girls. J Clin Endocrinol Metab. 1999;84(12):4541–4.
143. De-Regil LM, Palacios C, Ansary A, Kulier R, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev. 2012; 2:CD008873.
144. Bassir M, Laborie S, Lapillonne A, Claris O, Chappuis MC, Salle BL. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. Acta Paediatr. 2001;90(5):577–9.
145. Ainy E, Ghazi AA, Azizi F. Changes in calcium, 25(OH) vitamin D3 and other biochemical factors during pregnancy. J Endocrinol Invest. 2006;29(4): 303–7.
146. Molla AM, Al Badawi M, Hammoud MS, Molla AM, Shukkur M, Thalib L, et al. Vitamin D status of mothers and their neonates in Kuwait. Pediatr Int. 2005;47(6):649–52.
147. Serenius F, Elidrissy AT, Dandona P. Vitamin D nutrition in pregnant women at term and in newly born babies in Saudi Arabia. J Clin Pathol. 1984;37(4):444–7.
148. Kimball S, El-Hajj Fuleihan G, Vieth R. Vitamin D: a growing perspective. Crit Rev Clin Lab Sci. 2008;45(4):339–414.
149. Biale Y, Shany S, Levi M, Shainkin-Kestenbaum R, Berlyne GM, et al. 25 Hydroxycholecalciferol levels in Beduin women in labor and in cord blood of their infants. Am J Clin Nutr. 1979;32(12): 2380–2.
150. Sabour H, Hossein-Nezhad A, Maghbooli Z, Madani F, Mir E, Larijani B. Relationship between pregnancy outcomes and maternal vitamin D and calcium intake: a cross-sectional study. Gynecol Endocrinol. 2006;22(10):585–9.
151. Kumar GT, Sachdev HS, Chellani H, Rehman AM, Singh V, Arora H, et al. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomized controlled trial. BMJ. 2011;342:d2975.
152. Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. Clin Chem. 2004;50(11):2195–7.
153. Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. J Clin Endocrinol Metab. 2004;89(7): 3152–7.
154. Yetley EA, Pfeiffer CM, Schleicher RL, Phinney KW, Lacher DA, Christakos S, et al. NHANES monitoring of serum 25-hydroxyvitamin D: a roundtable summary. J Nutr. 2010;140(11):2030S–45.
155. Barake M, Daher RT, Salti I, Cortas NK, Al-Shaar L, Habib RH, et al. 25-hydroxyvitamin D assay variations and impact on clinical decision making. J Clin Endocrinol Metab. 2012;97(3):835–43.
156. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, Vitamin D Standardization Program (VDSP). Vitamin D status as an international issue: national surveys and the problem of standardization. Scand J Clin Lab Invest Suppl. 2012;243:32–40.
157. Lai JK, Lucas RM, Banks E, Ponsonby AL, Ausimmune Investigator Group. Variability in vitamin D assays impairs clinical assessment of vitamin D status. Intern Med J. 2012;42(1):43–50.

Sexual Dimorphism in Bone and Body Composition in Rural Gambian Prepubertal Children Habituated to a Low Calcium Intake

28

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Abstract

Gender differences in bone during childhood and adolescence are well described in countries with moderate to high calcium intakes; there are few data from countries where children have delayed puberty and low habitual calcium intakes. The aim of this study was to determine whether gender differences in bone and body composition exist in prepubertal Gambian children accustomed to a low calcium intake.

Four hundred and forty-seven prepubertal children (216 males, 231 females) were recruited between the ages of 7.8 and 11.9 years. Bone mineral content (BMC) and bone area (BA) were measured at whole body, lumbar spine, and hip using dual-energy X-ray absorptiometry (GE Lunar Prodigy). There were no significant differences between males and females in age, body weight, and height or body mass index.

Gender differences existed in BMC and BA both before and after size adjustment. Body composition also differed between males and females. Regular follow-up measurements in these children have commenced to assess whether these differences persist during and after puberty and implications for future bone health.

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Children • Africa • Males • Females • Dual-energy X-ray absorptiometry • Bone mineral content • Bone area • Growth • Body composition • Calcium intake

Introduction

Skeletal growth and mineral accumulation during childhood and adolescence is crucial for bone health. Peak bone mass is attained by the late second and early third decade, in females and males, respectively. Thereafter, no net change of bone mineral occurs until menopause and aging. The magnitude of peak bone mass is therefore related to fragility fractures of old age.

Differing fracture rates in males and females during growth and aging are in part due to sexual dimorphism in bone accrual and loss. Gender differences in bone phenotype emerge during puberty [1–4]. Some studies report differences in prepuberty, while others report no differences [5–8]. The majority of data on bone growth are provided from studies in children with good growth and moderate to high calcium intakes. However, few data exist from countries where stunting is common, and children have delayed puberty and low habitual calcium intakes, yet fragility fracture risk is low [9–14]. Studying bone development in different populations may help to understand the underlying basis of skeletal fragility.

Therefore, the aim of this study was to determine whether gender differences exist in bone mineral and body composition in prepubertal Gambian children accustomed to a low calcium intake.

Methods

Subjects

This study was conducted at MRC Keneba, West Kiang, The Gambia Prepubertal children aged 7.8–11.9 years were recruited.

The study was approved by the MRC/Gambian Government Ethics Committee (SCC 1073), and informed written consent was obtained from the parent or guardian of the child, with the assent of the child.

Anthropometry

Standing height was measured to the nearest 0.1 cm using a stadiometer (SECA 225). Weight was measured on electronic scales (Tanita HD310) to the nearest 0.1 kg, with the subject wearing light clothing and no shoes.

Dual-Energy X-Ray Absorptiometry (DXA)

Bone and body composition measurements were obtained using a GE Lunar Prodigy DXA scanner using software version 10.51.006 (GE Medical Systems, GE Lunar Corporation, Madison, USA). Whole body, lumbar spine, total hip, and femoral neck bone mineral content (BMC, g), areal bone mineral density (aBMD, g/cm^2), and bone area (BA, cm^2) were measured. The scanner automatically selected the appropriate scan mode based on the subject's weight and height (thin, standard, or thick). Lean and fat mass (g) measurements were obtained from the whole body scan. Whole body bone measurements were analyzed less head (WBLH) as recommended for children [15]. The precision of repeated measurements of aBMD at different skeletal sites in adults (n=35 measured twice) using this scanner was as follows: whole body 0.6 %, lumbar spine 0.8 %, total hip 0.7 %, and femoral neck 1.1 %.

Data Handling and Analysis

Statistical analysis was performed using the Linear Model facility in Data Desk 6.1.1 (Data Description, Ithaca, NY).

Sex effects were tested for using univariate and multivariate regression. BMC was adjusted for BA, weight and height (size-adjusted BMC, SA-BMC), BA for weight and height, and lean and fat mass for height [16]. Variables were converted to natural logarithms whereby differences × 100 correspond closely to percentage differences [(difference/mean) × 100] [17].

Results

Four hundred and forty-seven participants (216 male, 231 female) were recruited. There were no significant differences between males and females in age, height, weight, or BMI (Table 28.1). Males had significantly greater lean mass and less fat mass than females of the same size. These significant differences remained after adjustment for height: lean mass 7.3 ± 0.7 % and fat mass 45.2 ± 3.3 % ($p < 0.001$).

Table 28.2 shows the unadjusted and adjusted results for gender differences in BMC, BMD, and BA. For the same body size, males had wider bones at the whole body, lumbar spine, and femoral neck. Size-adjusted BMC was also higher (mean [SE]) in males at total hip (10.8 [1.0]%) and femoral neck (10.2 [1.0]%), and marginally lower at WBLH (0.9 [0.4]%). Lumbar spine size-adjusted BMC was lower in males (−3.4 [0.1]%).

Table 28.1 Subject characteristics

	Males $n = 216$ (mean ± SD)	Females $n = 231$ (mean ± SD)	% (SE) diff M vs. F
Age (year)	9.3 ± 0.1	9.2 ± 0.1	0.4 (0.9)^{NS}
Height (m)	1.3 ± 0.1	1.3 ± 0.1	−0.5 (0.5)^{NS}
Weight (kg)	23.8 ± 0.2	24.0 ± 0.3	−0.6 (1.5)^{NS}
BMI (kg/m^2)	14.5 ± 1.5	14.6 ± 1.2	−5.3 (12.6)^{NS}
Lean mass (kg)	20.5 ± 0.2	19.2 ± 0.2	6.5 (1.3)*
Fat mass (kg)	2.2 ± 0.1	3.6 ± 1.1	−47.3 (3.9)**

NS no significant difference
$^{*}p \le 0.05$; $^{**}p \le 0.0001$

Table 28.2 Unadjusted and adjusted gender differences in BMC, BA, and BMD in study cohort

	Region of interest	Males (mean ± SD)	Females (mean ± SD)	Unadjusted % diff (SE) M vs. F	Size-adjusted[a], [16] % diff (SE) M vs. F
Whole body, less head	BMC (g)	607 ± 9	590 ± 9	3.0 (2.1)^{NS}	−0.9 (0.4)*
	BA (cm^2)	862 ± 9	843 ± 9	2.3 (1.5)^{NS}	3.2 (0.5)**
	BMD (g/cm^2)	0.689 ± 0.004	0.693 ± 0.003	0.7 (0.7)^{NS}	
Lumbar spine	BMC (g)	17.0 ± 0.2	17.2 ± 0.2	−1.2 (1.8)^{NS}	−3.4 (0.1)**
	BA (cm^2)	27.8 ± 0.2	27.0 ± 0.2	2.5 (1.1)*	3.2 (0.7)**
	BMD (g/cm^2)	0.609 ± 0.005	0.632 ± 0.005	−3.9 (1.1)*	
Total hip	BMC (g)	12.1 ± 0.2	11.1 ± 0.2	9.3 (2.1)**	10.8 (1.0)**
	BA (cm^2)	14.9 ± 0.2	15.1 ± 0.2	−1.4 (1.5)^{NS}	0.3 (0.6)^{NS}
	BMD (g/cm^2)	0.810 ± 0.006	0.728 ± 0.006	10.7 (1.1)**	
Femoral neck	BMC (g)	2.50 ± 0.04	2.12 ± 0.03	16.1 (1.5)**	10.2 (1.0)**
	BA (cm^2)	2.97 ± 0.04	2.79 ± 0.03	6.4 (1.7)*	7.0 (1.5)**
	BMD (g/cm^2)	0.837 ± 0.006	0.760 ± 0.006	9.7 (1.1)**	

NS no significant difference
$^{*}p \le 0.05$; $^{**}p \le 0.0001$
[a]BMC adjusted for weight, height, and bone area; BA for weight and height

Discussion

In rural Gambia, stunting is common and habitual calcium intakes are low [12–14]. Despite this, the incidence of fragility fracture is low during childhood and adulthood [18]. We explored gender differences in prepubertal children to begin to characterize bone development in this population.

The males and females did not differ significantly in weight and height. Males had wider bones than females at whole body, lumbar spine, and femoral neck. Size-adjusted BMC was greater at both hip sites but lower at lumbar spine. There were no differences in WBLH BMC between the genders before or after adjusting for size. The magnitude of differences at femoral neck and proximal femur was comparable to those reported in black children from South Africa of similar age [9]. Spine data were also similar between the Gambia and South Africa, with lower BMC and greater BA in males compared to females. This apparent BMC deficit in males may be due to differences in rates of bone accrual at the axial and appendicular skeletal regions.

Despite these similarities in gender differences in bone, this rural Gambian population differed in body weight to urban South Africans. The Gambian children were of similar height but lighter (~5 kg) with 2 kg/m^2 lower BMI. The differences may indicate asynchrony in pubertal timing between rural Gambians and urban South Africans [19, 20]. Such differences may have implications for peak bone mass timing and magnitude and thus bone health in these populations.

Weight and BMI were similar in male and female Gambian children. However, lean mass was greater and fat mass lower in males than females. These data highlight the importance of separating tissue compartments for complete understanding of the relationship between body mass and bone growth. The differences in fat mass were much greater than previous reports in US or South African black children [19, 21]. The contribution of the compartments to gender differences in BMC or BA requires further investigation. These body composition differences may have important implications for bone in later life.

The data demonstrate that sex differences exist in BMC, bone size, and body composition in Gambian prepubertal children. Regular follow-up measurements in these children have commenced to assess whether these differences persist during and after puberty and implications for future bone health.

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References

1. Ashby R, et al. Gender differences in the functional muscle-bone unit in growing children by DXA and pQCT. Osteoporos Int. 2004;15 Suppl 2:S6.
2. Bailey D. The Saskatchewan pediatric bone mineral accrual study: bone mineral acquisition during the growing years. Int J Sports Med. 1997;18(3):191–4.
3. Garn S, Poznanski A, Nagy J. Bone measurement in the differential diagnosis of osteopenia and osteoporosis. Radiology. 1971;100:509–18.
4. Gilsanz V, et al. Differential effect of race on the axial and appendicular skeletons of children. J Clin Endocrinol Metab. 1998;83(5):1420–7.
5. Kroger H, et al. Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. Bone Miner. 1992;17(1):75–85.
6. Nelson D, et al. The accumulation of whole body skeletal mass in third- and fourth-grade children: effects of age, gender, ethnicity and body composition. Bone. 1997;20(1):73–8.
7. Gilsanz V, et al. Gender differences in vertebral body sizes in children and adolescents. Radiology. 1994; 190(3):673–7.
8. Schoenau E, et al. The development of bone strength at the proximal radius during childhood and adolescence. J Clin Endocrinol Metab. 2001;86(2):613–8.
9. Micklesfield LK, et al. Comparison of site-specific bone mass indices in South African children of different ethnic groups. Calcif Tissue Int. 2009;85(4): 317–25.
10. Patel DN, et al. The effect of ethnic group on appendicular bone mass in children. J Bone Miner Res. 1992;7(3):263–72.
11. Nyati LH, et al. Effect of ethnicity and sex on the growth of the axial and appendicular skeleton of children living in a developing country. Am J Phys Anthropol. 2006;130(1):135–41.

12. Lo C, et al. Delayed development of bone mass in West African adolescents. In: Christiansen C, Overgaards H, editors. Osteoporosis 90. Aalborg: Handelstrykveriat; 1991.
13. Dibba B, et al. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. Am J Clin Nutr. 2000; 71(2):544–9.
14. Prentice A, Cole TJ, Whitehead RG. Impaired growth in infants born to mothers of very high parity. Hum Nutr Clin Nutr. 1987;41(5):319–25.
15. Gordon CM, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. J Clin Densitom. 2008;11(1):43–58.
16. Prentice A, Parsons T, Cole T. Uncritical use of bone mineral density in absorptiometry may lead to size- related artifacts in the identification of bone mineral determinants. Am J Clin Nutr. 1994;60:837–42.
17. Cole TJ. Sympercents: symmetric percentage differences on the 100 log scale simplify the presentation of log transformed data. Stat Med. 2000;19: 3109–25.
18. Aspray TJ, et al. Low bone mineral content is common but osteoporotic fractures are rare in elderly rural Gambian women. J Bone Miner Res. 1996; 11(7):1019–25.
19. Prentice S, et al. Evidence for a downward secular trend in age of menarche in a rural Gambian population. Ann Hum Biol. 2011;37(5):717–21.
20. Jones LL, et al. Age at menarche and the evidence for a positive secular trend in urban South Africa. Am J Hum Biol. 2009;21(1):130–2.
21. Nelson DA, Barondess DA. Whole body bone, fat and lean mass in children: comparison of three ethnic groups. Am J Phys Anthropol. 1997;103(2): 157–62.

29 The Likely Importance of Specific Dairy Foods in Relation to Bone Health: Current Knowledge and Future Challenges

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

Abstract

Osteoporosis is a major public health problem affecting over 200 million people worldwide. The 2010 dietary guidelines for Americans recommend consuming 3 cups/day of fat-free or low-fat dairy products for adults. Although individual nutrients usually found in dairy products may be beneficial for bone health, few studies have directly compared specific types of dairy foods. Yet it has been suggested that dairy foods are not equivalent vehicles of calcium due to their different nutrient profile. While studies of milk intake and bone mineral density (BMD) are plentiful and mostly positive, the evidence for hip fracture risk reduction remains weak. Studies on yogurt intake and bone health have been very few but promising though the role of probiotics in yogurt on bone is unclear. It is unclear how other dairy foods may relate to bone health. Few studies that have examined cheese intake have focused either on intake of total cheese or a specific low-fat variety. High-fat/high-sugar dairy foods like cream and ice cream have low nutrient density and are widely consumed, yet very little is known about their impact on the skeleton. The additive and synergistic role of sodium and saturated fats along with other bone-specific nutrients (e.g., calcium, vitamin D, phosphorus, protein) in cream and cheese is complicated and needs further clarification. Future studies should (i) aim to resolve the disparate findings from BMD studies versus fracture studies on milk intake, (ii) clarify the role of probiotics in calcium absorption and bone health, (iii) focus on the skeletal effects of low-fat cheese and the influence of

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sodium content of the different cheeses, and (iv) focus on dairy food products instead of single nutrients within dairy while at the same time considering nutrient profiles of specific dairy foods. Overall, the studies that are highlighted in this chapter suggest a significant role for dairy intake in maintaining bone health.

Keywords
Dairy • Milk • Yogurt • Cheese • Cream • Bone mineral density • Hip fracture • Dietary intake • Bone health

Introduction

Osteoporosis is a major public health problem affecting more than 200 million people worldwide [1]. While many risk factors have been described, dietary factors represent an important but less investigated area of research for bone health. The 2010 dietary guidelines for Americans recommend consuming three cups per day of fat-free or low-fat milk and milk products for adults [2]. Yet dairy foods are a complex source of essential nutrients, including protein, carbohydrates, fatty acids, calcium, phosphorus, potassium, and magnesium, and in the USA are often fortified with vitamin D. It has been suggested that the health effects of dairy foods may be due to more than a single nutrient, and in fact, the effects of dairy foods may be greater than the sum of their parts. A review by Heaney [3] and a report by the US Dietary Guidelines advisory committee [4] both reported that 25 out of 32 observational studies and 11 out of 11 randomized controlled trials showed a significantly positive association between dairy food intake and bone mineral density (BMD) or bone mineral content (BMC).

In one observational study using the Framingham Offspring cohort, most dairy intake (defined as the sum of milk, yogurt and cheese intake) was positively associated with hip and spine BMD [5]. In two randomized controlled trials, high dairy intake was protective against bone loss at the total hip [6] and the vertebrae [7]. Individual nutrients usually found in dairy products, such as calcium and vitamin D [8], protein [9, 10], and potassium [11], have been shown to be beneficial for bone health, while high intakes of sodium [12] may have a negative impact. However, few studies have directly compared different types of dairy foods even though it has been suggested that dairy foods are not equivalent vehicles of calcium due to their different protein, sodium, potassium, and vitamin A contents [13]. As the nutrient profile changes a great deal with the different types of dairy foods, consequently not all dairy foods should be expected to have a similar impact on bone health. For example, a diet rich in milk or yogurt rather than cheese or cream can increase the intakes of potassium, vitamin A, and vitamin D and decrease intakes of sodium, cholesterol, and saturated fatty acids. Thus, Weinsier and Krumdieck raised an important question in their (2000) review paper [13] as to whether the dairy options in the US Dietary Guidelines are nutritionally equivalent and exchangeable for optimal bone health. Based on the nutrient profiles of dairy foods, it can be predicted that milk (which has the largest calcium to sodium ratio of 2.4) and yogurt may be beneficial for the skeleton, whereas certain cheeses, which have high sodium and polyphosphate, may be less advantageous [13]. Due to its low nutrient density, cream may actually be disadvantageous to bone health. Furthermore, calcium from yogurt has higher bioavailability than from milk due to its acidic pH, which ionizes the calcium, facilitating absorption [14]. Whereas the manufacture of fresh, aged, and processed cheeses results in products that differ markedly in their content of dairy-specific nutrients compared to milk [13]. In light of these factors, it is important to examine the impact of individual dairy foods upon bone health. Of the dairy foods, milk remains the most studied; far less is known about the asso-

ciations of other dairy foods, such as yogurt, cheese, and cream, with bone health. The objective of this book chapter is to highlight findings from previous studies on this topic to underscore our current understanding and emphasize the importance of examining the individual dairy foods and their role in skeletal health.

Milk Intake and Bone Health

Evidence from Observational Studies

Several cross-sectional studies have reported a positive link between childhood milk consumption and bone density later in life [15–19]. Evidence for a beneficial role of milk intake on osteoporotic fracture is less convincing [18, 20–22]. A cross-sectional study of women (aged 44–74 years) found consistent associations between self-reported milk consumption before the age of 25 years and higher bone mineral density (BMD) of the total hip, femoral neck, trochanter, intertrochanter ($P<0.05$ each), and Ward's triangle ($P<0.005$) [15]. Results from 581 older white women (mean age, 71 years) in the Rancho Bernardo Study showed that regular milk consumption in youth was associated with higher BMD values at cortical and trabecular sites [23]. In 2,479 men and women (mean age, 54.9 ± 9.6 years) from the Framingham Offspring Study, participants in the highest quartile of current milk intake tended to have significantly higher BMD of the femoral neck (P trend = 0.08) and trochanter (P trend = 0.05) compared to participants in the lowest quartile of milk intake. No significant associations were observed for lumbar spine BMD (P trend = 0.29). In this study, after adjusting for other dairy foods, milk intake remained weakly but positively associated with femoral neck BMD ($P=0.06$) [5]. In the third National Health and Nutrition Examination Survey, women aged 20–49 years who consumed <1 serving of milk per week during childhood (ages 5–12 years) were found to have 5.6 % less bone mineral content (BMC) than those who consumed >1 serving per day. Similarly, women who consumed <1 serving of milk per week during adolescence were found to have ~3 % lower hip BMC and BMD ($P<0.02$) than those who consumed >1 serving per day [18]. In the same study, women aged 50 years or older with low milk intake during childhood were found to have twice the risk of lifetime fracture [OR, 2.02 (95 % CI; 1.13, 3.59), $P<0.05$] [18]. On the other hand, a 12-year prospective study of 77,761 women (aged 34–59 years) from the Nurses' Health Study found no evidence that higher adult milk intake (≥2 glasses of milk per day versus ≤1 glass per week) reduced incidence of hip fracture [RR, 1.45 (95 % CI; 0.87–2.43)] or forearm fracture [RR, 1.05 (95 % CI; 0.88–1.25)] [22]. An 18-year prospective analysis in 72,337 postmenopausal women from the same study also showed no association between milk intake and hip fracture risk (RR for ≥1.5 glasses of milk per day versus ≤1 glass per day, 0.83; 95 % CI; 0.61–1.10) [21]. With higher daily intakes of (2.5 glasses) milk, there was still no evidence of a protective effect (RR = 0.86; 95 % CI; 0.63, 1.18) [21]. Furthermore, a (2011) meta-analysis of milk consumption and hip fracture found no overall association [Pooled RR for each additional glass of milk per day for men, 0.91 (95 % CI; 0.8–1.01) and for women, 0.99 (95 % CI; 0.96–1.02), Q test $P=0.37$] [20].

Evidence from Randomized Controlled Trials

A 2-year randomized trial of fortified milk supplementation in older men (mean age, 61.9 ± 7.7 years) showed that the net beneficial effect of fortified milk supplementation on BMD persisted at the femoral neck and ultradistal radius at the end of the intervention (1.8 and 1.5 %, respectively; $P<0.01$ for both) and was sustained at 18-month follow-up ($P<0.05$ for both), during which time no fortified milk was provided. However, no lasting benefits were observed at the lumbar spine [24]. In another randomized controlled trial of 240 healthy men and women (aged 55–85 years), who typically consumed fewer than 1.5 servings of dairy a day, increasing intake to 3 servings of milk per day for 12 weeks decreased bone resorption, with a serum parathyroid hormone decrease of 9 % and a decrease in urinary excretion of N-telopeptide of 13 % [25]. In a prospective crossover trial of 16 weeks, 30 healthy postmenopausal women aged

59.3±3.3 years were provided a 600 mg calcium diet and randomized to receive either 500 ml semi-skimmed milk (containing 600 mg calcium and no vitamin D, thus providing a total of 1,200 mg calcium) or no milk. The authors concluded that a 6-week period of milk supplementation induced a decrease in several biochemical variables compatible with diminished bone turnover mediated by reduction in parathyroid hormone secretion [26]. Similar results have been reported from other randomized controlled trials, including postmenopausal women from South Australia [27], China [28], and Chinese women in Malaysia [29].

While studies of milk intake and BMD are plentiful and mostly positive, the evidence for hip fracture risk reduction remains weak. Results from two of the largest prospective cohort studies to date have shown no association between dairy intake and the risk of hip fracture [20, 22]. As noted by Bischoff-Ferrari et al., it is quite possible that adequate vitamin D exposure is required for a benefit of milk on hip fracture prevention [20], which may explain the overall null associations in their study. This may also explain null findings from the Nurses' Health Study, where vitamin D from foods and multivitamin supplements was 448 IU/day in the high milk intake group (≥2 glasses of milk/day). Similarly, in the follow-up study, mean dietary vitamin D intake from foods alone was 5 μg/day (or 200 IU/day) and 65.8 % of the women examined had total vitamin D levels <8.99 μg/day (or 359.6 IU/day) [21].

Yogurt Intake and Bone Health

Evidence from Observational Studies

In 2,479 men and women (mean age, 54.9±1.6; range 26–85) from the Framingham Offspring Study, participants with high yogurt intake (>4 servings per week) had higher BMD at the trochanter (P trend=0.05) compared to those with no intake, while no significant associations were observed for other bone sites (P range, 0.27–0.32) [5]. Even when intakes of milk, yogurt, cheese, and cream were simultaneously included in the final model, high yogurt intake (>4 servings per week) remained positively associated with trochanteric BMD (P=0.04), and statistically borderline associations were observed with femoral neck BMD (P=0.09) compared to no yogurt intake.

Evidence from Randomized Controlled Trials

An intervention study by Heaney et al., with 29 postmenopausal women (mean age, 61±4.3 years) not taking calcium supplements and with dietary calcium intake of <600 mg/day, showed that 3 servings of yogurt (versus 3 servings of jellied fruit snack) led to 22 % lower urinary excretion of N-telopeptide (P<0.03), a marker of bone resorption [30].

Yogurt is an important source of bone-specific nutrients, is low in sodium, and has high satiety factor. Yogurt is a good source of probiotics; consequently, yogurt is better tolerated by people who avoid dairy products due to lactose intolerance. Whether these probiotics also affect the absorption of bone-specific nutrients such as calcium is yet unknown. Studies on yogurt intake and bone health have been very few but promising.

Cheese Intake and Bone Health

Evidence from Observational Studies

In men and women from the Framingham Offspring Study (mean age, 54.9±1.6), no significant associations were observed for total cheese intake with BMD at the hip or spine [5].

Evidence from Randomized Controlled Trials

Two controlled trials were conducted by Bonjour et al. [31, 32] in France that proposed skimmed-milk soft cheese as an effective dietary intervention against bone loss. In a randomized controlled trial, 71 healthy postmenopausal women (mean age, 56.6±3.9 years) with low calcium and vitamin D intakes were randomized to consume two daily servings of skimmed-milk, soft plain cheese (2×100 g) for 6 weeks (n=36 intervention; 35

placebo controls). The vitamin D and calcium-fortified cheese product provided 661 kJ of energy, 2.5 μg vitamin D, 400 mg calcium, and 13.8 g protein per day. At the end of the intervention, the decrease in TRAP 5b (tartrate-resistant acid phosphatase, isoform 5b, a bone resorption biomarker) and the increase in insulin-like growth factor-I (IGF-I, serum bone anabolic factor) were greater in the treated than in the placebo group ($P<0.02$). The changes in the resorption marker, serum carboxy-terminal cross-linked telopeptide of type I collagen, did not differ significantly between the two groups [32]. The results of this trial were consistent with the results of a previous trial by the same group that used a crossover controlled study design in older institutionalized women (mean age, 87.2 ± 6.1 years) with low vitamin D status and calcium intake <700 mg/day [31, 33].

Several have speculated that intake of hard cheeses and processed cheese products are less advantageous to bone and that cottage cheese would also be disadvantageous [13]. A possible explanation for this is that the manufacture of cheese increases its sodium content, particularly processed cheese products and the acid-cured cheeses such as cottage cheese [34]. Thus, while the recommended 2–3 dairy servings taken as milk would provide ~315 mg Na, a comparable intake of calcium from American cheese would increase the sodium intake to ~2,500 mg and if consumed as cottage cheese would increase the sodium intake to ~5,000 mg [13]. However, few studies have examined the association of cheese intake with bone health, and these studies either examined total cheese intake or focused on a specific low-fat variety. Future studies should focus on the skeletal effects of low-fat cheese and the influence of sodium content of the different cheeses.

Cream Intake and Bone Health

Evidence from Observational Studies

In the Framingham Offspring Study, no significant associations were observed for dietary cream intake (defined as the sum of intake of cream, ice cream, sour cream, and cream cheese; *P* trend range, 0.39–0.42) and BMD at the hip or spine. However, after adjustment for other dairy foods such as milk, yogurt, and cheese, cream intake tended to be negatively associated with femoral neck BMD (β, –0.00062; SE, 0.0004; *P*, 0.08) [5].

Evidence from Randomized Controlled Trials

In a randomized controlled trial, 80 women (ages 20–39 years with calcium intake <750 mg/day) were randomized to consume lower saturated fat/sugar ice cream containing 96, 244, 459, or 676 mg calcium daily for 28 days. This study concluded that daily consumption of this low fat/sugar and calcium-fortified ice cream by premenopausal women may significantly reduce levels of serum carboxy-terminal collagen cross-links (CTX, a bone resorption marker), without stimulating weight gain [35].

Dairy foods like cream, ice cream, and sour cream are usually high in saturated fat or sugar and have low nutrient density. These dairy foods are widely consumed yet very little is known about their impact on the skeleton.

Conclusion

Although the evidence for a positive association between milk intake and BMD is strong and that of yogurt intake looks promising, it is unclear how other dairy foods may be related to bone health. Recent studies have highlighted the important role of various macro- and micronutrients as well as that of milk basic protein in bone health [36, 37]. However, the additive and synergistic role of sodium and saturated fats along with other bone-specific nutrients (e.g., calcium, vitamin D, phosphorus, and protein) in dairy foods such as cream and cheese is complicated and needs further clarification. There is a clear dearth of studies on specific dairy foods and hip fractures in older adults, and thus, the evidence for hip fracture remains weak. This highlights the need for future studies to resolve the disparate findings from BMD studies versus fracture studies. Single nutrient studies cannot account for synergistic interaction between nutrients. Therefore, future studies should

focus on dairy food products instead of single nutrients within dairy. At the same time, it is vital to consider nutrient profiles of specific dairy foods while investigating their associations with bone health. Dairy foods contribute 70.3 % of calcium, 16 % of magnesium almost all of the vitamin D, 18.2 % of vitamin B12, 15 % of zinc, and 25 % of riboflavin in the typical US diet [4]. Given that dairy intake is an essential resource of bone-building nutrients in the western diet and plays an important role in maintaining bone health, it is essential to address the issue of nutritional equivalency of dairy foods in order to optimize bone health.

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References

1. Cooper C, Campion G, Melton 3rd LJ. Hip fractures in the elderly: a world-wide projection. Osteoporos Int. 1992;2(6):285–9.
2. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary guidelines for Americans. 7th ed. Washington, D.C.: U.S. Government Printing Office; December 2010.
3. Heaney RP. Dairy and bone health. J Am Coll Nutr. 2009;28 Suppl 1:82S–90. 28/Supplement_1/82S [pii].
4. U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary guidelines for Americans. Dietary Guidelines Advisory Committee Report, 6th edn. Washington, D.C.: U.S. Government; 2005.
5. Sahni S, Tucker KL, Kiel DP, Quach L, Casey VA, Hannan MT. Total dairy intake and milk consumption are linked with higher bone mineral density but not with hip fracture: the Framingham Offspring Study. Arch Osteoporos. 2013;8:119. doi:10.1007/s11657-013-0119-2
6. Teegarden D, Legowski P, Gunther CW, McCabe GP, Peacock M, Lyle RM. Dietary calcium intake protects women consuming oral contraceptives from spine and hip bone loss. J Clin Endocrinol Metab. 2005;90(9):5127–33. doi:10.1210/jc.2004-0924. jc.2004-0924 [pii].
7. Baran D, Sorensen A, Grimes J, Lew R, Karellas A, Johnson B, Roche J. Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: a three-year prospective study. J Clin Endocrinol Metab. 1990;70(1):264–70.
8. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med. 1997;337(10):670–6. doi:10.1056/NEJM199709043371003.
9. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000;15(12):2504–12. doi:10.1359/jbmr.2000.15.12.2504.
10. Sahni S, Cupples LA, McLean RR, Tucker KL, Broe KE, Kiel DP, Hannan MT. Protective effect of high protein and calcium intake on the risk of hip fracture in the Framingham offspring cohort. J Bone Miner Res. 2010;25(12):2770–6. doi:10.1002/jbmr.194.
11. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr. 1999;69(4):727–36.
12. Devine A, Criddle RA, Dick IM, Kerr DA, Prince RL. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. Am J Clin Nutr. 1995;62(4):740–5.
13. Weinsier RL, Krumdieck CL. Dairy foods and bone health: examination of the evidence. Am J Clin Nutr. 2000;72(3):681–9.
14. Unal G, El SN, Kilic S. In vitro determination of calcium bioavailability of milk, dairy products and infant formulas. Int J Food Sci Nutr. 2005;56(1):13–22. doi:10.1080/09637480500081423. RR54364580742841 [pii].
15. Murphy S, Khaw KT, May H, Compston JE. Milk consumption and bone mineral density in middle aged and elderly women. BMJ. 1994;308(6934):939–41.
16. Kull M, Kallikorm R, Lember M. Impact of molecularly defined hypolactasia, self-perceived milk intolerance and milk consumption on bone mineral density in a population sample in Northern Europe. Scand J Gastroenterol. 2009;44(4):415–21. doi:10.1080/00365520802588117. 906066060 [pii].
17. McCabe LD, Martin BR, McCabe GP, Johnston CC, Weaver CM, Peacock M. Dairy intakes affect bone density in the elderly. Am J Clin Nutr. 2004;80(4):1066–74. 80/4/1066 [pii].
18. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am J Clin Nutr. 2003;77(1):257–65.
19. Teegarden D, Lyle RM, Proulx WR, Johnston CC, Weaver CM. Previous milk consumption is associated with greater bone density in young women. Am J Clin Nutr. 1999;69(5):1014–7.
20. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, Kanis JA, Orav EJ, Staehelin HB, Kiel DP, Burckhardt P, Henschkowski J, Spiegelman D, Li R, Wong JB, Feskanich D, Willett WC. Milk intake and risk of hip fracture in men and women: a meta-analysis of

prospective cohort studies. J Bone Miner Res. 2011;26(4):833–9. doi:10.1002/jbmr.279.
21. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. Am J Clin Nutr. 2003;77(2):504–11.
22. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. Milk, dietary calcium, and bone fractures in women: a 12-year prospective study. Am J Public Health. 1997;87(6):992–7.
23. Soroko S, Holbrook TL, Edelstein S, Barrett-Connor E. Lifetime milk consumption and bone mineral density in older women. Am J Public Health. 1994;84(8):1319–22.
24. Daly RM, Petrass N, Bass S, Nowson CA. The skeletal benefits of calcium- and vitamin D3-fortified milk are sustained in older men after withdrawal of supplementation: an 18-mo follow-up study. Am J Clin Nutr. 2008;87(3):771–7. 87/3/771 [pii].
25. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI, Rosen CJ. Dietary changes favorably affect bone remodeling in older adults. J Am Diet Assoc. 1999;99(10):1228–33. doi:10.1016/S0002-8223(99)00302-8. S0002-8223(99)00302-8 [pii].
26. Bonjour JP, Brandolini-Bunlon M, Boirie Y, Morel-Laporte F, Braesco V, Bertiere MC, Souberbielle JC. Inhibition of bone turnover by milk intake in postmenopausal women. Br J Nutr. 2008;100(4):866–74. doi:10.1017/S0007114508937429. S0007114508937429 [pii].
27. Cleghorn DB, O'Loughlin PD, Schroeder BJ, Nordin BE. An open, crossover trial of calcium-fortified milk in prevention of early postmenopausal bone loss. Med J Aust. 2001;175(5):242–5.
28. Kruger MC, Ha PC, Todd JM, Kuhn-Sherlock B, Schollum LM, Ma J, Qin G, Lau E. High-calcium, vitamin D fortified milk is effective in improving bone turnover markers and vitamin D status in healthy postmenopausal Chinese women. Eur J Clin Nutr. 2012;66(7):856–61. doi:10.1038/ejcn.2012.54. ejcn201254 [pii].
29. Chee WS, Suriah AR, Chan SP, Zaitun Y, Chan YM. The effect of milk supplementation on bone mineral density in postmenopausal Chinese women in Malaysia. Osteoporos Int. 2003;14(10):828–34. doi:10.1007/s00198-003-1448-6.
30. Heaney RP, Rafferty K, Dowell MS. Effect of yogurt on a urinary marker of bone resorption in postmenopausal women. J Am Diet Assoc. 2002;102(11):1672–4.
31. Bonjour JP, Benoit V, Pourchaire O, Ferry M, Rousseau B, Souberbielle JC. Inhibition of markers of bone resorption by consumption of vitamin D and calcium-fortified soft plain cheese by institutionalised elderly women. Br J Nutr. 2009;102(7):962–6. doi:10.1017/S0007114509371743. S0007114509371743 [pii].
32. Bonjour JP, Benoit V, Rousseau B, Souberbielle JC. Consumption of vitamin D-and calcium-fortified soft white cheese lowers the biochemical marker of bone resorption TRAP 5b in postmenopausal women at moderate risk of osteoporosis fracture. J Nutr. 2012;142(4):698–703. doi:10.3945/jn.111.153692. jn.111.153692 [pii].
33. Bonjour JP, Benoit V, Pourchaire O, Rousseau B, Souberbielle JC. Nutritional approach for inhibiting bone resorption in institutionalized elderly women with vitamin D insufficiency and high prevalence of fracture. J Nutr Health Aging. 2011;15(5):404–9.
34. Fox PF, O'Connor TP, McSweeney PL, Guinee TP, O'Brien NM. Cheese: physical, biochemical, and nutritional aspects. Adv Food Nutr Res. 1996;39:163–328.
35. Ferrar L, van der Hee RM, Berry M, Watson C, Miret S, Wilkinson J, Bradburn M, Eastell R. Effects of calcium-fortified ice cream on markers of bone health. Osteoporos Int. 2011;22(10):2721–31. doi:10.1007/s00198-010-1513-x.
36. Aoe S, Koyama T, Toba Y, Itabashi A, Takada Y. A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporos Int. 2005;16(12):2123–8. doi:10.1007/s00198-005-2012-3.
37. Aoe S, Toba Y, Yamamura J, Kawakami H, Yahiro M, Kumegawa M, Itabashi A, Takada Y. Controlled trial of the effects of milk basic protein (MBP) supplementation on bone metabolism in healthy adult women. Biosci Biotechnol Biochem. 2001;65(4):913–8.

Galacto-oligosaccharides: Prebiotic Effects on Calcium Absorption and Bone Health

30

Corrie M. Whisner and Connie M. Weaver

Abstract

Adolescence presents an opportune time to influence peak bone mass with prebiotic agents like galacto-oligosaccharides (GOS) that increase calcium absorption in the large intestine. Previous literature has helped elucidate the mechanisms by which prebiotics elicit their response which involves decreased luminal pH following bacterial fermentation. In addition to improved mineral absorption, dietary supplementation with GOS in rats has been associated with improved bone mineral content (BMC) during growth, reduced losses of BMC and bone mineral density (BMD) after ovariectomy, and increased cecal content weight. Similar bone-sparing results have been seen in postmenopausal women, while preliminary results in adolescents show that GOS increases fractional calcium absorption. This effect may be mediated by bacterial fermentation in the colon as bifidobacteria content of the feces was increased after GOS consumption. Further work is needed to fully elucidate the intestinal mechanism and understand the long-term effects of GOS consumption.

Keywords

Galacto-oligosaccharides • Prebiotic • Calcium absorption • Bone health • Microbiota • Intestinal flora • Bifidobacteria

Introduction

Pubertal growth is an important time period for building strong bones, during which 90 % of the adult skeletal mass is accumulated [1]. Peak bone mass acquired during adolescence has been identified as an important predictor of osteoporosis risk in later life [2–4]. Modifiable lifestyle factors during this time, such as dietary intake of important nutrients (calcium and vitamin D), can greatly influence bone health. Consuming adequate calcium is essential to bone health and the prevention of osteoporotic fracture [5, 6]. Milk being the primary source of dietary calcium has suffered significant declines in recent decades with only 48 % of American adolescents reporting

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milk consumption in 2005–2006 [7]. This decrease in milk consumption can pose a threat to bone health, as milk serves as a highly bioavailable source of calcium.

To combat habitually low calcium intakes among adolescent populations, the addition of bioactive compounds to the diet may help improve the bioavailability and utilization of calcium already in the diet. Prebiotics, which are non-digestible food components, work as functional food components that selectively stimulate the growth of beneficial gastrointestinal bacteria, ultimately resulting in health benefits for the host [8]. Non-digestible oligosaccharides (NDOs) are prebiotic carbohydrates that have beneficial effects on bone health. The influence of specific NDOs, such as fructo-oligosaccharides (FOS), have been studied in animals and humans showing significant increases in intestinal calcium absorption, decreases in bone resorption, and improved bone mass and strength [9–18]. Similar results have been reported for galacto-oligosaccharides (GOS) in rats and postmenopausal women, but little is known of their effect in growing adolescents.

Galacto-oligosaccharides

Galacto-oligosaccharides comprise a unique class of NDOs as they provide a number of structural and functional similarities with oligosaccharides occurring in human breast milk. Commercial production involves the conversion of lactose by bacteria expressing β-D-galactosidase activity [19]. GOS (Fig. 30.1) is composed of a galactose chain attached to a single glucose molecule which varies in chain length (2–8 monomers) and types of linkages (mainly β(1–4), β(1–2), β(1–6)). In breastfed infants GOS contributes to the protective microbiome that persists in the intestines [20, 21], specifically leading to the growth and proliferation of bifidobacteria and lactobacillus [22]. Studies have also shown positive effects of GOS on calcium absorption [23–27], but these effects vary depending on the dose given, treatment length, subject age, and habitual calcium intake.

Prebiotic Influence of GOS on the Gastrointestinal Tract

The benefit of prebiotics on calcium metabolism occurs in the lower gut and was confirmed when antibiotic-treated rats and ileostomy patients did not experience an increase in calcium absorption when their diets were supplemented with prebiotics [26, 28]. Ordinarily, the gut is mostly comprised of Cytophaga-Flavobacterium-Bacteroides and Firmicutes [29]. However, upon treatment with prebiotics, a shift in microbiota types and quantities occurs as the environment selects for those organisms that selectively ferment prebiotics. These compounds influence the growth of bacteria in the large intestine, favoring the

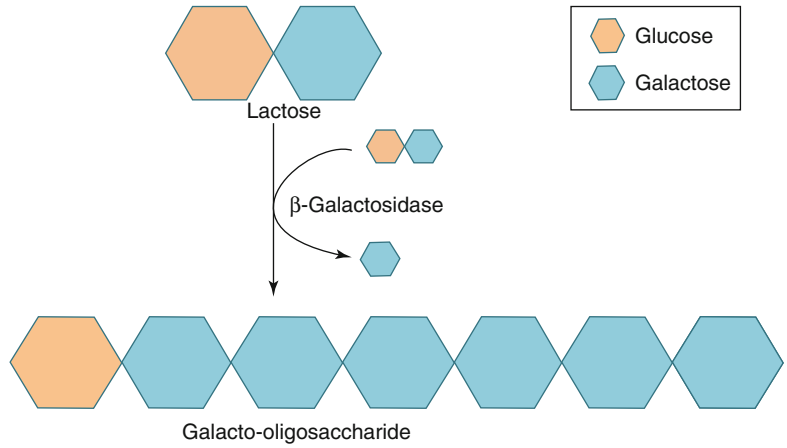

Fig. 30.1 Enzymatic production of galacto-oligosaccharides with β-galactosidase

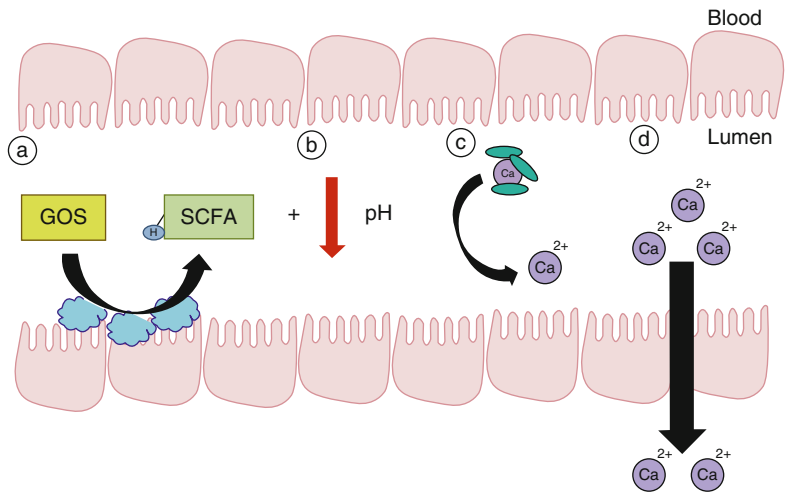

Fig. 30.2 Fermentation of galacto-oligosaccharide (*GOS*) is an important mechanism for calcium absorption in the colon. (*a*) Non-digestible oligosaccharides that have prebiotic effects in the colon such as GOS are selectively fermented by colonic microflora resulting in the production of short-chain fatty acids (*SCFA*). (*b*) Accumulation of SCFA results in a reduced pH. (*c*) This shift in colonic acidity increases the solubility of calcium. (*d*) Calcium is more readily absorbed across the enterocytes of the large intestine

beneficial strains that are able to elicit positive host effects such as increased calcium absorption and retention. Significant increases in the quantity of bifidobacteria and lactobacillus species have been reported in animals [30, 31] and humans [32–34] consuming prebiotic fibers. Recent in vitro work using a ^{13}C labeling technique has also confirmed these effects specifically for GOS. Administering ^{13}C-labeled GOS to a dynamic in vitro colonic model has shown that *Bifidobacterium bifidum*, *Bifidobacterium catenulatum*, *Lactobacillus gasseri*, and *Lactobacillus salivarius* were primarily involved in the fermentation of GOS [35].

GOS resists digestion in the stomach and small intestine allowing it to be fermented in the large intestine to produce short-chain fatty acids and a decrease in pH (Fig. 30.2). This increased level of acidity may enhance mineral solubility, thereby directly increasing calcium absorption through hydrogen ion exchange [36] or indirectly through hypertrophy of the intestinal mucosa to increase surface area for greater mineral diffusion. Bouhnik et al. [33] found that consumption of 10 g transgalacto-oligosaccharide for 21 days resulted in significant increases in fecal bifidobacteria content in healthy adults, while in vitro work has shown increased production of short-chain fatty acids with elevated acetate and lactate concentrations [35, 37]. In elderly, similar results were found after consumption of 4 g galacto-oligosaccharides twice daily [34]. Changes in intestinal surface area have also been associated with prebiotic consumption resulting in cecal hypertrophy [38, 39]. In rats fed GOS diets, this occurred as increased crypt depth and cell density in the proximal and distal colon [24]. Recently, work in rats has shown that GOS increased calcium absorption and bone mineral density (BMD) through decreased pH and increased bifidobacteria composition, cecal wall, and content weight [40]. Work in humans has also suggested the importance of colonic mechanisms in NDO-induced calcium absorption [41], but the specific effect of GOS needs to be confirmed in children.

Effects of GOS on Calcium Metabolism in Animals

GOS has beneficial effects on calcium absorption and bone density in both growing [23–26, 40] and ovariectomized rats [42, 43]. A number of

animal studies indicated that the cecum is an important site for calcium absorption [10, 39, 44, 45], while two others found no relationship [9, 46]. Given the coprophagic behavior of rats and their highly developed cecum, it is difficult to extrapolate these findings, positive or negative, to humans. Also, few studies have attempted to relate the changes in calcium absorption to physiological and/or morphological changes in the intestine.

We found a significant association between increased bifidobacteria counts and improved calcium absorption in a study with 75 four-week-old male Sprague-Dawley rats which were fed diets containing 0, 2, 4, 6, or 8 % GOS by weight [40]. Rats underwent treatment for 8 weeks and measures of mineral metabolism and cecal environment changes were assessed including measurements of cecal pH, content and wall weight, gut microbiota including bifidobacteria levels, bone geometry, bone mineral density, calcium absorption, and retention. Treatment with GOS increased tibial bone-breaking strength, total and trabecular volumetric bone mineral density (vBMD) of the distal femur, and area and vBMD of the proximal tibia. Results from this study suggested that trabecular-rich bones benefited the most from GOS supplementation, and regression analysis revealed that GOS improved vBMD through increased bifidobacteria content, trophic effects in cecal tissue, and decreased cecal pH (Fig. 30.3). These extensive findings strongly suggest the need for similar work in humans, specifically during periods of intense growth.

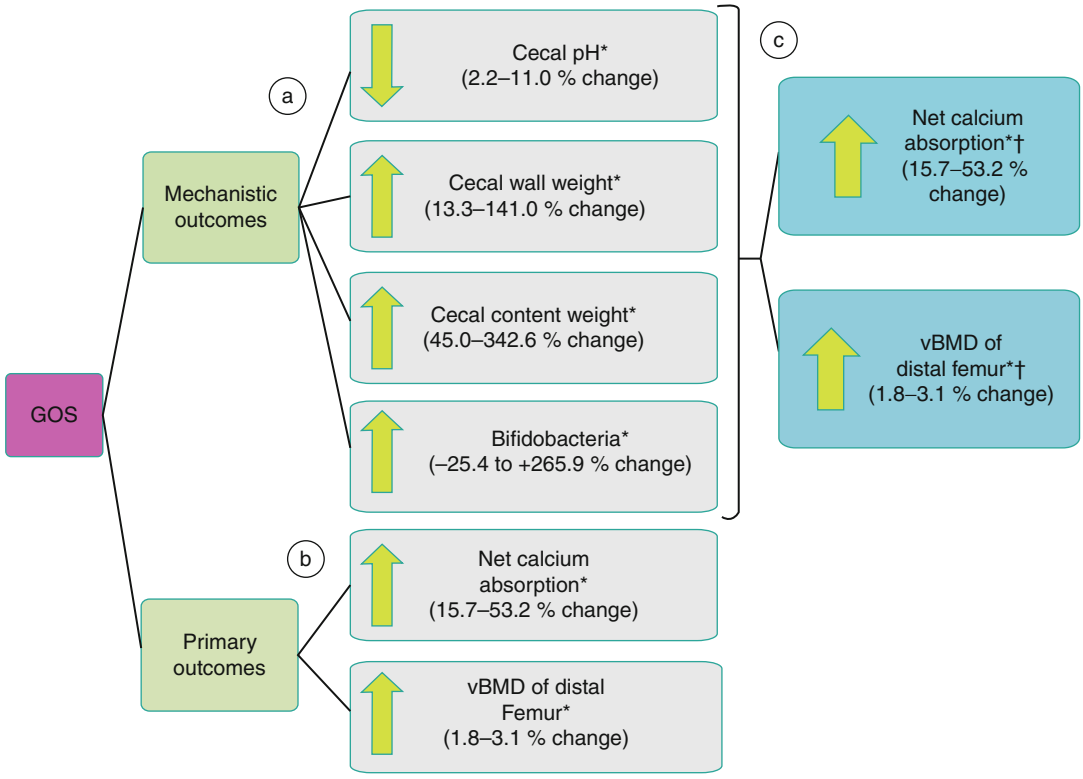

Fig. 30.3 Mechanistic outcomes predicted calcium absorption and BMD in growing rats in a multiple regression analysis. (*a*) Effect of GOS on mechanistic outcomes; (*b*) dose-response influence of GOS on primary outcomes that relate to skeletal health; and (*c*) influence of mechanistic outcomes as predictors of primary outcomes related to bone health. Cecal and bifidobacteria characteristics had a positive influence on calcium absorption and bone mineral density. The range for each variable as percent change is expressed below the variable name and superscripts represent significant influences from the variable(s) directly to the left of the respective variable. *$p<0.01$; †$p<0.05$ for bifidobacteria as a predictor of primary outcomes

Effects in Humans

The study of GOS in humans in relation to mineral metabolism has been scarce. In postmenopausal women, supplementation with 20 g transgalacto-oligosaccharide for 9 days increased calcium absorption by 16 % [27]. However, a study using a similar design in young men found no effect of GOS on calcium metabolism when 15 g/day was given for 21 days [47]. This lack of effect may be the result of an inadequate time period for urine collection (24 h) which is not long enough to capture the lower gut effect. No studies on GOS and calcium absorption have been reported in children and adolescents, but other prebiotic fibers, such as inulin, have proven beneficial for increasing calcium absorption during the pubertal growth spurt [12].

As adolescence is a time for rapid growth, this life stage presents an opportunity to influence peak bone mass with an agent like GOS that works to increase calcium absorption. To extend the results of the observations in rats [40] and to better understand the prebiotic effects of GOS during the adolescent growth spurt, we investigated the effect of GOS supplementation on calcium absorption in premenarcheal girls. The primary objective of our study was to assess the dose-response effect of GOS on fractional calcium absorption. Secondarily, we aimed to assess the potential dose response of GOS on intestinal microflora, specifically expecting to see a significant increase in bifidobacteria.

We designed a randomized, double-blind crossover trial with three treatments. Treatments consisted of two milk-based smoothie drinks each supplemented with 0, 2.5, or 5 g of GOS (total intakes of 0, 5, and 10 g GOS per day). Smoothie drinks were consumed twice a day (morning and night) for 3 weeks which was followed by a weekend clinical visit. During the clinic visit, subjects consumed a controlled diet containing approximately 1,300 mg calcium and 15 g fiber (not including the GOS from smoothies). A calcium absorption test was performed during this visit utilizing dual stable isotope methodology. Isotope enrichment was measured in urine collections pooled into four 12 h aliquots collected during the 48 h clinic visit. Fecal samples were collected and assessed for total microbial communities and bifidobacteria using 16S rRNA gene copies from PCR-DGGE and quantitative PCR, respectively. Variation between treatment profiles was assessed by principal components analysis (PCA) to identify major contributors to variation in PCR-DGGE banding patterns. Each phase was separated by a 2-week washout period to stop any effect on the gut colonization and avoid the potential for carryover effects between treatments.

Significant increases in fractional calcium absorption were observed but the effect was not dose dependent as twice daily treatment with 2.5 and 5 g GOS resulted in similar increases in absorption relative to control. Overall, bacteria community patterns assessed by PCA did not differ among the three treatments (Fig. 30.4), but bifidobacteria as a proportion of total bacteria increased with GOS treatment. This study was the first to assess the effects of GOS in young girls nearing the pubertal growth spurt and the results suggest that GOS may improve the bioavailability and absorption of calcium through the large intestine.

The increases in calcium absorption seen in this study were comparable to studies investigating the effects of similar prebiotic fibers. Inulin fiber (8 g/day) has been reported to improve calcium absorption by 8–18 % in adolescents [12, 13]. However, a study from our lab saw no effects with 9 g/day of the same inulin fiber [48], so further work is needed to understand the differences in response. Overall, our results with GOS suggest that modest consumption of NDO prebiotics could aid in the development of peak bone mass at a crucial time period for bone growth.

Adaptation

Novel dietary fibers with prebiotic effects elicit their beneficial effects by altering the gastrointestinal environment. These effects are transient resulting in a loss of benefits when consumption of the prebiotic is ceased. Little is known about the potential for adaptation or whether the

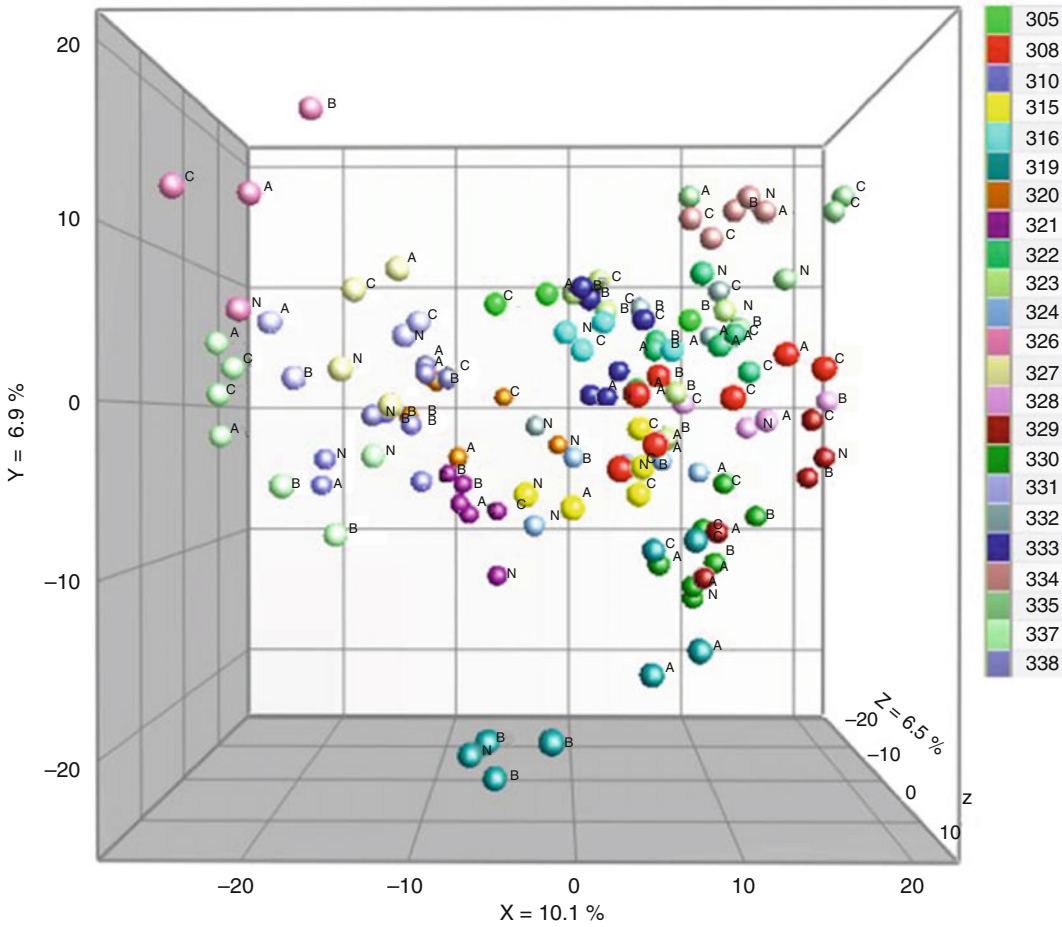

Fig. 30.4 Principal components analysis (PCA) of bacterial fingerprint profiles of adolescent girls before (N) and after 3 weeks of GOS treatment (A = 5 g/day GOS, B = 10 g/day GOS, C = control). PCR-DGGE profiles combined from two different gradients (35–56 %, 25–75 %) were used to generate the PCA using Bionumerics. Bacterial profiles did not separate by treatment but indicated slight similarities within each participant

positive health effects persist with prolonged use. Animal studies have indicated bone-sparing effects in animals undergoing longer-term interventions, of which a study in ovariectomized rats reported increases in BMC, BMD, and tibial microarchitecture after 4 months of treatment with FOS or FOS + Soy [49]. These results may not translate to humans though, and even fewer studies have been done to assess whether prebiotic effects persist in humans.

Currently, there is only one study that has assessed the long-term effects of a prebiotic on bone health. Abrams et al. completed a 1-year clinical trial in prepubertal girls and boys who received 8 g/day of ITF-mix (mixture of short and long-chain inulin-type fructans). Using dual stable isotope techniques, they found that calcium absorption in the fructan group was significantly higher after 8 weeks compared to the control group. This effect on absorption persisted throughout the entire intervention and also resulted in significant increases in whole body BMC and BMD after 1 year of treatment [12]. Further study of these children suggested that the effects of prebiotics may be modulated by genetic factors through vitamin D receptor (VDR) gene polymorphisms. Interaction between fructan supplementation and the Fok1 gene, a vitamin D receptor polymorphism that

affects bone mineralization during puberty, was a significant determinant of "responders" at 8 weeks after controlling for sex, tanner stage, and ethnicity [12]. In those children classified as responders (experiencing a 3 % increase in calcium absorption with treatment), calcium accretion was greater after 1 year compared to nonresponders and those on the control treatment [50].

Conclusions

Adolescence is a critical time for reaching peak bone mass. Novel dietary fibers such as GOS may improve mineral metabolism and bone health through their unique interactions with colonic microflora. Bacteria in the colon are able to ferment ingested prebiotics thereby altering the luminal environment of the colon, which has been associated with increased absorption of calcium. Previous research in animals and humans identified a relationship between GOS consumption and specific gut bacteria or improved calcium metabolism independently, but there is a need to better understand the mechanism connecting these two outcomes. Additionally, identifying an optimal dose to see effects would provide further understanding of the relationship between GOS and bone health. Studying these effects in important populations such as adolescents is equally important as GOS and other prebiotics may help make up for deficits in calcium intake to maximize bone mineral accretion and reduce the risk of osteoporosis later in life.

References

1. Henry YM, Fatayerji D, Eastell R. Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: relative contributions of bone size and volumetric bone mineral density. Osteoporos Int. 2004;15:263–73.
2. Teegarden D, Lyle RM, Proulx WR, Johnston CC, Weaver CM. Previous milk consumption is associated with greater bone density in young women. Am J Clin Nutr. 1999;69:1014–7.
3. Chevalley T, Rizzoli R, Hans D, Ferrari S, Bonjour J-P. Interaction between calcium intake and menarcheal age on bone mass gain: an eight-year follow-up study from prepuberty to postmenarche. J Clin Endocrinol Metab. 2005;90:44–51.
4. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am J Clin Nutr. 2003;77:257–65.
5. Bronner F. Calcium and osteoporosis. Am J Clin Nutr. 1994;60:831–6.
6. Nieves JW. Osteoporosis: the role of micronutrients. Am J Clin Nutr. 2005;81:1232S–9.
7. U.S. Department of Agriculture, Agricultural Research Service. Fluid milk consumption in the United States: what we eat in America, NHANES 2005–2006. Food Surveys Research Group Dietary Data Brief. 2010. http://ars.usda.gov/Services/docs.htm?docid=19476. Accessed 7th Aug 2012.
8. Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A. Dietary prebiotics: current status and new definition. Food Sci Technol Bull Funct Foods. 2010;7:1–19.
9. Brommage R, Binacua C, Antille S, Carrie AL. Intestinal calcium absorption in rats is stimulated by dietary lactulose and other resistant sugars. J Nutr. 1993;123:2186–94.
10. Ohta A, Ohtsuki M, Takizawa T, Inaba H, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of magnesium and calcium by cecectomized rats. Int J Vitam Nutr Res. 1994;64:316–23.
11. Takahara S, Morohashi T, Sano T, Ohta A, Yamada S, Sasa R. Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. J Nutr. 2000;130:1792–5.
12. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005;82:471–6.
13. Griffin IJ, Davila PM, Abrams SA. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. Br J Nutr. 2002;87:S187–91.
14. Griffin IJ, Hicks PMD, Heaney RP, Abrams SA. Enriched chicory inulin increases Ca absorption mainly in girls with lower Ca absorption. Nutr Res. 2003;23:901–9.
15. Zafar T, Weaver CM, Zhao Y, Martin BR, Wastney ME. Nondigestible oligosaccharides increase calcium absorption and suppress bone resorption in ovariectomized rats. J Nutr. 2004;134:399–402.
16. Zafar T, Weaver CM, Jones K, Moore DR, Barnes S. Inulin effects on bioavailability of soy isoflavones and their calcium absorption enhancing ability. J Agric Food Chem. 2004;52:2827–31.
17. Demigné C, Jacobs H, Moundras C, Davicco MJ, Horcajada MN, Bernalier A, et al. Comparison of native or reformulated chicory fructans, or non-purified chicory, on rat cecal fermentation and mineral metabolism. Eur J Nutr. 2008;47:366–74.
18. Lobo AR, Colli C, Filisetti TMCC. Fructooligosaccharides improve bone mass and biomechanical properties in rats. Nutr Res. 2006;26:413–20.

19. Macfarlane S, Macfarlane GT, Cummings JH. Review article: prebiotics in the pastrointestinal tract. Aliment Pharmacol Ther. 2006;24:701–14.
20. Bode L. Recent advances on structure, metabolism, and function of human milk oligosaccharides. J Nutr. 2006;136:2127–30.
21. German JB, Freeman SL, Lebrilla CB, Mills DA. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. Nestle Nutr Workshop Ser Pediatr Program. 2008;62:205–22.
22. Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, et al. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. Acta Paediatr Suppl. 2005;94:22–6.
23. Pérez-Conesa D, López G, Abellán P, Ros G. Bioavailability of calcium, magnesium and phosphorus in rats fed probiotic, prebiotic and symbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. J Sci Food Agric. 2006;86:2327–36.
24. Pérez-Conesa D, López G, Ros G. Effects of probiotics, prebiotic and synbiotic follow-up infant formulas on large intestine morphology and bone mineralization in rats. J Sci Food Agric. 2007;87:1059–68.
25. Chonan O, Watanuki M. The effect of 6′-galactooligosaccharides on bone mineralization of rats adapted to different levels of dietary calcium. Int J Vitam Nutr Res. 1996;66:244–9.
26. Chonan O, Takahashi R, Watanuki M. Role of activity of gastrointestinal microflora in absorption of calcium and magnesium in rats fed β1-4 linked galactooligosaccharides. Biosci Biotechnol Biochem. 2001;65: 1872–5.
27. van den Heuvel EG, Schoterman MH, Muijs T. Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women. J Nutr. 2000; 130:2938–42.
28. Ellegard L, Andersson H, Bosaeus I. Inulin and oligofructose do not influence the absorption of cholesterol, or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. Eur J Clin Nutr. 1997;51:1–5.
29. Backhed F, Hey RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915–20.
30. Tzortzis G, Goulas AK, Gee JM, Gibson GR. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. J Nutr. 2005;135: 1726–31.
31. Rodriguez-Cabezas ME, Camuesco D, Arribas B, Garrido-Mesa N, Comalada M, Bailón E, et al. The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats. Clin Nutr. 2010;29:832–9.
32. Ben X, Li J, Feng Z, Shi S, Lu Y, Chen R, et al. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal Bifidobacteria and Lactobacilli. World J Gastroenterol. 2008;14:6564–8.
33. Bouhnik Y, Flourié B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M, et al. Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J Nutr. 1997;127: 444–8.
34. Walton GE, van den Heuvel EG, Kosters MH, Rastall RA, Tuohy KM, Gibson GR. A randomised crossover study investigating the effects of galacto-oligosaccharides on the faecal microbiota in men and women over 50 years of age. Br J Nutr. 2012;107(10):1466–75.
35. Maathuis AJH, van den Huevel EG, Schoterman MHC, Venema K. Galacto-oligosaccharides have prebiotic activity in a dynamic in vitro colon model using a ^{13}C-labeling technique. J Nutr. 2012;142(7): 1205–12.
36. Raschka L, Daniel H. Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. Bone. 2005;37:728–35.
37. Durand M, Cordelet C, Hannequart G, Beaumatin P, Grivet JP. In vitro fermentation of a galacto-oligosaccharide by human bacteria in continuous culture. Proc Nutr Soc. 1992;51:6A.
38. Rémésy C, Levrat MA, Gamet L, Demigné C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. Am J Physiol. 1993;264:G855–62.
39. Levrat MA, Remesy C, Demigné C. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J Nutr. 1991;121:1730–7.
40. Weaver CM, Martin BR, Nakatsu CH, Armstrong AP, Clavijo A, McCabe LD, et al. Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. J Agric Food Chem. 2011;59:6501–10.
41. Abrams SA, Hawthorn KM, Aliu O, Hicks PD, Chen Z, Griffin IJ. An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. J Nutr. 2007;137:2208–12.
42. Chonan O, Matsumoto K, Watanuki M. Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. Biosci Biotechnol Biochem. 1995;59:236–9.
43. dos Santos EF, Tsuboi KH, Araújo MR, Andreollo NA, Miyasaka CK. Dietary galactooligosaccharides increase calcium absorption in normal and gastrectomized rats. Rev Col Bras Cir. 2011;38(3):186–91.
44. Demigné C, Levrat MA, Rémésy C. Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. J Nutr. 1989;119: 1625–30.
45. Chonan O, Watanuki M. Effect of galactooligosaccharides on calcium absorption in rats. J Nutr Sci Vitaminol. 1995;41:95–104.
46. Ohta A, Baba S, Ohtsuki M, Taguchi A, Adachi T. In vivo absorption of calcium carbonate and magnesium oxide from the large intestine in rats. J Nutr Sci Vitaminol. 1997;43:35–46.

47. van den Heuvel EGHM, Schaafsma G, Muys T, van Dokkum W. Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men. Am J Clin Nutr. 1998;67: 445–51.
48. Martin BR, Braun MM, Wigertz K, Bryant R, Zhao Y, Lee W, Kempa-Steczko A, Weaver CM. Fructo-oligosaccharides and calcium absorption and retention in adolescent girls. J Am Coll Nutr. 2010; 29:382–6.
49. Devareddy L, Khalil DA, Korlagunta K, Hooshmand S, Bellmer DD, Arjmandi BH. The effects of fructo-oligosaccharides in combination with soy protein on bone in osteopenic ovariectomized rats. Menopause. 2006;13:692–9.
50. Abrams SA, Griffin IJ, Hawthorn KM. Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. J Nutr. 2007;137: 2524S–6.

31 The Relationship of Weight-Bearing Physical Activity and Dietary Calcium Intake with Bone Mass Accrual in the Bone Mineral Density in Childhood Study Cohort

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Abstract

Background: Although it is widely accepted that dietary calcium intake (CaI) and weight-bearing physical activity (WBA) increase bone mass accrual during growth, few prospective studies have followed children from early childhood to sexual maturity to evaluate this relationship.

Aims: To describe the relationship between CaI and WBA and total body bone mineral content (TBBMC) accrual in a large, multiracial cohort of children followed prospectively.

Methods: Five US centers recruited 2014 healthy children (ages 5–19 years) and measured them annually for up to 7 years. Subjects with at least

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two annual visits are included in this analysis (944 boys, 973 girls). Assessments included TBBMC, Tanner stage, WBA, and CaI. Multiple regression was used to model annual increases in TBBMC, controlled for annualized overall height growth, Tanner stages, and baseline TBBMC. The effect of adding WBA and CaI to the model was evaluated for four subgroups: nonblack boys and girls and black boys and girls.

Results: WBA had a positive association with adjusted annual increases in TBBMC in all subgroups ($p < 0.05$), while CaI was positively related to TBBMC increase in nonblack males and nonblack females.

Conclusion: These findings support the importance of public health efforts to increase physical activity in children and adolescents while assuring adequate calcium intake.

Keywords

Dietary calcium intake • Physical activity • Children • Adolescents • Observational study • Bone mass accrual • Total body bone mineral content

Abbreviations

BMC	Bone mineral content
BMDCS	Bone Mineral Density in Childhood Study
CaI	Calcium Intake
CHGTO	Changed to
DXA	Dual Energy Absorptiometry
NICHD	National Institute for Child health and Human Development
RCT	Randomized Clinical Trial
SD	Standard Deviation
SS	Sums of Squares
TBBMC	Total Body Bone Mineral Content
WBA	Weight Bearing Activity

Introduction

Bone mineral accrual during childhood and adolescence is a critical determinant of osteoporosis later in life. In fact, peak bone mass, the maximum level of bone mass accumulated, is considered the best predictor of osteoporotic fracture [1–3]. Estimates are that a 5 % increase in peak bone mass at the population level would decrease the risk of fracture later in life by 50 % [4]. Thus, childhood and adolescence provide a window of opportunity to maximize peak bone mass and reduce the risk of osteoporosis later in life.

Adequate weight-bearing physical activity (WBA) to stimulate new bone formation and sufficient dietary calcium intake (CaI) to provide the substrate for new bone are essential for maximizing skeletal development. Numerous studies have been done to determine the levels of CaI and WBA required for maximal skeletal development. However, these levels are still not well defined.

Calcium is a threshold nutrient, that is, below a certain level, bone accrual varies with intake, while above that level, accrual is not dependent on intake [5]. Most observational studies of self-selected CaI and a large number of randomized trials of calcium supplementation or dairy food in growing children and/or adolescents show a positive effect of CaI on bone accrual [6, 7].

Bone adapts to varying levels of mechanical loading, that is, weight-bearing physical activity. The ability of bone to adapt to mechanical loading is significantly greater during growth than after maturity has been reached [8, 9]. Cross-sectional

studies have shown higher BMC in children and adolescents who are more physically active than their peers [10–12]. Similarly, prospective studies suggest the bone accrual advantage of WBA [13, 14]. Randomized trials show that an exercise intervention can increase BMC accrual by 2–4 % [15–18], and there is some evidence that this BMC advantage persists for several years [19].

However, prospective long-term studies that follow subjects from childhood into adulthood to determine the effects of self-selected CaI and WBA are sparse and have relatively small and homogenous samples. Few have provided an accurate assessment of pubertal stage even though biological maturation is one of the primary determinants of bone mass accrual. Thus, the purpose of this analysis of data from the Bone Mineral Density in Childhood Study (BMDCS) is to examine the effects of self-selected CaI and WBA on bone accrual in a large, multiethnic cohort in which sexual maturity was well characterized.

Methods

The US National Institute of Child Health and Human Development (NICHD) Bone Mineral Density in Childhood Study (BMDCS)

The BMDCS was a 6-year longitudinal study to develop reference data for dual energy absorptiometry (DXA) measures of bone mineral content and density in children and adolescents. A multiethnic sample of healthy children and adolescents was recruited from five centers across the USA: Children's Hospital of Los Angeles, Cincinnati Children's Hospital Medical Center, Creighton University, Children's Hospital of Philadelphia, and Columbia University.

Sample

Recruitment of girls (6–15 years) and boys (6–16 years) ($n=1{,}554$) occurred from July 2002 to November 2003. Entry criteria have been described previously [20]. Subjects were evaluated annually for 6 years (seven visits). A second wave of recruitment ($n=460$) occurred from August 2006 to November 2007 to increase the number of younger and older subjects to extend the reference percentiles to ages 5–20 years. These subjects were evaluated annually for 2 years (three visits). The final sample consisted of 2,014 subjects. Subjects with at least two annual visits are included in this analysis (944 males, 973 females). The racial/ethnic distribution of the sample was 6 % Asian, 23 % black, 48 % white, 16 % Hispanic, 6 % mixed, and 0 % American Indian.

Measurements

Total body bone mineral content (TBBMC, g) was assessed by DXA. Height (cm) was measured with a stadiometer and Tanner stages (1–5) of breast development and testicular volume were assessed by physician exam. Annual increases in TBBMC and height were calculated as the difference from the first to the last measurement divided by the years on study for each subject. Height growths less than 0.5 cm were set to 0.5 cm.

Weight-bearing physical activity (WBA, h/week) was assessed annually by self-report using the modified Slemenda questionnaire [21]. Slemenda reported correlations between children's reports of their activity 6 months apart as 0.62. Test-retest of the tool with a 2-week interval in ten adolescent females was 0.8 [22]. Dietary calcium intake (CaI, mg/day) was assessed annually by the Block semiquantitative food frequency questionnaire (Berkeley Ca). Parents assisted the younger children in completing both questionnaires. For each participant, the number of hours/week of WBA, on all activity reports submitted, was averaged to produce an overall indicator of activity level. Likewise, for each participant, the CaI (mg/day), from all dietary reports submitted, was averaged to produce an overall indicator of calcium intake. These averages provided continuous measures of WBA and CaI that were positively skewed, so both were log-transformed for comparisons among groups.

At each visit, subjects were scored on Tanner stage. These data were used to calculate five indicators of pubertal stage:

1. Tanner 1 or 2 at first visit (startT12)
2. Moved from a lower stage to Tanner 2 (chgtoT2)
3. Moved from a lower stage to Tanner 3 (chgtoT3)
4. Moved from a lower stage to Tanner 4 (chgtoT4)
5. Moved from a lower stage to Tanner 5 (chgtoT5)

Every subject was coded as 0 (no) or 1 (yes) for each of the five indicators.

Analysis

Descriptive statistics were calculated. Given that WBA and CaI were highly skewed, the median and confidence intervals were calculated by nonparametric rank-based methods.

Values of the continuous variables (baseline values, height and TBBMC change, average CaI, average WBA) were compared among the race/sex subgroups using multiple regression, with race (black, nonblack), sex, and race-sex interaction included in the model. Significance was evaluated using the type III sums of squares (SS). Correlations of CaI for each subject from one visit to the next and from the first visit to each subsequent visit were determined. Correlations between visits were determined similarly for WBA values.

Multiple regression was used to model predicted average annualized increases in TBBMC controlled for all five indicators of pubertal stage, baseline TBBMC value, and annualized change in height. The difference between the predicted and actual value (residual) was calculated so that positive values indicated greater increase in TBBMC than expected, while negative values indicated lesser increase than expected. The residuals reflect TBBMC increases adjusted for all of the factors in the basic model. The degree of association between this adjusted TBBMC increase and WBA and CaI, added both individually and together with an interaction term, was evaluated using a t-test (type III SS). In addition, to provide a readily interpretable estimate of the amount of additional TBBMC increase associated with a unit increase in either WBA or CaI, regression analysis was performed on the untransformed WBA/CaI measures.

All analyses were performed separately for four subgroups: nonblack males, black males, nonblack females, and black females. In hypothesis testing, probabilities ≤ 0.05 were taken as significant. Statistical analysis was performed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.)

Results

Table 31.1 provides the number of observations, the mean, and the standard deviation (SD) for all baseline values, change in TBBMC and height, and average WBA and CaI. Baseline age showed no differences among the groups. Baseline height and TBBMC and annual increase in height differed between sexes, with boys being larger. WBA and CaI both differed between sexes (both higher in males) and races (WBA higher in blacks, CaI higher in nonblacks). Annual increase in TBBMC showed significant sex and race differences and a significant interaction: males increased more than females and blacks more than nonblacks, with the latter difference larger in males than in females.

WBA and CaI were weakly but significantly intercorrelated overall and within each of the race/sex subgroups (data not shown). Both WBA and CaI were "tracked," that is, values from one visit to the next and from the first visit to each subsequent visit were significantly correlated, although moderate in size. Correlations from one visit to the next range from $r=0.42$ to $r=0.58$, while the correlation between the first and the last CaI and WBA observation are 0.28 and 0.24, respectively. Figures 31.1 and 31.2 show the average WBA and CaI, respectively, by age group. The average WBA varied among the age categories but remained relatively stable in all subgroups. However, CaI tends to be lower in older ages of all four subgroups. The mean CaI

Table 31.1 Descriptive statistics

	Nonblack boys ($N=716$)	Black boys ($N=228$)	Nonblack girls ($N=758$)	Black girls ($N=215$)
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Baseline age (year)	11.2 ± 4.4	11.5 ± 4.4	11.2 ± 4.4	11.2 ± 3.9
Baseline height (cm)	141.5 ± 19.4[a]	147.5 ± 22.7	141.5 ± 19.5	144 ± 18.56
Baseline TBBMC (g)	1475.3 ± 713.5[a]	1597.4 ± 740.4	1337.8 ± 547.9	1473.9 ± 579.8
Years on study	4.7 ± 1.8[b]	4.2 ± 1.9	4.7 ± 1.8	4.8 ± 1.8
Annual Δ height (cm/year)	4.1 ± 2.5[a]	4.2 ± 2.5	3.4 ± 2.6	3.1 ± 2.7
Annual Δ TBBMC (g/year)	131.8 ± 78.7[a,b]	159.3 ± 92.3	96.4 ± 64.2	108.6 ± 69.9
Physical activity (h/week)	13.2 ± 7.1[a,b]	15.5 ± 7.8	11.1 ± 5.6	13.1 ± 7.0
Median	11.9	13.9	10.3	12.3
Calcium intake (mg/day)	986.6 ± 438.5[a,b]	812.8 ± 369.6	776.6 ± 357.2	652.8 ± 321.7
Median	911.5	760.5	698.4	618.4

[a]Significant difference between sexes
[b]Significant difference between races

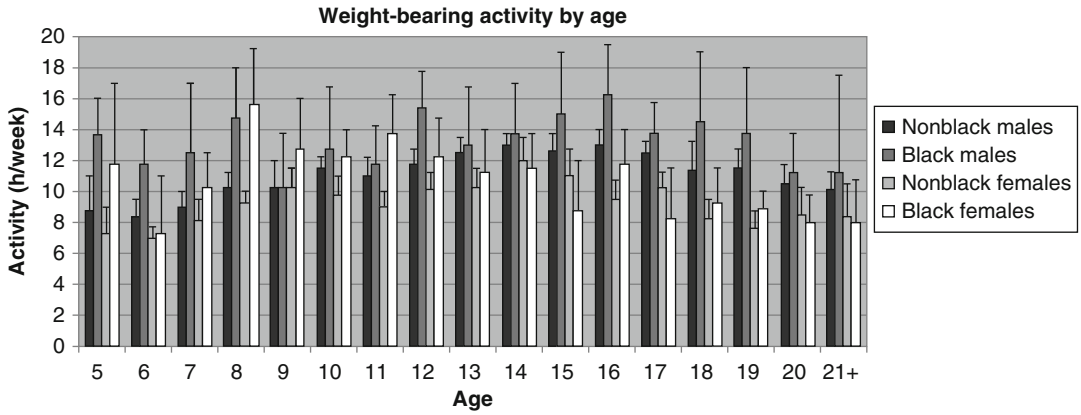


Fig. 31.1 Weight-bearing activity by age. *Bar height* shows median weight-bearing activity (h/week). *Error bar* shows upper limit of the 95 % confidence interval for the median. Due to low sample size of subjects, age 21 and over were pooled

ranged from 653 mg/day in black females to 987 mg/day in nonblack males.

Tanner staging was available on all subjects. During follow-up, some children transitioned between one or more Tanner stages, while others showed no pubertal development. The number and proportion of subjects who were positive for each of the Tanner stage indicators is provided in Table 31.2.

Log-transformed CaI was significantly and positively related to adjusted annual TBBMC increase in nonblack males ($P<0.006$) and nonblack females ($P<0.035$). Using the regression coefficient (of CaI on the original, untransformed TBBMC scale) as an estimate of the amount of annual increase in TBBMC added by each mg/day of CaI, we found that about 0.01 g of TBBMC is added per mg in both males and females. CaI was not associated significantly with TBBMC increase in either black males or black females.

Log-transformed WBA was significantly and positively related to adjusted annual TBBMC increase in all race/sex subgroups ($P<0.006$). Figure 31.3 shows the TBBMC increase by WBA in nonblack males. Using the regression coefficient (of WBA on the original, untransformed scale), we found that the amount of annual increase in TBBMC added by each hour of WBA is 1.0 g among black females and nonblack females, 1.4 g for white males, and 2.1 g for black males.

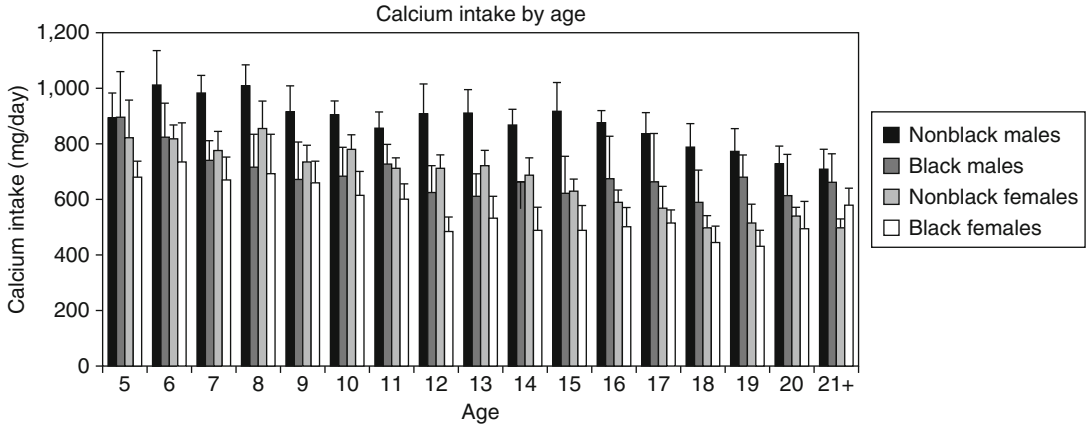
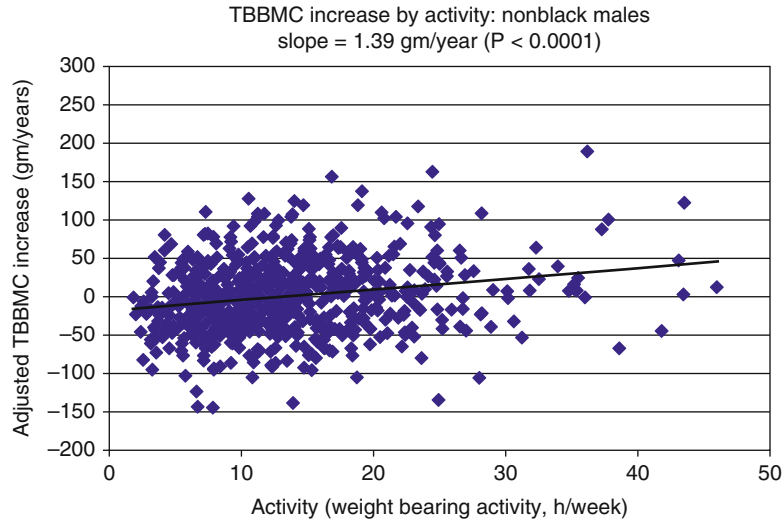


Fig. 31.2 Calcium intake by age. *Bar height* shows median calcium intake (mg/day). *Error bar* shows upper limit of the 95 % confidence interval for the median. Due to low sample size of subjects, age 21 and over were pooled

Table 31.2 Number of subjects who experienced each Tanner indicator during study

Tanner indicator	Total	Nonblack boys	Black boys	Nonblack girls	Black girls
StartT12	1,033	405	122	412	94
chgtoT2	342	34	10	241	57
chgtoT3	669	251	66	279	73
chgtoT4	717	257	80	306	74
chgtoT5	679	205	72	314	88

Fig. 31.3 TBBMC increase with activity: nonblack males. Log-transformed WBA was significantly and positively related to adjusted annual TBBMC increase



There was no interaction between log-CaI and log-WBA in any subgroup. In all race/sex subgroups, log-WBA was significantly and positively associated with adjusted TBBMC increase, after adjustment for log-CaI. In nonblack males and nonblack females, where a significant CaI effect was seen with no adjustment for activity, no significant CaI effect was seen after adjustment for WBA, indicating that the CaI effect observed in the simpler model may be accounted for by CaI's correlation with WBA. In black females, no significant calcium effect was seen, with or without adjustment for activity.

Discussion

In this cohort of 1,917 children and adolescents ranging from 5 to 19 years of age at study entry and followed for up to 7 years, self-selected weight-bearing physical activity contributed to greater bone accrual in both sexes and both racial subgroups. Self-selected dietary calcium intake was associated significantly with greater accrual in nonblack males and females, but not their black peers.

Subjects in this study were inactive compared to other reports of activity levels [19, 23], averaging about 1.5–2 h of WBA per day. Even so, activity had a positive impact on bone accrual. The average annual increase in TBBMC added by each hour/week of WBA was about 1 g for black and nonblack females, 1.3 g for white males, and 2.1 g for black males. Although this effect size is modest, the impact is biologically important. For example, the average nonblack girl accrued about 96 g/year of TBBMC and gained an additional 1 g/year for each h/week of WBA. If she increased her WBA by 3.5 h/week (½h/day), she theoretically could increase TBBMC accrual by about 3.5 g/year or about 4 %/year. If she could add an additional 3.5 g/year for each of 10 years until she reached the early third decade, when the mean TBBMC is about 2,100 g [24], she would have increased accrual by about 35 g or about 2 % of the young adult mean. Since estimates are that a 5 % increase in peak bone mass at the population level would decrease the risk of fracture later in life by 50 %, the increase in accrual added by an additional ½h/week of WBA is clinically significant. This hypothetical example using simplified estimates serves to demonstrate that small increments in WBA can help to maximize skeletal development.

Consistent findings from numerous observational, cross-sectional, and randomized interventional studies in children and adolescents show that weight-bearing physical activity increases bone mass accrual [14, 16, 18, 25–30]. The amount of BMC increase accounted for by physical activity in these reports varies but is similar to findings in the BMDCS cohort. For example, Janz et al. [13] showed that children ages 5–8 years with high activity levels accrued about 5 % more TBBMC than those with low activity levels. Slemenda et al. [31] reported 4–7 % greater bone mineral density in active prepubertal children compared to less active peers. Although a few studies find no exercise effect, the inconsistency is likely due to limitations in study design [32].

Calcium intake in this cohort was low compared to recommended intake levels, which are 1,000 mg/day for children ages 4–8 years, 1,300 mg/day for those 9–18 years, and 1,000 mg/day for those 19–30 years [33]. The mean CaI ranged from 653 mg/day in black females to 987 mg/day in nonblack males. Figure 31.1 shows that the only subgroup that consumed close to adequate levels was the group of nonblack boys ages 7–9. The percentage of subjects exceeding the recommended intake was as follows: 4–8 years, males 45 %, females 29 %; 9–18 years, males 20 %, females 9 %; and 19+ years, males 29 %, females 10 %. The proportion of BMDCS subjects in each category achieving recommended CaI intake is similar to reports from the 2005–2006 NHANES cohort [34]. Both studies show that calcium nutrition in USA is poor, especially during the period of rapid growth when the need for calcium is the greatest.

In our cohort, self-selected CaI was positively related to bone accrual in nonblack males and nonblack females. For every additional mg/day of CaI, 0.01 g/year of TBBMC is added. Based on this, a child who added one glass of milk/day (300 mg) to the diet could theoretically increase bone accrual by 3 g/year (~2 % in boys and ~3 % in girls). On average, the BMDCS subjects reported calcium intake below the threshold for calcium intake, that is, the lowest intake at which calcium retention is maximal [5]. Below the threshold bone, mass accrual varies with intake. Thus, it is not surprising that we see a calcium intake effect in these subjects.

CaI showed no significant effect on bone accrual in black subjects in this study. Black children had a higher baseline TBBMC than their nonblack peers and showed a greater absolute value (g) increase than nonblack subjects of their sex. Thus, they are building strong skeletons even though we did not detect a relationship between CaI and bone accrual. Weaver's group has shown that black adolescents

have greater net absorption of calcium and lower urinary calcium excretion than whites [35]. Thus, it may be that more black subjects than whites have reached their CaI threshold.

Numerous randomized clinical trials (RCTs) of calcium supplementation show a positive effect on bone accrual in children and adolescents that is about 1 %/year greater than the placebo group [36]. A recent meta-analysis of RCTs and observational studies found that dietary calcium increases bone accrual in children with low baseline intakes [6]. Most studies that do not find a calcium effect enrolled subjects who had calcium intakes at or above the threshold intake [36].

Limitations of this study are that CaI and WBA are self-reported and assessed only once a year. However, parents helped the younger children fill out both questionnaires. One of the strengths of this study is the accurate pubertal staging of subjects during follow-up and our controlling for maturation in the analysis. Bailey et al. point out that in studies of exercise in children and adolescents, "By far the greatest problem has to do with the failure to control for the wide range in maturational status for children of the same chronological age" [32]. Timing and rate of linear growth and biological maturation are highly variable among children and adolescents, and these events are the primary determinants of bone mass accrual. Thus, efforts to determine effects of exercise and nutrients on bone accrual will be confounded in studies that do not control for pubertal status. Other strengths include the large size of the cohort, the broad age span, and the length of follow-up. Also, the cohort included a large number of blacks and a diverse sample of nonblacks.

Conclusion

In this large sample of children and adolescents from across the USA, WBA showed a positive effect on bone accrual in boys and girls, blacks and nonblacks, while CaI was positively associated with bone accrual in nonblack subjects. Although the WBA and CaI effects were modest, small shifts in the mean of a population often result in large changes in the proportion of persons who may be above or below a specified level [37]. Thus, this study supports the benefits of WBA and CaI for maximizing skeletal development and suggests that small increases in these lifestyle behaviors may have a substantial impact on bone health.

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References

1. Seeman E, Hopper J, Back L, Cooper M, Parkinson E, McKay J, et al. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med. 1989;320: 554–8.
2. Sandler R, Slemenda C, LaPorte R, Cauley J, Schramm M, Barresi M, et al. Postmenopausal bone density and milk consumption in childhood and adolescence. Am J Clin Nutr. 1985;42:270–4.
3. NIH Consensus conference. Optimal calcium intake. NIH Consensus Development Panel on Optimal Calcium Intake. JAMA 1994;272(24):1942–8
4. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994;843:1–29.
5. Heaney R, Abrams S, Dawson-Hughes B, Looker A, Marcus B, Matkovic V, et al. Peak bone mass. Osteoporos Int. 2000;11:985–1009.
6. Huncharek M, Muscat J, Kupelnick B. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. Bone. 2008; 43(2):312–21.
7. Wosje K, Specker B. Role of calcium in bone health during childhood. Nutr Rev. 2000;58:253–68.
8. Forwood M, Burr D. Physical activity and bone mass: exercises in futility? Bone Miner. 1993; 21:89–112.
9. Parfitt A. The two faces of growth: benefits and risks to bone integrity. Osteoporos Int. 1994;4:382–98.
10. Mansfield M, Emans S. Growth in female gymnasts: should training decrease during puberty? J Pediatr. 1993;122(2):237–40.
11. Janz K, Burns T, Levy S, Torner J, Willing M, Beck T, et al. Everyday activity predicts bone geometry in children: the Iowa bone development study. Med Sci Sports Exerc. 2004;36:1124–31.
12. Tobias J, Steer CD, Mattocks CG, Riddoch C, Ness AR. Habitual levels of physical activity influence bone mass in 11-year-old children from the United

Kingdom: findings from a large population-based cohort. J Bone Miner Res. 2007;21:101–9.
13. Janz K, Gilmore J, Burns T, Levy S, Torner J, Willing M, et al. Physical activity augments bone mineral accrual in young children: the Iowa bone development study. J Pediatr. 2006;148:793–9.
14. Bailey D, McKay H, Mirwald R, Crocker PRE, Faulkner R. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J Bone Miner Res. 1999; 14:1672–9.
15. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone. 2007;40(1):14–27.
16. Fuchs R, Bauer JJ, Snow CM. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. J Bone Miner Res. 2001;16(1):148–56.
17. MacKelvie K, Petit MA, Khan K, Beck T, McKay H. Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. Bone. 2004;34:755–64.
18. MacKelvie K, Khan K, Petit MA, Janssen P, McKay H. A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. Pediatrics. 2003;112:e447–52.
19. Baxter-Jones AD, Kontulainen SA, Faulkner RA, Bailey DA. A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. Bone. 2008;43:1101–7.
20. Kalkwarf H, Gilsanz V, Lappe J, Zemel B, Horlick M, Oberfield S, et al. The bone mineral density in childhood study (BMDCS): bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab. 2007;92:2087–99.
21. Slemenda C, Miller J, Hui S, Reister T, Johnston C. Role of physical activity in the development of skeletal mass in children. J Bone Miner Res. 1991;6: 1227–33.
22. Lappe J, Rafferty K, Lypaczewski G, Haynatzki G, Davies M. Girls on a high calcium diet gain weight at the same rate as girls on a normal diet: a pilot study. J Am Diet Assoc. 2004;104:1361–7.
23. Nader P, Bradley R, Houts R, McRitchie S, O'Brien M. Moderate-to-vigorous physical activity from ages 9 to 15 years. JAMA. 2008;300:295–305.
24. Baxter-Jones ADG, Faulkner RA, Forwood M, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729–39.
25. Garcia A, Broda M, Frenn M, Coviak C, Pender N, Ronis D. Gender and developmental differences in exercise beliefs among youth and prediction of their exercise behavior. J Sch Health. 1995;65:213–9.
26. O'Brien K, Abrams S, Liang L, Ellis K, Gagel R. Bone turnover response to changes in calcium intake is altered in girls and adult women in families with histories of osteoporosis. J Bone Miner Res. 1998;13:491–9.
27. McKay H, Petit MA, Schutz RW, Prior J, Barr S, Khan KM. Augmented trochanteric bone mineral density after modified physical education classes: a randomized school-based exercise intervention study in prepubescent and early pubescent children. J Pediatr. 2000;136(2):156–61.
28. Bass S, Pearce G, Bradney M, Hendrich E, Delmas P, Harding A, et al. Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. J Bone Miner Res. 1998;13:500–7.
29. Kannus P, Haapasalo H, Sankelo M, Sievanen H, Pasanen M, Heinonen A, et al. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med. 1995;123:27–31.
30. Gustavsson A, Thorsen K, Nordstrom P. A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in health adolescent males. Calcif Tissue Int. 2003;73:108–14.
31. Slemenda CW, Reister T, Hui SL, Miller J, Christian J, Johnston jr CC. Influences on skeletal mineralization in children and adolescents: evidence for varying effects of sexual maturation and physical activity. J Pediatr. 1994;125:201–7.
32. Bailey D, Baxter-Jones A, Mirwald R, Faulkner R. Bone growth and exercise studies: the complications of maturation. J Musculoskelet Neuronal Interact. 2003;3(4):335–7.
33. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 dietary reference intakes for calcium and vitamin D: what dietetics practitioners need to know. J Am Diet Assoc. 2011;111(4):524–7.
34. Bailey R, Dodd K, Goldman J, Gahche J, Dwyer J, Moshfegh A, et al. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr. 2010;140(4):817–22.
35. Braun M, Palacios C, Wigertz K, Jackman L, Bryant R, McCabe L, et al. Racial differences in skeletal calcium retention in adolescent girls with varied controlled calcium intakes. Am J Clin Nutr. 2007;85:1657–63.
36. Weaver C, Osteoponosis: The early years. In: Coulstron A, Boushey C, editors. Nutrition in the prevention and treatment of disease. 2nd ed. New York: Academic; 2008.
37. Rose G, Day S. The population mean predicts the number of deviant individuals. BMJ. 1990;301: 1031–4.

32 Preventing Malnutrition to Reduce Fracture Risk in Aged Care Residents. A Dairy-Based Protein, Calcium, and Vitamin D Supplement Reduce Falls and Femoral Neck Bone Loss in Aged Care Residents: A Cluster Randomized Trial

Sandra Iuliano

Abstract

Falls and fracture rates are high in ambulatory aged care residents, and malnutrition may contribute to falls and fracture risk by influencing bone's material composition and structure. We aimed to test if a dairy-based protein (10 g/day), calcium (600 mg/day), and vitamin D (960 IU/day) supplement formulated to increase intakes to recommended levels would reduce falls and fracture risk in ambulatory low-level aged care residents.

This was a cluster-randomized, single-blind intervention involving 813 residents (mean age 86.1 ± 5.9 years, 76 % female) from 16 low-level aged care facilities in Melbourne, Australia. Twelve months of observation in all facilities was followed by 8 months of food-based supplementation (intervention) or usual intake (controls). Number of fallers and non-vertebral fractures was assessed in all residents, and serum 25(OH)D, PTH, osteocalcin, bone mineral density (BMD) by densitometry, bone structure and volumetric BMD at the distal radius and tibia using high-resolution pQCT, balance (Lord's balance test), and functionality (timed up and go, walking velocity) were tested in a subset of 84 residents. Repeated measures ANOVA and logistic regression models were used to compare cases and controls.

Among the whole sample, supplementation reduced the number of fallers by 42 % (OR = 0.58, 95 % CI: 0.44–0.78, $p < 0.001$). Among the 58 of 84 participants with follow-up data, supplementation slowed bone loss at

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the proximal femur, maintained serum 25(OH)D, and reduced PTH by 16 ± 8 %, $p < 0.03$.

Fortifying foods with protein, calcium, and vitamin D reduced falls in ambulatory aged care residents and is an accessible and inexpensive approach to reduce falls and slow the progression of bone fragility in the elderly.

Keywords

Aged care • Bone loss • Calcium • Dairy supplementation • Elderly • Falls • Fractures • Protein • Vitamin D

Malnutrition and Elderly in Aged Care

Institutionalized elderly are at high risk of malnutrition [1]. Malnutrition contributes to risk of morbidity and mortality and reduces the quality of life in this vulnerable group [2, 3]. Up to 50–75 % of residents in aged care facilities suffer from malnutrition. Malnutrition risk is high in elderly who have special dietary needs or require feeding assistance [4]. However, self-feeding, ambulatory low-level aged care residents (equivalent to residential care in the UK and assisted care in the USA) are also at risk of malnutrition [5]. We previously reported that foods served to residents did not provide sufficient fiber, calcium, magnesium, or folate in both sexes, zinc in men and energy for women, to meet recommended requirements, and 88 % of residents had one or more indicators of malnutrition [6]. Malnutrition is estimated to cost an additional €10,000 per patient for screening monitoring and treatment [7]. Numerous factors contribute to the risk of malnutrition including poor dentition and cognitive impairment, low staff numbers, and inadequate nutrition-related staff training [8–11].

Malnutrition is a risk factor for age-related loss of muscle mass, strength, and function, which is associated with increased falls [12–14]. The majority of fractures result from falls. Of all the hip fractures in the community, 30 % come from elderly in aged care, especially from ambulatory low-level care residents who have the highest fracture rates in the community [15, 16]. Targeting those in aged care is likely to assist in reducing the burden of fractures in the community and is likely to be cost-effective as this identifies a group at high risk of fracture likely to benefit from intervention.

As the population ages, there will be a greater reliance on institutionalized care for the elderly, and so the associated health costs will rise significantly. Preventing malnutrition in low-level aged care residents is important as the transfer of residents to high-level (nursing home) care, which provides more intensive 24-h nursing care, is associated with a substantial increase in health cost and a reduction in quality of life [17].

Malnutrition and Fracture Risk

Malnutrition contributes to fracture risk. Fractures in the elderly result in increased morbidity, mortality, and health costs. By 2051, the number of Australians aged over 65 years will double and will increase the absolute number of fractures and the burden of these fractures to the community. The annual cost of fractures in Australia is estimated at $7.4 billion [18–20]. Given the growth of the aging population, these costs will escalate. The aging of the population, and the increasing burden of fractures to the community, is occurring worldwide. About 65 % of all fractures in the community occur in persons over 65 years of age. The incidence of fractures is 9–14 % in aged care facilities that currently accommodate in excess of 150,000 elderly Australians [21–24].

Nutrient deficiencies, in particular deficiencies in protein, calcium, and vitamin D, are associated

with bone fragility. The burden of fractures attributable to calcium and protein under nutrition is large, because many people have low intakes. About 40 % of elderly living in the community receiving home assistance and 65 % of low-level care residents are malnourished [25, 26]. In ambulatory aged care residents, 100 % of women and 84 % of men consume less than the recommended amount of 1,300 mg/day of calcium, with 68 % of women and 16 % of men consumed less than 600 mg/day of calcium [26]. These rates of dietary calcium deficiency are similar to elderly in the community [27, 28]. In aged care, 77 % of residents consume below the suggested requirement of >1 g protein/kg body weight [29, 30]. In many cases, the actual foods served in aged care facilities do not provide sufficient nutrients [26]. Vitamin D deficiency is common in aged care residents [31].

Protein deficiency contributes to fracture risk. Low protein intake is associated with bone loss, reduced bone mass and strength, and increased fracture risk [32–35]. In the aging animal model, protein deficiency in males is associated with reduced bone formation, with a less marked change to bone resorption, while in females bone resorption was elevated with limited formation resulting in cortical thinning and loss of strength. Reduced IGF-1 and other factors were associated with the reduction in bone formation [34, 36, 37]. Protein deficiency reduces IGF-1 levels and low IGF-1 levels are associated with increased fracture risk in postmenopausal women [38, 39]. Hip fracture patients are often malnourished, and protein supplementation improved serum IGF-1, shortened recovery time, and reduced complications during hospitalization [40, 41].

Calcium deficiency also contributes to fracture risk. Women with reported lactose intolerance, who avoid consuming milk, have lower dairy calcium intakes than women who drink milk (570 mg/day vs. 850 mg/day) and a 33 % greater risk of fractures [42]. Dietary calcium deficiency is reported to increase bone remodeling intensity and results in a rapid reduction in BMD, as supported by the observed reversal of skeletal benefits after calcium (and vitamin D) supplementation is ceased [43]. The rapid loss of bone after acute calcium deficiency is reflective of the delay between bone resorption and the subsequent bone formation in the larger number of remodeling units [44]. With long-term calcium deficiency, remodeling steady state is eventually returned, but at a higher rate. The structural decay of bone and predisposition to fractures have not been well identified and would depend on the thickness and porosity of bone prior to calcium deficiency [44]. However, calcium and vitamin D supplementation reduced fall frequency, bone remodeling, and non-vertebral fractures in institutionalized elderly women [45, 46].

Targeting nutrient deficiencies in those in aged care may assist in reducing the burden of fractures in the community given that 30 % of hip fractures arise from those in aged care. Such a strategy may be cost-effective as it identifies a group at high risk of fracture and likely to benefit from intervention that is accessible to all persons, low cost, and free of adverse events. The optimal method of intervention and supplementation to overcome nutrient deficiencies in aged care residents is yet to be clearly defined.

Correcting Malnutrition in Aged Care Residents

The landmark study by Chapuy et al. demonstrated that fracture risk can be reduced in elderly aged care residents with calcium and vitamin D supplementation [23]. However, poor compliance with medications limits anti-fracture efficacy [47]. Two methods used to correct malnutrition in aged care residents are oral nutrition supplements (ONS) and food fortification. Most studies demonstrating efficacy of ONS are reported from hospital settings, where small but consistent gains in weight and improvements in health outcomes are reported [11, 40]. However, in aged care, it has been observed that supplements are often not delivered according to treatment schedules and waste can be considerable [48, 49]. Moreover, elderly with limited appetites may have difficulties consuming ONS in addition to regular meals, so normal intake may be compromised resulting in limited benefit to overall nutrient intake [50].

Food fortification to enhance nutritional content of foods has been trialed in aged care and evidence exists that specific indicators of nutrient status can be improved using food fortification. Smoliner et al. fortified dietary intakes of nursing home residents using energy- and protein-enriched soups and snacks and, relative to controls, observed an improvement in protein intake of ~12 g/day and a small but significant improvement in mini nutritional assessment score [51]. However, snacks were not readily consumed and total energy intake did not differ from controls, so benefits to overall nutrient intake remain unclear [51]. Bonjour et al. observed a 12.3 % decrease in PTH and a 16.9 % increase in IGF-1 in elderly institutionalized women after 1 month of supplementation using calcium and vitamin D fortified cheese [52].

To determine the effectiveness of food fortification to improve protein, calcium, and vitamin D intakes to recommended levels, and reduce falls and fracture risk in aged care residents, we conducted a 2-year randomized cluster trial involving 20 aged care facilities. Residents were supplemented with a dairy-based protein, calcium, and vitamin D supplement, which was provided in powdered form and mixed into various foods on the menu or added to 30 different pre-prepared foods that were provided to residents. Food service staff were trained on the incorporation of the supplement into the foods served to residents. The first year consisted of observation of falls and fractures in all facilities, followed by 8 months of food-based supplementation (intervention) or usual intake (controls). It was observed that among 813 residents (mean age 85.5 years), supplementation with protein, calcium, and vitamin D reduced the number of residents who fell by 42 % (OR = 0.58, 95 % CI: 0.44–0.78, $p < 0.001$). The benefit was confined to individuals who reported no falls in the prior year (OR = 0.35 95 % CI: 0.22–0.57, $p < 0.001$) or were infrequent fallers (1–2 falls during the observation year), (OR = 0.54 95 % CI: 0.32–0.91, $p < 0.05$). However, for frequent fallers (>2 falls during the prior year), supplementation did not reduce the number of fallers (OR = 0.86, 95 % CI: 0.42–1.76, $p = 0.68$). For facilities that received the supplement ($n = 7$), there was a trend for a dose effect of supplementation on falls reduction ($r = -0.6$, $p = 0.1$) in that higher supplement consumption was associated with fewer fallers. Supplementation slowed bone loss at the proximal femur, which declined in the control group by 2.5 % ($p < 0.05$), maintained serum 25(OH)D, which declined in the control group by 22 % ($p < 0.001$) and reduced PTH by 16 % $p < 0.05$).

Mean daily supplementation dose was approximately 4 g protein, 300 mg calcium, and 475 IU vitamin D3. Total calcium (537 ± 165 vs. 802 ± 258 mg/day, $p < 0.05$) and vitamin D (2.58 ± 0.24 vs. 13.62 ± 3.73 μg/day, $p < 0.01$) intakes increased with supplementation. During the supplementation period, calcium (802 ± 258 vs. 615 ± 297 mg/day, $p < 0.05$) and vitamin D (13.62 ± 3.73 vs. 2.23 ± 0.89 μg/day, $p < 0.01$) intakes were higher in the intervention group compared to controls. The dose received was approximately half of that prescribed and the major limitation to the study was the high staff turnover and difficulties in staff preparing the fortified foods. Pre-fortified foods improved administration of the supplement, but difficulties exist in the aged care setting in implementing interventions that require additional effort by staff.

Dairy Foods to Improve Nutritional Status in Aged Care Residents

Dairy foods are energy dense and a source of protein, calcium, vitamin D, magnesium, zinc, phosphorus, and riboflavin. Current intakes of dairy foods in low-level aged care residents are approximately two serves per day, half of recommended levels [6]. Given that dairy foods are readily available and require minimal time for staff to prepare, improving dairy food intake to provide the recommended intake of calcium (1,300 mg/day) may be a viable avenue to improve nutrient intake in elderly in aged care.

To determine the likelihood of improving nutrient intake in aged care residents, we conducted a feasibility study in two aged care facilities whereby

Table 32.1 Calcium content from example menu items before (regular) and after (high dairy) modifications provided to low-level aged care residents

Food item	Regular menu (mg Ca/serve)	Food item	High dairy menu (mg Ca/serve)
Beverages			
White coffee	24	Milk coffee	171
Cordial	2	Cold milk	212
Soups			
Tomato (water-based)	15	Tomato (milk-based)	122
Mushroom	25	Mushroom	173
Meal items			
Broccoli	11	Broccoli baked	122
Mashed potato	11	Scalloped potatoes	108
Desserts			
Jelly and cream	14	Rice pudding	279
Added cream	10	Added custard	283
Snacks			
Savory biscuit	10	Cheese and biscuit	146
Sweet biscuit	18	Yogurt	296

menu modifications were implemented to ensure residents were provided with at least four serves of dairy foods per day. Without food service support by a specialized catering consultant, mean dietary calcium intake only improved by ~100 mg/day (unpublished data). However, after providing on-site support with a dietician that specialized in food service in aged care, 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 (+18 %, $p<0.0001$) were observed, while proportion of energy from fat decreased (−3 %, $p<0.0001$). Increases in mean daily micronutrient intakes were observed for numerous nutrients including 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, $p<0.0001$), with recommended intake levels achieved on the higher-dairy diet. Mean sodium intakes remained unchanged. Dairy intake was enhanced in four ways: *substitution*, milk-based drinks offered in place of regular beverages (e.g., glass of milk); *modification*, inclusion of powdered or evaporated milk or cheese to recipes (e.g., soups); *addition*, adding larger serves of cheese to salads, sandwiches, and on savory biscuits; and *accompaniment*, cheese garnish on soups or including cheese-based sauces to vegetables. Changes to some menu items provided >1,000 % more calcium per serve than the previously served item (Table 32.1). Therefore, two additional serves of dairy food can significantly improve nutrient intake in aged care residents, and its ease of provision makes it a viable option to potentially prevent malnutrition (Iuliano, JNHA, accepted Nov 2012).

Preventing Malnutrition in Aged Care Residents: A Community-Based Approach

An effective means of reducing fracture risk is to shift the population mean for protein, calcium, and vitamin D intakes in the elderly so a smaller proportion of elderly people are deficient in these nutrients. Blood pressure has been lowered by reducing salt intake in the whole population and is seen as an effective means of reducing the burden of stroke and cardiac events [53, 54]. The principle of using dietary modifications to reduce cardiac events instead of using drugs can be applied to increasing intakes of protein, calcium, and vitamin D to recommended levels to reduce fractures and is a realistic option for fracture prevention in the community.

While fracture risk in an individual attributable to protein, calcium, or vitamin D deficiency alone is small, the high prevalence of deficiency confers a high attributable risk, so shifting the population to a higher level of intake will have a large net effect [27, 28]. For example, for a 2 % incidence rate of fractures and the estimation that 50 % of elderly Australians are calcium deficient, the relative risk of fractures associated with low calcium intake is 1.67 (RR = 1.67), or low calcium consumers have a 67 % greater risk of fractures. Assuming a 20 % efficacy (in relative risk reduction) of improving intakes of these nutrients to recommended levels, 12.5 % of fractures can be prevented. With nearly three million people over the age of 65 years currently in Australia, this equates to a reduction in the yearly medical cost of fractures alone of more than $40 million [20].

The additional costs associated with malnutrition in elderly aged care residents are high, both on a financial level to the community and on a personal level due to compromised quality of life. Improving nutrient intakes of residents using broad-based dietary interventions such as enhancing dairy food intake is a feasible option. Dairy foods are widely available, are relatively cheap, and, unlike pharmaceutical approaches, have minimal side effects, so may be a cost-effective approach to preventing fractures in aged care resident. Such interventions are warranted but lacking in the aged care setting.

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References

1. Gaskill D, Black LJ, Isenring EA, Hassall S, Sanders F, Bauer JD. Malnutrition prevalence and nutrition issues in residential aged care facilities. Australas J Ageing. 2008;27(4):189–94.
2. Correia MI, Waitzberg DL. The impact of malnutrition on morbidity, mortality, length of hospital stay and costs evaluated through a multivariate model analysis. Clin Nutr. 2003;22(3):235–9.
3. Rondanelli M, Opizzi A, Antoniello N, Boschi F, Iadarola P, Pasini E, et al. Effect of essential amino acid supplementation on quality of life, amino acid profile and strength in institutionalized elderly patients. Clin Nutr. 2011;30(5):571–7.
4. Volkert D, Pauly L, Stehle P, Sieber CC. Prevalence of malnutrition in orally and tube-fed elderly nursing home residents in Germany and its relation to health complaints and dietary intake. Gastroenterol Res Pract. 2011;2011:247315.
5. Isenring EA, Bauer JD, Banks M, Gaskill D. The Malnutrition Screening Tool is a useful tool for identifying malnutrition risk in residential aged care. J Hum Nutr Diet. 2009;22(6):545–50.
6. Woods J, Walker K, Iuliano-Burns S, Straus B. Malnutrition on the menu: nutritional status of institutionalised elderly Australians in low-level care. J Nutr Health Aging. 2009;13(8):693–8.
7. Meijers JM, Halfens RJ, Wilson L, Schols JM. Estimating the costs associated with malnutrition in Dutch nursing homes. Clin Nutr. 2012;31(1):65–8.
8. Fagerstrom C, Palmqvist R, Carlsson J, Hellstrom Y. Malnutrition and cognitive impairment among people 60 years of age and above living in regular housing and in special housing in Sweden: a population-based cohort study. Int J Nurs Stud. 2011;48(7):863–71.
9. Woo J, Chi I, Hui E, Chan F, Sham A. Low staffing level is associated with malnutrition in long-term residential care homes. Eur J Clin Nutr. 2005;59(4):474–9.
10. Nieuwenhuizen WF, Weenen H, Rigby P, Hetherington MM. Older adults and patients in need of nutritional support: review of current treatment options and factors influencing nutritional intake. Clin Nutr. 2010;29(2):160–9.
11. Leslie WS. Improving the dietary intake of frail older people. Proc Nutr Soc. 2011;70(2):263–7.
12. Roubenoff R, Hughes VA. Sarcopenia: current concepts. J Gerontol A Biol Sci Med Sci. 2000;55(12):M716–24.
13. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412–23.
14. Landi F, Liperoti R, Russo A, Giovannini S, Tosato M, Capoluongo E, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. Clin Nutr. 2012;11:22424775.
15. Bischoff-Ferrari HA, Can U, Staehelin HB, Platz A, Henschkowski J, Michel BA, et al. Severe vitamin D deficiency in Swiss hip fracture patients. Bone. 2008;42(3):597–602.
16. Chen JS, Sambrook PN, Simpson JM, Cameron ID, Cumming RG, Seibel MJ, et al. Risk factors for hip fracture among institutionalised older people. Age Ageing. 2009;38(4):429–34.
17. Australian Government Department of Health and Ageing. Aged care in Australia. 2006. www.health.gov.au. Accessed 22nd Aug 2012.

18. Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, et al. Long-term risk of osteoporotic fracture in Malm. Osteoporos Int. 2000;11(8): 669–74.
19. Sambrook PN, Seeman E, Phillips SR, Ebeling PR. Preventing osteoporosis: outcomes of the Australian Fracture Prevention Summit. Med J Aust. 2002;176 (Suppl):S1–16.
20. Randell A, Sambrook PN, Nguyen TV, Lapsley H, Jones G, Kelly PJ, et al. Direct clinical and welfare costs of osteoporotic fractures in elderly men and women. Osteoporos Int. 1995;5(6):427–32.
21. Sanders KM, Seeman E, Ugoni AM, Pasco JA, Martin TJ, Skoric B, et al. Age- and gender-specific rate of fractures in Australia: a population-based study. Osteoporos Int. 1999;10(3):240–7.
22. Chang KP, Center JR, Nguyen TV, Eisman JA. Incidence of hip and other osteoporotic fractures in elderly men and women: Dubbo Osteoporosis Epidemiology Study. J Bone Miner Res. 2004;19(4): 532–6.
23. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D3 and calcium to prevent hip fractures in the elderly women. N Engl J Med. 1992;327(23):1637–42.
24. Australian Institute of Health &Welfare. Residential aged care in Australia 2005–06: a statistical overview. 2006. www.aihw.gov.au/publications. Accessed 22nd Aug 2012.
25. Visvanathan R, Macintosh C, Callary M, Penhall R, Horowitz M, Chapman I. The nutritional status of 250 older Australian recipients of domiciliary care services and its association with outcomes at 12 months. J Am Geriatr Soc. 2003;51(7):1007–11.
26. Woods JL, Walker KZ, Iuliano Burns S, Strauss BJ. Malnutrition on the menu: nutritional status of institutionalised elderly Australians in low-level care. J Nutr Health Aging. 2009;13(8):693–8.
27. Pasco JA, Sanders KM, Henry MJ, Nicholson GC, Seeman E, Kotowicz MA. Calcium intakes among Australian women: Geelong Osteoporosis Study. Aust N Z J Med. 2000;30(1):21–7.
28. Bannerman E, Magarey AM, Daniels LA. Evaluation of micronutrient intakes of older Australians: The National Nutrition Survey–1995. J Nutr Health Aging. 2001;5(4):243–7.
29. Kurpad AV, Vaz M. Protein and amino acid requirements in the elderly. Eur J Clin Nutr. 2000;54 Suppl 3:S131–42.
30. Morais JA, Chevalier S, Gougeon R. Protein turnover and requirements in the healthy and frail elderly. J Nutr Health Aging. 2006;10(4):272–83.
31. Sambrook PN, Cameron ID, Cumming RG, Lord SR, Schwarz JM, Trube A, et al. Vitamin D deficiency is common in frail institutionalised older people in northern Sydney. Med J Aust. 2002;176(11):560.
32. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Protein consumption and bone mineral density in the elderly: the Rancho Bernardo Study. Am J Epidemiol. 2002;155(7):636–44.
33. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000;15(12): 2504–12.
34. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. J Bone Miner Res. 2000;15(4):683–90.
35. Wengreen HJ, Munger RG, West NA, Cutler DR, Corcoran CD, Zhang J, et al. Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. J Bone Miner Res. 2004;19(4): 537–45.
36. Ammann P, Laib A, Bonjour JP, Meyer JM, Ruegsegger P, Rizzoli R. Dietary essential amino acid supplements increase bone strength by influencing bone mass and bone microarchitecture in ovariectomized adult rats fed an isocaloric low-protein diet. J Bone Miner Res. 2002;17(7):1264–72.
37. Bourrin S, Toromanoff A, Ammann P, Bonjour JP, Rizzoli R. Dietary protein deficiency induces osteoporosis in aged male rats. J Bone Miner Res. 2000;15(8):1555–63.
38. Garnero P, Sornay-Rendu E, Delmas PD. Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women. Lancet. 2000;355(9207): 898–9.
39. Bourrin S, Ammann P, Bonjour JP, Rizzoli R. Dietary protein restriction lowers plasma insulin-like growth factor I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats. Endocrinology. 2000;141(9):3149–55.
40. Schurch MA, Rizzoli R, Slosman D, Vadas L, Vergnaud P, Bonjour JP. Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1998;128(10):801–9.
41. Delmi M, Rapin CH, Bengoa JM, Delmas PD, Vasey H, Bonjour JP. Dietary supplementation in elderly patients with fractured neck of the femur. Lancet. 1990;335(8696):1013–6.
42. Honkanen R, Kroger H, Alhava E, Turpeinen P, Tuppurainen M, Saarikoski S. Lactose intolerance associated with fractures of weight-bearing bones in Finnish women aged 38–57 years. Bone. 1997;21(6): 473–7.
43. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of withdrawal of calcium and vitamin D supplements on bone mass in elderly men and women. Am J Clin Nutr. 2000;72(3):745–50.
44. Seeman E. Evidence that calcium supplements reduce fracture risk is lacking. Clin J Am Soc Nephrol. 2010;5 Suppl 1:S3–11.
45. Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum S. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. N Engl J Med. 1990;323(13): 878–83.

46. Recker RR, Hinders S, Davies KM, Heaney RP, Stegman MR, Lappe JM, et al. Correcting calcium nutritional deficiency prevents spine fractures in elderly women. J Bone Miner Res. 1996;11(12): 1961–6.
47. Compston JE, Seeman E. Compliance with osteoporosis therapy is the weakest link. Lancet. 2006; 368(9540):973–4.
48. Simmons SF, Patel AV. Nursing home staff delivery of oral liquid nutritional supplements to residents at risk for unintentional weight loss. J Am Geriatr Soc. 2006;54(9):1372–6.
49. Gosney M. Are we wasting our money on food supplements in elder care wards? J Adv Nurs. 2003;43(3):275–80.
50. Manders M, de Groot CP, Blauw YH, Dhonukshe-Rutten RA, van Hoeckel-Prust L, Bindels JG, et al. Effect of a nutrient-enriched drink on dietary intake and nutritional status in institutionalised elderly. Eur J Clin Nutr. 2009;63(10):1241–50.
51. Smoliner C, Norman K, Scheufele R, Hartig W, Pirlich M, Lochs H. Effects of food fortification on nutritional and functional status in frail elderly nursing home residents at risk of malnutrition. Nutrition. 2008;24(11–12):1139–44.
52. Bonjour JP, Benoit V, Pourchaire O, Ferry M, Rousseau B, Souberbielle JC. Inhibition of markers of bone resorption by consumption of vitamin D and calcium-fortified soft plain cheese by institutionalised elderly women. Br J Nutr. 2009;102(7): 962–6.
53. Bibbins-Domingo K, Chertow GM, Coxson PG, Moran A, Lightwood JM, Pletcher MJ, et al. Projected effect of dietary salt reductions on future cardiovascular disease. N Engl J Med. 2010;362(7): 590–9.
54. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med. 1997;336(16):1117–24.

Effects of Vitamin D and Calcium Supplementation on Heart Rate and Blood Pressure in Community-Dwelling Older Individuals

33

Michael Pfeifer

Abstract

Objectives: Vitamin D deficiency has been linked to hypertension and cardiovascular events in observational studies. It is unclear whether vitamin D and/or calcium supplementation can reduce blood pressure, and if so, by how much.

Methods: This prospective study was undertaken to test the influence of latitude, seasonal variations, possible threshold effects, and duration of vitamin D efficacy after cessation of therapy. Two hundred and forty-two healthy male and female subjects with a mean age of 77 ± 4 years and a 25-hydroxyvitamin D serum level below 75 nmol/l were recruited in Bad Pyrmont and Graz and were randomly assigned to two treatment groups: one group receiving 1,000 mg calcium per day (Ca) and the other group 1,000 mg calcium and 800 IU vitamin D (Ca + D) over 12 months. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured under standardized conditions every 4 months. Statistical evaluation was carried out using the statistics software of IDV, Gauting (Test + Estimation, version 5.2, "CRO" Dr. Heinz and Partner, Vienna, Austria).

Results: We performed an intention-to-treat analysis and found the following results:

In the (Ca + D) group, 25-hydroxyvitamin D increased significantly ($p < 0.01$) from 57 ± 20 nmol/l at baseline (BL) to 84 ± 18 nmol/l at month 12 (M12), whereas in the (Ca) group, there was no change (54 ± 19 versus 55 ± 18 nmol/l).

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In the (Ca+D) group, SBP decreased significantly ($p<0.01$) from 134±17 mmHg at BL to 124±14 mmHg at M12, whereas in the (Ca) group, there was no change (137±17 versus 133±16 mmHg).

In the (Ca+D) group, DBP decreased significantly ($p<0.01$) from 76±7 mmHg at BL to 72±7 mmHg at M12, whereas in the (Ca) group, there was no change (79±8 versus 78±9 mmHg).

In the (Ca+D) group, HR decreased significantly ($p<0.01$) from 74±4 beats per minute at BL to 70±4 beats per minute at M12, whereas in the (Ca) group, there was no change (74±4 versus 75±4 beats per minute).

Conclusion: Despite a relatively high inclusion criterion for vitamin D (75 nmol/l) and independent of latitude, we observed a significant reduction of blood pressure and heart rate after supplementation with vitamin D and calcium. This effect of nutritional supplements may be comparable to the efficiency of antihypertensive drugs.

Keywords

Vitamin D deficiency • Heart rate • Blood pressure • Calcium and vitamin D supplementation • Randomized controlled trial (RCT)

Introduction

Vitamin D insufficiency is common, especially in populations living at high latitudes [1, 2]. Vitamin D has been known to regulate calcium and bone homeostasis for many decades, but recent investigations have revealed that vitamin D receptors exist on a very wide range of tissues, including the endothelium and the myocardium, suggesting a much wider range of biological functions for vitamin D [3]. Of particular cardiovascular interest are the observations that vitamin D can suppress renin production via effects on the juxtaglomerular apparatus [4] and the fact that endothelial cells contain vitamin D receptors, thus providing a direct vascular substrate for vitamin D to exert effects [5].

Furthermore, vitamin D is known to suppress parathyroid hormone (PTH) production, itself associated with cardiovascular disease, and can suppress proinflammatory cytokine production, including tumor necrosis factor alpha (TNFα). In addition, hyperparathyroidism has been associated with hypertension [6], and it increases calcium uptake in human cells [7]. Parathyroidectomy after primary hyperparathyroidism improves blood pressure and arterial smooth muscle [8]. Likewise, chronic PTH infusion results in hypertension in normal subjects [9].

Observational data suggest that low 25-hydroxyvitamin D levels are associated with higher blood pressure, with a greater chance of developing hypertension in the future [2, 4] and with increased mortality rates and higher rates of cardiovascular events [10, 11].

Patients with hypertension and low vitamin D levels appear to be at particular risk of cardiovascular events [12]. Meanwhile one meta-analysis of interventional studies suggests that vitamin D supplementation may reduce mortality [13] and the effect of vitamin D and calcium on blood pressure reduction has recently been systematically reviewed in two articles [14, 15]. Both reviews, however, do not show consistent results.

Eleven years ago, we demonstrated in a first randomized, placebo-controlled, and double-blind clinical trial that a short-term supplementation with vitamin D and calcium is significantly more effective in reducing systolic blood pressure (SBP) than calcium alone ($p=0.04$). No statistical difference, however, was observed in the diastolic blood pressures (DBP) of the calcium-treated and calcium plus vitamin D-treated groups ($p=0.10$), [16]. In contrast, long-term

supplementations with vitamin D and calcium may show very different results.

In the following, we, therefore, present effects of a long-term vitamin D and calcium supplementation on blood pressure and heart rate in community-dwelling older individuals. However, it is important to mention that in this randomized, controlled, double-blind clinical trial, blood pressure and heart rate were secondary parameters only, while the primary study endpoint was on falls [17].

Methods

Study Subjects

We studied healthy ambulatory women and men 70 years of age or older who were recruited through newspaper advertisements and mailing lists. The inclusion criterion was a 25-hydroxyvitamin D serum level below 75 nmol/l (30 ng/ml) [18]. The exclusion criteria included hypercalcemia or primary hyperparathyroidism; fractures of the extremities due to osteoporosis; therapy with a thiazide, bisphosphonate, calcitonin, vitamin D, and vitamin D metabolites; estrogen and antiestrogen in the past 6 months; or fluoride treatment in the past 2 years. Furthermore, known intolerance to study medication, chronic renal failure (serum creatinine above 20 % of the upper limit of the reference range), history of drug or alcohol abuse, nicotine abuse (more than 20 cigarettes per day), more than seven cups of coffee daily, scheduled holidays along the geographic longitude during the study period, diabetes mellitus, and severe cardiovascular disease were exclusion criteria. Three hundred and fifteen subjects were invited for screening out of which 242 (77 %) could finally be enrolled. The protocol was approved by the responsible ethics committees of Graz (Austria) and Hanover (Germany), and written informed consent was obtained from each subject.

Study Design and Supplements

During a 20-month, double-blind, controlled trial, subjects were randomly assigned to either the calcium mono or the calcium plus vitamin D group. Following month 12, treatment was stopped and study subjects were followed without treatment for an additional 8 months without unblinding the initial treatment code. At study entry, a complete physical examination and assessment of the subjects' medical history, diet, and physical activity were performed. The subjects were advised to maintain their usual diets and to avoid taking supplemental calcium and vitamin D on their own. The study participants took either one tablet containing 500 mg of elemental calcium in the form of calcium carbonate or one tablet with 500 mg of elemental calcium and 400 IU cholecalciferol at breakfast and dinner together with the meals. Study medication was provided by Meda Pharma Inc., Vienna, Austria. The study took place in Bad Pyrmont, Germany (latitude 52°N), and Graz, Austria (latitude 46°N), and commenced in May, when vitamin D levels are starting to rise in spring, and terminated 20 months later in March at the end of winter, when vitamin D levels to be at their lowest.

Status of Subjects and Compliance

Treatment compliance was estimated by recording the number of tablets handed out at the visits and the number of tablets returned at visits. Overall compliance was then calculated as a percentage of tablets actually taken by the patient and the number of treatment days between baseline visit and month 12. Eighteen subjects with an overall compliance below 80 % were rated as noncompliant. One subject started treatment with raloxifene, and another 12 subjects did not return to all visits. Therefore, 31 subjects were excluded from the per protocol data set.

Measurements

The calcium, vitamin D, and salt intakes of the subjects were assessed semiquantitatively by a food frequency questionnaire. Physical activity and alcohol consumption were also determined

by questionnaires. Height was measured with a stadiometer, and weight was determined by a digital scale. Concomitant medication was classified according to Anatomical Therapeutic Chemical groups and anatomical regions depending on the active compound and the indication (Anatomical Therapeutic Chemical classification index 1994). Blood pressure and pulse rate were measured after at least 5 min of supine rest in a quiet room using a sphygmomanometer with an appropriate cuff. Systolic and diastolic blood pressures were taken at Korotkov sounds I and V.

Laboratory Analysis

Blood was drawn between 8:00 and 9:00 a.m. after the subjects had fasted for at least 8 h. Serum 25-hydroxyvitamin D was measured by radioimmunoassay following extraction (Immunodiagnostic Systems, Bolton, UK). To validate the in-house performance of the assay system, we also measured 102 patient samples with HPLC which yield an overall correlation of $r=0.96$ and $r=0.92$. Parathyroid hormone was analyzed using Elecsys Intact PTH (Roche Diagnostics). Serum creatinine was determined by the Jaffé method, gamma-GT by the Szasz method, and albumin by the bromkresolgreen method. The coefficients of variation for the assays range from 5.5 to 7.9 %. All samples, except for the screening samples, were frozen at −80 °C and analyzed at the same time.

Quality Assurance and Statistical Analyses

Quality assurance was conducted by the contract research organization (CRO) Dr. Robert Heinz & Partners, Medical Consulting (Vienna. Austria). A start-up meeting with investigators and monitors took place before the start of the clinical trial. Follow-up meetings were performed after 12 months and at the end of the study. Periodical monitoring was conducted every 2 months by designated monitors.

Statistical calculations were conducted with the statistical software IDV, Gauting, Germany, using Testimate part of the program (Test and Estimation, version 5.2). All study subjects, who were initially randomized and received study medication, had been included into the analysis (intention-to-treat analysis).

The sample size was calculated based on the primary parameter "number of falls." Statistical comparisons of secondary target parameters comprised intergroup and intragroup calculations. Both comparisons were conducted with the Wilcoxon-Mann–Whitney U-test.

Results

Of the 315 subjects who underwent screening, 242 (77 %) fulfilled all inclusion and exclusion criteria and their 25-hydroxyvitamin D level was below 75 nmol/l (30 ng/ml). The baseline characteristics of the 242 subjects enrolled in this trial are shown in Table 33.1. Both treatment groups did not differ in terms of age, height, weight, gender, serum 25-hydroxyvitamin D, nutritional calcium intake, and intact parathyroid hormone (PTH) levels. An intense homogeneity analysis of this data set did not reveal any clinically relevant differences between the two centers. For that reason a combined data analysis of the two sites was performed.

Compared to baseline, an expected increase in mean serum 25-hydroxyvitamin D level was observed after 12 months of treatment in the calcium plus vitamin D-treated group, whereas 25-hydroxyvitamin D levels were similar to baseline in the calcium mono group. By the end of the treatment-free observation period, 25-hydroxyvitamin D had decreased to below baseline levels in both groups and was still significantly higher in the calcium plus vitamin D group. In both groups, serum PTH levels showed a similar decline by month 12 and were no longer different to baseline values by the end of the observation period. The data are shown in more detail together with the results of blood pressure and heart rate measurements in Table 33.2.

Table 33.1 Baseline characteristics of study subjects

	Calcium mono ($N=121$)	Calcium + vitamin D ($N=121$)
Age (years)	77 ± 4	76 ± 4
Age range (years)	70–91	70–94
Height (cm)	162 ± 7	161 ± 7
Weight (kg)	72 ± 11	70 ± 10
Subjects recruited in Graz (in %)	41	41
Male participants (in %)	25	26
Nutritional calcium intake (mg)	628 ± 42	608 ± 38
25-Hydroxyvitamin D (nmol/l)	54 ± 18	55 ± 18
Parathyroid hormone (pg/ml)	53 ± 19	48 ± 18

Values are mean ± SD
No significant difference among baseline characteristics was found between the two groups

Table 33.2 Blood pressure and heart rate in 242 study subjects at baseline and month 12

25-Hydroxyvitamin D (nmol/l)			
Calcium mono (nmol/l)	54 ± 19	55 ± 18	n.s.
Calcium plus vitamin D (nmol/l)	54 ± 18	84 ± 18	$p<0.01$
Systolic blood pressure (mmHg)			
Calcium mono (nmol/l)	137 ± 17	133 ± 16	n.s.
Calcium plus vitamin D (nmol/l)	134 ± 17	126 ± 14	$p<0.01$
Diastolic blood pressure (mmHg)			
Calcium mono (mmHg)	79 ± 8	78 ± 9	n.s.
Calcium plus vitamin D (mmHg)	76 ± 7	72 ± 7	$p=0.04$
Heart rate (beats per minute)			
Calcium mono (beats/min)	74 ± 4	75 ± 4	n.s.
Calcium plus vitamin D (beats/min)	74 ± 4	70 ± 4	$p=0.05$

Discussion

In this randomized, controlled, and double-blind trial, a long-term supplementation with vitamin D and calcium was more effective in reducing SBP, DBP, and heart rate than calcium alone. Inadequate vitamin D and calcium intake could play a contributory role in the pathogenesis and progression of hypertension and cardiovascular disease in elderly women. Essentially, these results confirm data from our previous publication in 2001 [16].

So far nine trials reporting the effect of vitamin D supplementation have been identified by the latest systematic review [15] concerning vitamin D and cardiometabolic outcomes [16, 19–26]. Vitamin D was given either alone (seven trials) or in combination with calcium (two studies) at doses equivalent to 400–8,571 IU per day. Eight studies used D_3 and one D_2. Another trial compared UV-B (which increases cutaneous synthesis of vitamin D) with UV-A (which does not), [27]. Follow-up varied from 5 to 52 weeks in most studies and was 7 years in the Women's Health Initiative study [28]. The total number of participants was 37,162 with the Women's Health Initiative trial contributing 36,282 participants. The study populations were heterogeneous, including healthy participants [28], participants with established hypertension [27], and heart failure [5].

Most trials found no statistically significant effects on either systolic or diastolic blood pressure. One trial reported relatively large net effects of vitamin D supplementation on systolic blood pressure of about −7 mmHg [16]. Another trial that compared UV-B with UV-A exposure also reported a large net effect on systolic blood pressure favoring UV-B (−6 mmHg), but it was not clear whether the net effect was statistically significant [27]. The latter study was also the only one that found a large net difference in diastolic blood pressure with UV-B exposure [27]. In the largest and longest duration trial, the Women's Health Initiative, combined low-dose vitamin D_3 (400 IU/day) and calcium carbonate supplementation (1,000 mg) had no effect on self-reported incident hypertension after 7 years of follow-up (28). Several subgroup analyses in this trial did not change this outcome. In meta-analysis of all trials, there was no statistically significant effect of vitamin D supplementation versus placebo on systolic blood pressure (weighed mean difference 1.9 mmHg; 95 % CI 4.2–0.4).

Cross-sectional studies have reported consistent associations between lower vitamin D status and prevalent cardiometabolic outcomes [1, 29]. In longitudinal observational studies, lower vitamin D status was associated with increased risk of incident hypertension and possibly cardiovascular disease, but the strengths of associations were attenuated compared to cross-sectional studies. In trials, there was no statistically significant effect of vitamin D supplementation on systolic or diastolic blood pressure. However, there was a suggestion of lower systolic blood pressure (by 2 mmHg) with vitamin D supplement use. Further data are necessary to adjudicate this observation.

Several possible reasons may explain the lack of apparent concordance between the cross-sectional, longitudinal, observational, and randomized studies. The inverse association between vitamin D status and cardiometabolic outcomes may be confounded by a variety of factors. First, vitamin D status is an excellent marker of good health, including positive associations with young age, normal body weight, and a healthy lifestyle [30] and negative associations with smoking, parental history of myocardial infarction, and alcohol intake [23]. Second, lower vitamin D status may reflect chronic nonspecific illness. Therefore, the inverse association seen in cross-sectional studies may be due to reverse causation. Third, additional components in foods rich in vitamin D (e.g., fish or fortified dairy products) may have direct effects on cardiometabolic disease (e.g., fortified milk replacing sweetened drinks) [31]. Finally, observational studies have used single measurements of serum or plasma 25-hydroxyvitamin D as a proxy of vitamin D status, which may not reflect long-term vitamin D status.

The Women's Health Initiative, which is the largest trial on vitamin D and calcium supplementation to date, reported no statistically significant effects for all cardiometabolic outcomes examined. However, this trial used a relatively small dose of vitamin D (400 IU/day), had difficulties with compliance over 7 years, and allowed participants in both intervention groups to take supplemental vitamin D. Based on dose and compliance, the effect of supplementation on 25-hydroxyvitamin D concentration in the Women's Health Initiative trial has been estimated to be only 5 nmol/l [32], an increment very unlikely to be associated with any change in risk of cardiometabolic outcomes based on data from the observational studies.

The current evidence base for blood pressure reduction is based on a small number of studies of variable quality, many of which did not explicitly set out to study patients with hypertension. Further interventional studies are therefore needed to define the optimum dose, dosing interval, and type of vitamin D to administer. This is a highly interesting scientific topic with potentially considerable public health relevance. Studies are required to confirm magnitude of effect of vitamin D on blood pressure reduction, particularly in patients with hypertension, followed by large studies examining the effects of vitamin D on cardiovascular events and death.

References

1. Hypponen E, Power C. Hypovitaminosis in British adults at age 45 years: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr. 2007;85:860–8.
2. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination. Am J Hypertens. 2007;20:713–9.
3. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. Osteoporos Int. 2002;13:187–94.
4. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworogger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007;49:1063–9.
5. Schleithoff SS, Zitterman A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006;83:754–9.
6. Langford HG, Nainby-Luxemoore JC, Melson JC, et al. Hyperparathyroidism is associated with hypertension and may be causal. Clin Res. 1980;28:333–8.
7. Bogin E, Massry SC, Levi J, et al. Effect of parathyroid hormone on osmotic fragility of human erythrocytes. J Clin Invest. 1982;69:1017–25.
8. Stefenelli T, Mayr H, Bergler-Klein J, et al. Primary hyperparathyroidism: incidence of cardiac abnormalities and partial reversibility after successful parathyroidectomy. Am J Med. 1993;95:197–202.
9. Hulter HN, Melby JC, Petersen JC, Cooke CR. Chronic continuous PTH infusion results in hypertension in normal subjects. J Clin Hypertens. 1986;4:360–70.
10. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med. 2008;168:1629–37.
11. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D with all-cause and cardiovascular mortality. Arch Intern Med. 2008;168:1340–9.
12. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117:503–12.
13. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med. 2007;167:1730–7.
14. Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: a systemic review and meta-analysis. J Hypertens. 2009;27:1948–54.
15. Pittas AG, Chung M, Trikalinos T, et al. Vitamin D and cardiometabolic outcomes: a systematic review. Ann Intern Med. 2010;152:307–14.
16. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab. 2001;86:1633–7.
17. Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H. Effects of a long-term vitamin D and calcium supplementation on falls and other parameters of muscle function in community-dwelling older individuals. Osteoporos Int. 2009;20:315–22.
18. Bischoff-Ferrari HA, Willett WC, Orav EJ, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367:40–9.
19. Hsia J, Heiss G, Ren H, et al. Calcium/vitamin D supplementation and cardiovascular events. Circulation. 2007;115:846–54.
20. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007;49:1063–9.
21. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. Hypertension. 2008;52:828–32.
22. Wang L, Manson JE, Buring JE, Lee IM, Sesso HD. Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. Hypertension. 2008;51:1073–9.
23. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med. 2008;168:1174–80.
24. Pilz S, Dobnig H, Fischer JE, et al. Low vitamin D levels predict stroke in patients referred to coronary angiography. Stroke. 2008;39:2611–3.
25. Marniemi J, Alanen E, Impivaara O, et al. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. Nutr Metab Cardiovasc Dis. 2005;15:188–97.
26. Scragg R, Khaw KT, Murphy S. Effects of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. Eur J Clin Nutr. 1995;49:640–6.
27. Krause R, Buhring M, Hopfenmüller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet. 1998;352:709–10.
28. Margolis KL, Ray RM, Van Horn L, et al. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. Hypertension. 2008;52:847–55.
29. Nemerovski CW, Dorsch MP, Simpson RU, Bone HG, Aaronson KD, Bleske BE. Vitamin D and cardiovascular disease. Pharmacotherapy. 2009;29:691–708.
30. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. Am J Clin Nutr. 2008;88:500S–6.
31. Jacobs DR, Steffen LM. Nutrients, foods and dietary patterns as exposures in research: a framework for food synergy. Am J Clin Nutr. 2003;78:508S–13.
32. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of serum 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84:18–28.

Calcium Metabolism in Mexican American Adolescents

34

Cristina Palacios and Connie M. Weaver

Abstract

Hispanics are a growing segment of the US population and will soon comprise one-fourth of the population. Mexican Americans (MA) are the majority of Hispanics in the USA. MA have been reported in some studies to have significant differences in several health outcomes that could impact calcium metabolism. Determining racial differences is imperative as this information can then be used to make racial- and ethnic-specific recommendations for behavior changes to reduce risk of osteoporosis, particularly during adolescence, the period of rapid bone accumulation that accounts for up to half of adult peak bone mass. Calcium intake is critical for adequate bone mineralization and increases in calcium intake result in higher bone mass, which, if sustained, may result in a lower risk of osteoporotic bone fracture later in life. Determining the influence of calcium intake on calcium retention and bone metabolism requires metabolic studies on a range of calcium intakes. Here we describe metabolic studies conducted in MA, white, black, and Asian adolescents while consuming controlled diets with various levels of calcium intakes. Our results showed that Mexican American girls had higher calcium retention compared to white girls but similar to Asians and blacks. However, Mexican American boys had similar calcium retention compared to white, Asian, and black boys. Future work is needed to calculate the minimal calcium intake leading to the maximal calcium retention in this group, kinetic analysis, and

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multiple regression models to quantify the effects of calcium intake, race/ethnic group, sex, sexual maturity, body composition, and hormonal and bone biomarkers on calcium retention. Our results will allow us to determine if MA adolescents have different calcium needs compared to the other groups studied.

Keywords
Mexican American • Calcium metabolism • Calcium intake • Calcium excretion • Calcium retention • Adolescents

Introduction

Hispanics, Mexican Americans in particular, are an ever-growing segment of our population. Hispanics will soon comprise one-fourth of the US population (Fig. 34.1). The 2000 US Census documented 32.8 million Hispanics, or 12 % of the population [1]. Of these, 66.1 % were of Mexican origin, 14.5 % Central and South American, 9 % Puerto Rican, 4.0 % Cuban, and 6.4 % other. It is projected that the US population of people of Hispanic origin will increase by 67 million people between 2000 and 2050, doubling their share of the total population to 24.4 %. By 2050, Hispanics will be the largest minority group, as blacks are expected to have 14.6 % of the nation's population and Asians 8 %. Mexican Americans comprise the majority of Hispanics in the USA. Mexican Americans have been reported in some studies to have significant differences in several health outcomes that could impact calcium metabolism and bone health.

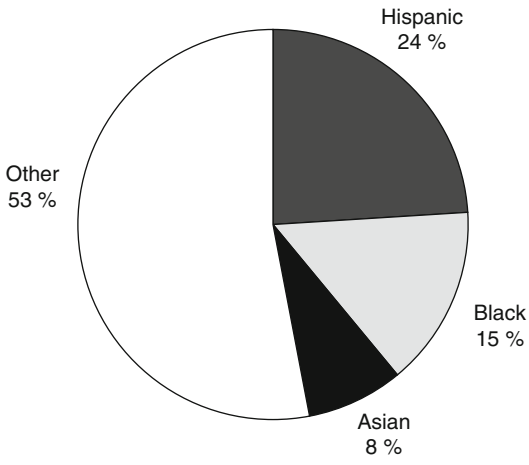

Fig. 34.1 Projections of the US population by the year 2050. Hispanics are projected to comprise 24 % of the US population by 2050 (Based on data from Ref. [1])

Hispanics are an admixture of European, Native American, and African origin. Although it has been estimated that there is only 8 % of genetic variation between major races, recent genetic marker cluster analysis of 326 markers from the Family Blood Pressure Program study showed distinct, nonoverlapping clustering of Hispanic, white, black, and East Asian peoples that nearly perfectly matched their self-described classification [2]. Thus, genetic marker cluster analysis would indicate that differences among genetic marker allele frequencies exist that sort individuals into at least four major racial groups and that create major differences in conducting genetic association studies in samples containing members from more than one group. Our current understanding of genetic variation in Hispanics is inadequate; therefore, there is a need to further study this particular group.

Differences in calcium metabolism among major population groups may be affected as much or more by ethnicity as race. Ethnicity, like race, implies a shared genealogy but encompasses a broader construct that takes into account shared experiences including cultural, linguistic, religious, historical, and sometimes geographical traits [3]. Dietary intake, physical activity, and cultural lifestyles, such as limited sun exposure, are examples of ethnic behaviors that could have major influences on calcium metabolism and, consequently, on bone health. Race and ethnicity

should be taken into account in clinical studies to avoid the risk of not identifying helpful discoveries in biomedical research [3].

Bone Mass in Mexican American Adolescents

The adolescent period is crucial for optimizing future bone health because bone accumulates rapidly during these years and accounts for up to half of adult peak bone mass. In addition, peak bone mass is a strong predictor of bone fragility later in life [4]. It is important to understand racial differences in bone mass in adolescents to implement race-specific strategies for future bone health.

There is limited data in bone mass in Mexican American adolescents (Table 34.1). A study by Weaver et al. [5] in 748 early pubertal Hispanic, Asian, and white girls showed that bone measures in Hispanic girls were generally similar to those in white girls and greater than those in Asian girls. However, Hispanic girls had lower total body bone mineral density (BMD) and lumbar spine bone mineral content (BMC) than white but greater than Asian girls, even after adjusting for differences in body fat and Tanner score. Models adjusting for race, Tanner score, status of menarche, and bone area of relevant bone site from dual-energy X-ray absorptiometry explained 96 % of the variance in total body BMD, 73 % the variance in one-third radius BMC, 88 % the variance for lumbar spine, and 77 % the variance for total hip.

Racial differences in BMC could be explained by dairy calcium intake, bone area, and physical activity. In the study mentioned above by Weaver et al. [5], dairy calcium intake made a significant additional contribution to the model and this study showed that Hispanic girls consumed the lowest proportion of calcium intake as dairy. Others have also shown that calcium intakes are particularly low among Mexican Americans, which has been attributed to greater incidence in lactose malabsorption in Mexican Americans (53 %) compared to white populations (15 %) [6]. In focus groups conducted in public schools, early pubertal and teenage Hispanic girls had more negative attitudes

Table 34.1 Studies on bone mass in Hispanic children and adolescents

Study	Population	Results
Wang et al. [25]	Children and young adults (9–25 years): 102 Hispanics 103 whites 103 Asians 115 blacks	BMD: similar among Hispanics, whites, and Asians Femoral neck BMAD: blacks > other groups
Bachrach et al. [26]	Children and young adults (9–25 years): 103 Hispanics 103 whites 103 Asians 114 blacks	Total BMD: blacks > other groups *Males* Total hip BMD: whites and Asians > Hispanics *Females* Spine BMD: Hispanics > whites and Asians
Weaver et al. [5]	Adolescents (10–14 years): 234 Hispanics 326 whites 188 Asians	Total body BMD: whites > Hispanics > Asians Total body BMC: Hispanics and whites > Asians Spine BMD: whites > Hispanics > Asians Total hip BMD: Hispanics and whites > Asians

BMD bone mineral density, *BMC* bone mineral content, *BMAD* bone mineral apparent density

toward milk consumption than Asian and white girls [7]. Milk was associated with fat and was perceived as more favorable by boys than by girls. Symptoms of lactose intolerance played a lesser role than attitudes about milk and influence of parents as models for drinking milk among Hispanics. Among Hispanic and white adolescents, calcium intake averaged 850 mg/day and milk and milk products were the source of 50–65 % of the calcium [8].

Optimal Calcium Intakes in Adolescents

Calcium intake is critical for adequate bone mineralization. An increasing body of knowledge suggests that increases in calcium intake result in higher bone mass, which, if sustained, will result in a lower risk of osteoporotic bone fracture later in life [9]. Therefore, maximizing calcium retention by the skeleton within the genetic potential during growth is a key strategy to prevent osteoporosis. Racial differences in calcium metabolism between blacks and whites during the pubertal growth period correspond with differences in adult bone mass [10].

Calcium requirements are determined as the calcium intake that maximizes calcium retention. This is best determined by the response of calcium retention to calcium intake through balance studies, since 99 % of calcium is in the bone and reflects bone accretion. Changes in BMC would be another appropriate outcome measure as calcium is a constant proportion of bone, but it is not practical to control diet, i.e., calcium intakes' for the months to years required to measure changes in BMC.

Calcium requirement for adolescents aged 9 through 18 years in the USA was set as 1,300 mg/day by the Institute of Medicine based on the minimal intake required to achieve maximal calcium retention in white girls [11], assuming that this would optimize development of peak bone mass and reduce the risk of osteoporosis later in life [12]. This requirement remained the same for North America in the recent revision by the Institute of Medicine [13].

It is unknown if this recommended level is appropriate for Mexican American adolescents. Studies in white children and adolescents demonstrated that dietary intakes less than the requirements of 1,300 mg/day failed to result in maximal bone accretion [14–18]. Since there are known differences in calcium metabolism in children of different races and ethnic groups, determining racial differences is imperative as this information can then be used to make racial- and ethnic-specific recommendations for behavior changes to reduce risk of osteoporosis [19–21].

Calcium Metabolism in Mexican American Adolescents

The effect of dietary calcium in building peak bone mass during growth in Mexican Americans is understudied compared to other major racial groups. Mexican Americans have been reported in some studies to have lower habitual calcium intakes than white adolescents but higher than Asian adolescents [7, 22, 23]. They also have lower serum 25(OH)D and parathyroid hormone values than whites but higher biochemical markers of bone turnover [24]. These differences are similar to those reported in black children [24], yet blacks have higher bone density than Hispanics [25, 26].

In terms of calcium absorption, a study in prepubertal Mexican American girls ($n=19$) aged 7–8.9 years showed no racial differences in calcium absorption or 24-h urinary excretion using a double stable calcium isotope test with a calcium load of ~350 mg despite lower serum 25(OH)D levels (27.6 vs. 43.8 ng/mL) and higher parathyroid hormone (4.01 vs. 1.96 pmol/L) in the Mexican American girls [24]. However, only one calcium load was studied, diet was not completely controlled, and bone balance could not be determined.

Determining the influence of calcium intake on calcium retention and bone metabolism requires metabolic studies on a range of calcium intakes. We have performed such studies in adolescents 11–15 years old of different races over several years with similar methods. In these

metabolic studies, we controlled calcium intake and measured all excretion during 3 weeks to calculate maximal retention during this rapid growing phase. These 21-day randomized crossover controlled feeding studies were conducted as summer camps, with educational and recreational components, simulating a free living environment. Most subjects have been studied twice, once on a low and once on a high calcium intake, to cover a range of dietary calcium intakes of 600–2,300 mg/day. In these studies, diet was completely controlled, with fixed amounts of sodium, phosphorus, protein, fat, fiber, and vitamin D but adjusting for calories with beverages and candies to maintain body weight. Basal diets usually provided 600 mg of calcium per day and the additional calcium was accomplished through fortified foods or supplements. All the foods were carefully weighed and subjects were strictly supervised to ensure compliance. Urine and feces were collected daily during the study periods and compliance was measured with creatinine for urine and polyethylene glycol for feces. Bone biomarkers, bone measures, and anthropometrics have been measured in all studies similarly.

Results from such metabolic studies show that in white girls, a calcium intake of 1,300 mg/day was necessary to maximize calcium retention during the adolescent years of rapid skeletal growth in American white girls [11]. We have also shown that American black girls, matched for weight and postmenarcheal age to white girls, retain significantly more calcium on the same dietary calcium intakes than whites [10]. In addition, retention was found to be more efficient in black girls compared to white girls; therefore, this group does not require higher calcium intakes to achieve their higher bone mass [27]. In Asians, calcium absorption efficiency has been found to be much higher than in whites, suggesting that recommendations may be lower for this group [28]. In white boys, we found that they develop larger skeletons than girls but have similar requirements for maximal skeletal accretion because they retain calcium more efficiently than girls across a broad range of calcium intakes [29]. In white adolescents, calcium absorption efficiency was not influenced by calcium intake between 800 and 2,300 mg/day, but Asian adolescents responded like white adults [28].

In Mexican Americans (23 boys and 23 girls), we recently found that fecal calcium increased with calcium intake for both boys and girls, with no difference in the slopes ($p<0.0001$); urinary calcium increased for both boys and girls, but the slope was greater for girls ($p<0.05$). Calcium retention increased with calcium intake ($p<0.0001$), with similar retention in boys and girls. When comparing with our previous studies in whites and Asians girls, retention was significantly lower in the white girls ($p<0.05$), with no significant differences in calcium retention between Asians and Hispanics. In boys, no significant differences in calcium retention were found between blacks, Hispanics, Asians, and whites.

Future analyses include the calculation of the minimal calcium intake leading to the maximal calcium retention to compare with previous results in white, black, and Asian adolescents of both genders. In addition, kinetic analysis will allow us to compare the relative contribution of bone, gut, and kidney to calcium retention for Mexican American adolescents and to compare these in white, black, and Asian adolescents. In addition, we will develop a statistical model to quantify the effects of calcium intake, race/ethnic group, sex, sexual maturity, BMC and density, anthropometrics, and hormonal and bone biomarkers, such as sex hormones, parathyroid hormone, 25(OH)D, 1,25(OH)D, total alkaline phosphatase, and osteocalcin, on calcium retention.

Conclusions

The adolescent period is crucial for optimizing future bone health because bone accumulates rapidly during these years. Calcium intake is particular critical during these years and maximizing calcium retention by the skeleton within the genetic potential during growth is a key strategy to prevent osteoporosis. Determining the influence of calcium intake on calcium retention and bone metabolism requires metabolic studies on a range of calcium intakes. Metabolic studies performed by

our group while consuming a controlled diet with various levels of calcium intakes showed that Mexican Americans girls had higher calcium retention compared to white girls but similar to Asians and blacks. However, Mexican American boys had similar calcium retention compared to white, Asian and black boys. These results may help define racial- and ethnic-specific recommendations for behavior changes to maximize peak bone mass during adolescence.

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References

1. U.S. Bureau of the Census, Bureau of Labor Statistics. Current Population Survey, March 2000 [machine-readable data file]. Washington: Bureau of the Census [producer and distributor], 2000.
2. Tang H, Quertermous T, Rodriguez B, Kardia SL, Zhu X, Brown A, Pankow JS, Province MA, Hunt SC, Boerwinkle E, Schork NJ, Risch NJ. Genetic structure, self-identified race/ethnicity, and confounding in case–control association studies. Am J Hum Genet. 2005;76(2):268–75.
3. Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, Mountain JL, Perez-Stable EJ, Sheppard D, Risch N. The importance of race and ethnic background in biomedical research and clinical practice. N Engl J Med. 2003;348(12):1170–5.
4. Parfitt AM. Genetic effects on bone mass and turnover-relevance to black/white differences. J Am Coll Nutr. 1997;16(4):325–33.
5. Weaver CM, McCabe LD, McCabe GP, Novotny R, Van Loan M, Going S, Matkovic V, Boushey C, Savaiano DA, ACT Research Team. Bone mineral and predictors of bone mass in white, Hispanic, and Asian early pubertal girls. Calcif Tissue Int. 2007;81(5):352–63.
6. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. N Engl J Med. 1997;337(2):77–82.
7. Auld G, Boushey CJ, Bock MA, Bruhn C, Gabel K, Gustafson D, Holmes B, Misner S, Novotny R, Peck L, Pelican S, Pond-Smith D, Read M. Perspectives on intake of calcium-rich foods among Asian, Hispanic, and white preadolescent and adolescent females. J Nutr Educ Behav. 2002;34(5):242–51.
8. Looker AC, Loria CM, Carroll MD, McDowell MA, Johnson CL. Calcium intakes of Mexican Americans, Cubans, Puerto Ricans, non-Hispanic whites, and non-Hispanic blacks in the United States. J Am Diet Assoc. 1993;93(11):1274–9.
9. Weaver CM, Peacock M, Johnston Jr CC. Adolescent nutrition in the prevention of postmenopausal osteoporosis. J Clin Endocrinol Metab. 1999;84(6):1839–43.
10. Bryant RJ, Wastney ME, Martin BR, Wood O, McCabe GP, Morshidi M, Smith DL, Peacock M, Weaver CM. Racial differences in bone turnover and calcium metabolism in adolescent females. J Clin Endocrinol Metab. 2003;88(3):1043–7.
11. Jackman LA, Millane SS, Martin BR, Wood OB, McCabe GP, Peacock M, Weaver CM. Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females. Am J Clin Nutr. 1997;66(2):327–33.
12. Institute of Medicine (IOM). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, D.C.: National Academy Press; 1997.
13. Institute of Medicine (IOM). Dietary reference intakes for calcium and vitamin D. Washington, D.C.: The National Academy Press; 2010.
14. Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, Eggli DF, Kieselhorst K, Kulin HE. Calcium supplementation and bone mineral density in adolescent girls. JAMA. 1993;270(7):841–4.
15. Johnston Jr CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, Peacock M. Calcium supplementation and increases in bone mineral density in children. N Engl J Med. 1992;327(2):82–7.
16. Lee WT, Leung SS, Wang SH, Xu YC, Zeng WP, Lau J, Oppenheimer SJ, Cheng JC. Double-blind, controlled calcium supplementation and bone mineral accretion in children accustomed to a low-calcium diet. Am J Clin Nutr. 1994;60(5):744–50.
17. Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, Rizzoli R. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest. 1997;99(6):1287–94.
18. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ. 1997;315(7118):1255–60.
19. Orces CH, Casas C, Lee S, Garci-Cavazos R, White W. Determinants of osteoporosis prevention in low-income Mexican-American women. South Med J. 2003;96(5):458–64.
20. Pothiwala P, Evans EM, Chapman-Novakofski KM. Ethnic variation in risk for osteoporosis among women: a review of biological and behavioral factors. J Womens Health (Larchmt). 2006;15(6):709–19.
21. Yarbrough MM, Williams DP, Allen MM. Risk factors associated with osteoporosis in Hispanic women. J Women Aging. 2004;16(3–4):91–104.

22. Novotny R, Boushey C, Bock MA, Peck L, Auld G, Bruhn CM, Gustafson D, Gabel K, Jensen JK, Misner S, Read M. Calcium intake of Asian, Hispanic and white youth. J Am Coll Nutr. 2003;22(1): 64–70.
23. Abrams SA, Griffin IJ, Hicks PD, Gunn SK. Pubertal girls only partially adapt to low dietary calcium intakes. J Bone Miner Res. 2004;19(5):759–63.
24. Abrams SA, Copeland KC, Gunn SK, Stuff JE, Clarke LL, Ellis KJ. Calcium absorption and kinetics are similar in 7- and 8-year-old Mexican-American and Caucasian girls despite hormonal differences. J Nutr. 1999;129(3):666–71.
25. Wang MC, Aguirre M, Bhudhikanok GS, Kendall CG, Kirsch S, Marcus R, Bachrach LK. Bone mass and hip axis length in healthy Asian, black, Hispanic, and white American youths. J Bone Miner Res. 1997;12(11):1922–35.
26. Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. J Clin Endocrinol Metab. 1999;84(12):4702–12.
27. Braun M, Palacios C, Wigertz K, Jackman LA, Bryant RJ, McCabe LD, Martin BR, McCabe GP, Peacock M, Weaver CM. Racial differences in skeletal calcium retention in adolescent girls with varied controlled calcium intakes. Am J Clin Nutr. 2007;85(6):1657–63.
28. Wu L, Martin BR, Braun MM, Wastney ME, McCabe GP, McCabe LD, DiMeglio LA, Peacock M, Weaver CM. Calcium requirements and metabolism in Chinese-American boys and girls. J Bone Miner Res. 2010;25(8):1842–9.
29. Braun M, Martin BR, Kern M, McCabe GP, Peacock M, Jiang Z, Weaver CM. Calcium retention in adolescent boys on a range of controlled calcium intakes. Am J Clin Nutr. 2006;84(2):414–8.

Calcium Is Not Only Safe but Important for Health

35

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Abstract

Concern about calcium supplementation causing cardiovascular events can widen the gap between calcium intakes and calcium recommendations. At this time, the association between calcium intake and cardiovascular disease is inconsistent, lacks a plausible mechanism, has no dose–response effect, and is not associated with cardiovascular mortality. In fact, calcium and vitamin D supplementation is systematically associated with decreased all-cause mortality. It is prudent to obtain recommended intakes of three servings of dairy products each day or to include fortified foods or supplements containing 300 mg calcium for every serving of dairy missed in order to meet calcium requirements without exceeding upper levels.

Keywords

Calcium • Calcium supplements • Cardiovascular disease • Myocardial infarction • Ossabaw pig

Introduction

Publicity surrounding the reports of Ian Reid, Mark Bolland, and collaborators regarding increased risk of cardiovascular events has led to a substantial public concern over calcium intake as evidenced by questions to medical professionals and falling sales of calcium supplements and milk. At stake is the likelihood of many individuals falling even further from their recommended calcium intakes compared to the possible risk of increased adverse cardiovascular events. How are individuals to meet their recommended intakes of calcium if they do not drink much milk and what are health professionals to recommend? What is the strength of the evidence for both benefit and

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risk for consuming too little and too much calcium? Does the answer depend on whether calcium comes from diet or supplements?

Strength of Evidence of Calcium Supplements and Risk of Cardiovascular (CV) Events

The recent meta-analysis of randomized controlled trials (RCTs) of calcium supplementation (>500 mg/day) with ≥100 participants with a mean age >40 years and study duration >1 year included 15 trials, 5 with patient level data (n = 8,151) and 11 with trial level data (n = 11,921) [1]. Of the several cardiovascular end points, only myocardial infarction was significant in the studies contributing patient level data and results from trial level data were not consistent across end points. The authors failed to appropriately adjust their P value for significance for multiple comparisons. In some 32 letters to the editor to this article (available online with BMJ), this analysis was criticized for none of the studies having CV end points as a primary outcome, no apparent dose–response relationship, no increase in mortality, no correlation between total calcium intake and myocardial infarction (MI), unreported baseline CV status of participants, predominantly unpublished data from the trials and events not adjudicated, and use of intention-to-treat (ITT) analysis rather than per protocol analysis was used. The RECORD trial provided the highest number of MI events, but compliance was only 45 % with calcium supplementation and MI events were self-reported. Neither ITT nor adjusted for compliance analysis resulted in significant CV end points in the RECORD trial [2]. In the Women's Health Initiative (WHI) with 36, 282 women randomized to 1,000 mg Ca and 400 IU vitamin D daily or placebo, there was no effect of treatment on coronary or cerebrovascular events and no interaction with baseline total calcium intake [3]. In contrast, a meta-analysis of 17 prospective and RCTs found no effect of calcium supplementation on CV events [4].

Bolland and others [5] redid their meta-analysis including the WHI trial. They stratified by baseline and found significant interaction between treatment and personal calcium use for myocardial infarction and stroke. However, subgroup analysis destroys the protection of randomization against unequal confounders between the treatment and placebo groups. An important potential confounder is baseline CV health. Another important confounder likely was asymptomatic chronic kidney disease (CKD) which increases risk of calcification due to loss of renal function [6]. Women in the WHI were not excluded for CKD as evidenced by their serum cystatin C levels [7]. Nevertheless, there were no adverse CV events or calcification associated with calcium supplementation in the WHI [8].

An important report published subsequently to the meta-analyses of Bolland et al. [1, 5] comes from the CAIFOS study, a 5-year RCT in 1,400 (85 % women) individuals aged 70 years or more [9]. There was no association between calcium supplementation and CV events in this study in which events were determined by hospital records. Several other large associational studies have shown an inverse relationship between calcium supplements and mortality including the Iowa Women's Health Prospective study in over 38,000 women [10] and the Finnish study in over 23,000 hip fracture patients [11]. An inverse relationship was also reported between milk consumption and overall CV disease risk [12].

Plausible Mechanisms

The concern regarding calcium supplementation and CV events suffers from lack of a good plausible mechanism or demonstrated dose–response effects. Ian Reid argues that a bolus dose of calcium can transiently increase serum calcium levels which could lead to calcification of soft tissues including coronary arteries. Serum total and ionized calcium do increase following bolus dose of calcium whether as food or calcium salts. Doses have to be rather large, i.e., >300 mg Ca, to measure these changes. In a study comparing change in serum Ca^{2+} area under the curve (AUC) from 500 mg Ca doses from dairy products and calcium carbonate with a crossover design in healthy young adults,

AUC were similar between yogurt and calcium carbonate and 37 % greater than with liquid milk [13]. These transient increases in serum calcium for <4 h are argued without evidence by Reid to lead to increased risk of soft tissue calcification. However, calcium deposits as $CaHPO_4$ and this product is only half saturated in the serum and not vulnerable to precipitation in the arteries. Furthermore, New Zealand white rabbits fed with high dietary calcium (3 %) had 41 % fewer lesions and 62 % less calcification in aortas compared to rabbits fed with control calcium levels (1 %) [13]. The authors attributed this to lower levels of soluble calcium and phosphorus in the aorta.

Animal models have the advantage for directly assessing causal relationships feeding protocols of known dietary intake for sufficiently long periods to develop the disease and, thereby, enabling disease outcome measures. The disadvantage with animal models is that most have a different pathogenesis of coronary disease than humans. For example, the rabbit is very vulnerable to atherosclerosis, and rodents develop plaque within the intima rather than on the surface as do humans. Another difficulty in studying the effect of calcium supplementation on calcification of soft tissue has been the methodological barrier of needing advanced calcification in order to detect abnormalities by visual imaging and histology. We are working on both a novel animal model and approach to overcome these disadvantages. We are using the Ossabaw miniature pig model that more closely mimics human coronary artery calcification and ^{41}Ca uptake to detect early calcium accumulation [6]. When the Ossabaw miniature pigs are fed an energy excess atherogenic diet, they develop metabolic syndrome that progresses to coronary atherosclerosis. ^{41}Ca is a rare isotope that can be detected at the extremely low concentrations expected in soft tissues by accelerator mass spectrometry. We have shown that pigs fed high-fat diets with metabolic syndrome accumulate measureable amounts of ^{41}Ca in contrast to barely detectible levels in lean pigs [14]. A study of pigs fed high-calcium diets as calcium carbonate or milk is underway.

In contrast to the lack of a good plausible mechanism to support a concern for calcium intake and CV events, there are plausible mechanisms for protective effects. Calcium supplementation has been shown to lower total serum cholesterol in both animal models [15] and humans [16]. In humans, 1 year of calcium supplementation improved serum HDL/LDL cholesterol ratios by both increasing HDL cholesterol and lowering LDL cholesterol. Calcium can bind fatty acids in the intestine and the insoluble soaps are excreted in the stools [17] thereby decreasing lipid absorption. Furthermore, dietary calcium lowers serum PTH which is associated with lower blood pressure and, ultimately, a decrease in risk of myocardial infarction.

Getting Enough Calcium

Calcium intakes around the world nearly universally fall below the recommended intakes for their citizens despite a rather wide range in recommended intakes [18].

In the USA where calcium intakes have been assessed in a representative sample of the population, 64 % of the population met the Estimated Average Requirement and a significant portion of what is consumed is as dietary supplements [19, 20]. Calcium and vitamin D are standard therapies to supplement the diet as safe and effective for prevention and treatment of osteoporosis [21, 22]. Calcium suppresses PTH and, thereby, suppresses bone remodeling. Reduction of hip fracture has been estimated at 12 % and double that if adjusted for compliance [23].

The evidence for risk of calcium supplements and CV events has been considered insufficient to change current calcium recommendations considering the evidence for benefit to bone health, hypertension, stroke, and other disorders [24, 25]. In fact overall mortality over 3 years was reduced by calcium and vitamin D supplementation in a systematic review of 13 trials involving 70,528 participants [26]. Individuals should avoid exceeding the recommend upper level for calcium. Fewer than 3 % of Americans do exceed their upper level of calcium intake [19]. Most countries have dietary guidelines that recommend two to three cups of milk a day [27]. To get enough calcium while

avoiding too much, it is prudent to follow the dietary guidance for three cups of dairy product equivalents per day or a 300 mg calcium supplement for every serving missed.

References

1. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ. 2010;341:c3691.
2. Avenell A, MacLennan GS, Jenkinson DJ, McPherson GC, McDonald AM, Pant PR, Grant AM, Campbell MK, Anderson FH, Cooper C, Francis RM, Gillespie WJ, Robinson CM, Torgerson DJ, Wallace WA, RECORD Trial Group. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D3 and/or calcium (RECORD trial). J Clin Endocrinol Metab. 2012;97:614–22.
3. Hsia J, Heiss G, Ren H, Allison M, Dolan NC, Greenland P, Heckbert SR, Johnson KC, Manson JE, Sidney S, Trevisan M. Calcium/vitamin D supplementation and cardiovascular events. Circulation. 2007;115:846–54.
4. Wang L, Manson JE, Song Y, Sesso HD. Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. Ann Intern Med. 2010;152:315–23.
5. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ. 2011;342:d2040.
6. Spence LA, Weaver CM. Calcium intake, vascular calcification, and vascular disease. Nutr Rev. (in press).
7. LaCroix AZ, Kotchen J, Anderson G, Brzyski R, Cauley JA, Cummings SR, Gass M, Johnson KC, Ko M, Larson J, Manson JE, Stefanick ML, Wactawski-Wende J. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. J Gerontol A Biol Sci Med Sci. 2009;64:559–67.
8. Manson JE, Allison MA, Carr JJ, Langer RD, Cochrane BB, Hendrix SL, Hsia J, Hunt JR, Lewis CE, Margolis KL, Robinson JG, Rodabough RJ, Thomas AM, Women's Health Initiative and Women's Health Initiative-Coronary Artery Calcium Study Investigators. Calcium/vitamin D supplementation and coronary artery calcification in the Women's Health Initiative. Menopause. 2010; 17(4):683–91.
9. Lewis JR, Zhu K, Prince RL. Adverse events from calcium supplementation; relationship errors in myocardial infarction self-reporting in randomized controlled trials of calcium supplementation. J Bone Miner Res. 2011. doi:10.1002/jbmr.1484.
10. Mursu J, Robien K, Harnack LJ, Park K, Jacobs Jr DR. Dietary supplementation and mortality rate in older women: the Iowa Women's Health Study. Arch Intern Med. 2011;171(18):1625–33.
11. Nurmi-Lüthje I, Sund R, Juntunen M, Lüthje P. Post-hip fracture use of prescribed calcium plus vitamin D or vitamin D supplements and antiosteoporotic drugs is associated with lower mortality; a nationwide study in Finland. J Bone Miner Res. 2011;26(8):1845–53.
12. Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engbrink MF, Willett WC, Geleijnse JM. Milk and dairy consumption and incidence of cardiovascular disease and all-cause mortality: dose–response meta-analysis of prospective cohort studies. Am J Clin Nutr. 2011;93:158–71.
13. Talbot JR, Guardo P, Seccia S, Gear L, Lubary R, Saad G, Roberts ML, Fradiinger E, Marino A, Zanchetta JR. Calcium bioavailability and parathyroid hormone acute changes after oral intake of dairy and nondairy products in healthy volunteers. Osteoporos Int. 1999;10:137–42.
14. Wastney M, Lee WH, Jackson GS, Alloosh M, Sturek M, Lachcik P, Peacock M, Martin B, Weaver CM. Soft tissue calcification in the Ossabaw miniature pig: experimental and kinetic modeling studies. Osteoporo Int. (in press).
15. Hsu HHT, Culley NC. Effects of dietary calcium on atherosclerosis, aortic calcification, and icterus in rabbits fed a supplemental cholesterol diet. Lipids Health Dis. 2005;5:16. doi:10.1186/1476-511x5-16.
16. Reid IR, Mason B, Horne A, Ames R, Clearwater J, Bava U, Orr-Walker B, Wu F, Evans MC, Gamble GD. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. Am J Med. 2002;112:343–7.
17. Denke MA, Fox MM, Shulte MC. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J Nutr. 1993;123:1047–53.
18. Looker AC. Dietary calcium intake. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totawa: Humana Press; 2006. p. 105–27.
19. Fulgoni III VL, Keast DR, Bailey RL, Dwyer J. Foods, fortificants, and supplements: where do Americans get their nutrients? J Nutr. 2011;141(10):1847–54. doi:10.3945/jn.111.142257.
20. Bailey RL, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR, Betz JM, Sempos CT, Picciano MF. Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr. 2010;140:817–22.
21. U.S. Department of Health and Human Services. Bone health and osteoporosis: a report of the surgeon general. Rockville: U.S. Department of Health and Human Services; 2004.
22. National Osteoporosis Foundation. (2012). http://www.nof.org/ Accessed 14 June 2012.
23. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet. 2007;370:657–66.

24. American Society for Bone and Mineral Research statement on potential cardiovascular risks associated with calcium supplements. August 12, 2010. http://www.asbmr.org/About/detail.aspx?cid=80efb4d9-d696-46c8-b83b-3e0940c9dddd. Accessed 13 Sept 2011.
25. Consumer Health Products Canada: advancing evidence-based self-care. http://www.chpcanada.ca/index.cfm?fuseaction=main.DspPage&PageID=65&fkMainPage=0Accessed. Accessed 14 June 2012.
26. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, Salovaara K, Cooper C, Smith HE, Jacobs ET, Torgerson D, Jackson RD, Manson JE, Brixen K, Mosekilde L, Robbins JA, Francis RM, Abrahamsen B. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528, patients from eight major vitamin D trials. J Clin Endocrinol Metab. 2012;97(8):2670–81: PMID: 22605432.
27. World Health Organization Energy and protein requirements. Report of a joint FAO/WHO/UNO expect consultation, vol. 724. Geneva: World Health Organization [In press]

Cardiovascular Safety of Calcium Supplements

36

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Abstract

Analyses of all cardiovascular event data available from all trials using calcium supplements alone have demonstrated a significant increase in the risk of myocardial infarction. When data from the Women's Health Initiative (WHI) is evaluated, the same adverse effect is apparent in those who were not already taking calcium supplements at the time of randomization. Meta-analyses of these WHI data with those of other trials which studied calcium with or without vitamin D confirm that the risk of myocardial infarction is increased by 24 % and that of stroke by 15 % with the use of calcium supplements. While there is not a comparable set of trials using calcium-rich foods as an intervention, observational data do not suggest that dietary calcium is a risk for cardiovascular disease. Therefore, the use of calcium supplements for prevention of osteoporosis is no longer appropriate in most situations, since it causes more adverse events than it prevents. Instead, we should advise our patients as to how they can obtain calcium intakes in the range of 600–1,000 mg/day from a balanced diet.

Keywords

Calcium • Myocardial infarction • Stroke • Vascular calcification

Introduction

The news that calcium supplements might increase the risk of cardiovascular disease arrived completely unheralded in 2008. The first study in the osteoporosis literature to seriously suggest this was the Auckland Calcium Study, which set out to investigate cardiovascular outcomes as secondary endpoints with the hypothesis that calcium supplements would have a beneficial effect [1]. We had previously shown improvements in circulating cholesterol fractions in women randomized to calcium [2] and confirmed the substantial literature that calcium supplements produced a small beneficial change in blood pressure [3]. Therefore, we hypothesized that with a large enough study, decreases in cardiovascular event rates may well be demonstrable. To our surprise, we found trends in the opposite direction, with increases in

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myocardial infarction being about 40 % higher in the calcium group than in those randomized to placebo. Initial response was to dismiss this as a chance finding (like the significant increase in hip fractures we had found in the same study) [4], but the mounting evidence that calcium supplements increased coronary artery calcification and cardiovascular event rates in patients with varying degrees of renal failure [5–7] was one of the specific findings that caused us to take this more seriously. While some have dismissed the renal literature as having no relevance to patients at risk of osteoporosis, this is not correct. Studies have been done in patients with pre-dialysis renal impairment, with average glomerular filtration rates in the range of 30–50 mL/min, which overlap substantially with the levels of renal function commonly found in the older population requiring prophylaxis against fractures.

Meta-analyses

Having found this adverse outcome, our next challenge was to determine how best to test this question further. While some are still advocating for the conduct of a definitive study, we note that there have been no serious moves to undertake the 20,000-person, 5-year trial that would be necessary to confirm this finding, and we believe that such an undertaking is most unlikely to proceed. The size of the anti-fracture effects of calcium is already adequately established from other studies, so the primary question to be tested in such a further study would be whether there truly is an increase in cardiovascular risk. To fund, gain approval for, and enroll patients into a study with a primary question that relates to the harm that the intervention might cause is unlikely to be a viable proposition and is without precedent. Therefore, we decided that a collation of cardiovascular events from the substantial trial database that already exists was more likely to move the area forward. We drafted a protocol for such a meta-analysis, identifying trials that had at least 100 participants and lasted for at least 1 year, in subjects aged greater than 40 years. We identified trials comprising almost 12,000 subjects that fitted these criteria, and we were able to access the adverse event data on 93 % of these subjects, as a result of the active support of the principal investigators of these studies. This meta-analysis of studies in which the intervention was calcium alone was published in 2010 [8] and showed a remarkably consistent increase in risk of myocardial infarction, with a hazard ratio of 1.27 (95 % CI 1.01–1.59). The major trials all showed the same adverse trend, though this was not statistically significant within any single study. There were similar but less marked adverse trends in the risk of stroke which did not reach statistical significance (hazard ratio 1.12, 95 % CI 0.92, 1.36).

We believed these data compellingly made the case that calcium supplements, as opposed to a calcium-rich diet, were associated with increased cardiovascular risk. However, the Women's Health Initiative (WHI) investigators had already documented the effect of combined intervention with calcium and vitamin D on cardiovascular event rates in that study, and though they found upward trends for some endpoints, they did not have a statistically significant increase in their primary cardiovascular endpoint [9]. There were several reasons why the WHI might have produced a different result from our meta-analysis of calcium monotherapy. Firstly, the intervention in the WHI was calcium plus vitamin D, and there is a growing literature suggesting that vitamin D may have beneficial effects on cardiovascular events and on mortality. Secondly, the average age of the participants in the WHI was more than 10 years less than that of our meta-analysis, so it could be hypothesized that the greater prevalence of vascular disease in the elderly and their poorer renal function could both conspire to accentuate the adverse effects of calcium supplements. A third possibility, however, was that the widespread use of self-prescribed calcium supplements throughout the duration of the WHI had obscured the effect of calcium on cardiovascular events. Fifty-five percent of women were self-administering calcium supplements at the time of randomization in the WHI, and this figure rose to more than two-thirds throughout the duration of the study.

The size of the WHI study, however, meant that there were still 16,000 women who were

calcium naive at the time of randomization, so we proposed an analysis to the NHLBI, the body holding the database, which primarily sought to determine whether there was an interaction between calcium supplement status at randomization and the effect of the trial intervention on cardiovascular event rates. This analysis demonstrated a significant interaction for clinical myocardial infarction, stroke, and the composite of the two [10]. Within the calcium-naive group, the increase in the hazard ratio of clinical myocardial infarction was 1.22 (95 % CI 1.00, 1.50) and that for stroke was 1.17 (95 % CI 0.95, 1.44), the latter being not quite significant. Our conclusions from these analyses were that the adverse trend of cardiovascular events in the WHI was essentially the same as that in the studies which used calcium monotherapy as the intervention, implying that this small dose of vitamin D did not have a significant cardioprotective effect and that it was the contamination from self-administration of calcium which had led to the earlier analysis from the WHI being negative.

In the light of calcium plus D appearing to have comparable effects to calcium alone, Mark Bolland has now performed a comprehensive meta-analysis of studies which used either of these as the intervention [10]. This now comprises more than 28,000 individuals, 676 incident myocardial infarctions, and a mean weighted trial duration of 5.7 years. In the trial-level meta-analysis, the relative risk for myocardial infarction is 1.24 (95 % CI 1.07, 1.45) and the comparable figures for stroke are a relative risk of 1.15 (95 % CI 1.00, 1.32) based on 764 incident strokes (Fig. 36.1). This analysis provides robust evidence for an adverse effect, particularly on myocardial infarction but probably also for stroke. Because of the size of this database and the number of events, it will be little influenced by any of the relatively small studies of calcium that are currently underway. Therefore, the hope that some new data will become available in the foreseeable future which will substantially reverse the findings here is likely to be a forlorn one, and practitioners need to make decisions about their use of calcium supplements, based on the data which is currently available.

Mortality

Since that meta-analysis, one further randomized, controlled trial has provided further insight into this issue. Sambrook et al. carried out a trial in very frail elderly individuals who were randomized to a control group, to have daily sunlight exposure, or to have daily sunlight exposure plus calcium [11]. During the follow-up period, one-third of the 602 individuals died, and the hazard ratio for death was 1.47 (95 % CI 1.05, 2.06) when those randomized to calcium plus sunlight were compared to those randomized to sunlight alone [12]. Most of this excess mortality appeared to be cardiovascular, and the hazard ratio for cardiovascular death was 1.76 (95 % CI 1.10, 2.82). The Bolland meta-analyses have not shown significant increases in death and some have taken comfort from this, but the Sambrook study suggests that if calcium supplements are used in a trial in which the death rate is sufficiently high, a significant adverse effect on this endpoint is also demonstrable.

Observational Studies

Two groups have used existing observational databases to determine whether the effects of the randomized trials can be reproduced. In the Kuopio Osteoporosis Study, 10,555 older women were followed for 7 years, and the hazard ratio for coronary heart disease associated with calcium use was 1.24 (95 % CI 1.02,1.52) [13]. More recently the EPIC-Heidelberg cohort of 23,980 adults followed for 11 years showed a hazard ratio for myocardial infarction associated with calcium supplement use of 1.86 (95 % CI 1.17, 2.96) [14]. Interestingly, this analysis suggested that *dietary* calcium intake was protective, the opposite of what was found for supplement use. This particular finding focuses attention on why a supplement could be different from diet and what the potential mechanisms are that underlie the adverse effect of supplements. There is a precedent, of course, for calcium supplements having an adverse effect when dietary calcium is beneficial, and that is in the case of renal

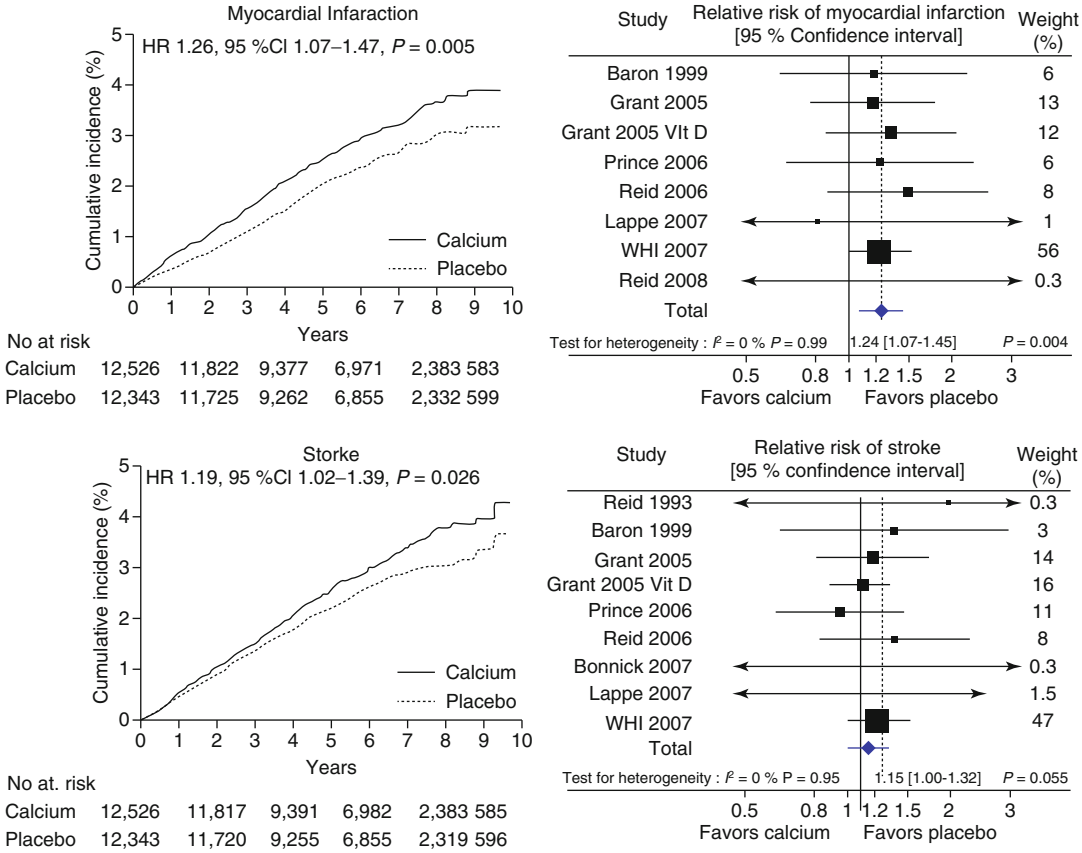

Fig. 36.1 Patient-level meta-analyses of the effect of calcium supplements with or without vitamin D on cardiovascular events (*left-hand panels*) and corresponding trial-level meta-analyses (*right-hand side*). Patient-level data show the time-to-first event analyses for 24,869 participants in five trials of calcium supplements, and Women's Health Initiative (WHI) calcium and vitamin D (CaD) participants not taking personal calcium supplements at baseline. Trial-level analyses show data for 28,072 participants in eight trials of calcium supplements where complete trial-level data were available, together with data for WHI CaD participants not taking personal calcium supplements at baseline. One study randomized participants to calcium, CaD, or placebo [37]. For this analysis, we pooled the outcomes from both the calcium and CaD arms. Abbreviations: Grant 2005 is the RECORD study calcium versus placebo arms, and Grant 2005 Vit D is the RECORD study calcium plus vitamin D versus vitamin D plus placebo arms [38] (Reprinted from Bolland et al. [10]. With permission from BMJ Publishing Group Ltd)

calculi [15]. It is possible that somewhat similar mechanisms may be implicated.

Mechanisms

The mechanism of the adverse effect of calcium supplement on cardiovascular disease is unknown, but it is likely to be mediated by the increases in serum calcium which have been frequently documented in studies of calcium supplement administration (Fig. 36.2). This contrasts with the effects of a calcium-rich meal, which causes much smaller elevations of serum calcium, presumably because the protein and fat in such a meal slow the meal's transit to the small intestine. In addition, dietary calcium is usually taken in boluses much smaller than those used in supplements and which are spread throughout the day. While serum calcium is only pushed to the upper end of the normal range or slightly above following calcium supplements, this is not necessarily free of risk. There is now a substantial amount of evidence indicating that,

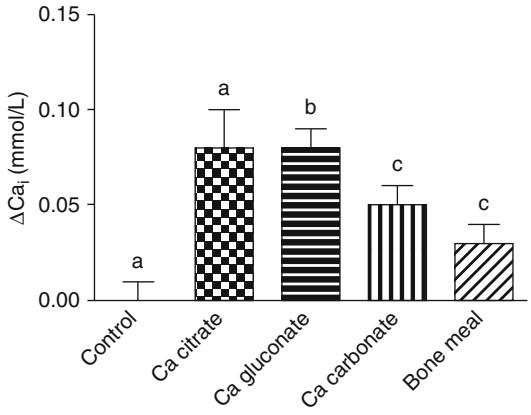

Fig. 36.2 Change in serum ionized calcium 3 h after taking 1 g of calcium in the form indicated. Data are mean ± SEM. Bars with different letters are significantly different (Based on data from Reid et al. [39]. Copyright IR Reid, used with permission)

in normal populations, serum calcium levels in the upper quintile or quartile are associated with increased thickness of carotid artery plaques [16], increased likelihood of abdominal aortic calcification [17], increased extent of coronary artery calcification [18], increased risk of incident cardiovascular events [19–21], and increased mortality [22–24]. If these associations are causally related to high normal calcium levels, then the propensity of calcium supplements to raise serum calcium to the upper end of the normal range could well mediate the increase in cardiovascular risk.

If increases in serum calcium mediate the adverse effects of calcium supplements, then one would expect to see a difference between large doses (e.g., 1 g) of soluble calcium salts administered on an empty stomach, when compared with smaller doses (e.g., 500 mg) of less soluble salts, taken fasting. Unfortunately, there are no adequate data available to address this important question. Many studies do not indicate what dosing instructions were given to subjects, and many subjects in trials will have devised their own strategies for taking the calcium. In our meta-analysis of calcium monotherapy, only one study used a dose as low as 500 mg/day [8]. In that analysis, we detected no significant effect of supplement type, though the majority of studies used carbonate. The WHI used carbonate and advised participants to take it in doses of 500 mg with meals [25]. Despite this, that study showed the same adverse trends as the other major trials reviewed. This is clearly an area that requires further investigation.

These data raise the further question as to why high normal calcium concentrations should increase cardiovascular risk. There are calcium-sensing receptors on vascular smooth muscle cells and endothelial cells, as well as on platelets [26, 27]. Therefore, changes in the arterial wall or in blood coagulability are both possible. The potential importance of the calcium-sensing receptor in the genesis of vascular disease is suggested by the association of increased cardiovascular risk with polymorphisms of this receptor [28]. Calcium, of course, is a critical factor in blood coagulation and its chelation is a commonly used way of preventing blood from clotting. There is in vitro evidence that perturbations of plasma calcium within the normal range can affect blood coagulation [29]. In vitro studies of vascular smooth muscle cells have demonstrated that increases in ambient calcium concentration increase calcium deposition in cultured cells [30]. One of the key regulators of vascular calcification is pyrophosphate. Calcium may directly complex pyrophosphate, but it has also recently been demonstrated that 1,25-dihydroxyvitamin D increases tissue pyrophosphate levels through effects on pyrophosphate production and metabolism. Thus, high levels of 1,25-dihydroxyvitamin D tend to inhibit tissue calcification [31, 32]. Calcium supplements reduce circulating 1,25-dihydroxyvitamin D, reducing pyrophosphate, and, thus, potentially facilitating soft tissue calcification. These putative mechanisms are summarized in Fig. 36.3.

Clinical Implications

The accumulating evidence that calcium supplements increase cardiovascular risk creates a conundrum for clinicians and for patients concerned about their bone and vascular health. Obviously, it is important that further research is

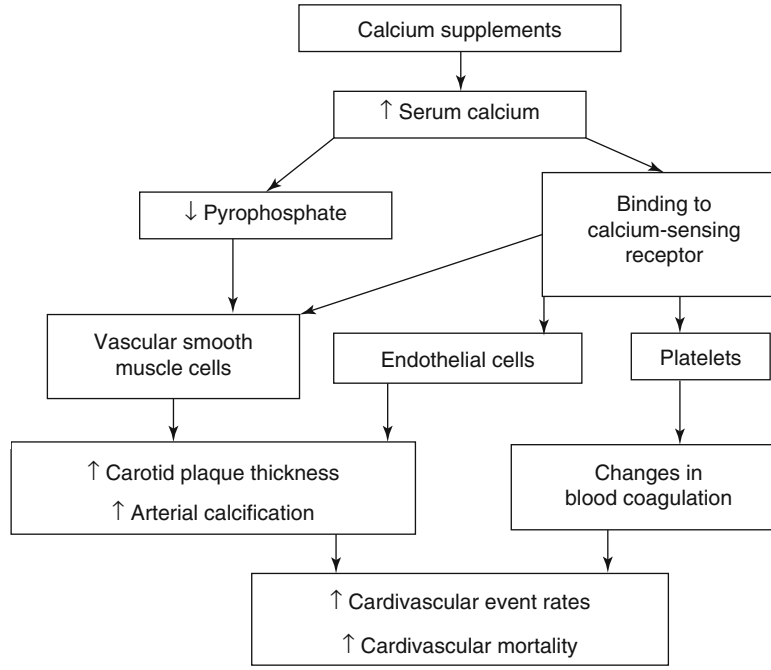

Fig. 36.3 Possible sequence of events by which calcium supplements increase cardiovascular risk. Clinical studies have demonstrated the effect of supplements on blood calcium, and on the clinical endpoints shown, there is in vitro evidence of calcium effects on coagulation and for regulation of pyrophosphate production by 1,25-dihyroxyvitamin D, and calcium-sensing receptors have been identified on these cell types (Copyright IR Reid, used with permission)

carried out so that we can understand the mechanisms of this adverse effect. In turn, this might lead to strategies for circumventing this problem. In the meantime, we need to balance the possible cardiovascular risk against the possible bone benefit from calcium supplementation. For the most part, those studies which have demonstrated fracture prevention from calcium supplementation are the same which have demonstrated cardiovascular risk, and analyses of event rates in these studies indicate that the balance of risk versus benefit is negative. This reflects the fact that while the absolute increase in cardiovascular event rates from calcium supplements is small, the absolute decrease in fracture rates from supplement use is even smaller. Therefore, the responsible course to follow would seem to be to encourage patients to obtain adequate calcium from their diet and to aim for an achievable intake in the range of 600–900 mg/day. Vitamin D supplementation in those who are at risk of deficiency is a sensible adjunctive measure, and there is no suggestion that this carries any adverse effects on cardiovascular risk.

In individuals whose fracture risk is higher, pharmaceutical intervention is indicated. It has frequently been stated that current anti-osteoporosis medications have not been shown to be effective unless given with calcium and vitamin D. This is not strictly a correct description of the literature. A randomized, controlled trial of alendronate with or without calcium showed its effects on bone density to be exactly the same whether or not a supplement was given [33]. Clodronate without calcium supplementation has been shown to prevent fractures as efficiently as other bisphosphonates when given with supplements [34]. While zoledronate has not been studied with and without calcium in the context of a head-to-head trial, the effects of zoledronate without calcium supplementation appear to be very comparable to those found when a supplement is coadministered [35, 36]. Thus, it is likely that we can virtually abandon the use of calcium supplements, place greater emphasis on a balanced, calcium-rich diet, and achieve the same benefits for bone health which we do at the present time, without increasing our patients' risk of serious cardiovascular consequences.

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References

1. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, et al. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. BMJ. 2008;336(7638):262–6.
2. Reid IR, Mason B, Horne A, Ames R, Clearwater J, Bava U, et al. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. Am J Med. 2002;112(5):343–7.
3. Reid IR, Horne A, Mason B, Ames R, Bava U, Gamble GD. Effects of calcium supplementation on body weight and blood pressure in normal older women: a randomized controlled trial. J Clin Endocrinol Metab. 2005;90(7):3824–9.
4. Reid IR, Mason B, Horne A, Ames R, Reid HE, Bava U, et al. Randomized controlled trial of calcium in healthy older women. Am J Med. 2006;119(9):777–85.
5. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. Kidney Int. 2007;71(5):438–41.
6. Di Iorio B, Bellasi A, Russo D. Mortality in kidney disease patients treated with phosphate binders: a randomized study. Clin J Am Soc Nephrol. 2012;7(3):487–93.
7. Russo D, Miranda I, Ruocco C, Battaglia Y, Buonanno E, Manzi S, et al. The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. Kidney Int. 2007;72(10):1255–61.
8. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ. 2010;341:C3691.
9. Hsia J, Heiss G, Ren H, Allison M, Dolan NC, Greenland P, et al. Calcium/vitamin D supplementation and cardiovascular events. Circulation. 2007;115(7):846–54.
10. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium/vitamin D supplements and cardiovascular events: a re-analysis of the Women's Health Initiative limited-access dataset, and meta-analysis of calcium with or without vitamin D. BMJ. 2011;342:d2040. doi:10.1136/bmj.d2040.
11. Sambrook PN, Cameron ID, Chen JS, Cumming RG, Durvasula S, Herrmann M, et al. Does increased sunlight exposure work as a strategy to improve vitamin D status in the elderly: a cluster randomised controlled trial. Osteoporos Int. 2012;23(2):615–24.
12. Reid IR, Bolland MJ, Sambrook PN, Grey A. Calcium supplementation: balancing the cardiovascular risks. Maturitas. 2011;69(4):289–95.
13. Pentti K, Tuppurainen MT, Honkanen R, Sandini L, Kroger H, Alhava E, et al. Use of calcium supplements and the risk of coronary heart disease in 52-62- year-old women: the Kuopio Osteoporosis Risk Factor and Prevention Study. Maturitas. 2009;63(1):73–8.
14. Li K, Kaaks R, Linseisen J, Rohrmann S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg). Heart. 2012;98:920–5.
15. Curhan GC, Willett WC, Speizer FE, Spiegelman D, Stampfer MJ. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. Ann Intern Med. 1997;126(7):497–504.
16. Rubin MR, Rundek T, McMahon DJ, Lee H-S, Sacco RL, Silverberg SJ. Carotid artery plaque thickness is associated with increased serum calcium levels: the Northern Manhattan study. Atherosclerosis. 2007;194(2):426–32.
17. Bolland MJ, Wang TKM, van Pelt NC, Horne AM, Mason BH, Ames RW, et al. Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. J Bone Miner Res. 2010;25(3):505–12.
18. Shin S, Kim K-J, Chang H-J, Cho I, Kim YJ, Choi B-W, et al. Impact of serum calcium and phosphate on coronary atherosclerosis detected by cardiac computed tomography. Eur Heart J. 2012;33(22):2873–81. doi:10.1093/eurheartj/ehs152.
19. Slinin Y, Blackwell T, Ishani A, Cummings SR, Ensrud KE. Serum calcium, phosphorus and cardiovascular events in post-menopausal women. Int J Cardiol. 2011;149:3350.
20. Lind L, Skarfors E, Berglund L, Lithell H, Ljunghall S. Serum calcium: a new, independent, prospective risk factor for myocardial infarction in middle-aged men followed for 18 years. J Clin Epidemiol. 1997;50(8):967–73.
21. Foley RN, Collins AJ, Ishani A, Kalra PA. Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J. 2008;156(3):556–63.
22. Leifsson BG, Ahren B. Serum calcium and survival in a large health screening program. J Clin Endocrinol Metab. 1996;81(6):2149–53.
23. Durup D, Jørgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B. A reverse j-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice, the CopD Study. J Clin Endocrinol Metab. 2012;97(8):2644–52. doi:10.1210/jc.2012-1176.
24. Larsson TE, Olauson H, Hagstrom E, Ingelsson E, Arnlov J, Lind L, et al. Conjoint effects of serum calcium and phosphate on risk of total, cardiovascular, and noncardiovascular mortality in the community. Arterioscler Thromb Vasc Biol. 2010;30(2):333–9.
25. Jackson RD, LaCroix AZ, Cauley JA, McGowan J. The Women's Health Initiative calcium-vitamin D trial: overview and baseline characteristics of participants. Ann Epidemiol. 2003;13(9):S98–106, Suppl. S.

26. Alam M, Kirton JP, Wilkinson FL, Towers E, Sinha S, Rouhi M, et al. Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. Cardiovasc Res. 2009;81(2):260–8.
27. House MG, Kohlmeier L, Chattopadhyay N, Kifor O, Yamaguchi T, Leboff MS, et al. Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. J Bone Miner Res. 1997;12(12):1959–70.
28. Marz W, Seelhorst U, Wellnitz B, Tiran B, Obermayer-Pietsch B, Renner W, et al. Alanine to serine polymorphism at position 986 of the calcium-sensing receptor associated with coronary heart disease, myocardial infarction, all-cause, and cardiovascular mortality. J Clin Endocrinol Metab. 2007;92(6):2363–9.
29. James MFM, Roche AM. Dose–response relationship between plasma ionized calcium concentration and thrombelastography. J Cardiothorac Vasc Anesth. 2004;18:581–6.
30. Yang H, Curinga G, Giachelli CM. Elevated extracellular calcium levels induce smooth muscle cell matrix mineralization in vitro. Kidney Int. 2004;66:2293–9.
31. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Dresselaers T, et al. Vitamin D regulates serum calcium by inhibiting skeletal calcium deposition. Bone. 2011;48:S102.
32. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. J Clin Invest. 2012;122(5):1803–15.
33. Bonnick S, Broy S, Kaiser F, Teutsch C, Rosenberg E, DeLucca P, et al. Treatment with alendronate plus calcium, alendronate alone, or calcium alone for postmenopausal low bone mineral density. Curr Med Res Opin. 2007;23(6):1341–9.
34. McCloskey EV, Beneton M, Charlesworth D, Kayan K, Detakats D, Dey A, et al. Clodronate reduces the incidence of fractures in community-dwelling elderly women unselected for osteoporosis: results of a double-blind, placebo-controlled randomized study. J Bone Miner Res. 2007;22(1):135–41.
35. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med. 2007;356(18):1809–22.
36. Grey A, Bolland MJ, Wattie D, Horne A, Gamble G, Reid IR. The antiresorptive effects of a single dose of zoledronate persist for two years: a randomized, placebo-controlled trial in osteopenic postmenopausal women. J Clin Endocrinol Metab. 2009;94(2):538–44.
37. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007;85(6):1586–91.
38. Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet. 2005;365(9471):1621–8.
39. Reid IR, Schooler BA, Hannon S, Ibbertson HK. The acute biochemical effects of four proprietary calcium supplements. Aust N Z J Med. 1986;16:193–7.

37 Blueberry in Calcium- and Vitamin D-Enriched Fermented Milk Is Able to Modulate Bone Metabolism in Postmenopausal Women

Marie-Jeanne Davicco, Caroline Puel, Patrice Lebecque, and Véronique Coxam

Abstract

Research in the field of nutrition allows considering the establishment of a real prevention of osteoporosis. The value of fruits is discussed. Indeed, red fruits are particularly interesting for their high content in anthocyans, endowed with antioxidant and anti-inflammatory properties.

Fifty-six postmenopausal women (less than 6 years) aged 50–65 years, without HRT, were included in a controlled, randomized, double-blind placebo, prospective study, after a medical examination and a blood test. Throughout the 3-month study period, they kept their eating habits, limiting however consumption of red fruits. They were randomized into two groups of 28 subjects receiving either 0 or 120 mg of anthocyans daily, from blueberry extract. Those polyphenols were provided at the dose of 0 or 60 mg of active molecule in 100 ml of fermented milk (two bottles of

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100 ml/day covering 25 and 20 % RDA for calcium and vitamin D, respectively).

Consumption of the milk enriched in polyphenols significantly improved serum bALP activity (an osteoblastic marker), without significant modification of CTX, a marker for bone resorption. This favorable orientation of bone metabolism could be explained by the contribution of anthocyans, the only noticeable difference between the two test foods. This finding is independent of the initial calcium and vitamin D consumption.

In conclusion, consumption of fermented milk enriched with calcium and vitamin D, containing blueberry, for 3 months, has corrected the insufficiency of vitamin D of postmenopausal women and resulted in improved bone formation, as indicated by the rise of a biomarker of osteoblastic activity. This benefit is probably related to the presence of blueberries (rich in polyphenols and phenolic acids).

Keywords

Postmenopausal women • Blueberry extract • Bone biomarkers • Calcium- and vitamin D-enriched milk

Abbreviations

bALP	Bone alkaline phosphatase
CRP	C-reactive protein
CTX	C-telopeptide cross-links
DPD	Deoxypyridinoline
IU	International unit
25(OH)D	25-hydroxyvitamin D
PYD	Pyridinoline
RDA	Recommended dietary allowance
SEM	Standard error to the mean

Introduction

Among the manifestations of senescence, impairment of the musculoskeletal system is particularly debilitating and greatly speeds up entry into the dependence. Thus, given the aging of populations globally and in the industrialized countries specifically, osteoporosis, the primary skeletal disorder, is considered to be a major public health and socioeconomic problem worldwide [1, 2].

So far, hormone replacement therapy was currently used as prophylaxis. Nevertheless, because of side effects, health professionals strongly advocate new strategies of proven clinical value for prevention, to provide a wide array of treatments. For that purpose, research in nutrition over the past 30 years has led to an exciting progress supporting the hypothesis that, by modulating specific target functions in the body, diet can help to achieve optimal health by reducing the risk of disease. In this context, although calcium and vitamin D are required nutrients for bone development [3], it has been recognized that the human diet contains a complex array of naturally occurring bioactive molecules, the phytochemicals endowed with antioxidant and anti-inflammatory properties. Actually, antioxidant nutrients may enhance bone formation and reduce the production of free radicals which contribute to bone resorption, leading to a bone-sparing effect. This is why they could play a strategic role in the prevention of osteoporosis as well [4–6]. Indeed, recent observations suggest that consumption of dried plum, rich in phenolic compounds, is associated with increased markers for bone formation in postmenopausal women [7]. Besides, a prospective study has shown a positive correlation between tea consumption and bone mineral density in elderly [8].

In this regard, red fruits (red grapes, blueberry, cassis, cherry, cranberry, elderberry) are particularly interesting for their high anthocyanin content (from a few 100 mg to several g/kg of fresh weight; blueberry 25–495 mg/kg) and their widespread consumption (leading to a mean daily of intake of such compounds from 25 to 215 mg) [9]. Moreover, recent studies have demonstrated the benefit of blueberries to prevent various age-related chronic diseases such as cancer, diabetes, hyperlipidemia, hypertension, neurodegeneration, obesity through their apoptosis, antioxidant, anti-inflammation, and antiangiogenesis effects [10].

The objective of this study was thus to evaluate the effect of consumption of a fermented milk (providing 25 and 20 % RDA of calcium and vitamin D) enriched with a blueberry extract rich in anthocyanins, on bone biomarkers in postmenopausal women.

Subjects and Methods

Subjects

For this prospective randomized, double-blind, placebo-controlled trial, 56 postmenopausal women were recruited. They were 50–65 years old (postmenopausal for at least 1–5 years), without hormone replacement therapy. The volunteers were informed of the study procedures and gave informed written consent before enrollment in the clinical trial. Throughout the study period, they kept their eating habits, limiting however red fruits. They were randomized into two groups (a placebo group vs. an intervention group) of 28 subjects each and received either 0 or 120 mg of anthocyanins/day.

Study Design

The duration of the study was 3 months and was conducted at the Clinical Investigation Center (CIC INSERM Clermont-Ferrand France) which has the ministerial approval for biomedical research without direct individual benefit (N° 03046MHC; 2007-A00940-53).

The anthocyanins (amount equivalent to 22.5 g of blueberries) were provided at the dose of 0 or 60 mg of active drug/100 ml of calcium- and vitamin D-enriched fermented milk (allowing covering 25 and 20 % of the RDA for calcium and vitamin D, respectively). The volunteers were asked to consume two bottles per day.

A food record and a questionnaire on lifestyle, to assess tobacco and alcohol consumption and physical activity level as well, were established at baseline. A full clinical examination was also performed at the screening visit.

Throughout the study, biomarkers to evaluate bone metabolism and inflammatory status were assessed. Blood was collected at 0, 45, and 90 days, after an overnight fasting. The record of intercurrent events was carried out at the same time (45 and 90 days), to ensure good tolerance of the food test.

Biochemical Analysis

Blood samples were collected at baseline, 45 and 90 days. The samples were stored at −80 °C and analyzed. Biochemical tests were performed by using electrochemiluminescence-based immunoanalysis (Roche Diagnostics GmbH, Germany), according to standard methods. Concentrations of bone-specific alkaline phosphatase (a marker of bone formation) were measured by enzyme immunoassay on paramagnetic particle DXI; serum concentrations of C-telopeptide cross-links (a marker of bone resorption), serum CRP (a marker of inflammation), and serum 25-hydroxyvitamin D (25(OH)D) with the Roche Modular Analytics E immunoassay system.

Statistical Analysis

The significance of the treatment effects on the main criteria between the supplemented and control group was performed by Student's *t*-test (after log transformation of data, if necessary). *P* values <0.05 were considered significant.

Results are expressed as mean ± SEM. Analyses of repeated measurements for bone

Table 37.1 Characteristics of subjects and baseline values of measured biochemical variables (Mean ± SEM)

	Supplemented group	Placebo control group	
Variables	Mean ± SEM	Mean ± SEM	*P*
Age at screening (years)	53.9 ± 0.5	54.5 ± 0.5	NS
Months since menopause (months)	37.0 ± 3.3	35.5 ± 2.9	NS
Weight (kg)	65.4 ± 1.6	61.3 ± 1.8	NS ($P < 0.087$)
Height (m)	1.63 ± 0.01	1.62 ± 0.01	NS
BMI (kg/m^2)	24.67 ± 0.63	23.34 ± 0.62	NS
Current smoker (%)	0.18 ± 0.07	0.18 ± 0.07	NS
Alcohol intake (%drinker)	0.50 ± 0.10	0.21 ± 0.08	$P < 0.026$
Total alkaline phosphatase (IU/l)	71.50 ± 3.15	71.04 ± 2.57	$P < 0.047$
25(OH)D (nmol/l)	38.67 ± 2.61	46.17 ± 2.90	NS
FSH (IU/l)	76.14 ± 4.25	91.31 ± 6.53	NS ($P < 0.057$)
Bone alkaline phosphatase (μg/l)	13.06 ± 0.58	13.76 ± 0.70	NS
CTX (ng/ml)	0.54 ± 0.04	0.57 ± 0.04	NS
CRP (mg/l)	2.06 ± 0.45	1.95 ± 0.61	NS

Data was analyzed using the XLSTAT software (Addinsoft, Paris, France), by ANOVA
BMI body mass index, *FSH* follicle-stimulating hormone, *CTX* cross-linked telopeptide of type I collagen, *CRP* C-reactive protein

markers were performed using SAS version (SAS Institute Inc.). The percent changes in serum bALP and in serum CTX were calculated [(post intervention value − baseline value)/baseline value × 100 %] for each group.

Results

Of the 56 subjects included in the study, 1 dropped out for personal reasons.

Baseline characteristics of the subjects who completed the study are presented for each group in Table 37.1. No difference was observed between the two groups at baseline, except for alcohol consumption which is slightly higher in the supplemented group ($p < 0.026$) and which could explain the higher plasma levels of total alkaline phosphatase (71.50 ± 3.15 vs. 71.04 ± 2.90 IU/l) in this group. Indeed, anthropometric data, plasma biomarker for bone metabolism, and vitamin D status were similar.

The analysis of food records completed over 5 days and collected before the start of the study showed that the two groups were similar in their protein, carbohydrates, fats, vitamins, and trace element consumption. With regard to calcium intake, the average consumption (mg/day) was 827 ± 228 for women in the treated group and 854 ± 297 mg for the placebo group, which is far below the RDA in postmenopausal women (1,200 mg/day). Nevertheless, whatever the experimental group, a significant dispersion was observed (from 338 to 1,423 mg/ day).

At the end (after 3 months of test foods consumption), vitamin D status was significantly improved. Indeed, serum 25(OH)D levels were increased by 72 % in the placebo group and 101 % in the intervention group. Regarding the primary outcome, i.e., bone remodeling, consumption of the milk enriched in polyphenols significantly increased osteoblast activity (as shown by higher serum bALP concentrations), without significant modification in CTX values, a marker for bone resorption (Fig. 37.1). CRP did not significantly change throughout the study, whatever the test food was.

Discussion

Broad-based preventive strategies designed to lower the risks of osteoporosis need to be established and implemented. This is why the concept of a healthy diet providing adequate amounts of

various potent micronutrients deserves mention [11]. We targeted anthocyanins from blueberry as a possible way to achieve this goal. Indeed, a recent interest in food phenolics has increased greatly owing to their anti-inflammatory and free radical scavenging abilities. We thus assessed the effect of consumption of a fermented milk (providing 25 and 20 % RDA of calcium and vitamin D) enriched with a blueberry extract rich in anthocyanins, on bone biomarkers in postmenopausal women.

The volunteers enrolled in the present study are representative of the general population of this age. Indeed, their vitamin D status was relatively low, since 50 % of them had a level <40 nmol/l, which is consistent with the results found in other studies. Actually, in France, prevalence of vitamin D insufficiency (defined as 25(OH)D concentrations below 30 nmol/l) reached 15–30 % in the SUVIMAX study [12] (general population) and more than 59 % in the DHE age study [13] (60–79 years healthy subjects), with the lowest values being seen in winter. As a matter of fact, it is considered that serum level from 100 to 150 nmol/l would be necessary to maintain the integrity of bone in osteoporosis prevention strategies [12] and at least 50 nmol/l to optimize intestinal calcium absorption and to avoid secondary hyperparathyroidism (clinical chemistry laboratories now defining vitamin D insufficiency as a serum 25(OH)D level less than 75 nmol/l) [14–16]. In this study, conducted over 3 months, consumption of fermented milk fortified with calcium and vitamin D (20 % of the RDA) resulted in an improvement of vitamin D status, whatever the group, plasma 25 (OH)D level reaching levels above 75 nmol/l. These results agree with those published by Golombick and Diamond [17] who obtained a normalization of serum 25(OH)D (70 nmol/l) after a supplementation providing both calcium (500 mg/day) and vitamin D (500 UI/day) for 3 months in postmenopausal women. This is also consistent with the study conducted in the elderly by Romagnoli et al. [18]. In the same way, 25(OH)D has been shown to play a role in determining bone density, not just in elderly people, but in peri- and postmenopausal women as well [19]. Indeed, healthy postmenopausal women with vitamin D intakes of 100 IU daily can significantly reduce late wintertime bone loss and improve net bone density of the spine over 1 year by increasing their intake of vitamin D [20]. Again, in women in late menopause [21] or in pre- and postmenopausal women (<5 years), Di Daniele et al. [22] confirmed the preventive role of calcium and vitamin D supplementation on bone loss, especially when baseline level is low. On another hand, these changes in vitamin D status were not associated with any variation of plasma CRP concentrations in both groups. In fact, in the NHANES study, vitamin D supplementation elicited reduced CRP levels only among adults with low serum 25(OH)D (<21 ng/ml) [23].

With regard to the primary outcome, bone remodeling, dynamic changes in bone turnover, estimated by measurement of bone biochemical markers, can account for a major portion of anti-fracture efficacy of antiresorptive agents [24]. These parameters are known to be early and predictive of fracture risk, from 3 to 6 months of treatment [25]. In the present work, compared to what is observed in the placebo group, consumption of the fermented milk enriched with calcium, vitamin D, and a blueberry extract significantly improved serum bALP (a marker for osteoblast activity), without any significant change in CTX levels (a marker of bone resorption), only a trend toward a decrease being observed for this parameter. This positive effect on bone metabolism could be explained by the contribution of anthocyanins in the blueberry extract, i.e., the only difference between the two test foods. Effectively, in a population from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) of Scottish women 54.8 years old, between 1997 and 1999, high dietary anthocyanin intakes (22 μg/day) were associated with increased BMD and decreased markers of bone resorption (PYD and DPD) [26]. Actually, such a bone-sparing activity of blueberry has recently been demonstrated in an ovariectomized rat model for menopausal osteoporosis as well [27]. According to this study, this effect could be due to suppression of the ovariectomy-induced increase in bone turnover, as evidenced by

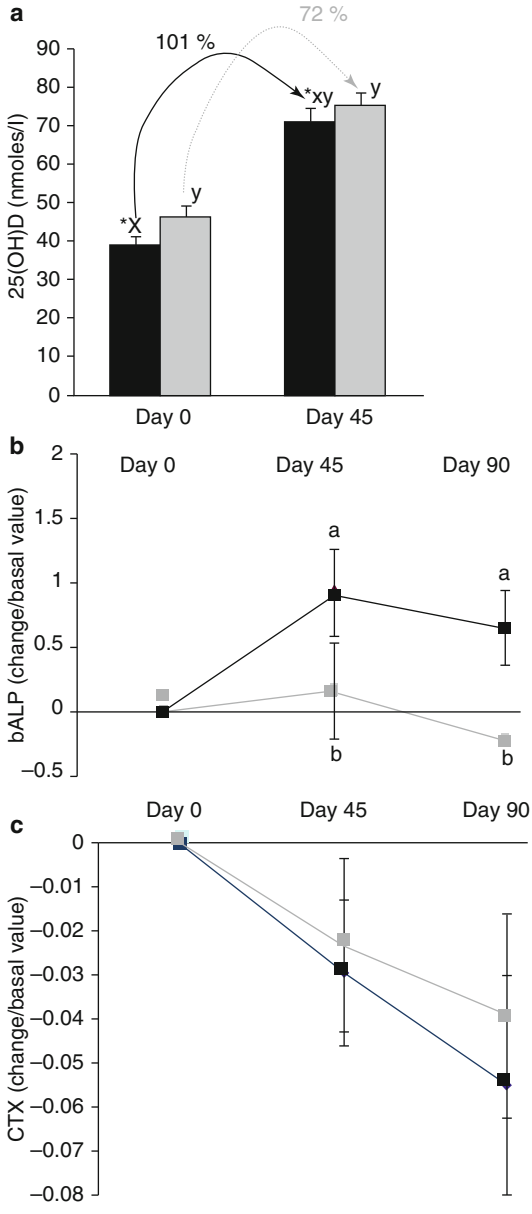

Fig. 37.1 Mean plasma levels of 25(OH)D (**a**), bALP (**b**), and CTX (**c**) in the placebo group (in *gray*), i.e., in postmenopausal women who were given for 3 months a fermented milk enriched with calcium (400 mg/day) and vitamin D (1.6 μg/day), and in the treated group (in *black*) corresponding to consumption of a fermented milk enriched with calcium (400 mg/day), vitamin D (1.6 μg/day), and blueberry (providing 120 mg/day anthocyanins). (**a**) * $p<0.029$; x, y $p<0.0001$. (**b**, **c**) mean percentage changes from baseline for bALP and CTX (a, b: $p<0.017$). Results are expressed as mean ± SEM. Mean values within a row with unlike superscript letters were significantly different

lowered femoral mRNA levels of alkaline phosphatase and type 1 collagen, and increased bone mineral density (compared to what is measured in Sham animals). More precisely, such a protection could result from the phenolic compounds of blueberry such as flavonoids (anthocyanins, catechin, quercetin, epicatechin) or phenolic acids (gallic and caffeic acids) [28, 29]. As a matter of fact, the effect of grape seed proanthocyanidin extract on bone formation has already been documented using mandibular condyles of rats [30–32]. Indeed, a mixture of phenolic acids found in the serum of young rats fed with blueberries was able to significantly stimulate osteoblast differentiation through Wnt signaling pathway, and the p38 MAPK/b-catenin signaling cascade appeared to be a critical molecular determinant of the positive skeletal effects of blueberry [33]. Furthermore, blueberry polyphenol-derived phenolic acids may also contribute to the prevention of osteoblast senescence. Indeed, short-term feeding of a diet containing blueberry, just prior to puberty, can prevent OVX-induced bone loss later on, in adult rats, and early exposure of osteoblastic cells or mesenchymal stromal cells to dietary blueberry can maintain long-term cytoskeletal stability by regulation of myosin and Runx2 genes [34]. The molecular targets (i.e., Sirt 1) have been identified in the senescence pathway whereby blueberry prevents senescence and differentiation to adipocytes [35]. Finally, it has been shown, in healthy adults, that anthocyanin supplementation isolated from bilberries (300 mg/day for 3 weeks) significantly reduced plasma concentration of NF-kB-related proinflammatory cytokines and chemokines, suggesting that anthocyanins possess anti-inflammatory effects as well [36].

Conclusion

In conclusion, this is the first clinical trial demonstrating the bone-sparing potential of blueberry in postmenopausal women. More precisely, consumption of fermented milk enriched with calcium and vitamin D, and such red fruits, for 3 months:

- Has corrected vitamin D status (initially low)
- Has resulted in a favorable orientation of bone metabolism, as indicated by the rise of a biomarker of osteoblast activity, whereas CTX tended to decrease

This benefit is most likely related to the presence of blueberries (rich in polyphenols); the only difference with the placebo group in which bALP was lower. Consequently, these original results demonstrate that, in addition to calcium and vitamin D, other nutrients must be considered in the nutritional prevention strategies of osteoporosis. The nutritional prevention of osteoporosis must thus evolve to new concepts which include the potential exerted by certain micronutrients, even though these promising data need to be implemented by longer term trials allowing bone mineral density assessment.

References

1. Seeman E. Pathogenesis of bone fragility in women and men. Lancet. 2002;359(9320):1841–50.
2. Maravic M, Le Bihan C, Landais P, Fardellone P. Incidence and cost of osteoporotic fractures in France during 2001. A methodological approach by the national hospital database. Osteoporos Int. 2005; 16(12):1475–80.
3. Rizzoli R, Boonen S, Brandi ML, Burlet N, Delmas P, Reginster JY. The role of calcium and vitamin D in the management of osteoporosis. Bone. 2008;42(2): 246–9.
4. Dew TP, Day AJ, Morgan MR. Bone mineral density, polyphenols and caffeine: a ressaissement. Nutr Res Rev. 2007;20:89–105.
5. Das AS, Mukherjee M, Mitra C. Evidence for a prospective anti-osteoporosis effect of black tea (*Camellia sinensis*) extract in a bilaterally ovariectomized rat model. Asia Pac J Clin Nutr. 2004;13(2):210–6.
6. Horcajada MN, Coxam V. Flavonols and isoflavones prevent bone loss in the ovariectomized rat a model for postmenopausal osteoporosis. In: Burckard F, editor. Nutritional aspects of osteoporosis. San Diego: Academic; 2001. p. 325–40.
7. Arjmandi BH, Khalil DA, Lucas EA, et al. Dried plums improve indices of bone formation in postmenopausal women. J Womens Health Gend Based Med. 2002;11(1):61–8.
8. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. Am J Clin Nutr. 2000;71(4):1003–7.
9. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006;54(11):4069–75.
10. Chen CF, Li YD, Xu Z. Chemical principles and bioactivities of blueberry. Yao Xue Xue Bao. 2010;45(4): 422–9.
11. Wauquier F, Leotoing L, Coxam V, Guicheux J, Wittrant Y. Oxidative stress in bone remodelling and disease. Trends Mol Med. 2009;15(10):468–77.
12. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int. 1997;7(5):439–43.
13. Souberbielle JC, Cormier C, Kindermans C, et al. Vitamin D status and redefining serum parathyroid hormone reference range in the elderly. J Clin Endocrinol Metab. 2001;86(7):3086–90.
14. Heaney RP. Vitamin D, and calcium interactions: functional outcomes. Am J Clin Nutr. 2008;88(2): 541S–4.
15. Holick MF. Vitamin D, deficiency. N Engl J Med. 2007;357(3):266–81.
16. Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, Flicker L, Wark J, Jackson RD, Cauley JA, Meyer HE, Pfeifer M, Sanders KM, Stähelin HB, Theiler R, Dawson-Hughes B. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012;367(1):40–9.
17. Golombick T, Diamond T. The effect of a combined oral calcium and vitamin D supplement for treating mild to moderate vitamin D deficiency in postmenopausal women. Clin Interv Aging. 2008;3(1):183–6.
18. Romagnoli E, Mascia ML, Cipriani C, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;93(8):3015–20.
19. Khaw KT, Sneyd MJ, Compston J. Bone density parathyroid hormone and 25-hydroxyvitamin D concentrations in middle aged women. BMJ. 1992;305(6848): 273–7.
20. Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Ann Intern Med. 1991;115(7):505–12.
21. Dawson-Hughes B, Harris SS, Dallal GE, Lancaster DR, Zhou Q. Calcium supplement and bone medication use in a US Medicare health maintenance organization. Osteoporos Int. 2002;13(8):657–62.
22. Di Daniele N, Carbonelli MG, Candeloro N, Iacopino L, De Lorenzo A, Andreoli A. Effect of supplementation of calcium and vitamin D on bone mineral density and bone mineral content in peri- and post-menopause women; a double-blind, randomized, controlled trial. Pharmacol Res. 2004;50(6):637–41.
23. Amer M, Qayyum R. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol. 2012;109(2):226–30.

24. Srivastava AK, Vliet EL, Lewiecki EM, et al. Clinical use of serum and urine bone markers in the management of osteoporosis. Curr Med Res Opin. 2005; 21(7):1015–26.
25. Garnero P. Biomarkers for osteoporosis management: utility in diagnosis, fracture risk prediction and therapy monitoring. Mol Diagn Ther. 2008;12(3):157–70.
26. MC Donald CJ, Fraser WD, Reid DM, MC Donald HM. Dietary anthocyanidin intakes are associated with increased bone mineral density and decreased markers of bone resorption in a population of Scottish women. In: 8th international symposium on nutritional aspects of osteoporosis (Isnao) proceedings, Lausanne; 2012, p. 35.
27. Devareddy L, Hooshmand S, Collins JK, Lucas EA, Chai SC, Arjmandi BH. Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. J Nutr Biochem. 2008;19(10):694–9.
28. Sellappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J Agric Food Chem. 2002;50(8):2432–8.
29. Taruscio TG, Barney DL, Exon J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest Vaccinium berries. J Agric Food Chem. 2004;52(10):3169–76.
30. Kamitani Y, Maki K, Tofani I, Nishikawa Y, Tsukamoto K, Kimura M. Effects of grape seed proanthocyanidins extract on mandibles in developing rats. Oral Dis. 2004;10(1):27–31.
31. Kojima K, Maki K, Tofani I, Kamitani Y, Kimura M. Effects of grape seed proanthocyanidins extract on rat mandibular condyle. J Musculoskelet Neuronal Interact. 2004;4(3):301–7.
32. Ishikawa M, Maki K, Tofani I, Kimura K, Kimura M. Grape seed proanthocyanidins extract promotes bone formation in rat's mandibular condyle. Eur J Oral Sci. 2005;113(1):47–52.
33. Chen JR, Lazarenko OP, Wu X, et al. Dietary-induced serum phenolic acids promote bone growth via p38 MAPK/beta-catenin canonical Wnt signaling. J Bone Miner Res. 2010;25(11):2399–411.
34. Zhang J, Lazarenko OP, Blackburn ML, et al. Feeding blueberry diets in early life prevent senescence of osteoblasts and bone loss in ovariectomized adult female rats. PLoS One. 2011;6(9):e24486.
35. Zhang J, Lazarenko OP, Blackburn ML, Badger TM, Ronis MJ, Chen JR. Blueberry consumption prevents loss of collagen in bone matrix and inhibits senescence pathways in osteoblastic cells. Age (Dordr). 2012; [Epub ahead of print].
36. Karlsen A, Retterstol L, Laake P, et al. Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. J Nutr. 2007;137(8):1951–4.

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