Review

Nutrition in Bone Health Revisited: A Story Beyond Calcium

Jasminka Z. Ilich, PhD, RD, and Jane E. Kerstetter, PhD, RD

University of Connecticut, School of Allied Health, Storrs, Connecticut

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Osteoporosis is a complex, multi-factorial condition characterized by reduced bone mass and impaired micro-architectural structure, leading to an increased susceptibility to fractures. Although most of the bone strength (including bone mass and quality) is genetically determined, many other factors (nutritional, environmental and life-style) also influence bone. Nutrition is important modifiable factor in the development and maintenance of bone mass and the prevention and treatment of osteoporosis. Approximately 80–90% of bone mineral content is comprised of calcium and phosphorus. Other dietary components, such as protein, magnesium, zinc, copper, iron, fluoride, vitamins D, A, C, and K are required for normal bone metabolism, while other ingested compounds not usually categorized as nutrients (e.g. caffeine, alcohol, phytoestrogens) may also impact bone health. Unraveling the interaction between different factors; nutritional, environmental, life style, and heredity help us to understand the complexity of the development of osteoporosis and subsequent fractures. This paper reviews the role of dietary components on bone health throughout different stages of life. Each nutrient is discussed separately, however the fact that many nutrients are co-dependent and simultaneously interact with genetic and environmental factors should not be neglected. The complexity of the interactions is probably the reason why there are controversial or inconsistent findings regarding the contribution of a single or a group of nutrients in bone health.

Key teaching points:

- With prolonged life expectancy and the increasing number of elderly, it is predicted that osteoporotic fractures will reach epidemic proportions.
- A substantial effort has been made toward understanding the effect of nutrients, particularly Ca and vitamin D, on bone accretion during youth and bone loss during aging.
- Bone health depends on the whole range of other nutrients and foods as well as the environmental factors.
- The interactions of nutrients among themselves and with other pharmacological, environmental and life-style factors need to be considered when recommendations regarding bone health are given.
- A prolonged deficiency or excess of one or combination of several nutrients, as well as the changes in requirements of some nutrients due to physiological (growth, development, aging, pregnancy) and/or metabolic (disease, reactions to medications) causes might contribute to the osteoporotic problem.

INTRODUCTION

About sixty years ago, Fuller Albright defined osteoporosis as a disease where there is "too little bone in the bone, but what bone there is, is normal" [1]. By this he meant that, although some bone mass is lost, the chemical composition of the remaining bone is normal. At that time, we knew little about osteoporosis and had no means to treat or prevent it, other than mending the fractures, the ultimate clinical sequelae of the disease. Bone mass (most often expressed as areal bone mineral density) is only one of the components of bone strength. Others include bone quality, structure and turnover. However, it is well established that bone density and bone strength are highly, although imperfectly, correlated [2]. Since it is hard to measure

Address reprint requests to: Jasminka Z. Ilich, PhD, RD, Associate Professor, University of Connecticut, School of Allied Health, 358 Mansfield Road, U-101, Storrs, CT 06269. E-mail: ernst@uconnvm.uconn.edu

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bone quality or structure precisely *in vivo*, particularly in humans, we depend on the measurement of bone mass in assessing overall bone strength and fragility.

In the past few decades, our understanding of bone metabolism and pathogenesis of fractures has grown tremendously because of the improved technology in measuring bone mineral density (BMD) and in identifying and quantitating the markers of bone turnover. In addition, we now understand the importance of maximizing peak bone mass (PBM) during the first few decades of life. The words of endocrinologist, Charles Dent, given 30 years ago in his key-note address to the International Symposium on Clinical Aspects of Metabolic Bone Disease, that "senile osteoporosis is a paediatric disease," are now fully vindicated [3].

It is worth noting that, like in many other conditions, there is a continuum in bone health for any given age group or segment of population [4], which is genetically determined and possibly environmentally modified. Because bone mass in population conforms to a continuum rather than to a sharp bimodal distribution, it is often hard to distinguish between healthy or osteoporotic bone, based simply on a BMD measurement. The quality of a bone and other risk factors have to be taken into consideration. Table 1 presents the current World Health Organization (WHO) criteria for the diagnosis of osteoporosis [5].

Osteoporosis is currently diagnosed based on the level of the patient's BMD compared with the average peak BMD of young adult women [6] and expressed as T-score. The criteria presented in Table 1 are rather arbitrary and have several pitfalls. For example, only white young women are used as the reference population. Difficulty also lies in defining the cut-off point for osteoporosis, given the continuum of bone health and some of the bone strength components not accounted for when measuring only bone density. The criteria for defining osteoporosis are, therefore, being reviewed.

Using the definitions above and the Third National Health and Nutrition Examination Survey (NHANES) III data, conducted between 1988 and 1994, it becomes clear that the prevalence of low femoral BMD is reaching epidemic proportions (Table 2) [7]. The frequently quoted "25 million Americans" affected by this disease have now grown close to 30 million, and even larger numbers of people will be affected by osteoporosis because of increasing life expectancy.

Table 1. World Health Organization (WHO) Criteria for the Diagnosis of Osteoporosis

Category	Criteria (expressed as T-score)
Normal	Patient BMD $\leq$ 1 SD of average peak young adult BMD (T-score, 0 to -1)
Osteopenia	Patient BMD between 1 SD and 2.5 SD below average peak young adult BMD (T-score, -1 to -2.5)
Osteoporosis	Patient BMD $\geq$ 2.5 SD below average peak young adult BMD (T-score, ≥ -2.5)
Severe Osteoporosis	Patient BMD $\geq$2.5 SD below average peak young adult BMD with fragility fractures

BMD = bone mineral density. Osteoporosis is diagnosed by comparing a patient's BMD (expressed as a T-score) with an average peak bone mass of young, normal adult women.

Table 2. Prevalence of Low Femoral Bone Mineral Density in US Non-Hispanic White Adults over the Age of 50 Years [7]

Category	Women	Men
Osteopenia	37–50% or 13–17 million	28–47% or 8–13 million
Osteoporosis	13–18% or 4–6 million	3–6% or 1–2 million

Nutrition is one of the important modifiable factors in the development and maintenance of bone mass and the prevention and treatment of osteoporosis. The nutrients of most obvious importance to bone health are Ca and phosphorus (P), since they compose roughly between 80% to 90% of the mineral content of bone. Protein is incorporated into the organic matrix of bone for collagen structure upon which mineralization occurs. Other minerals and vitamins are crucial in carrying out reactions and metabolic processes in bone.

A new system of defining optimal nutrient intakes for healthy populations in the United States and Canada has been developed and is known as the Dietary Reference Intakes (DRIs) [8]. Unlike the previous Recommended Dietary Allowances (RDAs) [9], where only one level of a nutrient was defined, the DRIs delineate different levels of intakes including the Estimated Average Requirement (EAR), the Recommended Dietary Allowances (RDA), the Adequate Intake (AI), and the Tolerable Upper Intake Level (UL) (Table 3). The DRIs for the bone-related nutrients (Ca, P, Mg, F and vitamin D) were initially published in 1997 and will be updated as scientific knowledge expands [8].

The purpose of this paper is to review the main nutritional determinants of bone health throughout different stages of life and discuss nutritional strategies for primary (during young age) and secondary (later in life) prevention of osteoporosis. Each nutrient is discussed separately; however, many nutrients are co-dependent, and they may interact not only among themselves, but with other genetic and environmental factors. The complexity of these interactions is probably the reason many studies have controversial or inconsistent findings regarding the effects of single nutrients or groups of nutrients in bone health.

BONE CHANGES THROUGHOUT LIFE

Fig. 1 shows the changes that occur in bone mass in various stages of women's lives, along with the principal influences on those changes. During the first two decades of life, bone grows in both length and width. From infancy through young adulthood, bone formation predominates over resorption, resulting in a steady accumulation of bone mass, toward the formation of PBM. Almost half of the adult skeletal mass is gained during the pubescent growth spurt. Ca balance during this time is most

Table 3. Dietary Reference Intakes (DRI) for the Bone Related Nutrients, with the Term "DRI" Encompassing Several Levels of Nutrient Requirements Including the Adequate Intake (AI), Recommended Dietary Allowance (RDA) and Upper Limit (UL)

Life Stage Group	Calcium (mg)		Phosphorus (mg)		Magnesium (mg) (Male/Female)		Vitamin D (mg)		Fluoride (mg) (Male/Female)	
	AI[a]	UL[b]	AI[a] or RDA[d]	UL	AI or RDA	UL	AI	UL	AI	UL
0–6 months	210	ND[c]	100[a]	ND	30/30[a]	ND	5	25	0.01/0.01	0.7
6–12 months	270	ND	275[a]	ND	75/75[a]	ND	5	25	0.5/0.5	1.9
1–3 years	500	2,500	460[d]	3,000	80/80[d]	65	5	50	0.7/0.7	1.3
4–8 years	800	2,500	500[d]	3,000	130/130[d]	110	5	50	1.1/1.1	2.2
9–13 years	1,300	2,500	1,250[d]	4,000	240/240[d]	350	5	50	2.0/2.0	10
14–18 years	1,300	2,500	1,250[d]	4,000	410/360[d]	350	5	50	3.2/2.9	10
19–30 years	1,000	2,500	700[d]	4,000	400/310[d]	350	5	50	3.8/3.1	10
31–50 years	1,000	2,500	700[d]	4,000	420/320[d]	350	5	50	3.8/3.1	10
51–70 years	1,200	2,500	700[d]	4,000	420/320[d]	350	10	50	3.8/3.1	10
>70 years	1,200	2,500	700[d]	3,000	420/320[d]	350	15	50	3.8/3.1	10
Pregnancy										
<19 years	1,300	2,500	1,250[d]	3,500	−/400[d]	350	5	50	−/2.9	10
19–30 years	1,000	2,500	700[d]	3,500	−/350[d]	350	5	50	−/3.1	10
31–50 years	1,000	2,500	700[d]	3,500	−/360[d]	350	5	50	−/3.1	10
Lactation										
<19 years	1,300	2,500	1,250[d]	4,000	−/360[d]	350	5	50	−/2.9	10
19–30 years	1,000	2,500	700[d]	4,000	−/310[d]	350	5	50	−/3.1	10
31–50 years	1,000	2,500	700[d]	4,000	−/320[d]	350	5	50	−/3.1	10

[a] Adequate Intake (AI) is the goal intake for an individual or a group to sustain health and reduce disease risk.

[b] Tolerable Upper Intake Levels (UL) is defined as the maximal level of daily nutrient intake that is unlikely to pose a risk of adverse health effects for any but a few individuals within the life stage group. This term connotes the amount that, with high probability, can be tolerated biologically.

[c] ND = not determined because of lack of data.

[d] Recommended Dietary Allowance (RDA) is the nutrient intake level that is sufficient to meet the requirements of 97% to 98% of the population.

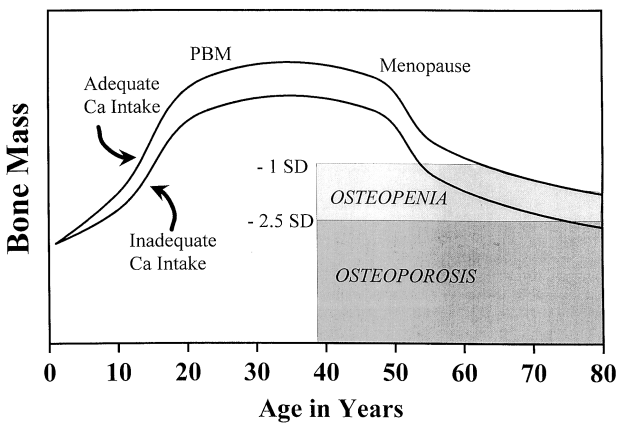

Fig. 1. Changes in bone mass in women (arbitrary units) with age. Principal influences on bone mass are genetics, hormonal status, mechanical loading and Ca intake (throughout life) and structural errors (mostly later in life). Ca = calcium, PBM = peak bone mass.

positive and ranges from 200 to 300 mg/day [8, 10]. At the completion of puberty, bone continues to increase in thickness and density into young adulthood, when PBM is achieved. It has been assumed that individuals with higher PBM achieved in early adulthood will be at lower risk for developing osteoporosis later in life. Therefore, since rapid skeletal mineral acquisition occurs relatively early in life, the exogenous factors that might optimize peak bone mass to its maximal genetic potential need to be identified.

After PBM is reached, bone loss begins and persists until the end of life. Loss of bone with advancing age is an universal phenomenon present in women and men of all populations studied so far. From the time of middle adulthood, bone resorption predominates over formation, resulting in steady loss of bone mass, particularly pronounced in women in first 5 to 15 years after menopause due to estrogen cessation. Therefore, the main determinants of osteoporosis are the level of PBM and the subsequent rate of bone loss. Accordingly, these are two areas in which preventive strategies can be developed: primary prevention aimed at maximizing the PBM in formative years and secondary prevention, in adulthood and after menopause, aimed at reducing bone loss with age.

CALCIUM

The adult human body contains about 1000 to 1500 g of Ca (depending on gender, race, size of the body) of which 99% is found in the bones in the form of hydroxyapatite. For this reason, Ca is probably the most studied nutrient in the area of bone health. Dietary Ca requirement is determined mostly by skeletal needs, and it exerts a threshold behavior. This means that the skeletal response (in this case, skeletal accretion) will occur only when Ca is increased from the deficiency level to a threshold zone. Adding more Ca when the level of dietary intake already exceeds the threshold will not likely improve

bone mass. Matkovic and Heaney determined threshold Ca levels for children, adolescents and young adults based on a review of 34 published balance studies [11]. The threshold behavior of Ca in adolescent females was confirmed more recently in a study examining the relationship between Ca intake and urinary Ca excretion [12]. The later study showed that, at Ca intakes of about 1500 mg/day (threshold level for adolescents, as determined by Matkovic and Heaney [11]), urinary Ca starts to rise more rapidly, indicating that skeletal saturation with Ca was reached. Ca threshold for adults is approximately 1100 mg. Therefore, the typical baseline Ca intake of the subjects becomes important when evaluating the literature for efficacy of dietary Ca on bone. For example, if baseline intake was already at the threshold level, additional Ca would not be expected to improve bone.

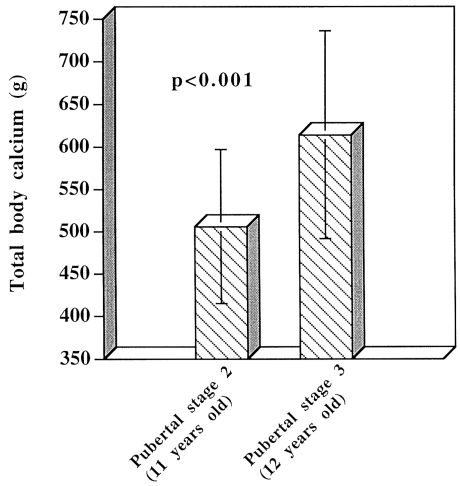

Fig. 2. Total body calcium (mean ± SD) measured by dual energy X-ray absorptiometry over a one-year period in 364 teenage girls progressing from pubertal stage 2 to 3. (Adapted from [10].)

Growth and Development

There is tremendous interest in Ca intake in American youth, since the skeleton matures at a relatively early age. In young American women, 90% of total bone mineral content was attained at age 17 and 99% was achieved by age 26 [13]. The peak bone density in hip and vertebrae is achieved between ages 17 and 20 [14]. Recent studies by Molgaard *et al.* [15] suggest that bone accretion is significantly associated with pubertal stages in girls and boys. The peak annual accretion of bone mineral content was reached earlier in girls (12.5 years) than in boys (14.2 years). Assuming that Ca composes 38% of bone mineral, Ilich *et al.* [10] showed that young girls are able to accumulate approximately 108 g of Ca in one year when progressing from pubertal stage two to pubertal stage three. This amount requires a daily positive Ca balance of about 300 mg, demonstrating the importance of adequate Ca during growth [10] (Fig. 2).

The studies (both cross-sectional and longitudinal) that relate dietary Ca intake to bone health are relatively consistent and show a positive effect of Ca on BMD. Those which do not either were not specifically designed to evaluate the effect of Ca on bone or contained a small sample size in each age group [16, 17]. Cross-sectional studies conducted in children (Caucasian and Chinese) [18–20], adolescents [21, 22] and young women [23] indicate that higher Ca intakes are associated with higher bone mass at almost all measured skeletal sites. African American children and adolescents show higher Ca absorptive efficiency than Caucasians [24], a circumstance which may contribute to their higher bone density [25].

Most of the Ca intervention studies performed in children and adolescents also show a positive effect of Ca on bone accretion [26–35]. There have been at least nine longitudinal Ca intervention studies done in girls and boys published to date [26–29, 31, 32, 34–36]. In general, when Ca is supplemented (either as food or a supplement) in girls and boys, bone mineral accretion improves between 1% and 5% at all sites measured. The increment is improved up to 10% when dairy products are used as a source of supplemental Ca [28], and the improvement is more pronounced when baseline Ca intake is low. The length of Ca intervention in most of these studies ranges from 6 to 18 months. The long term effect of the gain in accretion on peak bone mass is not well understood.

Some of the longitudinal clinical trials in children and adolescents showed that the difference in bone mass gained as a result of Ca supplementation disappears when supplementation is terminated [37–40]. A possible explanation for the diminishing difference between placebo and Ca groups could be the "bone remodeling transient" phenomenon. It is speculated that Ca supplementation suppresses bone turnover (due to the suppression of parathyroid hormone), leading to a transient increase in measurable bone mass which then disappears after Ca is withdrawn [41]. In children and adolescents one remodeling cycle might last for about six months. Therefore, this should be the time to begin measurement of the rate of BMD increase, presumably resulting from Ca supplements, and compare it with BMD in the placebo group. If supplementation continues, bone remodeling suppression will continue as well. The above clinical trials lasted from 6 to 18 months, and the final BMD measurements (revealing the diminished difference between groups) were usually performed one year after the study termination. Therefore, some of the studies might not have been long enough to establish a real increase in bone mass caused by Ca supplements. It is likely that, for the difference in bone mass to persist throughout puberty, high Ca intake should be maintained all the time to suppress bone turnover within the expanding periosteal envelope.

The gain in bone density throughout the first several decades translates to lower risk of fracture later in life, and there are two epidemiologic studies that support this contention. They examined bone mass in populations accustomed to different Ca intakes over a lifetime [42, 43]. Both studies were

cross-sectional: one in a Croatian and another in a Chinese population. Differences in bone mass in both men and women living in high and low Ca regions were present during young adulthood and continued into old age. These studies indicate that Ca was an important agent for skeletal formation affecting PBM and subsequent rates of bone fractures. Retrospective studies in adults support the above conclusions. Dietary Ca from the distant past (childhood and adolescence) was a significant predictor of current adult bone mass [44–49].

Overall, it is likely that variations in Ca nutrition early in life can account for as much as a 5% to 10% difference in peak adult bone mass. Such a difference, although small, could potentially contribute more than 50% to the hip-fracture rates later in life [42, 50].

Young and Middle Adulthood

Because BMD is relatively stable between the ages of 20 and 50, there are relatively few studies evaluating the effect of Ca on bone health during young and middle adulthood. A meta-analysis of the effect of Ca intake on bone mass in women and men (aged 18 to 50 years) was performed by Welten *et al.* [51]. They analyzed 33 eligible studies: 27 cross-sectional, two longitudinal and four interventional studies and found significant positive correlation between dietary Ca and bone mass. In the two interventional studies, supplementation of 1000 mg Ca in premenopausal women prevented bone loss of about 1% per year in all measured skeletal sites, except the ulna. The overall bone loss for this population is about 0.5% to 1.5% per year.

The conclusions from the above analysis are consistent with those from the meta-analysis conducted by Anderson and Rondano [52]. They summarized the effects of dietary Ca on PBM accrued by premenopausal women during their 20s and 30s and found that both prospective and cross-sectional studies showed a positive effect of Ca intake on bone mineral content. In five interventional studies, the inclusion of Ca-rich foods or Ca supplementation increased or maintained PBM in comparison to control or nonsupplemented groups. Likewise, in 20 cross-sectional studies, there was a beneficial effect of adequate Ca intake on PBM. Presumably, in all these cases the PBM reached was at or close to the maximal PBM within each subject's genetic potential.

Pregnancy and Lactation

Of particular interest are the changes in Ca and bone metabolism that occur during pregnancy and lactation. There is speculation that increased physiological requirements for Ca in pregnancy and lactation might lead to hazardous and lasting changes on maternal skeletal integrity. An infant is born with about 25 to 30 g of skeletal Ca, which is mostly diverted from maternal stores. Lactational output of milk to meet the infant's needs in the first four months is about 720 to 750 mL/day, leading to the maternal loss of approximately 250 mg of Ca/day, resulting in a temporary bone loss [53–56]. However, earlier cross-sectional and epidemiological studies (some conducted on women accustomed to low Ca intakes, some on Bantu women with up to six closely spaced pregnancies) did not find an association between pregnancy and bone mass [57, 58]. Pregnancy is associated with hyperestrogenemia and weight gain, both of which have protective effect on bone. In addition, the later part of pregnancy is characterized by increased levels of the active form of vitamin D and increased Ca absorption, all leading toward protection of bone integrity. Some recent studies were more or less in agreement that women do lose bone during lactation, but return to baseline level after weaning [53–56], especially if the lactation is confined to three months or less (a common breast feeding practice in the United States) [59].

The source of Ca during these periods of great physiologic demand of pregnancy and lactation has not been entirely elucidated. Theoretically, the Ca could originate from increased dietary intake, higher intestinal absorption, increased bone resorption, decreased urine Ca excretion or any combination thereof. Two recent, well-conducted studies [54, 60] suggest that dietary Ca is not a major contributor to the changes in bone observed throughout pregnancy and lactation. Ritchie *et al.* [60] found that fetal demand for Ca was met by an increase in maternal intestinal Ca absorption, and, during lactation, the additional Ca is provided by maternal renal Ca conservation. Five months after menarche resumed in the post-lactating women, trabecular bone density (but not total body bone mineral) was restored to pre-pregnancy values [54]. Both studies agree that the dramatic changes in maternal bone and Ca metabolism occur independently of dietary Ca supply; hence, there is no need for additional Ca intake above current recommendations [8].

While the bone loss in pregnancy and lactation in mature women is self-limiting and a transient phenomenon, the same may not be true in pregnant teenagers, particularly if Ca intake is low. This is the time when skeletal growth and bone consolidation of a pregnant teenage female pose extra demand for Ca beside just mineralization of the fetal skeleton or provision for milk during lactation. Preliminary data of Chan *et al.* [61] and Scholl *et al.* [62] point in that direction. Definitive data, particularly with regard to the long-term effect on peak bone mass and adult height, are lacking. Since the number of teenagers, as well as the number of teenage pregnancies, is rising in the US, it would be important to clarify these issues.

Later Adulthood

The gonadal hormones have a tremendous impact on bone health, and this becomes most clear in the postmenopausal woman. The cessation of estrogen secretion in women at the menopause (or testosterone secretion in men) contributes to accelerated bone loss. If untreated, a woman can lose 20% to 30% of cancellous bone (also known as trabecular or spongy

bone) and 5% to 10% of cortical bone (also known as compact bone, found primarily in long bones) between age 50 and 60 [63].

When evaluating the effect of dietary Ca on BMD, it is important to distinguish early from late menopause. For the most part, interventional studies done during the early postmenopausal period (within the first five to eight years after menopause) demonstrate that the effects of supplemental Ca are relatively small and appear to be confined to cortical, rather than trabecular bone. A meta-analysis in early postmenopausal women was performed by Cumming [64] and included 49 separate mostly cross-sectional studies. There was a positive correlation between bone mass and Ca intake, such that for each 500 mg increase in dietary Ca, there was a 0.5% to 1% less cortical bone loss, but not trabecular. As expected, the effect was greatest when the baseline Ca intake was low, supporting the threshold hypothesis. Subsequent interventional studies support Cumming's conclusions [65–68]. Given the large impact of estrogen withdrawal on bone during the early menopausal period, the effect of Ca is small but, nevertheless, an important one.

In general, the effect of dietary Ca on bone loss in the late postmenopausal woman is more pronounced than during the early postmenopausal period. There are at least six studies documenting an increase or maintenance in BMD in mid to late postmenopausal women when additional Ca was given either as a food or supplement [65, 66, 69–72]. Again, not surprisingly the largest improvement is observed when the baseline Ca intakes are the lowest [65]. The combination of estrogen and dietary Ca is more effective than either treatment alone in the late postmenopausal women [73], particularly if Ca intake is low [74]. Conversely, it is quite clear that the bone loss observed in untreated menopausal women is exacerbated by a dietary Ca deficiency [75].

Although the increase in BMD from additional Ca intake is encouraging, the most important outcome variables are bone fractures. An increase in BMD alone would not be all that helpful, if there were no concurrent decrease in fractures. There are at least four studies showing around a 30% reduction in fracture risk in postmenopausal women taking 1000 mg Ca supplement per day [72, 76–78]. In a meta-analysis of 16 observational studies of dietary Ca and hip fractures, there was a small but consistent reduction of fractures [79]. The data suggested that 1 g of dietary Ca/day is associated with a 24% reduction in the risk of a hip fracture. Whether we consider both the BMD and the fracture data, most of these studies are consistent and support the public health policy for increasing Ca intake in older adults. It is worth mentioning that some of the studies were done with simultaneous vitamin D supplementation; as a result, the benefits are probably due to the combined effects.

For more on Ca and bone health and evaluation of published scientific data, readers should refer to the most recent monumental review by Robert Heaney [80].

Calcium Intake

Despite the abundance of evidence supporting the positive effects of dietary Ca on bone, national surveys indicate that Ca intakes in females of all age groups in the US are consistently lower than current recommendations. The 1994 USDA Continuing Survey of Food Intakes by Individuals (CSFII) showed that mean Ca intake in males over the age of nine years is 925 and females over the age of nine years is 657 mg/day. Data from the HANES III survey are consistent with the CSFII. Increasing Ca intake was a primary objective in the Healthy People 2000 and remains a primary objective in the newest version of Healthy People 2010 [81].

Our national Ca deficient diet, particularly for women, places a significant financial burden on our health care system. Bendich *et al.* [82] estimated the cost-effectiveness of daily Ca supplementation for the prevention of primary osteoporotic hip fractures using the HANES III data. The authors estimate that 2.6 billion dollars in direct medical costs would be avoided if individuals over the age of 50 would consume approximately 1200 mg of supplemental Ca. It is important to note that the effect of dietary Ca on bone is weaker than that of estrogen, bisphosphonates or calcitonin, and Ca alone should not be considered a sole therapy for osteoporosis. However, adequate Ca intake is the basis from which any other therapy or treatment should start [83].

On an opposite note, we need to bear in mind that, on average, about 15% to 40% of people (varying with age, race or gender) are taking mineral and/or vitamin supplements [84]. Due to the growing awareness and attention that osteoporosis has received in recent years, Ca supplement intake has increased. In addition, many foods are now being fortified with Ca (among them, orange juice, breakfast cereals and margarine). Whether it is Ca or any other mineral and/or vitamin, supplements should be taken with caution. While Ca supplementation is justified for most women, there is a possibility that it may cause some adverse effects and imbalance with other cations if taken in excess. In general, the upper tolerable limit of 2,500 mg/day (including diet and supplements) should not be exceeded for a prolonged period of time [8]. Persons at risk for developing milk-alkali syndrome (antacids as Ca supplements are very popular), thiazide users and those with renal failure should be most cautious. The possible interactions of Ca with other minerals are discussed separately in the sections below.

The safety of supplements is not determined just by the amount of intake. Potential contamination of Ca supplements with lead and/or aluminum is a problem that was first recognized in the early sixties when bonemeal, dolomite and fossilized oyster shells and other "natural" supplements became popular [85]. This prompted US Food and Drug Administration warnings and enactment of numerous state and federal measures toward reducing permissible levels of environmental and industrial lead and aluminum exposure [86]. Based on the recent laboratory analysis of lead content in 136 brands of Ca

supplements purchased in 1996, it seems that the levels of lead are lower now than some 10 to 20 years ago [87]. That is particularly true for synthesized and refined Ca supplements and infant formulas, while "natural" products still might contain higher amount of lead and more often exceed federal limits [87].

PHOSPHORUS

As an inorganic element, phosphorus (P) is second to Ca in abundance in the human body with 85% of the body's P bound to the skeleton. P is widely distributed in foods including meat, poultry, fish, eggs, dairy products, nuts, legumes, cereals, grains and cola beverages. It is also added in processing foods. The primary purpose of dietary P is to support growth and to replace losses. Dietary P intakes have risen 10% to 15% over the past 20 years because of the increased use of phosphate salts in food additives and cola beverages [8]. It should be noted that nutrient databases may not reflect these changes and may underestimate actual P intake, particularly when processed foods are a mainstay [88]. Dietary P intakes in US adults range between 1000 and 1500 mg/day, a level well above current recommendations of about 700 mg/day.

Although P is an essential nutrient, there is concern that excessive amounts may be detrimental to bone. For example, a rise in dietary P increases serum P concentration, producing a transient fall in serum ionized Ca resulting in elevated parathyroid hormone (PTH) secretion and potentially bone resorption. The primary function of PTH is to prevent hypocalcemia by increasing bone resorption of calcium. The hypothesis that excess dietary P is harmful to bone was tested in young adults consuming controlled diets containing 1660 mg P and 420 mg Ca. Within 24 hours, the diet resulted in elevated indexes of PTH action [89] that persisted for four weeks [90]. Animal data confirm that the combination of high P and low Ca diets is deleterious to bone mass [91]. However, it is difficult to differentiate the detrimental effects of low Ca from that of high P.

On the other hand, there are data that show the transient decline in serum Ca induced by a P load is caused by an inhibition of PTH-mediated Ca release from bone, thus conferring beneficial effects on bone [92]. Human studies using Ca kinetic methodology showed no effect on bone turnover from doubling P [93], a conclusion supported by a nonisotopic study done in young men and women [94, 95]. The P intake typically consumed in the US diet probably does not adversely affect bone health [8].

A frequently raised issue is the potential adverse effect of consumption of carbonated beverages. Some studies have shown decreased bone mass and elevated fracture rates with the consumption of carbonated beverages [96–98], while others have not shown such a relationship [99]. A possible explanation for the adverse effect of carbonated beverage on bones could be due to the resulting acid load caused by the ingestion of phosphoric acid used as an acidulant. However, other available studies do not differentiate between the beverages made with phosphoric acid or other acidulants, thereby making the proton load effect unclear. The reported adverse effect of carbonated beverages on bones might be due simply to the displacement of milk from the diet and thus to lower Ca intake, rather than to any other plausible mechanism.

MAGNESIUM

There are approximately 25 grams of magnesium (Mg) in the human body, two-thirds of which are in the skeleton and the rest in soft tissue. The Mg in bone is not the integral part of the hydroxyapatite crystal structure (like Ca and P); rather, it is adsorbed on the surface of the crystal. Only a small fraction of Mg in bone is freely exchangeable with extracellular Mg [100]; however, it plays an important role in Ca and bone metabolism. Magnesium deficiency alters calcium metabolism, resulting in hypocalcemia, vitamin D abnormalities and neuromuscular hyperexcitability. The primary reason for the hypocalcemia commonly observed in Mg deficiency is impaired PTH secretion [101].

Animal studies show that Mg deficiency results in decreased bone strength and volume, poor bone development [101, 102] and uncoupling of bone formation and resorption [103, 104]. For these reasons, it is thought that Mg deficiency may be a risk factor for osteoporosis. Consistent with the animal studies, numerous populations' studies demonstrate a positive association between magnesium intake and BMD [105–108]. Most recently, Tucker *et al.* [109] found that Mg intake was positively associated with hip BMD in both men and women of the original Framingham Heart Study cohort. Several studies found no correlations between Mg intake and bone density [110, 111].

The effect of Mg supplementation in humans is poorly understood because of few well-controlled clinical trials. In osteoporotic postmenopausal women, Mg supplementation for one year improved radial bone mass [112]. There were no further increases in BMD at two years on these Mg supplemented women. A second intervention study showed that 600 mg Mg, 500 mg Ca and a multivitamin-mineral supplement improved calcaneous BMD in postmenopausal women in less than a year [113]. The subjects were also taking estrogen, so it is difficult to tease out the potential benefit of the hormones, calcium or multivitamin-mineral preparation.

National surveys consistently show low intakes of Mg among females of all age groups, but particularly among teenagers [114]. The deficiency becomes even more pronounced with the new increased recommendations for Mg [8]. A recent report on Mg balance by Andon *et al.* [33] showed that teenage girls with low Mg intake ($<$177 mg/day) were in negative Mg balance. It is noteworthy that the 1997 RDA for teenage girls increased from 280 mg/day (1989 RDAs) to 360 mg/day. Andon *et al.*'s study [33] did not show any adverse effect of Ca

supplementation (total intake approximately 1700 mg Ca/day) on any components of Mg metabolism.

Good sources of Mg in food are whole grains, vegetables (broccoli, squash), nuts and seeds. Dairy products and meats also contribute magnesium to a diet, as well as chocolate and coffee, depending on the amount consumed. "Hard" water contains high concentrations of Mg, and can be considered a dietary source. Although our diets are marginally low in magnesium, we know very little about how Mg affects bone health in humans.

FLUORIDE

Fluoride (F) is an ultratrace element, occurring in minute amounts in food and water supplies. It is completely and readily absorbed in the gastrointestinal tract by passive diffusion. Once absorbed, F readily crosses cell membranes and becomes incorporated into the teeth and bones. There is a strong affinity toward F in bones, particularly during growing period. Because F is not easily released from bone, F toxicity (fluorosis) may be a problem.

An early survey in North Dakota showed the incidence of osteoporosis was lower in an area where F was naturally high in the water [115]. Drinking water fluoridation (at 1 mg/L) is well known to prevent dental caries, but its effect on bone is unknown. Optimal drinking water fluoridation does not appear to alter bone mass in humans (as evidenced from epidemiological studies), nor is the effect on hip fractures clear [116–118].

In the process of bone mineralization, or deposition of minerals into the collagenous matrix of bone to form hydroxyapatite crystals, other species (other than Ca and P) may be incorporated, substituting for Ca in the crystal lattice and usually yielding crystals that are smaller, more soluble and imperfect in size (e.g., with incorporation of Mg or strontium) [119]. This is not a case with F. F incorporation into bone increases the size and, therefore, decreases the solubility of the apatite crystals [120]. This was a rationale for using F supplements for treatment of osteoporosis, as larger crystals are more resistant to osteoclastic attack. However, if the crystals are excessively large, as in the case of skeletal fluorosis, bones may become brittle and more fragile [120].

Do sodium fluoride (NaF) supplements benefit bone and treat osteoporosis? In addition to increasing hydroxyapatite crystals, fluoride seems to be a potent stimulator of osteoblastic bone formation acting primarily on trabecular bone [121] and resulting generally in the 5% to 10% annual increase in spinal bone mass. Although the precise mechanism of F action on bones is not completely clear, it seems that it exerts its effect by sensitizing various skeletal growth factors through inhibition of osteoblastic acid phosphatase [122] or stimulation of osteoblastic replication [123] or both. These properties triggered a great enthusiasm for NaF as a very cheap, relatively safe and effective drug for osteoporosis. However, the results of the pioneering clinical trials conducted in parallel in mid eighties at Mayo Clinic, Rochester, MN, [124] and Henry Ford Hospital, Detroit, MI, [125] were disappointing. Both studies, four-year randomized, double blind, placebo-controlled trials of NaF supplementation in postmenopausal, osteoporotic women showed increase in vertebral bone densities but no decrease in vertebral fracture rates and increase in non-vertebral fractures. Both studies were criticized for a possible high levels of NaF (75 mg/day) used and that better results could have been achieved with lower doses.

Many other similar trials with different NaF dosages were subsequently conducted; however, the data on the effect of F on bone fractures are still inconsistent [126, 127]. Some authors found decreases in fracture rates in the fluoride supplemented subjects [128, 129], while others found negative or inconclusive results [127, 130]. Slow release NaF as a therapy for osteoporosis is used in many European countries; however, its approval in the United States by the Food and Drug Administration is still pending.

IRON

Iron (Fe) may play an important role in bone formation acting as a cofactor for enzymes involved in collagen synthesis [131]. Bone breaking strength was lower in Fe deficient rats, suggesting that Fe deficiency may play a role in bone fragility [132]. We recently examined the relationship between bone mass and ferritin in a four-year clinical trial of Ca supplementation in adolescent girls [133]. There was a trend for a positive association between BMD of forearm and serum ferritin at baseline. A similar trend was noticed between the total body bone mineral density and content and serum ferritin in year 4 of the study, but only in the placebo group [133]. Further studies are necessary to clarify the above trend, particularly in people who are Fe deficient.

Fe absorption may be inhibited by the high intakes of other minerals and trace elements, particularly Ca. Numerous studies have shown the inhibitory effect of Ca on Fe from different supplements (salts) or Ca-containing foods [134–138]. However, when Ca consumption occurs separately from the meal containing Fe, the effect is less clear [139, 140]. Prather and Miller [141] used a rat hemoglobin repletion assay to determine if the inhibitory effect of Ca was due to the Ca, the accompanying anion or a combination of the two. Low, medium and high doses of Ca carbonate, Ca sulfate, Na carbonate or Na sulfate were added to the repletion diet. Ca carbonate reduced iron bioavailability in a dose related manner. Ca sulfate and Na carbonate also decreased iron bioavailability, but only at the highest dose. Based on these observations, it was concluded that both the cation and the anion contribute to the inhibitory

effect. Since Ca inhibits both heme and non-heme Fe absorption, Hallberg *et al.* [142] suggested that the inhibition is occurring in the intestinal mucosal cells where Ca interferes with Fe transport.

However, it is not clear to what extent, if any, higher Ca intake (even when it interferes with Fe absorption) might influence Fe stores in population and what would be the consequences of lower Fe stores on bone mass. There is no effect of Ca on serum ferritin (indicator of Fe stores) in infants [143], adolescent girls, even after a long term supplementation with Ca [133], adults [138] or lactating women [53].

On an opposite note, Fe might act as a toxin to bone cells and contribute to osteoporosis or other bone diseases in people with impaired Fe metabolism and Fe overload. Most typical such cases are in hemochromatosis, African ("Bantu") hemosiderosis, chronic renal diseases (including renal osteodystrophy) and any case of Fe overload with prolonged and repeated Fe therapy or hemotransfusion. It is not always clear whether the insult to bone comes from iron itself, Fe overload-induced hypovitaminosis C or both [144]. Conte *et al.* [145] compared BMD and bone histomorphometric analyses among patients with primary hemochromatosis, alcoholic cirrhosis and controls. Densitometric and histomorphometric results indicated impairment of trabecular bone in both patient groups compared with controls, while cortical impairments were limited only to hemochromatotic patients. Similar findings resulted from the study of osteoporosis in African hemosiderosis patients [144]. However, in the situations where both Fe overload and alcohol are involved, to what extent the pathological changes are caused by iron alone, by chronic alcoholism or by the associated nutrient disturbances is not known. In chronic kidney diseases, various bone-histomorphometric lesions are attributed to iron and/or other trace mineral overloads, usually as a result of dialysis [146, 147].

Although most breakfast cereals and flour are fortified with Fe, its bioavailability from those sources is low. It is also found in dark green vegetables, like spinach (with the lower bioavailability as well). The best Fe sources are red meats, particularly liver and other organ meats.

ZINC

The human body contains one to two grams of zinc (Zn) and about 90% is found in muscle, bone, skin and hair, while blood contains less than 1%. Zn plays an important role in connective tissue metabolism, acting as a cofactor for several enzymes, such as alkaline phosphatase (necessary for bone mineralization), and collagenase (essential for the development of the collagenous structure of bone) [148].

Zn deficiency results in impaired DNA synthesis and protein metabolism, which lead to negative effects on bone formation [148]. The role of Zn in bone formation is well documented in animal models [149], and low serum levels of Zn and excessive urinary excretion are related to osteoporosis in humans [150, 151]. Zn concentration in bone is greatly reduced during Zn deficiency [152]. A beneficial effect of Zn supplementation was observed in vertebral and femoral bone mass in rats during strenuous treadmill exercise [153].

Zn is abundant in animal protein foods (red meat, poultry, fish, oysters, eggs), legumes, whole-grain breads and milk. However, the population that may be susceptible to a mild to moderate Zn deficiency are infants and adolescents, due to increased requirements for growth and, in the case of latter, poor eating habits [154, 155]. Several dietary constituents may decrease the bioavailability of Zn including phytic acid, dietary fiber, low dietary protein and Ca [156]. Although animal studies show that Ca interferes with the intestinal absorption of Zn [157], human studies are less convincing [158–160]. Long term supplementation of 1000 mg Ca/day (from Ca citrate malate) did not affect any components of Zn metabolism (balance, urinary or fecal excretion) in adolescent girls already consuming low amounts of Zn [161].

COPPER

The body contains about 75 to 100 mg copper (Cu) which is mostly accumulated during growth. Deficiency of Cu is rare as Cu is present in nearly all foods, particularly legumes, nuts, whole grains, beef liver and shellfish. The intake of Cu in the US ranges widely (from 0.7 to 7.5 mg/day) and the safe and adequate intake ranges from 2 to 3 mg/day [9].

Because Cu influences collagen maturation, it could influence bone composition and structure. The enzyme lysyl oxidase is a copper-containing enzyme that catalyses crosslinking of lysine and hydroxyproline in collagen, contributing to the mechanical strength of collagen fibrils [162]. Cu deficiency results in decreased bone strength in rats [132, 163] and chicks [164].

SODIUM

There is a positive relationship between urinary sodium (reflecting Na intake) and urinary Ca excretion. Since the late fifties and early sixties [165, 166], it has been repeatedly shown in animals and humans that dietary sodium, in the form of salt (NaCl) increases urinary Ca excretion [12, 167, 168]. On average, for every 100 mmol (2,300 mg) of Na excreted in urine, there is about 0.6 to 1 mmol (24–40 mg) loss of Ca, in free-living healthy population of various ages [12, 167].

Although it is clear that Na is an important determinant of obligatory Ca loss in urine and causes bone loss in animals (especially at lower Ca intakes) [169, 170], there are only a few studies examining its effect on bone mass in humans. Forearm BMD was significantly and negatively correlated with 24-hour urinary Na excretion in a cross-sectional study of 440 healthy postmenopausal women [171]. Results from cross-sectional

studies, one in elderly men and women [172] and another in preadolescent females [12], show strong correlation between urinary Na and Ca. However, there were no direct effects of urinary Na on BMD at spine, hip, forearm or whole body [12, 172]. In the only longitudinal study examining bone mass and urinary Na, Devine *et al.* [173] showed that changes in urinary Na were negatively correlated with changes in BMD of the hip and ankle in postmenopausal women.

Other, indirect evidence of adverse effects of Na on bone comes from short-term interventional studies with Na loading or restriction and markers of bone turnover. Evans *et al.* [174] showed that postmenopausal, but not premenopausal women, responded to a one-week high Na intake of 300 mmol/day by an increase in deoxypyridinoline (bone resorption marker). In a cross-sectional study of free-living Japanese men and women ranging in age from 20 to 79 years, Itoh *et al.* showed that the excessive Na intake was associated with higher deoxypyridinoline concentrations, thereby increasing bone resorption [175]. Other studies evaluating the effect of dietary Na on bone markers are inconclusive [176, 177].

The interaction between Ca and Na becomes even more important when considering the trends in intakes of each: Ca intake is lower than recommendations, and Na intake remains consistently high. The estimated minimal requirements for adults are 500 mg/day [9], and the American Heart Association recommendations are at 2,400 mg/day or less [178]. Yet dietary Na intake is generally much higher than recommendations [179]. While the influence of Na on blood pressure is still controversial, its hypercalciuric effect is well established. However, whether habitual salt excess decreases bone mass and presents a risk factor for fracture incidence is still not established. There is a need for prospective, longitudinal studies with repeated bone mass measurements and the assessment of markers of bone turnover after a longer period of intervention with sodium within the range of usual intake (1,500–7,500 mg/day).

VITAMIN D

The vitamin D endocrine system influences Ca and P metabolism by affecting the target organs: intestine, bone and kidney. The active metabolite, 1,25$(OH)_2$vitamin D_3 (calcitriol) facilitates active Ca absorption in the intestine by stimulating the synthesis of Ca binding protein (calbindin). Vitamin D is also involved in bone turnover, and a deficiency may cause rickets in children and osteomalacia in adults (both characterized by defective mineralization of bone).

We recently demonstrated the important role of calcitriol on bone mass accretion in pubertal girls. Calcitriol concentration was the highest during peak growth (pubertal stages 3 and 4), probably due to the high skeletal demands for Ca. Baseline calcitriol levels also predicted annual change in total body and forearm bone mass of adolescent girls [180].

Vitamin D status declines with age for many reasons: lower exposure to sunlight (particularly in the northern latitudes during winter months), decreased ability to activate precursors in the skin, decreased ability of the kidney and liver to hydroxylate vitamin D, lesser end-organ response to calcitriol itself, reduced dietary intake and diminished absorption from food, as well as the use of anticonvulsant and/or steroid drugs. Consequently, vitamin D deficiency in the aged is not uncommon, particularly in the frail elderly living in northern industrialized cities. Approximately half of the medical inpatients in the Boston-area had low levels serum 25(OH)vitamin D [181]. A similar trend was observed in homebound elderly [182]. However, apparently healthy noninstitutionalized adults in the US have a much lower incidence of hypovitaminosis D [183].

Blood level of 25(OH)vitamin D (an indicator of vitamin D status) varies seasonally. As recently shown, the increase in 25(OH)vitamin D from winter to summer is much lower in black than in white women and inversely related to PTH [184]. Although black women have denser bones despite the lower 25(OH)vitamin D [185], there may be negative consequences that contribute to their higher rate of bone loss at more advanced age. The results from the Study of Osteoporotic Fractures showed no benefits of vitamin D supplements to fracture rates [186]. It was reported earlier that substantial proportion of patients with hip fractures also have osteomalacia, caused by vitamin D deficiency [187]. Vitamin D deficiency may also be associated with reduced muscular function [188], which may increase risk for falling. There are just a few foods that are naturally rich in vitamin D, like butter, margarine, liver and eggs. Therefore, milk in the US (and in Canada) is fortified with vitamin D to the level of 2.5 μg (100 IU) per serving. However, considering the high prevalence of lactose intolerance or maldigestion—25% of the US population and 75% of adults worldwide [189] (although according to some this is an overestimation)—fortifying milk by vitamin D might not be beneficial for that group. When compared to the previous RDAs, the 1997 Requirements [8] for vitamin D were decreased (by half) for adolescents and children, while they were doubled or even tripled for the aged. Ca supplements in the elderly should always be accompanied by vitamin D.

VITAMIN K

Vitamin K, originally recognized as a factor required for normal blood coagulation, is now receiving more attention for its role in bone metabolism. Vitamin K is a coenzyme for glutamate carboxylase, an enzyme that mediates the conversion of glutamate to gamma carboxyglutamate, (known as a Gla protein). In this conversion, the Gla (dicarboxylic glutamyl) residues attract positive Ca ions and, by that, enhance its incorporation into the hydroxyapatite crystals. There are three Gla proteins associated with bony tissue, of which osteocalcin is the most studied and best known [190]. Osteocalcin is the

major non-collagenous protein incorporated in bone matrix during bone formation. However, around 30% of the newly produced osteocalcin stays in circulation, where it serves as an indicator of bone formation. During bone resorption and erosion of bone matrix, osteocalcin is released back into circulation. Therefore, the blood contains osteocalcin that may be a component of bone formation and/or resorption. Although osteocalcin is generally regarded as a bone formation marker, a more appropriate label would be a bone turnover marker. An exact function of osteocalcin is not clear; however, deficiency of vitamin K will result in an increase in undercarboxylated osteocalcin, a protein with low biological activity and detected in osteoporotic patients [191].

There are several population studies showing that low dietary or circulating vitamin K levels are associated with low BMD or increased fractures [192, 193]. Additionally, other studies show that vitamin K supplementation reduces undercarboxylated osteocalcin [194, 195], reduces urinary Ca excretion [195] and improves bone turnover profile [196, 197]. There are at least two studies showing that high levels of undercarboxylated osteocalcin (presumably, as the consequence of low vitamin K) are associated with low BMD and increased hip fractures [198, 199]. Warfarin and other anticoagulants (vitamin K antagonists) should, in theory, lower BMD; however, the scientific evidence on this issue is unclear [200, 201].

Recent data from the Framingham Heart Study cohort showed an increased incidence of hip fractures with lower dietary vitamin K (assessed by food frequency) but no association of vitamin K with BMD in elderly men and women [202]. The authors also examined the association with apolipoprotein E genotype. Phylloquinones are transported by lipoproteins and are strongly influenced by apolipoprotein E genotype. In previous research, the highest phylloquinone concentration was noticed in individuals with E2 allele and the lowest with E4 allele; the incidence of fractures was higher [203] and BMD lower [204] in individuals with E4 allele. However, the data from Framingham study did not support the above association.

Vitamin K is supplied to the body from two sources: the diet and intestinal bacterial synthesis. Dietary vitamin K is provided primarily by green vegetables, particularly broccoli, cabbage, spinach, brussel sprouts, turnip greens and lettuce, all of which contain more than 100 μg vitamin K/100 g serving. The 1989 RDA for dietary vitamin K ranges from 55–70 μg per day for the adult or in the range of 1 μg/kg weight/day. The usual diet contains around 300–500 μg vitamin K, so an overt deficiency under normal circumstances would be unusual. Those at risk for a subclinical deficiency would be newborns, those with fat malabsorption or chronic antibiotic use. At this point, there is every reason to recommend high vegetable intakes for bone health, although there is much research to be completed on this interesting topic.

ANTIOXIDANT VITAMINS

Interest in vitamin C has been traditionally directed toward diseases or conditions such as cancer, cardiovascular disease, the common cold, cataracts and now we may add bone health to the list. Vitamin C is required for collagen crosslinking, and in the extreme case of vitamin C deficiency, scurvy, there is a weakening of the collagenous structure in bone [205]. Vitamin C along with other antioxidant vitamins may serve to protect the skeleton from the oxidative stress from smoking. Smoking is known to increase the relative risk of hip fracture between 1.5 and 2.0, possibly due to increased free radical generation and bone resorption. In a recent study of women, high intakes of vitamin E and C (but not beta carotene or selenium) significantly decreased the odds ratio for hip fracture in current smokers [206]. The study supports the hypothesis that certain antioxidant vitamins are protective against oxidant-mediated bone loss in smokers. Many fruits and vegetables are high in vitamin C, while meats and dairy products contain small amounts and grains contain none.

Vitamin A is important in the bone remodeling process because both osteoblasts and osteoclasts contain nuclear receptors for retinoic acid [207, 208]. It appears that too high or too low levels of vitamin A are detrimental to bone. Hypervitaminosis A in animals results in accelerated bone resorption, increased bone fragility and spontaneous fractures [209, 210]. In a group of Swedish women, high dietary intake of retinol was negatively associated with BMD and increased hip fracture risk [211]. On the other hand, in a vitamin A deficiency, osteoclasts are reduced in number resulting in excessive deposition of periosteal bone from unchecked osteoblast activity [205]. As long as vitamin A is consumed within recommended levels, it is both safe and beneficial to bone health [212]. There are various forms of vitamin A in the diet: animal products and red, orange and yellow vegetables are excellent sources. Fast foods are very low in vitamin A.

ENERGY

There is a consistent positive association between body weight and BMD. Increased energy intake favors weight gain and higher BMD. The strong positive effect of excess weight on BMD may be due to weight-bearing forces exerted on the skeleton [213, 214]. Likewise, moderate weight loss of 10% typically results in 1% to 2% bone loss [215–217]. More severe weight loss or conditions of malnutrition are considered a risk factor for osteoporosis, and there could be many contributing factors: low macronutrient intake (including protein), low micronutrient intake (including Ca, vitamin D, vitamin K), increased propensity to fall due to poor muscle strength and less protective soft padding on the hip region (reviewed in [218]).

Bone loss (from osteopenia to severe osteoporosis) and increased fragility are well established in those with eating

disorders, particularly in those with anorexia nervosa [219]. Once thought to be only affecting women, bone loss from eating disorders is now recognized in men [220]. Typically in anorexia nervosa, there is an increased bone resorption and decreased bone formation leading to bone loss and impaired structure [221]. The etiology of bone loss and demineralization in anorexia nervosa is multifactorial. It is mostly attributed to prolonged amenorrhea (and accompanying) hypoestrogenemia, hypercortisolemia, low body mass index and low fat and lean mass, as well as very limited nutrient intake (particularly Ca, vitamin D and protein) [219]. Pathological fractures of spine, hip or long bones may occur within 7 to 15 years after the onset of a disorder. Crucial for increase of BMD and reversal of osteoporosis in those patients are restoration of weight and resumption of menstrual cycles [219].

A special case is the "Female Athlete Triad," a common clinical sequela in female athletes, characterized by disordered eating, amenorrhea and osteoporosis. The consequences of osteoporosis and accompanying frequent stress and/or regular fractures, amplified by strenuous exercise, may be devastating for these athletes. Nevertheless, this disorder often goes unrecognized and neglected [222].

PROTEIN

Increasing dietary protein increases urine Ca excretion such that for each 50 g increment of protein consumed, an extra 60 mg of urinary Ca is excreted [223]. It follows that the higher the protein intake, the more urine Ca is lost and the more negative Ca balance becomes. Since 99% of the body's Ca is found in bone, one would hypothesize that high protein induced hypercalciuria would result in high bone resorption and increased prevalence of osteopenia or osteoporotic-related fractures.

The epidemiological and clinical data addressing this hypothesis are controversial. On one hand, most [224–226], but not all [227] epidemiological studies found a positive association between protein intake and BMD. On the other hand, many [228–230], but not all [231] report higher fractures in groups consuming a high protein diet.

Clinical intervention trials generally support the hypothesis. Most [232–234], but not all [235] report an increase in bone resorption when animals or humans were fed a high protein diet. A summary of the controversy that surrounds the influence of dietary protein on Ca metabolism and bone health was presented by Massey and colleagues [236–238].

There is growing evidence that a low protein diet has a detrimental effect on bone. We have reported that in healthy young women, acute intakes of a low-protein diets (0.7 g protein/kg) decreased urinary Ca excretion accompanied by secondary hyperparathyroidism [239]. The etiology of the secondary hyperparathyroidism is due, in part, to a significant reduction in intestinal Ca absorption during the low protein diet [240]. Intestinal Ca absorption on the low protein diet (0.7 g protein/kg) was 19 ± 3% which was significantly lower than on the high diet (2.1 g protein/kg) which averaged 26 ± 3%. As expected, a moderate protein diet (1.0 g protein/kg) does little to perturb Ca homeostasis.

In a recent short term intervention trial, we evaluated the effect of graded levels of dietary protein (0.7, 0.8, 0.9, and 1.0 g protein/kg) on Ca homeostasis (Fig. 3). Secondary hyperparathyroidism developed by day 4 of the 0.7 and 0.8 g protein/kg diets (due to decreased intestinal Ca absorption), but not during the 0.9 or 1.0 g protein/kg diets in eight young women [241]. Similarly, when Giannini *et al.* [242] restricted dietary protein to 0.8 g protein/kg, he observed an acute rise in serum PTH in 18 middle aged hypercalciuric adults. Taken together, both of these studies suggest, at least in the short term, the RDA for protein (0.8 g/kg) does not support normal Ca homeostasis.

The long-term consequences of a protein restriction are unknown, but there is suggestion that it may affect bone. In two epidemiological studies, the lowest quartile of dietary protein intake was associated with a significantly reduced BMD [243, 244]. We also know that the addition of protein in conditions of protein undernutrition improves bone health. Bonjour and colleagues studied the effects of six months' protein supplementation in a group of elderly subjects post hip fracture. The additional protein (+20 g) over a rather low baseline protein intake (approximately 40 g) in these subjects was associated with attenuation of proximal femur bone loss and almost a 50% reduction in proximal femur bone loss after one year [245].

Overall, it appears that both low and high protein diets may

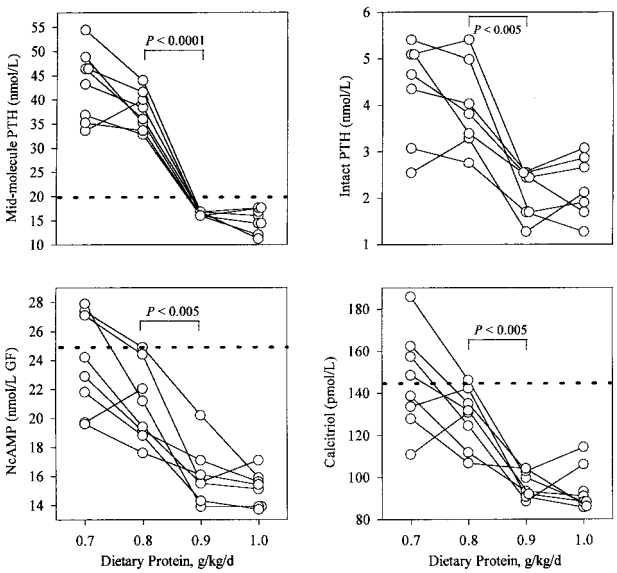

Fig. 3. Individual responses in calcitropic hormones at day 4 in response to graded intakes of dietary protein (n = 8 healthy young women). The order of treatments was randomized. The upper limits of normal are represented by horizontal dashed lines. NcAMP = nephrongenous cyclic adenosine monophosphate, a bioindicator of PTH activity. GF = glomerular filtration. (Reproduced with permission from [241].)

be detrimental to bone health. Low protein diets interfere with intestinal Ca absorption and IGF-1 levels, and high protein diets induce excess urine Ca loss. Diets containing moderate protein levels (approximately 1.0–1.5 g/kg) are probably optimal for bone health.

FOOD AND FOOD COMPONENTS

Acid-Alkaline Ash of a Diet

When discussing whole foods containing multiple nutrients (instead of one isolated nutrient), the situation becomes more complex. For example, the acid or alkali ash generated by the diet may affect bone by altering acid-base status. It was proposed in late sixties that the skeleton may serve as an ion-exchange or buffering system for neutralizing acid or alkaline challenges of food and maintaining constant pH of blood [246]. In response to metabolic acidosis caused by the acid ash of a diet, bone might undergo increased resorptive processes and release Ca to neutralize generated protons. Therefore, the acid ash producing diets like meats (especially if consumed for a long time) might contribute to the depletion of calcium and increased risk of osteoporosis, as opposed to fruits and vegetables with an alkaline and dairy products with a neutral ash. Consistent with this notion is a study by Sebastian *et al.* [247] who reversed meat induced increased urinary Ca and negative Ca balance with potassium carbonate. Therefore, consumption of meat based diets might contribute to the depletion of calcium and increased risk of osteoporosis as supported by a cross sectional study by Abelow *et al.* [230].

Based on the above, it could be speculated that vegetarians, who would be consuming less of the acid inducing and more of the alkaline inducing foods, would have stronger or denser bones than omnivores. There are many epidemiological reports addressing the relationship between source of protein intake (animal *versus* vegetable) and bone health. Almost all of them report no differences in BMD between vegetarians and non-vegetarians [248–254]. A few report a greater rate of bone loss after age 50 in lacto-ovovegetarians [255, 256] or lower BMD at the hip [257], putting them at higher fracture risk [258]. An epidemiological approach to answering the question of animal/vegetable protein and bone health is limited by the heterogeneity in vegetarian groups and the differences in health and lifestyle factors that are not easily quantified or controlled. For example, Eskimos [259, 260] that consume very high amounts of animal protein (approximately 200 g/day) and a very low calcium diet lose bone faster over the age of 50 than do Caucasians living in the mainland US. However, Eskimos are also known as a group with extremely harsh living conditions, yet engaged in strenuous exercises all their lives and with relatively high intakes of vitamin D (via organ meats they consume). In general, it is hard to tease out the negative effect of high protein from that of low dietary Ca, or other special conditions, thus limiting the applicability of some of the results.

It is difficult if not impossible to differentiate the positive effects of the alkaline nature of fruits and vegetables *versus* the other bone related nutrients commonly found in these food groups (such as vitamin K, magnesium, potassium, Zn and fiber). New *et al.* [107, 261] found various positive associations between BMD in spine and hip and present and past fruit and vegetable intakes, as well as with magnesium, potassium, zinc and fiber in premenopausal women. Moreover, they found an inverse relationship between some of the above nutrients and markers of bone resorption, all indicating the positive effect of fruits and vegetables on bone, presumably due to the more favorable acid-base balance produced by those foods. Similarly, Tucker *et al.* [109] reported greater BMD in hip and forearm among surviving members of the Framingham Heart Study cohort who had higher intake of fruits, vegetables, potassium and magnesium. It might be possible that mixed diets with higher emphasis on fruits and vegetables have favorable impact on bone (besides numerous other benefits). More carefully designed and controlled studies are needed to examine and clarify these associations.

Phytochemicals

Phytochemicals are naturally occurring, plant-derived compounds that may have biological activity. One such group, the phytoestrogens, are a broad assortment of about 20 compounds (derived from more than 300 plants, including soy and grains, fruits, coffee and herbs), nonsteroidal in structure, that mimic estrogens. The main classes of phytochemicals include isoflavones, coumestans, and lignans.

Because of their phenolic ring structure, phytoestrogens may act as estrogen agonists or antagonists [262]. Isoflavones, a type of phytoestrogen derived from soy beans, include genistein and daidzein, have been studied in postmenopausal osteoporosis and generally found to have positive effect in maintaining bone density and reducing fractures. The threshold intake of dietary isoflavones in humans to achieve biological activity is thought to be 30 to 50 mg/day, an amount that is attainable by the inclusion of several soy containing foods per day [262]. Intakes in the 30 to 50 mg/day range may influence serum lipids, but higher doses may be required to influence bone.

For example, moderate amounts of isoflavones (56 mg/day) did not affect BMD over a six-month period; however, a 90 mg/day dose (taken as a supplement) was needed to show increase in BMD of the lumbar spine in a group of postmenopausal women [263]. When isoflavones were added to the diets of ovariectomized rats, they prevented bone loss to varying degrees [264–266], perhaps by interfering with osteoclast acid transport [267] or by enhancing bone formation [266].

A related synthetic phytoestrogen, ipriflavone, is used in several countries for the prevention and treatment of osteoporosis and has been tested extensively in clinical trials in Italy, Japan and Hungary [268]. A recent review of interventional

clinical trials with isoflavones/ipriflavone in animals and humans by Scheiber and Rebar [269] suggests that they have antiresorptive properties and are safe alternatives to estrogen therapy in postmenopausal osteoporosis. However, since they are relatively new agents, the long term benefits or consequences, as well as their impact on fracture reduction, need more clarification.

Caffeine

It was once thought that caffeine simply increased urinary loss of Ca, and as such, was considered a risk for bone loss. However, the long term effect of caffeine on Ca and bone metabolism is more complex, probably affecting intestinal Ca absorption from endogenous sources. In an analysis of 560 balance studies carried out in 190 adult women, caffeine surprisingly does little to affect urinary Ca excretion or total Ca entry into the gut [270]. Caffeine is negatively correlated with intestinal Ca absorption with the net result being a more negative Ca balance. In adult women, for each 6 fl oz serving of coffee (containing an estimated 103 mg of caffeine), Ca balance was more negative by 4.6 mg/day. An additional 40 mg Ca (e.g., by adding about two tablespoons of milk) will offset the amount of Ca lost from one cup of caffeinated coffee [270]. Additionally, there was an inverse relationship between caffeine intake and Ca intake [270] such that as caffeine containing coffee increased, milk consumption decreased.

However, the epidemiological data addressing the association between coffee consumption and bone status are quite contradictory. There are those that show detrimental associations between caffeine consumption [271, 272] and those that do not [273–276]. It appears that the deleterious effect of caffeine becomes most pronounced when dietary Ca is inadequate and less harmful when dietary Ca is high [271, 277, 278].

Alcohol

Chronic alcoholism leads to lower BMD and higher fracture risk due to a combination of factors: 1) poor nutrition and malabsorption of critical nutrients, particularly calcium, magnesium and zinc, 2) liver disease, abnormal vitamin D metabolites and parathyroid function, 3) direct toxicity to osteoblasts (bone forming cells) and 4) increased propensity to fall thereby increasing chances for fractures.

Heavy drinkers are also at risk for bone loss. A prospective study in almost 85,000 middle aged women showed that those who consumed more than 25 g alcohol/day had increased risk for hip and forearm fractures compared to those who did not drink [279]. (Note, one 12 fl oz of beer has about 13 g of alcohol, 3.5 fl oz of wine about 10 g and 1.5 fl oz of liquor contains about 15 g of alcohol.) Similarly, Hoidrup *et al.* showed increased risk for hip fractures in 18,000 men who consumed more than 27 drinks/week, particularly in those who preferred beer over wine or other spirits [280].

However, moderate alcohol consumption appears to be beneficial for bone. For example, studies have found a positive association between moderate alcohol consumption and bone mass of different skeletal regions in premenopausal [261] and postmenopausal [281] women. In the Framingham Heart Study cohort, both men and women who drank (women 7 and men 14 oz alcohol drinks/week) had higher BMD in femur, spine and forearm, compared to those who had <1 oz/week of alcohol drinks [282]. On a similar note, positive association between moderate alcohol intake and BMD was also revealed from the Copenhagen Center for Prospective Population Studies [280]. Postmenopausal women who were on hormone replacement therapy (HRT) and drank alcohol had lower incidence of hip fractures than non-drinkers (also on HRT). In other words, the protective effect of HRT on hip fractures was stronger in women who drank alcohol [283].

The possible explanation for why moderate alcohol intake improves bone status may be that alcohol stimulates androstenedione conversion into estrone [284]. The aromatization of androgens to estrogens in postmenopausal women is the only source of their estrogen. The results from a study in postmenopausal women which revealed higher estradiol levels in women with moderate alcohol consumption are consistent with this hypothesis [284].

Differentiating the beneficial effects of alcohol from the other beneficial compounds in alcoholic drinks such as flavonoids, antioxidants and hydroxystilbenes is difficult. More precise data and case-control studies are necessary to establish the amount of alcohol that is beneficial as opposed to detrimental for bone. Additionally, compared to abstainers, moderate drinkers tend to demonstrate improved mental status, decreased stress and depression, lower absenteeism from work and lower incidence of dementia [285]. It is difficult to give general recommendations for alcohol consumption in the name of potential health benefits when its abuse and addiction is easily achieved by some individuals.

HERBALS AND BOTANICALS

The use of herbal, non-mineral and non-vitamin supplements has expanded tremendously [286]. Since these products are not regulated and FDA has limited control over them, many are sold with glamorous claims of curing various ailments, but only few are subjected to the scientific evaluation and have been studied in animals or humans to prove their potency. In the area of bone health there have been several attempts, mostly with animal models or *in vitro*, to evaluate potency of various herbal products. For example, traditional Chinese medicines (different Kampo formulae, which are combinations of several herbs) show similar effects to estrogen in preventing bone loss in ovariectomized rats [287] or inhibiting bone resorption *in vitro* [288]. Japanese herbal medicine, Chujo-to [289], and Chinese Guizhou epimedium [290] both show improved bone

mass and increased mineral content in ovariectomized rats. Research in this area is in its infancy, and before any official recommendations can be given for the herbal effects on bone, more controlled clinical trials have to be performed.

CONCLUSIONS

With prolonged life expectancy and the increasing number of elderly, it is predicted that osteoporotic fractures will reach epidemic proportions. Therefore, osteoporosis prevention and treatment as well as Ca nutrition remain of high priority in the latest health goals for the United States presented in Healthy People 2010 [81]. A substantial effort is being made toward understanding the effect of nutrients, particularly Ca and vitamin D, on bone accretion during youth and bone loss during aging. A wealth of new knowledge is now available. Osteoporosis is a multifactorial disorder, and, despite the considerable influence of heredity, bone health depends on the whole range of other nutrients and foods as well as the environmental factors. The prolonged deficiency or excess of one or the combination of several, as well as the changes in requirements of those nutrients caused by physiological and metabolic changes, might contribute to osteoporosis. It is also necessary to account for the interaction between different factors, nutritional, environmental, life style and heredity, to understand the complexity of bone, development of osteoporosis and subsequent fractures. Although our understanding of nutrients and other components affecting bone health continues to grow, the process of acquiring knowledge is not over. Referring to the Oscar Wilde's adage: "Truth is seldom pure and never simple," we realize that what is considered a truth now might change, but as long as we keep with our quest, the more certain we will become that what we know is true.

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