THE STANDARD

Volume 6, No.2 2004

A CONCISE UPDATE OF IMPORTANT ISSUES CONCERNING NATURAL HEALTH INGREDIENTS

Thomas G. Guilliams Ph.D.

OSTEOPOROSIS

PROTECTING AND STRENGTHENING BONES NATURALLY

It should come as no surprise that as the population gets increasingly older and life expectancy continues to increase, age-related diseases such as osteoporosis will impact more individuals. Reduced bone mineral density and resulting fractures is not inevitable, however. Diet and lifestyle factors, as well as numerous specific natural agents have tremendous impacts on preserving bone mass, preventing fractures, and even building bone mass. This review will outline strategies for preventing and treating osteoporosis using these factors.

Osteoporosis is a systemic skeletal disease involving decreased bone mass, weakened bone tissue and eventually leads to increased risk of bone fractures. Disease severity is defined by the World Health Organization (WHO) by an individual's bone mineral density (BMD) compared to mean peak young-adult BMD. Bone mass which is less than 1 standard deviation (SD) from the mean is considered osteopenia, while BMD less than 2.5 SD from the mean is diagnostic for osteoporosis. It should be noted that this diagnosis implies the bone is normal in every other respect, unlike osteomalacia, a metabolic disorder resulting in faulty mineral deposition in bone.

Osteoporosis Risk Factors

Osteoporosis is more than simply a disease of aging. While it is true that the prevalence of this condition is age-dependent, other factors such as peak bone mass while young (see below), nutrition, exercise and hormonal status (for women) play a significant role in determining an individual's risk for osteoporosis.

Genetic factors also play a role and it is known that Caucasian women have an increased risk compared to African American (typically higher BMD) and Asian women (typically lower BMD). Endogenous hormone profile, as well as age of menopause will affect risk for osteoporosis in all women. In some studies oral contraceptive use had slight adverse effects on bone mineral density. Certain other drugs, especially the use of glucocorticoids, often lead to a reduction in BMD and increased risk of fractures. Additionally, high alcohol intake, smoking and "thin body type" are all linked to increased risk for osteoporosis (16,17).

The onset of menopause is typically seen as leading factor in a rapid decline of bone mineral density (see Figure 1). This is seen in nearly all women and magnifies the importance of peak bone mass prior to this decline. For a complete discussion of menopause and natural treatments please see *The Standard* Volume 4 No. 1.

Peak Bone Mass

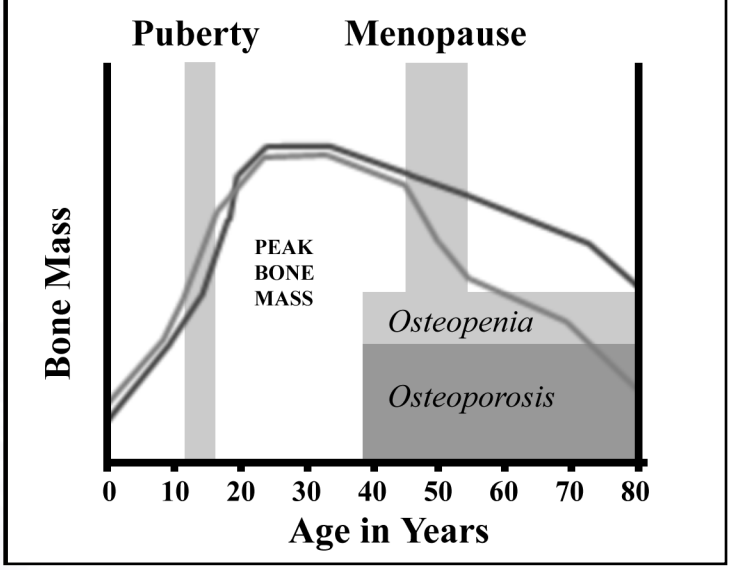
"Osteoporosis is a paediatric disease" - so said Charles Dent over thirty years ago, and to a great extent he was right (1,2). A high peak bone mass (PBM) may be one of the most important factors in maintaining strong bones in ones elderly years (See Figure 1). Reaching sufficient peak bone mass is accomplished in the first few decades of life and is influenced by genetics (some say 75%) and by many modifiable factors; the two most studied are diet (especially calcium and

(continued from page 1)

protein intake) and weight-bearing activities. Only in recent decades have these factors been analyzed critically; and it seems that while calcium intake is an important contributor, especially when woefully inadequate, weight-bearing activities during and just after the onset of puberty seems to be more important- even compensating for less than adequate calcium intake (3-7). Other factors that also influence PBM are age of puberty (esp. menarche in girls)(8), subsequent amenorrhea and eating disorders like anorexia nervosa (9).

The role of soft drink consumption by young women and subsequent osteoporosis risk has been controversial. It seems to be true that increased carbonated soft drink consumption by young girls reduces BMD and dramatically increases fractures, although the causative nature may be an indirect relationship with reduced calcium intake (from milk) or from a depletion of nutrients related to consuming sugar sweetened soft drinks (6, 10-13). The impact this has on future osteoporotic risk is unknown, but is unlikely to be insignificant. Since there are numerous reasons to recommend reduction (or elimination) of soft drink consumption by young girls- this serves only to add to the growing list (nutrient depletion, obesity and diabetes risk (14)). Calcium supplementation in young girls, conversely, is known to improve bone mineral content and improve bone health (15).

Figure 1: Bone Mass Lifecycle



Measuring Bone Density and Turnover

Measuring bone density and bone turnover is important in the assessing of an individual's risk for fractures and in determining the effectiveness of various therapies. Perhaps the most common analysis is the Dual Energy X-ray absorption (DEXA) scan. This method is considered more reliable and consistent than simple x-ray and is now considered the best validated method for determining bone mineral density. Typically these are done at the femur/hip and various lumbar vertebrae and computer analyzed for comparisons to mean peak bone mass (T score) and age adjusted mean bone mass (Z score). Most clinical trials that seek to measure therapies for osteoporosis risk will include DEXA scan data.

Several other markers are also used to measure bone turnover and therapy function. Various serum and urine tests can be used to measure bone specific alkaline phosphatase, osteocalcin, collagen and other proteins and peptides related to bone resorption and formation. Often these biochemical markers will be used to confirm DEXA scan data or follow the patients for more frequent intervals between the much more expensive DEXA scans.

Nutritional Factors (18,19)

Bone turnover is a life-long process (see article page 5). The need for proper nutritional factors in the form of vitamins and minerals has been recognized as an important area of study. The population most susceptible to osteoporosis, the elderly, is also the most likely to be deficient in many key nutrients. Below is an outline of many of the key nutrients, their mechanism for improving bone health and data supporting their use in the prevention or treatment of osteoporosis.

Minerals

Calcium and Phosphorus

Nearly 99% of the 1-1.5 kilograms of calcium in the adult is located within the skeletal system as a complex with phosphorus called hydroxyapetite. The obvious importance of these minerals for bone strength often overshadows the importance the bone minerals play as repositories for total body calcium and phosphorus and maintaining homeostasis of these important biochemical minerals. The sheer volume of calcium found within bone tissue and its importance in building bone in adolescents has made it the most studied nutritional component related to bone health and osteoporosis (22). It is commonly advised that individuals past early adolescence should consume

(continued from page 2)

1,000 to 1,500 mg/day of calcium from dietary or supplement sources to prevent inadequate bone formation.

While there seems to be a consensus that calcium intake seems to be one of the more important factors leading to peak bone mass development, there is much less consensus on the use of calcium alone to prevent or treat osteoporosis in peri and postmenopausal women. One recent meta-analysis suggests that calcium alone generally favors bone density improvements but rarely has shown a decreased risk of fractures after 2 years in well controlled trials (20). That said, nearly every trial comparing agents (drugs or supplements) intended to prevent or treat osteoporosis includes calcium supplementation as part of the regimen. Because the forms of calcium used in these trials was not consistent (or often not reported) it makes it quite difficult to assess the true benefit of calcium in this population.

Few well designed studies have compared clinical outcomes derived from different forms or sources of calcium. Calcium carbonate, one of the least expensive forms of calcium, is often used in the published trials leading many to believe it is superior to other forms. However, in 1990 the USDA published a trial comparing calcium carbonate with calcium citrate-malate with respect to improved bone mineral density in postmenopausal women. They found that the citrate-malate form was significantly better at preventing bone-loss in this trial than was the carbonate form (21). Another recent report showed calcium citrate improved several biochemical markers of bone resorption when compared to equivalent amounts of calcium carbonate (23), perhaps owing to a greater bioavailability (24); although not all reports agree (25).

One important factor often omitted in selecting calcium supplement sources is its relationship with phosphorus. Calcium, like most minerals and vitamins should be consumed with a meal. However, when high doses of calcium are ingested with food phosphorus, nearly all the phosphorus is complexed by unabsorbed calcium and is non-available for bone formation. Consuming forms of calcium like dicalcium phosphate or tricalcium phosphate (hydroxyapatite) maintains available phosphorus for bone formation (26,27). At this time there does not seem to be one particular calcium form which meets every potential benefit (high absorption, phosphorus retention, cost effectiveness) and combinations of salt forms, hydroxyapatite forms and amino acid chelate forms should be considered as a balanced way to combine these benefits.

Magnesium

Unlike calcium and phosphorus, magnesium is not part of the hydroxyapatite crystals themselves but nearly 2/3 of the magnesium in the human body is located within the bones. Magnesium is a vital cofactor for nearly all the reactions involving calcium, including those involved in calcium absorption and bone formation. Magnesium deficiency is often associated with decreased bone density (28,29), although magnesium alone is rarely studied in clinical trials for improved BMD in osteoporotic women (30,33). Medical conditions and poor dietary habits are often associated with hypomagnesemia leading to poor BMD and increased osteoporotic risk (31,32).

Strontium (34-37)

For many, the use of strontium to prevent and treat osteoporosis is rather new- following the recent publication of a large three year phase III clinical trial. However, others have known about strontium for some time. Strontium is a mineral which is closely related to calcium and is metabolized in the body in nearly identical ways. While the use of strontium for improved bone mineral strength in both animals and humans has been known for almost 50 years, the confusion between normal stable strontium with its radioactive isotopes has led many to shy away from using it. The unique benefit of strontium is its ability to incorporate into bone (to a maximum of 1 out of 10 calcium atoms), strengthening the bone matrix while also stimulating osteoblast formation and inhibiting osteoclast activity.

The most recent clinical trials have focused on one particular salt form, strontium ranelate, for both the prevention and treatment of osteoporosis in postmenopausal women. The Prevention of Early Postmenopausal Bone Loss by Strontium Ranelate (PREVOS) (38) trial was a two-year double-blind dose-response trial using either 125 mg, 500 mg or 1gram/day of strontium ranelate in postmenopausal women without osteoporosis (T-Scores -1.3 to -1.5). Each participant was also given 500 mg of calcium carbonate/day at lunch. Only in the 1 gram/day group did they see significant increase in BMD of the lumbar (L2-L4), femoral neck and total hip; increased bone specific alkaline phosphatase levels and decreased urinary cross-linked C-terminal telopeptide of type 1 collagen- all measures of improved bone strength and reduced risk for future fractures. The authors conclude that 1 gram/day was sufficient to prevent bone loss in early postmenopausal non-osteoporotic women (39).

(continued from page 3)

A second dose response clinical trial was performed to determine the optimal dose for postmenopausal women with established osteoporosis (40). In this two-year trial, while 1 gram/day was statistically better than placebo, 2 grams/day was better in most categories and was set as the optimal dose for treating these women. This dose was then used in a larger, phase III clinical trial published in early 2004 in the New England Journal of Medicine with dramatic results (41). A total of 1649 postmenopausal women with osteoporosis and at least 1 vertebral fracture were randomly assigned 2/grams per day of strontium ranelate or placebo (calcium and vitamin D were also supplemented to both groups). After three years, the difference in BMD between the groups was significantly different in the lumbar spine (+14.4%), femoral neck (+8.3%) and total hip (+9.8%). Participants taking strontium also had 41% fewer new vertebral fractures over the three year study. These results are similar to some of the most powerful drugs which thwart bone loss by preventing bone turnover- a mechanism which eventually prevents new bone growth. Strontium, on the other hand, stimulates new bone growth by improving bone-turnover rather than inhibiting it.

It should be noted that the close relationship between calcium and strontium means that they compete for absorption and should be taken at separate occasions. Each of these clinical trials gave strontium either in a single dose (evening) or in divided doses (morning and evening) and instructed participants to consume their calcium supplements at lunchtime.

Many forms of strontium are available as dietary supplements: carbonate, citrate, chloride etc. Previous human, animal and in vitro research shows that each of these forms acts similarly with respect to bone metabolism, but no clinical trial has been conducted to compare their relative effectiveness.

Other Minerals

Other trace minerals/metals which are necessary nutrients for proper bone strength include boron, zinc, copper, manganese and molybdenum. In animal studies and a few human observational studies, boron was able to modify calcium, phosphorus and vitamin D interactions which has beneficial outcomes for bone mineralization (42-46). Deficiencies in boron, like copper, results in weakened bone strength. Supplemental copper and manganese are able to slow the progressive bone-loss of ovariectomized rats- a model for menopausal bone loss (47, 48). Low copper status can result in serious health consequences, while as little as 1 mg/day of supplemental copper is sufficient to prevent these concerns (49).

In one particular study (53), postmenopausal women were supplemented with calcium alone (1000 mg/day as calcium citrate malate) or given additional zinc (15 mg/day), copper (2.5 mg/day) and manganese (5 mg/day). Those given the trace mineral blend along with the calcium had significantly higher bone mineral density that those given only calcium. The role of each trace mineral, their interaction with one another and the levels which are necessary to prevent deficiency-related animal and human bone-loss is far from agreement in the scientific community. Many studies, however, have addressed various aspects of trace mineral bone metabolism (50-52).

Vitamins

B-Vitamins

The vitamins often considered to be the most important for bone health are the fat-soluble vitamins D and K. Two recent articles published in the New England Journal of Medicine (NEJM), however, have given us reason to consider the role of B-vitamins in the prevention of osteoporotic risk. Both of these articles linked increasing levels of the blood chemical homocysteine with elevated risk for osteoporotic fractures (54,55). Elevated homocysteine levels, more commonly known as a risk factor for cardiovascular disease (56), is commonly linked with low intake of folic acid, vitamin B6 and B12. Some studies suggest that the homocysteine relationship is merely a measure of folate deficiency, the true culprit of poor bone metabolism and increased risk of osteoporotic fractures in menopausal women (57, 58). In younger women, vitamin B12 deficiency, common in vegetarians, is known to decrease bone mineral density (59).

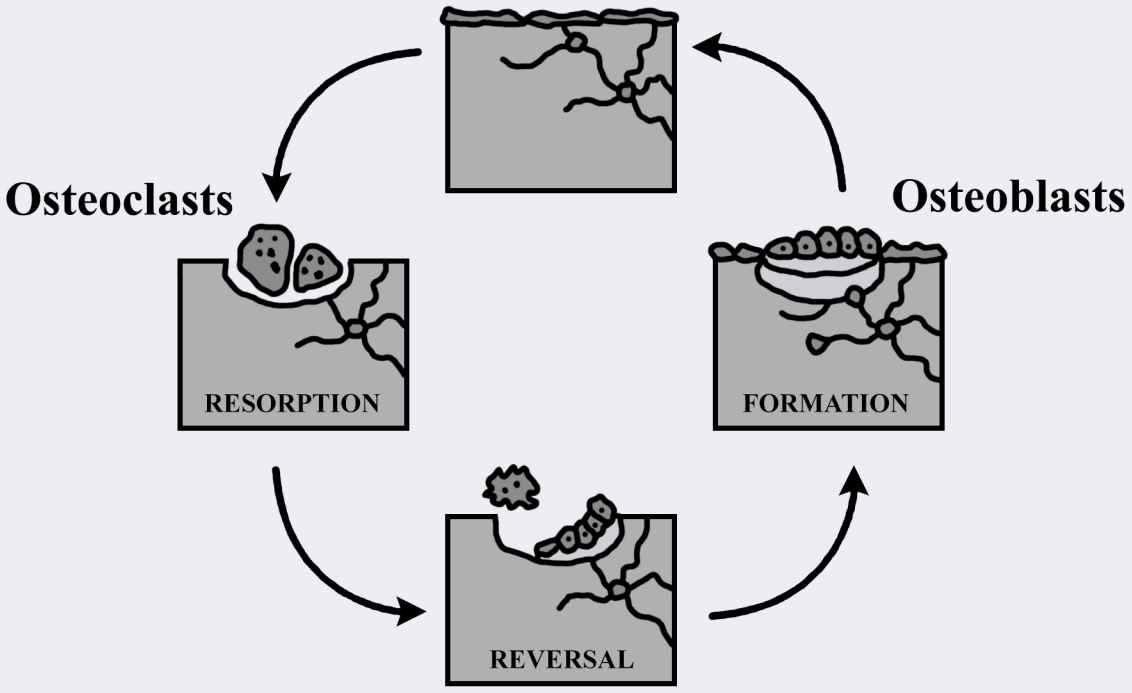
Vitamin D (60, 63)

The use and understanding of vitamin D in maintaining proper bone health is fairly ubiquitous. The active metabolite, 1,25 (OH) vitamin D3 (calcitriol) functions to facilitate calcium absorption in the intestines by stimulating calbindin synthesis. Its hormone-like activity, related to parathyroid hormone, modulates bone turnover and improves bone strength. Deficiencies of vitamin D manifest as the bone disease rickets. Most supplements use cholecalciferol (D3) or ergocalciferol (D2); while several vitamin D analogs have approved drug status.

It is now common practice to recommend high levels of vitamin D (800 IU or more orally per day or up to 300,000 IU injections once per year), with calcium for

Bone Remodeling Cycle

The notion that bone tissue is static is quite inaccurate. In fact, after the skeletal system has reached maturity there is a constant rebuilding and turnover of the mineralized portions of bone tissue. The process slows down with age, lack of exercise and poor nutrition as outlined in the main article. Various cells and cellular processes within the remodeling scheme have now become targets for pharmaceutical drugs which attempt to slow fracture rate by modifying these processes. Specific nutrients and dietary supplements are also able to reduce or stimulate cellular function which leads to improved bone mineral density and stronger bone tissues.



Osteoclasts-Bone Resorption

Bone resorption begins when osteoclasts differentiate and move to the surface of the bone. A portion of the bone is removed by these cells to be replaced later by the action of osteoblasts. While this stage is often seen as negative, it should be noted that it is a vital precursor and signaling step for bone formation.
Osteoclast activity is:

Enhanced by:
- Low Serum Calcium
- Parathyroid Hormone
- Corticosteroids

Decreased by:
- Strontium
- Vitamin D
- Ipriflavone
- Bisphosphonates
- Raloxifene
- Calcitonin

Osteoblasts- Bone Formation

Bone formation occurs when osteoblasts lay down collagen as well as mineral deposits over the area previously remodeled by osteoclasts. Osteoblast differentiation, maturation and recruitment is vital to maintain bone mineral density and bone strength in the aging years.
Osteoblast and Mineralization is:

Enhanced by:
- Calcium
- Calcitonin
- Vitamin K (via osteocalcin)
- Strontium
- Ipriflavone
- Genestein

Decreased by:
- Nearly all drugs that prevent resorption

(continued from page 4)

prevention and treatment of osteoporosis (65-67). Of course, proper exposure to sunlight which allows for natural conversion of pre-vitamin D within the skin is highly recommended in those for whom sunlight is not contraindicated. Large numbers of the elderly, already at risk for other reasons, are deficient in vitamin D because of diet and low sunlight exposure (61). In fact, vitamin D intake by most Americans, especially adolescent and adult women is below recommended levels (64).

Furthermore, a recent meta-analysis published in JAMA shows that elderly individuals are 20% less likely to fall if they are taking vitamin D supplements. This, the authors conclude, is due to the additional benefit vitamin D has on muscular strength (62). The benefit for proper vitamin D intake may then be even greater since falls combined with bone brittleness are the leading cause of fractures in the elderly.

Vitamin K (75)

Most people recognize vitamin K for its role in blood coagulation, although much more attention is now focused on its role in bone metabolism. The fat soluble vitamin is a coenzyme for specific carboxylating enzymes. Three proteins, the most important being osteocalcin, are responsible for enhancing calcium incorporation into hydroxyapatite crystals in the bone- only when carboxylated. Low vitamin K status will result in undercarboxylated osteocalcin (uOC), a marker for increased osteoporotic risk.

Two forms of vitamin K exist naturally; K1 (phylloquinone) produced primarily by plants and K2 (a family of menaquinones) produced by bacteria. Both forms seem to have similar functions. Vitamin K1 is the most commonly used in supplementation and foods although recent commercially available forms and research on vitamin K2 has piqued interest in this form.

It is fairly well documented that low levels of vitamin K intake (or serum levels) is directly related to reduced bone mineral density and increases the risk for osteoporotic fractures in women (68-72). Individuals with inflammatory bowel disorders like Crohn's disease may be particularly susceptible to vitamin K (as well as vitamin D) related BMD losses (73,74).

Intervention trials with vitamin K1 at 1 mg/day and K2 (typically at 45 mg/day) with or without vitamin D have been shown to increase carboxylation of osteocalcin and improve bone mineral density (76-80). Nearly all of the research on vitamin K2 comes from Japan where the K2-rich fermented soy product natto is consumed. More research needs to be conducted to determine how these two forms and doses compare and if either is more advantageous for certain populations.

Phytoestrogens (81)

Phytoestrogens are plant compounds that result in physiological changes characteristic of endogenous estrogens- regardless of their ability to bind to one or more type of estrogen receptor. As estrogen and its analogs are known to effect bone physiology, the question of whether phytoestrogens will have a positive impact on bone mineral density and osteoporosis requires investigation.

Soy Isoflavones

By far, the most commonly studied phytoestrogens are those derived from the soybean (*Glycine max*). The isoflavones genestein and daidzein are converted to active compounds by normal gut microflora before they enter the body to affect physiology.

For the most part, in vitro, animal and human epidemiological studies suggest that soy intake has a positive affect on bone mineral density and decreases incidence of postmenopausal fracture, but intervention trials using soy in postmenopausal women rarely show consistent positive results (82-87).

Ipriflavone

Ipriflavone is an isoflavone derivative studied for the past 20 years in humans for its ability to improve bone mineral density without affecting other estrogenic target tissues (88). Ipriflavone has been shown to improve calcium bioavailability and also improve bone formation while preserving bone biomechanics (89-91). Numerous positive clinical trials have shown 600 mg/day of ipriflavone to be an effective way to prevent bone mineral density loss in postmenopausal women (92-96). One study, however, questioned the role of ipriflavone in this population (97). This one negative study, while gaining prominence because it was published in JAMA, has several peculiarities. The greatest of which is the fact that in each of the previous trials the placebo group (calcium and vitamin D only) showed consistent decreases in bone mineral density and secondary markers for bone remodeling; while the JAMA publications placebo group showed no such changes and even improved in some of these categories. The overwhelming balance of the data suggests, however, that ipriflavone is both safe and effective at preventing BMD loss in postmenopausal women.

Summary

Decreased bone mineral density is not inevitable and certainly not irreversible. While the pharmaceutical companies continue to develop agents which unnaturally restrict the normal bone-turnover process in hopes of slowing bone loss, our bodies are designed to facilitate this necessary metabolic process with vitamins, minerals and other natural ingredients. Like many other chronic diseases, osteoporosis is exacerbated by our lifelong diet and lifestyle choices- making it difficult to rescue brittle bones in the sixth and seventh decade of life. Clinicians should encourage young women to eat a diet rich in calcium, magnesium, vitamins D and K and protein; while adding weight-bearing activities to their lifestyle. Improving peak bone mass into their middle-thirties is the greatest asset their bones will have during menopause. Supplementation of combination products, those including several of the nutrients described here, should be considered for all postmenopausal women as well as any pre-menopausal woman whose diet and lifestyle choices are likely to leave her vulnerable.

Osteoporosis Standard References

1. Dent C: Keynote address: problems in metabolic bone disease. In Frame B, Duncan H (eds): Clinical aspects of metabolic bone disease. 1973;Amsterdam: Exerpta Medica, pp1-7. (Quoted from Ref. 2)
2. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr. 2000 Nov-Dec;19(6):715-37.
3. Anderson JJ. Calcium requirements during adolescence to maximize bone health. J Am Coll Nutr. 2001 Apr;20(2 Suppl):186S-191S.
4. Welten DC, Kemper HC et al. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res. 1994 Jul;9(7):1089-96.
5. Rizzoli R, Bonjour JP. Determinants of peak bone mass and mechanisms of bone loss. Osteoporos Int. 1999;9 Suppl 2:S17-23
6. Whiting SJ, Vatanparast H, Baxter-Jones A, Faulkner RA, Mirwald R, Bailey DA. Factors that affect bone mineral accrual in the adolescent growth spurt. J Nutr. 2004 Mar;134(3):696S-700S.
7. Wallace LS, Ballard JE. Lifetime physical activity and calcium intake related to bone density in young women. J Womens Health Gend Based Med. 2002 May;11(4):389-98
8. Molgaard C, Thomsen BL, Michaelsen KF. Influence of weight, age and puberty on bone size and bone mineral content in healthy children and adolescents. Acta Paediatr. 1998 May;87(5):494-9.
9. Golden NH. Osteopenia and osteoporosis in anorexia nervosa. Adolesc Med. 2003 Feb;14(1):97-108.
10. McGartland C, Robson PJ et al. Carbonated soft drink consumption and bone mineral density in adolescence: the Northern Ireland Young Hearts project. J Bone Miner Res. 2003 Sep;18(9):1563-9.
11. Wyshak G, Frisch RE. Carbonated beverages, dietary calcium, the dietary calcium/phosphorus ratio, and bone fractures in girls and boys. J Adolesc Health. 1994 May;15(3):210-5.
12. Wyshak G. Teenaged girls, carbonated beverage consumption, and bone fractures. Arch Pediatr Adolesc Med. 2000 Jun;154(6):610-3.
13. Fisher J, Mitchell D, Smiciklas-Wright H, Birch L. Maternal milk consumption predicts the tradeoff between milk and soft drinks in young girls' diets. J Nutr. 2001 Feb;131(2):246-50.
14. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, Hu FB. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. JAMA. 2004 Aug 25;292(8):927-34.
15. Bonjour JP, Carrie AL Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest. 1997 Mar 15;99(6):1287-94.
16. Gourlay ML, Brown SA. Clinical considerations in premenopausal osteoporosis. Arch Intern Med. 2004 Mar 22;164(6):603-14.
17. Liggett NW, Reid DM. The incidence, epidemiology and aetiology of osteroporosis. Hospital Pharmacist. 2000; 7(3)
18. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr. 2000 Nov-Dec;19(6):715-37.
19. New SA. Bone health: the role of micronutrients. Br Med Bull. 1999;55(3):619-33.
20. Shea B, Wells G et al. Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. Endocr Rev. 2002 Aug;23(4):552-9.
21. 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 Sep 27;323(13):878-83.
22. Kass-Wolff JH. Calcium in women: healthy bones and much more. J Obstet Gynecol Neonatal Nurs. 2004 Jan-Feb;33(1):21-33.
23. Kenny AM, Prestwood KM et al. Comparison of the effects of calcium loading with calcium citrate or calcium carbonate on bone turnover in postmenopausal women. Osteoporos Int. 2004 Apr;15(4):290-4.
24. Heller HJ, Greer LG, Haynes SD, Poindexter JR, Pak CY. Pharmacokinetic and pharmacodynamic comparison of two calcium supplements in postmenopausal women. J Clin Pharmacol. 2000 Nov;40(11):1237-44.
25. Heaney RP, Dowell SD, Bierman J, Hale CA, Bendich A. Absorbability and cost effectiveness in calcium supplementation. J Am Coll Nutr. 2001 Jun;20(3):239-46
26. Shapiro R, Heaney RP. Co-dependence of calcium and phosphorus for growth and bone development under conditions of varying deficiency. Bone. 2003 May;32(5):532-40.
27. Heaney RP. Phosphorus nutrition and the treatment of osteoporosis. Mayo Clin Proc. 2004 Jan;79(1):91-7.
28. Stendig-Lindberg G, Koeller W, Bauer A, Rob PM. Experimentally induced prolonged magnesium deficiency causes osteoporosis in the rat. Eur J Intern Med. 2004 Apr;15(2):97-107.
29. Rude RK, Gruber HE, Wei LY, Frausto A, Mills BG. Magnesium deficiency: effect on bone and mineral metabolism in the mouse. Calcif Tissue Int. 2003 Jan;72(1):32-41
30. Abraham GE, Grewal H. A total dietary program emphasizing magnesium instead of calcium. Effect on the mineral density of calcaneous bone in postmenopausal women on hormonal therapy. J Reprod Med. 1990 May;35(5):503-7.
31. Habtezion A, Silverberg MS, Parkes R, Mikolainis S, Steinhart AH. Risk factors for low bone density in Crohn's disease. Inflamm Bowel Dis. 2002 Mar;8(2):87-92.
32. Launius BK, Brown PA, Cush EM, Mancini MC. Osteoporosis: The dynamic relationship between magnesium and bone mineral density in the heart transplant patient. Crit Care Nurs Q. 2004 Jan-Mar;27(1):96-100.
33. Stendig-Lindberg G, Tepper R, Leichter I. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. Magnes Res. 1993 Jun;6(2):155-63.
34. Marie PJ. Optimizing bone metabolism in osteoporosis: insight into the pharmacologic profile of strontium ranelate. Osteoporos Int. 2003;14 Suppl 3:S9-12
35. Marie PJ, Ammann P, Boivin G, Rey C. Mechanisms of action and therapeutic potential of strontium in bone. Calcif Tissue Int. 2001 Sep;69(3):121-9.
36. Dahl SG, Allain P, Marie PJ et al. Incorporation and distribution of strontium in bone. Bone. 2001 Apr;28(4):446-53.
37. Boivin G, Meunier PJ. The mineralization of bone tissue: a forgotten dimension in osteoporosis research. Osteoporos Int. 2003;14 Suppl 3:S19-24.
38. Reginster JY, Deroisy R, Dougados M, Jupsin I, Colette J, Roux C. Prevention of early postmenopausal bone loss by strontium ranelate: the randomized, two-year, double-masked, dose-ranging, placebo-controlled PREVOS trial. Osteoporos Int. 2002 Dec;13(12):925-31.
39. Reginster JY, Meunier PJ. Strontium ranelate phase 2 dose-ranging studies: PREVOS and STRATOS studies. Osteoporos Int. 2003;14 Suppl 3:S56-65.
40. Meunier PJ, Slosman DO, Delmas PD, Sebert JL, Brandi ML, Albanese C, Lorenc R, Pors-Nielsen S, De Vernejoul MC, Roces A, Reginster JY. Strontium ranelate: dose-dependent effects in established postmenopausal vertebral osteoporosis--a 2-year randomized placebo controlled trial. J Clin Endocrinol Metab. 2002 May;87(5):2060-6.
41. Meunier PJ, Roux C et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med. 2004 Jan 29;350(5):459-68.
42. Volpe SL, Taper LJ, Meacham S. The relationship between boron and magnesium status and bone mineral density in the human: a review. Magnes Res. 1993 Sep;6(3):291-6.
43. Sheng MH, Taper LJ et al. Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats. Biol Trace Elem Res. 2001 Summer;82(1-3):109-23
44. Rico H, Crespo E, Hernandez ER, Seco C, Crespo R. Influence of boron supplementation on vertebral and femoral bone mass in rats on strenuous treadmill exercise. A morphometric, densitometric, and histomorphometric study. J Clin Densitom. 2002 Summer;5(2):187-92.
45. Devirian TA, Volpe SL. The physiological effects of dietary boron. Crit Rev Food Sci Nutr. 2003;43(2):219-31.

46. Nielsen FH. The justification for providing dietary guidance for the nutritional intake of boron. Biol Trace Elem Res. 1998 Winter;66(1-3):319-30.
47. Rico H, Roca-Botran C et al. The effect of supplemental copper on osteopenia induced by ovariectomy in rats. Menopause. 2000 Nov-Dec;7(6):413-6.
48. Rico H, Gomez-Raso N et al. Effects on bone loss of manganese alone or with copper supplement in ovariectomized rats. A morphometric and densitomeric study. Eur J Obstet Gynecol Reprod Biol. 2000 May;90(1):97-101.
49. Klevay LM. Lack of a recommended dietary allowance for copper may be hazardous to your health. J Am Coll Nutr. 1998 Aug;17(4):322-6.
50. Gur A, Colpan L et al. The role of trace minerals in the pathogenesis of postmenopausal osteoporosis and a new effect of calcitonin. J Bone Miner Metab. 2002;20(1):39-43.
51. Southern LL, Ward TL, Bidner TD, Hebert LG. Effect of sodium bentonite or hydrated sodium calcium aluminosilicate on growth performance and tibia mineral concentrations in broiler chicks fed nutrient-deficient diets. Poult Sci. 1994 Jun;73(6):848-54.
52. Saltman PD, Strause LG. The role of trace minerals in osteoporosis. J Am Coll Nutr. 1993 Aug;12(4):384-9.
53. Strause L, Saltman P, Smith KT, Bracker M, Andon MB. Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. J Nutr. 1994 Jul;124(7):1060-4.
54. McLean RR, Jacques PF, Selhub J et al. Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med. 2004 May 13;350(20):2042-9.
55. van Meurs JB, Dhonukshe-Rutten RA et al. Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med. 2004 May 13;350(20):2033-41.
56. Guilliams, T. Homocysteine- A Risk Factor for Vascular Disease: Guidelines for the Clinical Practice. JANA. 2004; 7(1):11-24
57. Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A. Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women. Bone. 2003 Dec;33(6):956-9.
58. Golbahar J, Hamidi A, Aminzadeh MA, Omrani GR. Association of plasma folate, plasma total homocysteine, but not methylenetetrahydrofolate reductase C667T polymorphism, with bone mineral density in postmenopausal Iranian women: a cross-sectional study. Bone. 2004 Sep;35(3):760-5.
59. Dhonukshe-Rutten RA, Van Dusseldorp M, Schneede J, De Groot LC, Van Staveren WA. Low bone mineral density and bone mineral content are associated with low cobalamin status in adolescents. Eur J Nutr. 2004 Aug 30
60. Sahota O. Osteoporosis and the role of vitamin D and calcium-vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency. Age Ageing. 2000 Jul;29(4):301-4.
61. Deplas A, Debiais F, Alcalay M, Bontoux D, Thomas P. Bone density, parathyroid hormone, calcium and vitamin d nutritional status of institutionalized elderly subjects. J Nutr Health Aging. 2004;8(5):400-4.
62. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004 Apr 28;291(16):1999-2006.
63. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr. 2004 Mar;79(3):362-71.
64. Moore C, Murphy MM, Keast DR, Holick MF. Vitamin D intake in the United States. J Am Diet Assoc. 2004 Jun;104(6):980-3.
65. Grados F, Brazier M et al. Prediction of bone mass density variation by bone remodeling markers in postmenopausal women with vitamin D insufficiency treated with calcium and vitamin D supplementation. J Clin Endocrinol Metab. 2003 Nov;88(11):5175-9.
66. Grados F, Brazier M et al. Effects on bone mineral density of calcium and vitamin D supplementation in elderly women with vitamin D deficiency. Joint Bone Spine. 2003 Jun;70(3):203-8.
67. Lilliu H, Pamphile R et al. Calcium-vitamin D3 supplementation is cost-effective in hip fractures prevention. Maturitas. 2003 Apr 25;44(4):299-305.
68. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr. 1999 Jan;69(1):74-9.
69. Jie KG, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K status and bone mass in women with and without aortic atherosclerosis: a population-based study. Calcif Tissue Int. 1996 Nov;59(5):352-6.
70. Booth SL, Broe KE et al. Vitamin K intake and bone mineral density in women and men. Am J Clin Nutr. 2003 Feb;77(2):512-6.
71. Sato Y, Kaji M, Tsuru T, Satoh K, Kondo I. Vitamin K deficiency and osteopenia in vitamin D-deficient elderly women with Parkinson's disease. Arch Phys Med Rehabil. 2002 Jan;83(1):86-91.
72. Kanai T, Takagi T et al. Serum vitamin K level and bone mineral density in post-menopausal women. Int J Gynaecol Obstet. 1997 Jan;56(1):25-30.
73. Schoon EJ, Muller MC et al. Low serum and bone vitamin K status in patients with longstanding Crohn's disease: another pathogenetic factor of osteoporosis in Crohn's disease? Gut. 2001 Apr;48(4):473-7.
74. Jahnsen J, Falch JA, Mowinckel P, Aadland E. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. Scand J Gastroenterol. 2002 Feb;37(2):192-9.
75. Weber P. Vitamin K and bone health. Nutrition. 2001 Oct;17(10):880-7.
76. Douglas AS, Robins SP et al. Carboxylation of osteocalcin in post-menopausal osteoporotic women following vitamin K and D supplementation. Bone. 1995 Jul;17(1):15-20.
77. Braam LA, Knapen MH et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. Calcif Tissue Int. 2003 Jul;73(1):21-6.
78. Knapen MH, Hamulyak K, Vermeer C. The effect of vitamin K supplementation on circulating osteocalcin (bone Gla protein) and urinary calcium excretion. Ann Intern Med. 1989 Dec 15;111(12):1001-5.
79. Miki T, Nakatsuka K et al. Vitamin K(2) (menaquinone 4) reduces serum undercarboxylated osteocalcin level as early as 2 weeks in elderly women with established osteoporosis. J Bone Miner Metab. 2003;21(3):161-5.
80. Iwamoto J, Takeda T, Ichimura S. Effect of combined administration of vitamin D3 and vitamin K2 on bone mineral density of the lumbar spine in postmenopausal women with osteoporosis. J Orthop Sci. 2000;5(6):546-51.
81. Branca F. Dietary phyto-oestrogens and bone health. Proc Nutr Soc. 2003 Nov;62(4):877-87.
82. Chen YM, Ho SC, Lam SS, Ho SS, Woo JL. Beneficial effect of soy isoflavones on bone mineral content was modified by years since menopause, body weight, and calcium intake: a double-blind, randomized, controlled trial. Menopause. 2004 May-Jun;11(3):246-54.
83. Gallagher JC, Satpathy R, Rafferty K, Haynatzka V. The effect of soy protein isolate on bone metabolism. Menopause. 2004 May-Jun;11(3):290-8.
84. Lydeking-Olsen E, Beck-Jensen JE, Setchell KD, Holm-Jensen T. Soymilk or progesterone for prevention of bone lossA 2 year randomized, placebo-controlled trial. Eur J Nutr. 2004 Aug;43(4):246-57.
85. Kreijkamp-Kaspers S, Kok L et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA. 2004 Jul 7;292(1):65-74.
86. Chen YM, Ho SC, Lam SS, Ho SS, Woo JL. Soy isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. J Clin Endocrinol Metab. 2003 Oct;88(10):4740-7.
87. Setchell KD, 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 Sep;78(3 Suppl):593S-609S.
88. Gennari C. Ipriflavone: background. Calcif Tissue Int. 1997;61 Suppl 1:S3-4.
89. Civitelli R. In vitro and in vivo effects of ipriflavone on bone formation and bone biomechanics. Calcif Tissue Int. 1997;61 Suppl 1:S12-4.
90. Arjmandi BH, Khalil DA, Hollis BW. Ipriflavone, a synthetic phytoestrogen, enhances intestinal calcium transport in vitro. Calcif Tissue Int. 2000 Sep;67(3):225-9
91. Arjmandi BH, Birnbaum RS, Juma S, Barengolts E, Kukreja SC. The synthetic phytoestrogen, ipriflavone, and estrogen prevent bone loss by different mechanisms. Calcif Tissue Int. 2000 Jan;66(1):61-5.
92. Agnusdei D, Crepaldi G et al. A double blind, placebo-controlled trial of ipriflavone for prevention of postmenopausal spinal bone loss. Calcif Tissue Int. 1997 Aug;61(2):142-7.
93. Agnusdei D, Bufalino L. Efficacy of ipriflavone in established osteoporosis and long-term safety. Calcif Tissue Int. 1997;61 Suppl 1:S23-7.
94. Gennari C, Adami S et al. Effect of chronic treatment with ipriflavone in postmenopausal women with low bone mass. Calcif Tissue Int. 1997;61 Suppl 1:S19-22.
95. Katase K, Kato T, Hirai Y, Hasumi K, Chen JT. Effects of ipriflavone on bone loss following a bilateral ovariectomy and menopause: a randomized placebo-controlled study. Calcif Tissue Int. 2001 Aug;69(2):73-7.
96. Halpner AD, Kellermann G et al. The effect of an ipriflavone-containing supplement on urinary N-linked telopeptide levels in postmenopausal women. J Womens Health Gend Based Med. 2000 Nov;9(9):995-8.
97. Alexandersen P, Toussaint A et al. Ipriflavone in the treatment of postmenopausal osteoporosis: a randomized controlled trial. JAMA. 2001;285(11):1482-8.