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For Health & Wellness*

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July 8, 2004

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Office of Nutritional Products, Labeling and
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Center for Food Safety and Applied Nutrition
Food and Drug Administration
Harvey W. Wiley Federal Building
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To whom it may concern:

Enclosed is an original and one copy of a health claim petition entitled, "Authorization of a Health Claim For Vitamin D and/or Calcium and Reduced Risk of Osteoporosis." Each document consists of six volumes including appendices. This submission is in accordance with § 403(f)(4) of the Federal Food, Drug and Cosmetic Act and 21 CFR § 101.70.

Please note that the enclosed petition is seeking an unqualified health claim based on our contention that the significant scientific agreement standard has been met in this area.

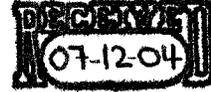
Correspondence and any questions regarding this petition should be directed to Dr. Carolyn Moore, Director of Nutrition and Health, The Beverage Institute for Health and Wellness, The Coca-Cola Company, (713/888-6101) or to Dr. Guy H. Johnson, Johnson Nutrition Solutions LLC (269/353-5903).

Sincerely,

Donald W. Short
President

2004P-0464

CP1



DATE: June 9, 2004

NAME OF PETITIONER: The Beverage Institute for Health & Wellness LLC

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Houston, TX 77056

SUBJECT OF PETITION: Authorization of a health claim for vitamin D and/or calcium and reduced risk of osteoporosis

SUBMITTED TO: Office of Nutritional Products, Labeling and Dietary Supplements (HFS-800)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
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I. INTRODUCTION

The undersigned, The Beverage Institute for Health & Wellness LLC (BIHW), an entity of The Coca-Cola Company, submits this petition pursuant to section 403(r)(4) of the Federal Food, Drug, and Cosmetic Act with respect to the ability of vitamin D and calcium to reduce the risk of osteoporosis. The claim would apply to eligible products that contain at least 20 percent of the Daily Value (DV) of vitamin D and/or calcium per reference amount customarily consumed (RACC).

The Food and Drug Administration (FDA) has authorized a health claim (21 CFR §101.72) on the ability of calcium to reduce the risk of osteoporosis among teen and young adult white and Asian women who engage in regular physical activity. The BIHW strongly believes that there is now significant scientific agreement that supports authorization of an expanded osteoporosis claim that includes vitamin D and eliminates the restrictive language regarding age, race, gender and physical activity. FDA has already proposed most of these changes (60 FR 66206, 66225, December 21, 1995) and we believe there is now compelling evidence to support even more simplification of the claim and to include vitamin D for applicable products. Model language for the proposed claim is, "Adequate vitamin D and calcium may reduce the risk of osteoporosis in later life."

This proposal is based on numerous statements published by authoritative bodies and academic experts that conclude adequate vitamin D and calcium are necessary to achieve maximal peak bone density during the developmental years, and to minimize the loss of

bone mineral density (BMD)¹ in adulthood. In addition, recommended intakes (i.e. Dietary Reference Intakes (DRIs)) for both nutrients have been increased for certain segments of the population since the original health claim was authorized in 1993, which indicates an increased realization of the importance of these nutrients for bone health. Unfortunately, inadequate nutritional status of calcium and vitamin D are widespread in the U.S. population. Finally, numerous observational studies, randomized clinical trials and meta-analyses have been published since authorization of the original health claim that show vitamin D and/or calcium (especially in combination) increases BMD in children and adolescents, reduces (or eliminates) bone loss in adults and/or reduces the incidence of fractures regardless of gender, race or physical activity level.

A. PROFESSIONAL ORGANIZATIONS/AUTHORITATIVE BODIES/ACADEMIC EXPERTS

There is a clear consensus in the professional and academic community that calcium and vitamin D are important to ensure optimal bone health and to reduce the risk of osteoporosis. Authorities who have expressed this point of view include:

1. The Food and Drug Administration

FDA concluded in the final rule of the current health claim, "The overwhelming concurrence among the experts in this area and the totality of publicly available evidence supports an association between adequate calcium intake and risk of osteoporosis." (58

¹ BMD is a direct measure of osteoporosis and quantitative diagnostic criteria using this measure have been established by the World Health Organization (Ilich *et.al.*, 2000). In addition, BMD correlates directly with fracture incidence in children (American Academy of Pediatrics, 1999) and in adults (Dawson-Hughes, 1998).

FR 2665, 2672, January 6, 1993). FDA also noted, “many nutrients [including vitamin D] are essential for normal bone growth and development” (*Id* at 2666). The agency reiterated this position in its 1995 proposed revisions to the claim (60 FR 66206 at 66216).

2. The Institute of Medicine

The DRI for calcium (Food and Nutrition Board, 1997) is based on the minimum amount of this nutrient that will maximize peak bone mass in children and minimize bone loss in adults. The ultimate goal is to reduce the risk of osteoporosis and fractures in later life. The FNB clearly recognized that adequate calcium at all stages of life is necessary to reduce the risk of subsequent osteoporosis. In addition, the DRI for vitamin D is based on ensuring a sufficient concentration of serum 25(OH)D to prevent elevated PTH levels and subsequent mobilization of calcium from the skeleton. In setting the DRIs for vitamin D, the Food and Nutrition Board (1997) concluded, “The ultimate effect of vitamin D on human health is maintenance of a healthy skeleton.”

3. The National Institutes of Health

An NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy (2001) concluded, “Adequate calcium and vitamin D intake is crucial to develop optimal peak bone mass and to preserve bone mass throughout life.” The Panel also concluded,

Vitamin D is required for optimal calcium absorption and thus is also important for bone health. Most infants and young children in the United States have adequate vitamin D intake because of

supplementation and fortification of milk. During adolescence when consumption of dairy products decreases, vitamin D intake is less likely to be adequate, and this may adversely affect calcium absorption. A recommended vitamin D intake of 400 to 600 IU/d has been established for adults.

4. The National Osteoporosis Foundation

The National Osteoporosis Foundation's clinical practice guide (Heinemann, 2000) recommends, "Advise all patients to obtain an adequate intake of dietary calcium (at least 1,200 mg/d, including supplements if necessary) and vitamin D (400 to 800 IU/d for individuals at risk of deficiency).

5. The Osteoporosis Society of Canada

The clinical practice guidelines of the Osteoporosis Society of Canada (Brown *et al.*, 2002) concluded, "Adequate calcium and vitamin D through diet or supplements are essential for the prevention of osteoporosis and, taken together, are essential adjuncts to preventative therapy."

6. The American Academy of Pediatrics

The Committee on Nutrition of the American Academy of Pediatrics (1999)² recommends,

Pediatricians should actively support the goal of achieving calcium intakes in children and adolescents comparable to those in recently recommended guidelines. The prevention of future osteoporosis, as well as the possibility of a decreased risk of childhood and

² This AAP statement also discusses the importance of calcium and vitamin D for the prevention of rickets and osteomalacia. Although these diseases are beyond the scope of the proposed health claim, it is likely that increased awareness of the benefits of vitamin D resulting from the proposed claim will call attention to these public health issues as well.

adolescent fractures, should be discussed as potential benefits to achieving these goals. Currently, relatively few children and adolescents achieve dietary calcium intake goals.

7. Academic experts in the area of calcium and vitamin D

Numerous review papers have been published during the past few years that conclude vitamin D and/or calcium reduce the risk of osteoporosis. Conclusions from some of the most thorough reviews are provided below:

Lips (2001) conducted a comprehensive review of the consequences of vitamin D deficiency in the elderly on bone loss and fractures. The paper concluded, "Vitamin D₃ supplementation causes a decrease of the serum PTH concentration, a decrease of bone turnover, and an increase of bone mineral density. Vitamin D₃ and calcium may decrease the incidence of hip and other peripheral fractures in nursing home residents."

Calvo (2000), a clinical researcher at the FDA, published a paper entitled, "Dietary Considerations to Prevent Loss of Bone and Renal Function." This paper observed, "Given the changing nature of the US food supply and changes in food-consumption patterns during the past century, two dietary recommendations for the 21st century come to mind that could help to optimize bone and kidney health. The first is to maintain adequate calcium and vitamin-D intakes from food sources at all stages of life...".

A review of the effects of calcium, vitamin D and vitamin K on osteoporosis by Meunier (1999) concluded, "If vitamin D 'insufficiency' is defined as a status of hypovitaminosis D influencing calcium homeostasis and bone remodeling through stimulation of PTH

secretion, this condition is much more common than was previously believed. This may indicate that a widespread increase in vitamin D and calcium intake is likely to have a greater effect on osteoporosis and fractures than many interventions.”

An assessment of the roles of calcium and vitamin D in the prevention of osteoporosis published by Reid (1998) concluded,

Calcium supplementation produces small beneficial effects on bone mass throughout postmenopausal life and may reduce fracture rates by as much as 50%. There is little reason to perform vitamin D supplementation in young populations replete in this compound, however, in the elderly at risk for vitamin D deficiency, the evidence suggests significant reductions in nonvertebral fracture rates with physiologic replacement regimens. Some of the most substantial reductions in fracture rates have been reported with combined therapy with calcium and vitamin D. In these protocols, it is not clear which substance is the principal active agent or whether, in fact, the combination is necessary for optimal antifracture efficacy.

Blank and Bockman (1999) conducted a review of clinical trials using fracture as an endpoint and concluded, “Fracture outcomes have been reported in clinical trials with calcium supplementation, vitamin D supplementation, estrogen replacement therapy (ERT), calcitonin, etidronate, alendronate, sodium fluoride (NaF), parathyroid hormone (PTH), and raloxifene. Compelling evidence for fracture prevention has been provided for calcium and vitamin D supplementation and alendronate treatment.”

Heaney (2000) reviewed 139 studies that explored the relationship between calcium intake and bone status and concluded,

It would not have been possible, within the space available here, to touch upon most of the foregoing studies individually. Manifestly, not all are of equal strength, nor are they all intercomparable. Some had samples sizes insufficient to find likely effects; others combined calcium with other nutritional interventions (indeed, this is inevitable when ever dairy foods, for example, are used as the calcium source). Others tested calcium effects in groups who already had relatively high calcium intakes. (Predictably, they found little effect). Nevertheless, the aggregate impact of them all and the congruence and internal consistency of the findings from various study types establish firmly the conclusions reached at the 1994 Consensus Development Conference on Optimal Calcium Intake, namely: 1) high calcium intakes are important throughout life, 2) the American people are not getting enough today, and 3) the need is greater than had once been thought.

Heaney (2001) reached a similar conclusion in a subsequent review of calcium needs in the elderly, "...many randomized, controlled trials have been performed, and the causal connection between augmented calcium intake and fracture reduction is now firmly established."

Hollick (2004) reviewed the importance of vitamin D in the prevention of cancers, type 1 diabetes, heart disease and osteoporosis and concluded,

Studies in both human and animal models add strength to the hypothesis that the unrecognized epidemic of vitamin D deficiency worldwide is a contributing factor of many chronic debilitating diseases. Greater awareness of the insidious consequences of vitamin D deficiency is needed. Annual measurement of serum 25(OH)D is a reasonable approach to monitoring for vitamin D deficiency.

An additional benefit of vitamin D was recently reported by Bischoff-Ferrari *et al.* (2004). A meta-analysis of randomized, controlled intervention trials show that vitamin D supplementation reduced the risk of falling among elderly subjects by 22% (corrected

odds ratio = 0.78; 95% confidence interval (CI), 0.64, 0.92) compared with patients that received calcium supplementation or a placebo. The authors concluded, "Vitamin D supplementation appears to reduce the risk of falls among ambulatory or institutionalized older individuals with stable health by more than 20%." The probable mechanism for this effect (increased muscle strength) is not directly related to osteoporosis, but these data provide yet another public health rationale from increasing the visibility of vitamin D to American consumers through the proposed claim.

Other review papers and editorials that conclude calcium and/or vitamin D are important to reduce the risk of osteoporosis have been published by Dawson-Hughes (1996), Weaver (1997), Meunier (1998), Shaota (2000), Anderson (2001), Fairfield and Fletcher (2002), Holick (2002), Heaney (2003) and Heaney and Weaver (2003). Only one editorial (Hegsted, 2001) was found that did not support the conclusion that high intakes of calcium are required to maintain bone health. This paper suggested that populations (including the US) can adapt to a low calcium intake and still maintain bone health, and criticized the use of calcium and vitamin D supplementation for this purpose.

Nevertheless, the overwhelming consensus of review papers and editorials published since authorization of the calcium and osteoporosis health claim supports the need for adequate vitamin D and calcium throughout life to maintain bone health and to reduce the risk of osteoporosis.

B. RECOMMENDED INTAKES OF VITAMIN D AND CALCIUM

As noted earlier, the recommended intakes of vitamin D and calcium have increased for certain segments of the North American population since the calcium and osteoporosis health claim was authorized. Calcium and vitamin D (along with magnesium, phosphorous and fluoride) were the first nutrients to be assigned DRIs using the IOM's revised process to define benchmarks of nutritional adequacy. Compared to the 1989 RDA (Food and Nutrition Board, 1989), the current Adequate Intake (AI) for calcium was increased from 1,200 to 1,300 mg/d for males and females 11 to 18 years of age, and from 800 to 1,200 mg/d for adults of both genders 51 years of age or older. The change in dietary recommendations for vitamin D was even more dramatic. The AI for this nutrient doubled (from 5 to 10 $\mu\text{g}/\text{d}$) for women and men aged 51-70, and tripled (to 15 $\mu\text{g}/\text{d}$) for those 71 years of age or more compared to the 1989 RDA (Food and Nutrition Board, 1997). The latter change was the largest increase ever made by the IOM for a nutrient intake recommendation and was prompted by considerations related to bone health (Heaney and Weaver, 2003). These changes reflect the strong consensus among academic experts that adequate vitamin D and calcium are needed by females and males of all ages and ethnic groups to reduce the risk of osteoporosis in later life. Although the DRIs for calcium were based primarily on data from white subjects, the committee concluded that there is not sufficient information to warrant a different DRI for African Americans (Bryant *et.al.*, 1999).

C. CURRENT INTAKES OF CALCIUM AND VITAMIN D

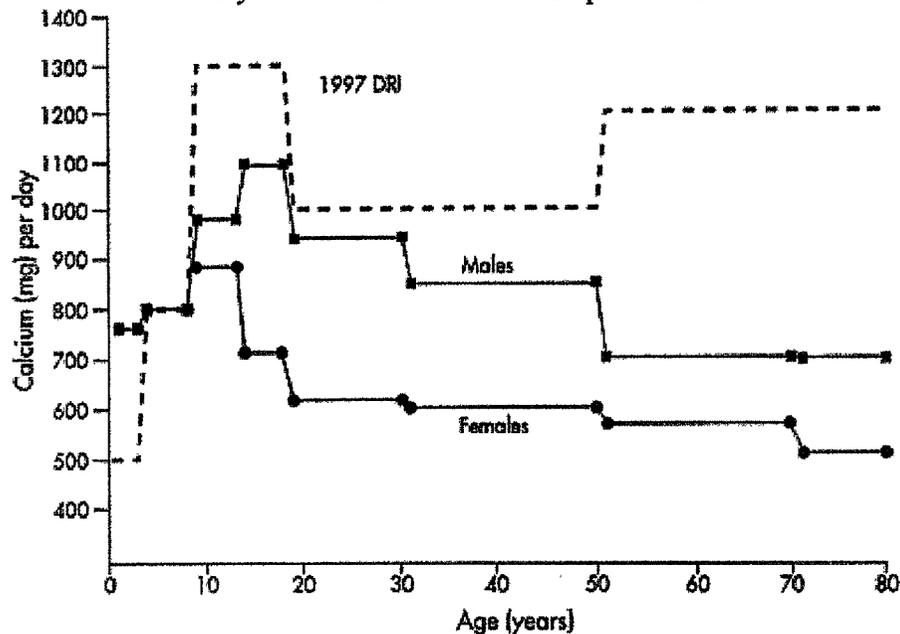
Unfortunately, at a time when calcium and vitamin D requirements are increasing, intake levels in the U.S. are suboptimal. The mean calcium intake in the U.S. is well below recommended intakes for virtually all age and gender segments of the population except infants and very young children. Data from the 1994-96 Continuing Survey of Food Intake by Individuals (CSFII) (USDA, 1996) show that only 44.6% of males and 22.0% of females 20 years of age and over received 100% of the 1989 RDA for calcium. Analogous data for men and women 70 years of age or greater are 38.8% and 20.8%, respectively. Calcium intakes in the U.S. compared to the current DRI are shown in Figure 1. These data clearly illustrate the need for compelling educational messages designed to increase consumption of calcium among virtually the entire U.S. population.

Current vitamin D intakes are also substantially lower than recommended amounts for most of the U.S. population. A recent analysis of the Third National Health and Nutrition Examination Survey (NHANES III) and the 1994-96 and 1998 CSFII databases (Moore *et al.*, in press) showed that fewer than 30% of male and female subjects age 50 or older received 100% of the AI of vitamin D from food and dietary supplements (see Figure 2). In addition, less than 10% of non-dietary supplement users in this age range met current dietary recommendations.

Suboptimal intakes of vitamin D do not necessarily reflect poor status of this nutrient because exposure of the skin to ultraviolet radiation can compensate for inadequate dietary sources. Nevertheless, the literature reflects a growing awareness that vitamin D

deficiency in the U.S. and other countries is a serious concern.

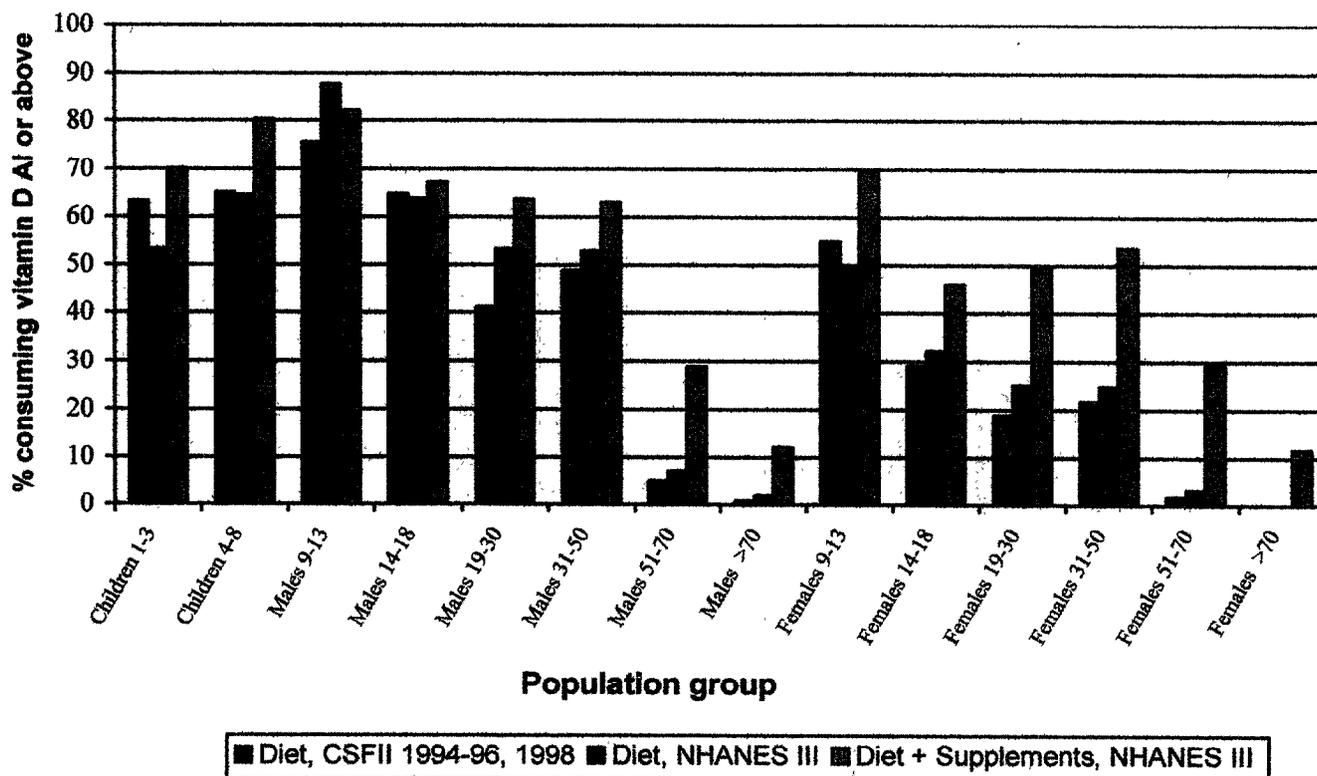
Figure 1
Mean Calcium Intakes of American Males and Females compared to the NAS 1997
Dietary Reference Intakes – Adequate Intakes



Source: Bryant, R.J. *et.al.* 1999. *J. Am. Col. Nutr.* 18:406S

The accepted indicator of vitamin D status in humans is the concentration of serum 25(OH)D. The optimal level of serum 25(OH)D necessary to prevent elevated PTH (which intern mobilizes calcium from bone) is a matter of some controversy. It has been proposed that serum 25(OH)D in the 25-50 nmol/L range reflect mild vitamin D deficiency, 12.5-25 nmol/L reflect moderate deficiency and values <12.5 nmol/L be considered severe deficiency (Lips, 2001). However several researchers including Holick (2002, 2004) and Hollis and Wagner (2004) have observed that values as high as 85 nmol/L may be necessary to reduce PTH to baseline values in some individuals.

Figure 2
Percent of U.S. Age/Gender Segments Consuming 100% or
More of the Adequate Intake of Vitamin D



Source: Moore *et.al.*, In press

Lips (2001) reviewed several studies that found prevalent vitamin D deficiency (serum 25(OH)D <30 nmol/L) in healthy adults, elderly residents of France, Australian hip fracture patients and inhabitants of Saudi Arabia who wear clothing that prevents exposure to sunlight. Other observations of vitamin D inadequacy have been reported in Chinese adolescents (Hu *et.al.*, 2001), Finish adolescents in the winter (Outila *et.al.*, 2001), U.S. adolescents and adults (Looker *et.al.*, 2002; Gordon and Nelson, 2003), U.S. young black and white women (Harris and Dawson-Hughes, 1998; Nesby-O'Dell *et.al.*, 2002), healthy French adults (Chapuy *et.al.*, 1997), postmenopausal black women living in the U.S. (Kyriakidou-Himonas *et.al.*, 1999), U.S. medical inpatients (Thomas, *et.al.*,

1998), healthy postmenopausal women (Need *et.al.*, 2000; Mezquita-Raya *et.al.*, 2001), postmenopausal women with femoral neck fracture (Punnonen *et.al.*, 1986), elderly women (Bischoff *et.al.*, 2003) and elderly men and women living in Baltimore (Gloth *et.al.*, 1995).

These studies demonstrate that vitamin D deficiency is likely to be a serious public health concern not only in vulnerable groups (e.g. elderly), but among healthy individuals as well. Holick (2004) has specifically noted the importance of evaluating vitamin D status among young healthy female adults to maintain bone health and to prevent other negative effects of vitamin D deficiency. Similar observations were made by Hollis (2004) in the case of pregnant and lactating women.

Unfortunately, there are practical obstacles to achieving optimal vitamin D status. Calvo (2000) noted that dietary sources of vitamin D in the U.S. food supply are limited (e.g. fortified milk, a few fortified yogurt and breakfast cereals as well as fatty fish).³ In addition, recommendations to limit exposure to the sun in order to reduce the incidence of skin cancer have the unintended consequence of compromising vitamin D status. The American Academy of Dermatology (Lim *et.al.*, 2000) and the U.S. Environmental Protection Agency (1998) have recommend that exposure to the sun be minimized by wearing a hat and other protective clothing and by applying sunscreen (SPF factor ≥ 15) 20 minutes prior to going outdoors. Lack of awareness of the suboptimal status of

³ Vitamin D has subsequently been added to a range of calcium-fortified juice products marketed in the United States.

vitamin D and misconceptions regarding its safety are also obstacles to addressing this issue.

D. CONCLUSION

The totality of scientific evidence as well as numerous professional and public health authorities agree that a broad segment of the U.S. population is at risk for osteoporosis due to inadequate intake of vitamin D and calcium. The need for additional public health measures designed to combat osteoporosis will become increasingly apparent as the population ages and incidence of the disease continues to rise. The BIHW strongly believes that the availability of a simplified, understandable health claim will allow food manufacturers to help address this public health issue by educating consumers about the importance of both vitamin D and calcium in reducing the risk of osteoporosis in later life.

II. PRELIMINARY REQUIREMENTS

Petitions for health claims pertaining to a component of a food to be consumed at other than decreased dietary levels are required by 21 CFR § 101.70 to demonstrate that certain preliminary requirements are met: that the object of the proposed claim conforms to the definition of a "substance" in § 101.14(a)(2); that the substance is eligible for a health claim according to § 101.14(b), which specifies it must be "associated with a disease or public health-related condition for which the general U.S. population, or an identified U.S. population subgroup...is at risk..."; that it contributes, "taste, aroma, or nutritive value, or any other technical effect listed in 21 CFR § 170.3(o); and that the substance is

safe and lawful at the level necessary to justify the claim under the food safety provisions of the Federal Food, Drug, and Cosmetic Act.

These requirements have already been established for calcium and osteoporosis as evidenced by the FDA authorized a health claim in this area. The BIHW believes that vitamin D also meets these requirements as discussed below.

A. Vitamin D is a substance under 21 CFR § 101.14 (a)(2)

The definition of a “substance” under 21 CFR § 101.14 (a)(2) is “...a specific food or component of food, regardless of whether the food is in conventional food form or a dietary supplement that includes vitamins, minerals, herbs, or other similar nutritional substances.” As its name indicates, vitamin D is a vitamin and therefore a substance eligible to be the object of a health claim.

B. Osteoporosis is a major public health concern in the United States

The National Osteoporosis Foundation (2002) estimates that approximately 44 million men and women in the United States currently have low bone density or osteoporosis (see Table 1). This value is projected to increase to more than 61 million by 2020. White and Asian women are the most susceptible to chronic bone disease, but the condition is also prevalent among African Americans. Five percent of the African American U.S. population (more than 13 million people) are currently thought to have frank osteoporosis compared to 20% for white and Asian women. The incidence of low BMD in these two groups is estimated to be 35 and 52%, respectively.

Table 1
Incidence of Osteoporosis and Low Bone Mineral Density in the United States

| | 2002 | 2010 | 2020 |
|---|------------|------------|------------|
| Osteoporosis and Low Bone Mass in Women and Men | 43,600,000 | 52,400,000 | 61,400,000 |
| Osteoporosis in Women and Men | 10,100,000 | 12,000,000 | 13,900,000 |
| Low Bone Mass in Women and Men | 33,600,000 | 40,400,000 | 47,500,000 |
| Women With Osteoporosis or Low Bone Mass | 29,600,000 | 35,100,000 | 40,900,000 |
| Women With Osteoporosis | 7,800,000 | 9,100,000 | 10,500,000 |
| Women With Low Bone Mass | 21,800,000 | 26,000,000 | 30,400,000 |
| Men With Osteoporosis and Low Bone Mass | 14,100,000 | 17,300,000 | 20,500,000 |
| Men With Osteoporosis | 2,300,000 | 2,800,000 | 3,300,000 |
| Men With Low Bone Mass | 11,800,000 | 14,400,000 | 17,100,000 |

Source: National Osteoporosis Foundation, 2002.

The importance of osteoporosis as a public health problem has increased since authorization of the original health claim in 1993. FDA noted in its proposed rule for this claim (56 FR 60689, 60690, November, 27, 1991) that the number of bone fractures in the U.S. due to osteoporosis was 1.3 million per year. Updated data from the National Osteoporosis Foundation (2004) show that this number has increased to 1.5 million. In addition, the direct medical cost required to treat osteoporosis has increased from approximately \$10 billion as noted by FDA in 1993 to an estimated \$17 billion per year at the present time.

C. Vitamin D contributes nutritive value to the diet

The definition of "nutritive value" under 21 CFR § 101.14 (a)(3) is "...a value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy." Vitamin D is itself an essential nutrient that has

been assigned Daily Reference Intakes (DRIs) by the Food and Nutrition Board of the IOM (1997) and, therefore, is eligible to be a substance that is the object of a health claim.

D. Vitamin D is safe and lawful

Vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) have been affirmed as generally recognized as safe (GRAS) when used as a source of this nutrient for breakfast cereals, grain products and pastas, milk, and milk products according to 21 CFR § 184.950(c)(1). Vitamin D may also be added to infant formula in accordance with section 412(a)(2) of the Food Drug and Cosmetic Act, and as an optional ingredient in margarine according to 21 CFR § 166.110. In addition, FDA recently approved vitamin D₃ as a food additive that may be added in amounts up to 100 IU per serving to 100% fruit juices (excluding those specifically formulated for infants) that are fortified with greater than 33% of the Recommended Daily Intake (RDI) of calcium per RACC and to fruit drinks (excluding those specifically formulated for infants) that are fortified with greater than 10% RDI of calcium per RACC (68 FR 9000, 9003, February 27, 2003). As part of this rulemaking, FDA concluded that the addition of vitamin D₃ to the beverages specified was safe in view of the overall intake of this nutrient in the U.S. and the tolerable upper intake level (UL) established by the Food and Nutrition Board (1997). The tightly regulated addition of vitamin D to food products provides a high level of assurance that authorization of the proposed claim will not result in excessive intakes by American consumers.

III. SCIENTIFIC EVIDENCE SUPPORTING THE CLAIM

As noted above, the BIHW strongly believes that the totality of available scientific evidence warrants expanding the existing health claim on calcium and osteoporosis to include vitamin D and to remove qualifying language regarding age, gender, race and physical activity. A comprehensive literature review was conducted to identify all human observational and intervention studies pertaining to vitamin D and/or calcium on bone health (see Appendix B). All such studies published in English were selected for possible consideration in this petition. Studies that were not applicable to the proposed health claim, did not provide enough information to be evaluated or were cited in FDA's Final Rule, "Food Labeling: Health Claims; Calcium and Osteoporosis (58 FR 2665) were eliminated from further discussion. The remaining studies are addressed individually below.

In accordance with 21 CFR § 101.70(c) and 21 CFR § 170(d) the BIHW declares that to the best of our knowledge, all non-clinical studies relied upon in our petition were conducted in compliance with the good laboratory practice regulations as set forth in 21 CFR Part 58, and all clinical or other human investigations relied upon were either conducted in accordance with the requirements for institutional review set forth at 21 CFR Part 56 or were not subject to such requirements in accordance with 21 CFR §§ 56.105 or 56.105, and were conducted in conformance with the requirements for informed consent set forth in 21 CFR Part 50.

The papers discussed below are segregated by age of the subjects studied in order to more concisely summarize the scientific findings pertinent to the proposed claim. Each section includes a summary of the applicable observational studies followed by a discussion of the human intervention trials⁴. The papers are discussed in chronologic order with studies published in the same year presented alphabetically according to the author's last name. "Summary and conclusions" sections are provided after the discussion of individual papers for each age segment. An overall assessment of the literature that demonstrates how the SSA standard has been met concludes this section of the petition.

All papers have been classified according to FDA's "Interim Evidence-based Ranking System for Scientific Data"⁵ by assigning each study a score that reflects its design type (1= randomized, controlled intervention trials; 2= prospective observational cohort studies; 3= nonrandomized intervention trials or case-control studies; and 4= cross-sectional observational studies) and quality ("+" adequate design and scientific quality; "Ø" some uncertainties with respect to scientific design and quality; "-" the report has not adequately addressed design and quality issues). The quality ratings were assigned according to specific questions provided in the ADA Evidence Analysis Guide⁶. Quality ratings were not assigned to cross-sectional observational studies because the BIHW believes such studies are unlikely to make a material difference in FDA's decision to authorize the claim regardless of their quality score. Nevertheless, all such studies that

⁴ Detailed summaries of the intervention trials, but not the observational studies, are also presented in tabular form (see Appendix C). This approach is being taken in accordance with FDA's "Interim Evidence-based Ranking System for Scientific Data," which places considerably more weight on randomized, controlled trials than it does on observational studies.

⁵ Food and Drug Administration, Center for Food Safety and Applied Nutrition. Interim Evidence-based Ranking System for Scientific Data. July, 2003. <http://www.cfsan.fda.gov/~dms/hclmgui4.html>

⁶ American Dietetic Association, Scientific Affairs & Research. ADA Evidence Analysis Guide. http://www.eatright.org/Public/Files/Evidence_Analysis_Manual.doc

met the criteria for discussion in this petition are summarized below to provide the agency with a comprehensive review of the available literature.

A. PRE-PUBERTAL CHILDREN

The available data discussed in this section clearly support the proposed claim for prepubertal children. Eight observational studies conducted with subjects in this age range (including a prospective study with the strongest design) support the notion that calcium intake is associated with bone health. In addition, two cross-sectional studies provide evidence that vitamin D has such an association. Furthermore, all of the randomized, controlled trials found that calcium supplementation improved bone mass in young children.

1. Observational studies

A cross-sectional study was conducted by Ruiz *et al.* (1995) to investigate the association between calcium intake and physical activity on BMD in a group of girls (n=81) and boys (n=70) (mean age = 12.4 and 12.3, respectively). Mean calcium intake was 810 mg/d with a wide range (157 – 2,033 mg/d). The percent of the subjects who failed to consume the French recommended intake of calcium at the time (1,000 mg/d) was 69% of the prepubertal children, 71% of the pubertal subjects and 57% of the postpubertal adolescents. Multivariate analysis found that calcium intake was independently associated with femoral and spine BMD ($p=0.05$) in the total sample of boys. Calcium intake was also associated ($p=0.02$) with spine BMD among prepubertal boys and girls. The authors concluded that dietary calcium influences the bone mass of children and

adolescents, and noted that calcium intakes below 1,000 mg/d before and during puberty may have deleterious consequences on vertebral and possibly femoral bone mass. [Study score = 4]

VandenBergh *et.al.* (1995) studied a group of Dutch boys (n=653) and girls (706) between 6.8 and 10.7 years of age. Mean calcium intake (as assessed by a FFQ of dairy products that covers approximately 80% of total dietary calcium) was 585 mg/d for boys and 620 mg/d for girls. There were no associations between dietary calcium intake and BMD of the index finger at two sites. The authors speculated that the negative results of this cross-sectional study might have been because 80% of the children had calcium intakes above a threshold value of 1,400 mg/d. [Study score = 4]

A cross-sectional study of 197 healthy adolescent Japanese girls (12-15 years of age) was reported by Tsukahara *et.al.* (1997). Mean calcium intakes, as estimated by 3-day dietary surveys, ranged from 521 mg/d for 15 year-olds to 639 mg/d for 13 year old subjects. Calcium intake was marginally associated ($p=0.0793$) with BMD of the second metacarpal as determined by digital image processing. The authors concluded that calcium is an important nutrient involved in bone growth. [Study score = 4]

Wang *et.al.* (1997) studied the association between dietary factors (i.e. calcium, protein and total energy), ethnicity and weight-bearing activity and parameters of bone mass in a cross-sectional cohort of 423 Asian (n=103), black (n=115), Hispanic (n=102) and non-Hispanic Caucasians (n=103) living in the U.S. The study population consisted of 229

females who were classified as pre/early puberty (n=39), midpuberty (n=81) or mature (n=109), and 194 males classified as pre/early puberty (n=54), midpuberty (n=65) and mature (n=75) based on self-reported Tanner stage assessments. Skeletal development was assessed at several sites by bone mineral content (BMC, g), bone mineral density (BMD, g/cm²) and bone mineral apparent density (BMAD, g/cm³) by dual-energy X-ray absorptiometry (DXA). With few exceptions, Asians and Hispanics had comparable bone mass to whites at all pubertal stages after controlling for gender, pubertal stage, weight and height. Black females had higher BMAD than their white counterparts at all pubertal stages, and black males had greater BMD and BMAD than white males at all sites in early puberty and at the femoral neck in maturity. Calcium intake, which was assessed by a 97-item National Cancer Institute (NCI) Food Questionnaire, was associated with BMAD at the femoral neck in early pubertal females, but there were no consistent associations with diet and bone mass among the other groups. There were no data reported on vitamin D intake or status of the subjects. This cross-sectional study suggests that bone density among racial groups tends to be similar after controlling for developmental stage, gender, weight and height. [Study score = 4]

Goulding *et.al.* (1998) conducted a case-control study in 100 white girls with recent distal forearm fractures and 100 age-matched controls. All female residents Dunedin, New Zealand aged 3 to 15 who experienced a fracture of the radius and/or ulna were recruited for the study. The participation rate was 83.7%. Friends of the fracture victims served as controls (93.6% participation rate). Current and past dietary calcium intakes were estimated by a questionnaire although very little information on the survey instrument

was provided. Vitamin D intake and/or dietary status was not reported. The study found that BMC and/or BMD were significantly lower at several skeletal sites among cases aged 11-15 compared to controls of a similar age. There were no differences in BMC or BMD in younger participants. Calcium intake from dairy products did not differ between cases and controls (858 and 829 mg/d, respectively) as a group, but fracture victims age 3-7 consumed less calcium from milk (372 mg/d) than controls (509 mg/d, $p < 0.02$) as did those aged 11-15 (787 vs. 947 mg/d, $p < 0.05$). The older group also reported consuming less milk when they were 6-10 years of age compared to controls (364 vs. 499 mg/d, $p < 0.05$). This study provides suggestive evidence that calcium intake in older girls (11-15) is associated with bone mass and fracture rates (detailed data on pubertal status was not provided), but no such observation was made in younger girls. Nevertheless, the dietary assessment was limited and there was no data on vitamin D. [Study score = 3+]

A cross-sectional study of 456 healthy white girls aged 8 – 13 was conducted to examine the association between anthropometric and growth parameters, nutritional factors and physical activity to indices of bone health (Ilich *et al.*, 1998). Mean calcium intake was 956 mg/d (range 220 – 2,300 mg/d) and mean vitamin D intake was 6 $\mu\text{g/d}$ (range 0 – 31 $\mu\text{g/d}$). Multiple regression analysis showed that the factors most predictive of TBBMD were total bone area, body fat, lean body mass, stature, skeletal age and dietary calcium. These factors explained 47.8% of the variability. The determinants for radius BMD, which explained 35.9% of the variability were total bone area, body fat, lean body mass, skeletal age and dietary calcium. This study supports the premise that dietary calcium is associated with bone mass. [Study score = 4]

Kardinaal *et al.* (1999) conducted a cross-sectional survey of 1,116 healthy, white girls (aged 11-15 years) and 526 women (aged 20-23 years) in six European countries. Mean calcium intake for girls and women varied between 609 mg/d in Italy to 1,267 mg/d in Finland. BMD was measured at the ultradistal and middistal radius using DXA. Calcium intake of both groups was not correlated to BMD at either site according to quartiles of calcium intake after adjustment of the data for height, weight, age, menarche (for women) and Tanner stage and BA (for girls). However, calcium intake among 424 pre-pubertal girls was significantly associated with middistal BMD after adjustment for age, height, weight, Tanner stage and BA ($p < 0.01$). Furthermore, multivariate analysis revealed that calcium intake was positively associated with middistal BMD for girls ($p = 0.02$) and for women ($p = 0.05$) who had calcium intakes < 600 mg/d. This study suggests that the effect of calcium on mass may be particularly important in pre-pubertal girls and among girls and women with low calcium intakes. Serum 25(OH)D data were available for four of the countries studied, but this indicator of vitamin D status was not correlated with bone mass at either site. [Study score = 4]

A cross-sectional study (Hoppe *et al.*, 2000) of 105 Danish 10-year old children (51 boys and 54 girls) was conducted to examine the association between lifestyle and dietary habits on whole body bone mineral content (BMC) and bone area (BA). Calcium intake as assessed by 7-day food records was close to amounts recommended for U.S. children (1,300 mg/d) and was similar for boys (1,239 mg/d) and girls (1,144 mg/d). Calcium intake correlated positively with BMC, size-adjusted BMC and BA. Vitamin D intake

was not assessed. The authors conclude that calcium intake in childhood is associated with size-adjusted bone mineral content. [Study score = 4]

Oliveri *et al.* (2000) conducted a cross-sectional study of 163 prepubertal children (mean age 8.9 years) living in two areas of Argentina. The purpose of the study was to compare BMC and BMD of the distal radius between children living in an area with sufficient sunlight to maintain adequate vitamin D status year round (Buenos Aires, 34° South latitude) with age- and gender-matched subjects in an area with limited winter sunlight (Ushuaia, 55° South). Calcium intake was estimated by a survey of dairy products, and none of the subjects had received supplemental vitamin D. Calcium intake for girls was similar between the two locations (798 mg/d in Ushuaia and 816 mg/d in Buenos Aires) but Ushuaia boys consumed less calcium (666 mg/d) compared to their Buenos Aires counterparts (966 mg/d, $p < 0.001$). There were no differences in BMC or BMD between the boys or girls living in the two locations. The conclusions that can be drawn from this study are limited because dietary intake data were limited and no objective parameters of vitamin D status (i.e. 25(OH)D) or calcium homeostasis (i.e. PTH) were reported. [Study score = 4]

A two-year prospective cohort study (Barr *et al.*, 2001) of 45 prepubertal girls (mean age 10.5 years at baseline) found that calcium intake predicted baseline, 2-year and 2-year change in total body BMC. Calcium was measured by 3-day food intake records and twice-yearly FFQs. Mean calcium intake at baseline was 831 mg/d. The authors

concluded that habitual calcium intake contributes to BM accretion during growth. No information on dietary vitamin D or its status was provided. [Study score = 2+]

Black *et.al.* (2002) conducted a cross-sectional study of bone mass in 50 pre-pubertal children (30 girls) with a history of milk avoidance compared to 200 age- and gender-matched controls from the same community. Dietary calcium was significantly lower in the milk avoiders (420 mg/d for girls and 478 mg/d for boys) than among milk drinkers (1,179 and 1,278 mg/d, respectively). Numerous parameters of bone mass (e.g. BA, total body BMC, BMD, fracture incidence) were lower among milk avoiders compared to control subjects. No information was provided on vitamin D intake or status. The authors concluded that milk avoiders are likely to have serious problems in skeletal development and should be given dietary counseling. [Study score = 4]

Cheng *et.al.* (2003) conducted a cross-sectional survey of 193 Finish girls aged 10-12 years to determine the effect of vitamin D status on BMC and BMD at different bone sites in early pubertal and prepubertal girls. Vitamin D deficiency ($25(\text{OH})\text{D} \leq 25$ nmol/L) occurred in 32% of the subjects while 46% of the participants were classified as vitamin D insufficient ($25(\text{OH})\text{D} = 26-40$ nmol/L). Serum intact PTH (iPTH) was significantly higher in the deficient and insufficient groups compared with vitamin D sufficient subjects. There were no significant differences in BMC and BMD of the total body, femur or lumbar spine as measured by DXA. However, when Tanner stage and BMI were controlled, the BMC of the total femur was significantly higher in the vitamin D deficient group compared to the sufficient group ($p < 0.04$). In addition, the volumetric

areal BMD (vBMD) and the cross-sectional area (CSA) of the distal radius measured by peripheral quantitative computed tomography (pQCT) was greater in the vitamin D sufficient group ($p < 0.001$) compared to the deficient group. Mean calcium intake of the group was 733 mg/d with 92.4% of the subjects failing to meet the Finish recommended intake of 900 mg/d. Subjects in the vitamin D deficient group had significantly higher calcium intakes compared to the sufficient group, but there was no difference after adjustment for total energy intake. The authors concluded that vitamin D insufficiency accompanied by high bone resorption might limit the accretion of bone mass in young girls. [Study score = 4]

2. Intervention studies

Johnston *et al.* (1992) conducted a randomized, placebo-controlled intervention trial on the effect of supplementing the diet with 1,000 mg of calcium citrate malate (CCM) daily for three years among 70 pairs of identical twins aged 6 to 14 years (mean age = 10 years). The calcium supplements did not contain vitamin D. The sample consisted of 86 pairs of girls and 54 pairs of boys. Mean calcium intake for girls and boys at baseline was 874 mg/d and 990 mg/d, respectively. There were no differences between the treatment and placebo groups in BMD at any skeletal site measured, nor were there differences at baseline in calcium intake or physical activity. Calcium intake and compliance with the study protocol were assessed through monthly food records. These records showed that the experimental group received a mean of 719 mg supplemental calcium during the three-year study. Calcium supplementation resulted in significant ($p < 0.05$) increases in mean BMD at the midshaft radius (5.1%), distal radius (3.8%) and

lumbar spine (2.8%) among twins who remained prepubertal throughout the study compared to the placebo group. There were no significant differences in BMD at three femoral sites. There were no significant differences in BMD due to supplementation among twin pairs who were pubertal at baseline or underwent puberty during the study (see discussion below in the section on adolescents). The authors concluded that dietary supplementation with calcium alone is associated with increased bone mineral acquisition among prepubertal children, which has the potential to reduce the risk of osteoporotic fractures later in life. [Study score = 1+]

Lee *et al.* (1995) conducted an 18-month randomized, placebo-controlled, double-blind study to determine the effect of calcium supplementation (300 mg/d from calcium carbonate) on BMD and height acquisition in a group of 109 male (n=63) and female (n=46) seven-year old children living in Hong Kong. There were no significant differences at baseline between the control and experimental group for any of the characteristics measured. Calcium intake was 571 and 563 mg/d for the treatment and placebo groups, respectively. Calcium supplementation resulted in significantly (p=0.035) greater BMC at the lumbar spine, lumbar spine area (p=0.049) and marginally greater BMC/BW at the distal radius compared to subjects given a placebo. There were no effects at the femoral neck or the spine. [Study score = 1Ø]

The effect of calcium supplementation on BMD and skeletal growth among 149 healthy prepubertal girls (mean age = 7.9 years) was assessed during a one-year randomized, double-blind, placebo-controlled trial (Bonjour *et al.*, 1997). Calcium supplements were

provided to the experimental group (n=55) as a variety of food products (cakes, biscuits, fruit juices, powdered drinking chocolate and yogurts) fortified with milk extract calcium. The placebo group (n=53) received the same foods in a non-fortified form. The paper did not indicate that vitamin D was added to the foods. Spontaneous calcium intake was estimated by averaging three FFQs made at 0, 24 and 48 weeks of the study and was similar for the experimental (916 mg/d) and placebo (879 mg/d) groups ($p>0.05$). Calcium intake from the fortified foods resulted in a mean increase of 916 mg/d. There were no differences in age, weight, height, BMI, or any measure of BMD between the experimental and control groups at the beginning of the study. Calcium supplementation resulted in significantly greater increases in BMD at the radial diaphysis ($p<0.02$), the femoral trochanter ($p<0.05$) and the femoral diaphysis ($p<0.01$) compared to the placebo group. There were no significant differences between the two groups for BMD of the radial metaphysis ($p<0.08$), the femoral neck ($p>0.05$) or the lumbar spine ($p>0.05$). In addition, there were significant differences between the experimental and placebo groups among subjects whose spontaneous calcium intake was below the median (880 mg/d) with respect to change in BMD of six skeletal sites ($p<0.02$) and total BMC ($p<0.05$). The authors concluded that calcium-enriched foods given to prepubertal girls with calcium intakes below the RDA significantly increased bone mass gain at several sites of the appendicular skeleton including the proximal femur. [Study score = 1+]

Dibba *et al.* (2000) studied the effect of supplementing the diet of 160 prepubertal Gambian children (80 girls and 80 boys) aged 8.3 to 11.9 years with 1,000 mg calcium carbonate per day for one year. Baseline nutrient intake was estimated by analysis of

foods consumed during a one-week assessment period. Calcium supplements were provided to the experimental group twice daily as chewable tablets. The placebo group received identical appearing tablets without calcium. The paper did not indicate that vitamin D was added to the supplements. There were no differences between the two groups at baseline with respect to age, weight, height or pubertal stage. Calcium intakes at baseline were low (342 mg/d in the experimental group and 334 mg/d in the placebo group) and did not differ between the two groups. Calcium supplementation increased intake by a mean of 714 mg/d to a total of 1,056 mg/d in the experimental group. Calcium supplementation resulted in significantly greater BMD of the midshaft radius ($p < 0.0001$) and distal radius ($p < 0.001$) compared to the placebo group after adjustment for bone width, body weight and height. Gain in BMD was not correlated with age, gender, pubertal status or spontaneous calcium intake. Calcium supplementation did not affect linear growth or weight gain. The authors concluded that supplementation with calcium carbonate increased bone mineral status, but did not alter growth or pubertal status in children with low customary calcium intakes who were lighter, shorter and less mature than reference children of the same age. [Study score = 1+]

A follow-up study to that of Bonjour *et.al.* (1997) (discussed previously in this section) was conducted by Bonjour *et.al.* (2001) to determine if the effects of calcium supplementation on bone mass persist after supplementation is discontinued. One hundred sixteen of the original cohort of 144 subjects (62 girls in the calcium-supplemented group and 54 non-supplemented controls) were studied 3.5 years after the original study concluded. There were no significant differences between the

experimental or placebo groups in age (mean age = 12.5 years), height, weight, BMI or pubertal stage upon reexamination. However, the supplemented group continued to have significantly greater BMD at the lumbar spine ($p=0.049$), radial diaphysis ($p=0.043$), femoral neck ($p=0.01$), femoral trochanter ($p=0.023$) and femoral diaphysis ($p=0.018$) than the placebo group. The radial metaphysis was the only site of those originally measured where differences in BMD between the experimental group and the controls did not persist ($p=0.20$). This effect was also seen in a subset of girls who remained at early pubertal stages (P1-P3) in the follow-up analysis. The authors concluded that milk-extracted calcium phosphate taken during the prepubertal period can modify the trajectory of bone mass growth and cause a long-standing increase in bone mass accrual, which lasts beyond the end of supplementation. [Study score = 1+]

3. Summary and conclusions

The observational studies conducted with prepubertal children provide overall support for the premise that calcium intake during this period is associated with bone mass. The prospective study by Barr *et.al.* (2001) provided the strongest evidence for this conclusion. A small case-control study (Goulding *et.al.*, 1998) found an association between calcium intake and fracture incidence in older girls but not in prepubertal subjects. The cross-sectional studies of Ruiz *et.al.* (1995), Wang *et.al.* (1997), Ilich *et.al.* (1998), Kardinaal *et.al.* (1999), Hoppe *et.al.* (2000) and Black *et.al.* (2002) also found associations between calcium intake and bone mass in prepubertal subjects. The cross-sectional study by Tsukahara *et.al.* (1997) did not find a significant association between calcium and BMD, but did report a borderline ($p=0.0793$) association. VandenBergh

et.al. (1995) did not find such an association but 80% of the participants had calcium intakes >1,400 mg/d. Cheng *et.al.* (2003) also did not report differences in bone parameters related to calcium, but found significant correlations with vitamin D status.

Only one study (Oliveri *et.al.*, 2000) was designed specifically to investigate a vitamin D-related exposure (i.e. sunlight) on bone health. Such an association was not found, but this study did not examine any objective parameters of vitamin D intake or status, and cannot be used to draw definitive conclusions.

The randomized, controlled studies provide very constant evidence that calcium supplementation increases BMD in prepubertal children of both genders. The most compelling results were provided by Bonjour *et.al.* (1997) who found positive effects among a cohort of girls at one year and in a subsequent follow-up study at 3.5 years Bonjour *et.al.* (2001). Other studies that found a positive effect of calcium supplementation on bone mass were Johnston *et.al.* (1992), Lee *et.al.* (1995) and Dibba *et.al.* (2000). No studies were found in the literature that failed to report a positive effect of calcium supplementation on bone health in prepubertal children.

In conclusion, as noted earlier, the studies reviewed in this section provide strong evidence in support of the proposed claim.

B. ADOLESCENTS AND YOUNG ADULTS

The available data discussed below clearly support the proposed claim for adolescent children. The cross-sectional studies conducted with adolescent subjects suggest that calcium intake is correlated with bone development if intakes are below recommended amounts, but that non-dietary parameters (especially hormonal changes) may overshadow dietary variables if intakes are adequate. Nevertheless, data from a retrospective cohort study and a case-control study suggest calcium is an important determinant of BMD among adolescents. In addition, three observational studies (including a prospective cohort study) found that vitamin D status is correlated with bone mass in this population. Most convincingly, all but one of the randomized, controlled clinical trials showed that supplementation of the diet with calcium improves bone mass among adolescent subjects.

1. Observational studies

A cross-sectional study of 162 girls aged 13 years (n=80) or 15 years (n=82) who resided in Reykjavik was reported by Kristinsson *et.al.* (1994). Calcium intake was estimated using a FFQ limited to milk, cheese and dairy products and BMD of the distal and ultradistal forearm was determined by single photon absorptiometry. Mean calcium intake from dairy products approximated recommended amounts for both the 13 and 15 year-old subjects (1,293 and 1,082 mg/d, respectively). Calcium intake was not related to BMD at either site among this population. Furthermore, univariate analysis showed that BMD was not significantly different among subjects in the lowest tertile of dairy calcium intake (<800 mg/d) compared to the highest tertile (>1,200 mg/d). However, the correlation coefficients between BMD and calcium intake were significantly greater

within the lowest tertile ($r=0.44$, $p<0.05$) compared to the other two tertiles ($r=0-0.2$). The results of this study are limited by its cross-sectional design, relatively small sample size and incomplete dietary assessment. Nevertheless, the authors concluded, "This study is consistent with the hypothesis that a threshold effect of calcium intake on BMD might exist". [Study score = 4]

Welten *et al.* (1994) conducted a 15-year prospective study on the association between physical activity and calcium intake among a cohort of 84 male and 98 female subjects living in Amsterdam. The subjects were 13 year of age at baseline and were followed until age 28. Mean calcium intake ranged from 941 mg/d among females aged 13-17 years to 1,204 mg/d to women at age 27 years. Similar results for males were 1,100 mg/d and 1,435 mg/d for younger and older subjects, respectively. There were no differences in BMD of the lumbar spine between male or female subjects in the lowest quartile of calcium intake compared to the highest. Furthermore, calcium intake was not associated with spine BMD among the entire cohort. The authors note that there may not have been enough subjects below the lower threshold of calcium intake to see an association on BMD, and that the vitamin D status of the participants was unknown. Other possible explanations were that the effect of puberty overshadowed dietary influences, or that such an association might have been observed at a site other than the spine. Spine is composed primarily of trabecular bone that the authors noted has been shown to be less responsive to calcium supplementation than cortical bone. [Study score = BØ]

The cross-sectional study by Ruiz *et al.* (1995) discussed in the previous section studied both pre- and post-pubertal boys and girls. Although calcium intake was independently associated ($p=0.05$) with BMD of the spine among all boys ($n=70$; mean age = 12.3 years) and among girls and boys at Tanner stage 1 ($n=49$; $p=0.02$), there was no such association among boys and girls at Tanner stage ≥ 2 ($n=70$; $p>0.05$). This study showed that puberty was significantly associated with increased BMD. Vertebral BMD was significantly greater among boys at Tanner stages 4 and 5 ($p<0.001$) and among girls at Tanner stage 5 ($p<0.001$) than children at earlier stages, and similar results were seen for femoral BMD among boys at Tanner stage 5 compared to their less developed counterparts ($p<0.01$). Although the authors concluded that calcium intake was important for development of bone mass in adolescent subjects, the data suggest that the effect of puberty was more pronounced in this group of children. [Study score = 4]

A prospective cohort study among 470 healthy Norwegian girls ($n=231$) and boys (mean age=8.2 to 16.5 years at baseline) was reported by Gunnes and Lehmann (1996). Calcium intake was assessed by a single 24-hour diet recall, and BMD was measured at baseline and after a one-year follow-up period. Mean calcium intake approximated recommended amounts and was significantly higher among boys (1,070 mg/d) than girls (913 mg/d) (p value not provided). Calcium was not associated with forearm BMD in this population, but there was a positive interaction of this mineral ($p<0.01$) with physical activity in girls, which suggests that dietary calcium increased the effect of physical activity on BMD. The greatest change in BMD occurred at age 14.0 in girls and 16.0 in boys. The lack of an association between dietary calcium and BMD in this study may be

due the relatively high dietary intakes of this nutrient. Results of the study may also have been affected by its limited dietary assessment (a single 24-hour recall) and short follow-up period. [Study score = 2-]

Moro *et.al.* (1996) conducted a cross-sectional study of 375 healthy adolescents and young adults aged 9-26 years from four ethnic groups (n=97 African-Americans; n=97 Asian-Americans; n=101 Caucasians; n=80 Hispanics). The sample consisted of 202 female and 173 male subjects. Energy adjusted calcium intake (calcium/energy) was estimated for the previous year using a food frequency questionnaire (FFQ). This parameter was significantly associated with BMC and BMD of the femur as well as femoral diaphyseal length, mid-diaphyseal diameter and an indicator of structural strength based on cross-sectional geometry. However, the greatest correlate of bone mass in these subjects was body weight. A multiple regression model accounting for age, pubertal group, lean body mass, weight-bearing activity level, calcium intake, gender, and ethnicity in addition to body mass did not substantially improved the predictive power of a model accounting for body mass alone. Nevertheless, this study provides support for the premise that calcium intake is a factor in promoting bone health in adolescent subjects. [Study score = 4]

As discussed above in the section on prepubertal children, the cross-sectional study by Wang *et.al.* (1997) found associations between dietary calcium and skeletal parameters for prepubertal subjects but not for adolescents. [Study score = 4]

In addition, a case-control study of girls with forearm fractures discussed in the previous section (Goulding *et.al.*, 1998) found that adolescent patients had lower calcium intakes in their current diet, and a history of low calcium intakes, than age- and gender matched controls. [Study score = 3+]

A cross-sectional sample of 249 healthy Icelandic girls age 16-20 (from an original cohort of 366) were studied for possible associations between diet, lifestyle factors and vitamin D status on BMC and BMD as determined by DXA (Kristinsson *et.al.*, 1998). The assessments were performed during the winter months (February – March) in Reykjavik (64° North latitude). Calcium intake was only modestly associated with skeletal parameters. Univariate analysis showed no significant correlation between calcium intake and BMC or BMD at any sight. However, one subgroup (21 girls aged 18 years) with calcium intakes <1000 mg/d had a significant correlation ($p=0.02$) between calcium intake and BMD of the hip. Multivariate analysis found significant correlations between calcium intake and spine BMD ($p=0.02$) in the youngest group and of forearm BMD ($p=0.015$) in the whole group. The lack of a strong association between calcium intake and bone mass may have been at least partially due to the fact that calcium intake tended to be high (lowest tertile <800 mg/d; highest tertile >1,200 mg/d) and may have been over the threshold of maximum effect for most subjects. Vitamin D intake was significantly associated with serum 25(OH)D ($p<0.001$) in univariate analysis, however this indicator of vitamin D status was not associated with BMC or BMD in the whole group. The only significant correlation with 25(OH)D was found for total forearm BMC and BMD in the 16-year old group ($p<0.05$). This lack of correlation occurred despite

the fact that 18.5% of the girls had serum 25(OH)D concentrations <25 nmol/L (87% of this group consumed less than 10 μ g vitamin D/d). Serum PTH was not reported. The authors speculated that factors other than vitamin D (e.g. growth hormone, estrogen) may have been effective in maintaining adequate calcium absorption in this sample of teenage girls. [Study score = 4]

Kardinaal *et.al.* (1999) (discussed in the prepubertal section above) reported that calcium intake was correlated with BMD of the middistal radius after adjustment for age, height, weight, Tanner stage and BA in a cross-sectional study of 424 premenarcheal girls, but not in older (11-15 year) menarcheal adolescents. However, calcium intake significantly contributed to middistal BMD ($p=0.01$) among 239 girls with low calcium intakes (<600 mg/d) after adjustment for age, height, weight, Tanner stage and BA. There were no associations between serum 25(OH)D and BMD reported. This study does not suggest that calcium is associated with bone density at the ultradistal or middistal radius in European girls and young women if dietary sources are adequate. [Study score = 4]

A similar cross-sectional study was reported by Maggiolini *et.al.* (1999) in a sample of 200 girls (aged 11-15) and 100 women (aged 20-23) living in southern Italy. This study found that calcium intake was not associated with BMD at the ultradistal or proximal radius in either age group, and that puberty was the major predictor of BMD. [Study score = 4]

Outila *et al.* (2001) conducted a cross-sectional study of 178 adolescent (mean age = 15.3 years) girls living in Finland in the winter. Mean calcium intakes were high (~1,200 mg/d) and were not correlated with BMD at the ulna or radius. However, subjects with serum 25(OH)D concentrations ≤ 40 nmol/L had significantly ($p=0.04$) lower radial BMD than girls with higher serum 25(OH)D concentrations. Serum 25(OH)D was associated with exposure to sunlight ($p<0.001$) and with use of vitamin D supplements ($p<0.001$). Serum iPTH was inversely correlated with serum 25(OH)D ($p=0.02$) in this group of young girls. The authors concluded that a large percentage of adolescent females have low vitamin D status during the winter in Finland, which seems to have negative effects on bone health. [Study score = 4]

Elgán *et al.* (2002) reported a cross-sectional study of 218 female college students (aged 16-24 years) living in Sweden. Calcium intake did not correlate with BMD of the heel as measured by DXA in this cohort. Mean calcium intake was 808 mg/d as estimated by a 34-item questionnaire on diet, physical activity and other physiological and lifestyle parameters. There were no data provided on vitamin D intake or status, but time spent outdoors in the summer correlated with serum concentrations of deoxypyridinoline (DPD) (a marker of bone remodeling). This paper suggests that effect of puberty (as measured by years since menarche) was a stronger predictor of heel BMD than dietary or other environmental factors. [Study score = 4]

A three-year prospective study of 191 healthy, Finnish girls aged 9-15 years was conducted to determine whether vitamin D status is associated with accrual of BMD and

BMAD (Lehtonen-Veromaa *et.al.*, 2002). All subjects were provided with 10 μ g supplemental vitamin D₂ from the beginning of October to February during the first two years of the study (the dosage was doubled during the third year), and subjects whose calcium intake was <1000mg/d were also given 500 mg of calcium carbonate per day. Baseline 25(OH)D correlated significantly with the unadjusted 3-year change in BMD at the lumbar spine ($p<0.001$) and femoral neck ($p<0.001$) in all participants. The authors observed that the last premenarcheal years are crucial to the prevention of osteoporosis, and concluded that vitamin D supplementation should be considered at this stage of development to help decrease the risk of not reaching maximum peak bone mass. [Study score = 2+]

A retrospective cohort study of 161 black and 180 white females aged 21-24 years who had participated in the National Heart, Lung, and Blood Institute (NHLBI) Growth and Health Study was conducted by Wang *et.al.* (2003). Mean dietary calcium was greater among prepubertal subjects (783 mg/d for blacks and 907 mg/d for whites) compared to those in mid-puberty (760 mg/d vs. 897, respectively) or post-puberty (686 mg/d vs. 882 mg/d, respectively). White subjects had higher calcium intakes compared to black girls at all stages of development. Multivariate analysis showed that calcium intake in mid-puberty was a positive predictor of whole body BMD ($p<0.001$) as well as BMD at the spine ($p<0.05$) and femoral neck ($p<0.05$). Calcium intake at this stage of puberty was more strongly associated with young adult peak bone mass (YABM) for the spine and whole body than was calcium intake in pre- or post-puberty. In addition, subjects who had consumed >1,000 mg calcium/d had significantly greater whole-body BMD ($p<0.01$)

than those who had received <800 mg/d, but there was no difference at the spine or femoral neck. The authors concluded that the skeleton is most responsive to dietary calcium during mid-puberty in both blacks and whites. [Study score = 2+]

Additional evidence for a positive association between vitamin D status and BMD was provided by the cross-sectional study by Cheng *et.al.* (2003) discussed in the previous section on prepubertal children. [Study score = 4]

2. Intervention studies

The study by Johnston *et.al.* (1992) (discussed in the previous section on prepubertal subjects) found that supplementing the diet of prepubertal girls and boys with 1,000 mg CCM/d resulted in significant increases in BMD of the midshaft radius, distal radius and lumbar spine, but that no such effects were seen in subjects who had achieved puberty before or during the study. The authors speculated that pubertal changes (e.g. secretion of growth hormone and sex steroids) may have dominated mineral accretion during this period so that bone is either maximally stimulated or changing so rapidly that the comparatively subtle effects of greater dietary calcium could not be detected. However, they noted that a balance study (Matkovic *et.al.*, 1990) reported a positive trend in bone accretion in 20 pubertal girls with an average intake of 1,640 mg calcium compared with nine girls of a similar age with low calcium intakes (750 mg/d). Other explanations noted were the difficulty of detecting small changes in BMD when skeletal growth is rapid, and the possibility that the size of the study was inadequate to detect small but important differences in pubertal children. [Study score = 1+]

Lloyd *et.al.* (1993) conducted a randomized, double-blind, placebo-controlled study on the effect of providing 500 mg/d CCM to 94 adolescent girls (mean age = 11.9 years) during a period of 18 months. The experimental group was instructed to consume two tablets containing 250 mg calcium and the placebo group was provided with indistinguishable tablets containing microcrystalline cellulose. The paper did not indicate that vitamin D was added to the supplements. Compliance was assessed by pill counts. Dietary calcium intake was estimated from prospective 3-day diet records completed at baseline and then every six months. There were no differences between the two groups at baseline in age, height, weight, BMI or percent body fat. Calcium supplementation resulted in a mean increase of 354 mg/d (compliance ranged from 64 – 77%). Mean total calcium intake during the study was significantly greater ($p=0.0001$) in the experimental group (1,370 mg/d) than the placebo group (935 mg/d). The supplemented group had greater increases in lumbar spine BMD (18.7% vs. 15.8%; $p=0.03$) and total body BMD (9.6% vs. 8.3%; $p=0.05$) compared to the placebo group. There was also a trend toward increased lumbar spine BMC (39.4% vs. 34.7%; $p=0.06$). The authors concluded that increasing daily calcium intake from 80% of the RDA to 110% via supplementation with CCM resulted in significant increases in TB and spine BMD in adolescent girls, which may provide protection against future osteoporotic fracture.

[Study score = 1+]

A randomized, controlled study of 48 healthy, white girls (mean age = 11.2) at the same stage of sexual development (stage 2) was conducted by Chan *et.al.* (1995). The experimental group consumed a diet supplemented with dairy products (e.g. milk,

American cheese, chocolate milk, yogurt) that were provided by the investigators and furnished at least 1,200 mg calcium per day. The control group consumed their usual diet. Dietary compliance was monitored by 3-day food records and a FFQ filled out at baseline and at 3, 6, 9 and 12 months duration. There were no differences in age, weight or height between the dairy and control groups at the beginning of the study. There were also no differences in weight, height, percent gain in weight or height, TBBMC or BMD of the lumbar spine, radius or femoral neck. The dairy group consumed significantly more protein compared to controls (70 vs. 52 g/d; $p=0.0003$). There were also significant differences in the intake of calcium (1,437 vs. 728 mg/d; $p=0.0001$), phosphorus (1,417 vs. 875 mg/d; $p=0.0001$) and vitamin D (288 vs. 128 IU; $p=0.0001$). Bone mineral values did not differ between the two groups at the end of the study, however, the percent increase in lumbar spine bone density and TBBMC were significantly greater ($p<0.001$) in the dairy group after 12 months. In addition, the average dietary calcium ($r=0.42$; $p<0.01$), vitamin D ($r=0.32$; $p<0.01$), phosphate ($r=0.39$; $p<0.01$) and protein ($r=0.36$; $p<0.01$) intakes, but not those of fat or calories, were associated with gains in TBBMC at the end of the study. The authors concluded that young girls who increased their dairy consumption had significantly greater gains in TBBMC and spine BMC and recommended that this population be encouraged to increase dairy consumption for better bone health and to prevent adult osteoporosis. The calcium and vitamin D provided in this study was in the form of dairy products so it is not possible to conclude that the significant effects on bone were due to these nutrients *per se*, nevertheless, the study provides additional support for the contention that calcium and vitamin D support optimal skeletal development. [Study score = 10]

The effect of calcium supplementation (500 mg/d from CCM) for 24 months on BMD of 112 premenarchal (mean age = 11.9 years), white girls was conducted by Lloyd *et al.* (1996) using a randomized, placebo-controlled, double-blind protocol. The percentage of girls who achieved menarche during the study was similar among the placebo (72.3%) and treatment groups (75.0%). There were no differences between the two groups at baseline in any of the parameters measured. Calcium supplementation resulted in significantly greater BMD of the total body ($p=0.0052$), lumbar spine ($p=0.0102$) and pelvis ($p=0.0071$) compared to the placebo group at the end of the study. The annualized increase in TBBMD was significantly greater ($p<0.006$) in the treatment group who were above the median Tanner score compared to those below median Tanner score. The authors concluded that modest calcium supplementation may provide significant long-term protection against fractures in later life. [Study score = 1+]

Cadogan *et al.* (1997) studied the effect of supplementing the diet of white girls (mean age = 12.2 years) with one pint of whole or reduced fat milk for 18 months on growth and bone mass. The subjects were randomized to the treatment group who were given 568 ml of whole, semi-skimmed or skimmed milk (115, 118 and 120 mg/dL calcium, respectively) or to the control group who were instructed to maintain their usual diet. A placebo was not provided. There were no significant differences between the two groups at baseline in any of the characteristics studied. Calcium intake was 739 mg/d in the treatment group and 753 mg/d in the control group. Intake of this nutrient increased to 1,125 mg/d in the treatment group ($p<0.01$) and remained constant in the control group (703 mg/d). The intake of vitamin D was not reported, nor did the authors specify if the

milk was fortified with this nutrient. TBBMC and TBBMD were significantly greater in the treatment group compared to the control group ($p=0.017$ and $p=0.009$, respectively). BMD of the pelvis ($p=0.003$) and legs ($p=0.005$) were also greater in the milk-supplemented group compared to the controls. The paper did not report the effect of milk supplementation on bone mass according to pubertal development during the study. The authors concluded that a modest increase in milk consumption augments bone mineral acquisition in adolescent girls and could have a substantial impact on future incidence of fractures, but noted that the results need to be confirmed with longer-term studies. [Study score = 10]

The effect of calcium supplementation (1,000 mg/d from 800 mg calcium carbonate and 5.23 g calcium lactate-gluconate) for up to 18 months on BMD among 42 mono- and dizygotic twin pairs (mean age 14 years) was studied by Nowson *et al.* (1997). A randomized, placebo-controlled, double-blind protocol was used. There were no significant differences between the placebo and treatment groups at the beginning of the study in any of the characteristics measured. Mean calcium intake at baseline was 692 mg/d in the placebo group and 776 mg/d in the supplemented group ($p>0.05$). Total calcium intake in the treatment group during the study was approximately 1,600 mg/d. Calcium supplementation resulted in significantly greater BMD at the lumbar spine ($p<0.01$) and the total hip ($p<0.05$) after six months. This effect was not accelerated during subsequent study periods (the difference in BMD between the treatment and placebo groups was not significant during the 6-12 month or 12-18 month periods at any site). However, the effect of calcium supplementation at the spine remained significant

throughout the study period. Calcium supplementation did not affect BMD among the 11 twin pairs who were pre-menarcheal at the beginning of the study, but there were significant effects on BMD of the spine ($p < 0.01$) and total hip ($p < 0.02$) for the twins who had achieved menarche at baseline. The authors concluded that calcium supplementation resulted in increased BMD of the spine in growing females with most of the benefit occurring during the first six months. [Study score = 1+]

Renner *et al.* (1999) studied the effect of providing dairy products to male and female adolescents aged 15 and 16 years. One hundred-ninety subjects were enrolled into the study and assigned to one of four groups: an “intervention group” of subjects with baseline BM values “below average”, a “control group” of subjects with similar BM values but no dietary intervention, a “medium” group with “average” BM values and no dietary intervention and an “optimum” group who had BM values “above average” with no dietary intervention. The intervention group was given dairy products to supply 1,000 mg calcium per day. The paper provided little information on anthropometric, dietary or lifestyle characteristics. The dairy supplemented group experienced a significantly greater increase in BMD ($p < 0.001$) than the non-supplemented groups. This study was difficult to interpret, and failed to provide a clear analysis of the results. Nevertheless, it suggests that supplementation of the diet with dairy products can have a positive effect on bone acquisition. [Study score = 1-]

Another randomized, controlled study using dairy products was conducted by Merrilees *et al.* (2000). Ninety-one girls (aged 15-16 years) were randomized (using forearm BMD

for stratification) to a dairy supplementation group or a control group. The dairy group was provided with dairy products (including milk, flavored milk, dairy dessert, cheese or yoghurt) that provided at least 1,000 mg calcium per day for two years. Seventy-three of these subjects were examined one year after supplementation ended. Dietary records were used to determine nutrient intake. There were no differences between the dairy and control groups for height, weight, body fat or lean muscle content at baseline, two years or at the end of the follow-up period (three years). At the end of the supplementation period, the dairy group had higher intakes of calcium (1,155 vs. 684 mg/d; $p < 0.001$), phosphorus (1,436 vs. 1,079 mg/d; $p < 0.001$) and protein (81.2 vs. 62.5 g/d; $p < 0.01$) compared to the control group. There were no significant differences in the intake of energy, fat, vitamin D or magnesium between the two groups. There were no differences in nutrient intake one year after the intervention, and the intake of calcium and other milk-related nutrients reverted to pre-supplementation levels. BMD was significantly higher in the experimental group at the end of the supplementation period for trochanter, lumbar spine and femoral neck ($p < 0.05$), but no differences were seen one year after supplementation ended. In addition, there was a significant increase in BMC ($p < 0.05$) of the trochanter and a borderline increase ($p = 0.054$) at the lumbar spine after at the end of the intervention, but these differences did not persist one year after supplementation ended. The study concluded that supplementation with dairy products had positive effects on the skeleton of teenage girls. The authors also observed that self-selection of high dairy containing diets may be difficult to achieve. As with the previous study (Chan *et al.*, 1995), it is not possible to attribute the positive changes attributed to a diet high dairy products to calcium *per se* because other nutrients in these foods may also have an

effect on BMD. Nevertheless, the study does provide evidence that calcium from dairy products is associated with improved skeletal development and is constant with the proposed claim. [Study score = 1Ø]

Bonjour *et al.* (2001) (discussed in the previous section on prepubertal subjects) found that calcium supplementation during the prepubertal period resulted in higher bone mass immediately following supplementation and 3 to 5 years later. This result did not appear to be due to the onset of puberty as evidenced by a separate analysis of prepubertal girls. This study suggests that calcium supplementation in prepubertal girls has a significant benefit for older adolescents as well. [Study score = 1+]

The effect of calcium supplementation (1,000 mg/d from calcium carbonate) for one year on bone mass in 100 adolescent (mean age = 14) postmenarcheal girls was reported by Rosen *et al.* (2003). The girls were randomized to the treatment or placebo group using a double-blind protocol. There were no significant differences between the two groups in any parameter measured at baseline. Calcium intake was low (578 and 586 mg/d in the placebo and experimental groups, respectively). The supplemented group had greater TBBMD ($p < 0.05$) and BMD at the lumbar spine ($p < 0.05$) but not the femoral neck compared to the control group at the end of the study. The authors concluded that calcium supplementation of postmenarcheal girls with low calcium intakes enhances bone mineral acquisition, especially in girls > 2 years past the onset of menarche. [Study score = 1+]

Stear *et al.* (2003) studied the effect of calcium carbonate supplementation (1,000 mg/d of elemental calcium) and exercise for 15.5 months on skeletal parameters in 131 adolescent (mean age = 17.3 years) girls using a randomized, placebo-controlled, double-blind design. Calcium supplements provided 500 mg calcium per chewable tablet. The placebo group received indistinguishable tablets without the calcium. The paper did not provide information on the vitamin D content of the supplements. The subjects were further randomized (stratified according to experimental or control group) to one of two exercise groups. One group was invited to attend three 45-minute exercise classes per week and the other group did not receive such invitations. Compliance with the study was determined by pill counts and dietary intake was estimated by FFQ administered at baseline and by a 7-day food diary completed during the study. There were no differences between the treatment groups at baseline in age, age at menarche, weight, height, calcium intake, BMC at any site, BA at any site, exercise, regular periods, contraceptive pill use or smoking. Mean calcium intake at baseline among all subjects was 938 mg/d. Calcium supplementation resulted in a significant increase in BMC of the femoral neck ($p < 0.01$) and trochanter ($p < 0.05$) compared to the placebo group. In addition, size adjusted (SA) BMC was significantly higher in the experimental group for spine ($p < 0.05$), ultradistal radius ($p = 0.002$), total hip ($p < 0.01$) as well as the femoral neck ($p < 0.01$), trochanter ($p < 0.01$) and intertrochanter ($p < 0.01$). These differences were more pronounced in a subset of the group with $>75\%$ compliance. The authors concluded that calcium supplementation enhanced bone mineral status in adolescent girls. [Study score = 1+]

A randomized, placebo-controlled, double-blind protocol was used to study the effect of calcium supplementation for seven years on BMD of the spine and proximal radius among 179 young females (mean age ~15 years at baseline) living in Ohio (Matkovic *et.al.* (2004). Subjects with baseline calcium intakes below a threshold of 1,480 mg/d (as determined by FFQ) at pubertal stage 2 were randomized to receive either 1,000 mg/d calcium (from CCM) or a placebo. The study also included a non-intervention “high dairy” group composed of subjects whose baseline calcium intake exceeded the threshold. The participants in the intervention groups were given a six-month supply of pills and visited the clinic twice a year. Subjects in the observational arm were also examined every six months. Dietary information was collected from all subjects at each clinic visit and compliance with the protocol was determined by pill counts. Seventy-nine subjects in the experimental group, 100 in the placebo group and 85 in the dairy group completed the study. The paper did not report the number of subjects originally recruited into the study or provide information on baseline characteristics of the subjects or the dropouts. There were no significant differences between the calcium and placebo groups at the final clinic visit in time since menarche, height, weight, pubertal stage calcium intake, protein intake or pill compliance. Mean dietary calcium intake was 881 mg/d in the calcium group, 785 mg/d in the placebo group and 1,213 mg/d in the dairy group ($p < 0.001$ vs. the intervention groups). The dairy group was significantly taller (mean height = 165.9 cm; $p < 0.01$) compared to the supplemented (163.4 cm) and placebo (163.2 cm) groups and consumed more protein (75 g/d vs. 63 g/d for the intervention groups; $p < 0.01$). There was no significant difference in lumbar spine BMD between the calcium and placebo groups, but the dairy group had significantly higher BMD at this site

compared to the intervention groups (p value not provided). Supplemented individuals had significantly higher BMD at the femoral trochanter (3%, $p=0.0024$) than subjects in the placebo group, but differences at the femoral neck (1.8%) were not significant ($p=0.234$). The supplemented group also had higher BMD at the proximal radius ($p=0.018$) compared to the placebo group when stratified by cumulative calcium intake. This study suggests that long-term calcium supplementation has beneficial effects on BMD of the trochanter and proximal radius in young girls, but lack of information on baseline characteristics of the subjects and dropout rates limits the conclusions that can be drawn. [Study score = 10].

3. Summary and conclusions

The observational studies that examined the association between calcium intake and bone health in adolescents are consistent with the premise that calcium and vitamin D are necessary for optimal development. The strongest support for the hypothesis that calcium is associated with BMD was provided by the retrospective cohort study by Wang *et.al.* (2003). This study found that calcium intake in mid-puberty was a positive predictor of BMD at the whole body, spine and femoral neck. The two other prospective studies provided equivocal results. Welten *et.al.* (1994) failed to find such an association in a small cohort living in Amsterdam, but intake of calcium was quite high, and likely exceeded the threshold for an effect on bone health. Similarly, Gunnes and Lehmann (1996) found that calcium intakes were unrelated to BMD among a cohort of 470 calcium-replete Icelanders although there was an interaction between the effect of calcium intake and physical activity on this parameter.

Additional support for a protective effect of calcium was provided by one case-control study (Goulding *et al.*, 1998) conducted with adolescent subjects. This study reported that calcium intakes were lower in female fracture victims aged 11-15 years compared to age- and gender-matched controls.

Three of the cross-sectional studies discussed in this section suggest that calcium is associated with bone health in adolescent females if intakes are below recommended levels (Kristinsson *et al.*, 1994; Kristinsson *et al.*, 1998; Kardinaal *et al.*, 1999), and Moro *et al.* (1996) reported such an association among boys and girls, but absolute calcium intakes were not reported. However, other cross-sectional studies failed to find a correlation between calcium and bone mass in this age range (Ruiz *et al.*, 1995; Wang *et al.*, 1997; Maggiolini *et al.*, 1999; Elgán *et al.*, 2002). The failure of cross-sectional studies to find positive results may be due to their inherent limitations to detect individual associations among the many factors that are known to effect bone mass – especially during the pubertal period when bone growth is strongly affected by hormonal changes.

Vitamin D status was shown to be associated with parameters of bone health in one prospective study (Lehtonen-Veromaa *et al.*, 2002) and two cross-sectional studies (Outila *et al.*, 2001; Cheng *et al.*, 2003). Such an association was not seen in the cross-sectional study of Kardinaal *et al.* (1999), but serum 25(OH)D values were not available for all subjects in this study and the authors did not report quantitative data on vitamin D status so it was not possible to determine whether there were enough deficient subjects to allow an association to be detected.

The available observational data also suggest that Vitamin D status (as measured by serum 25(OH)D concentrations) is associated with bone mass in healthy, female adolescents living in northern environments. Such data were reported in a prospective study (Lehtonen-Veromaa, *et.al.*, 2002) and in cross-sectional studies by Outila *et.al.* (2001) and Cheng *et.al.* (2003).

The randomized, controlled, intervention trials provide much more convincing evidence that calcium supplementation during adolescence results in improved bone mass. Clear evidence of this effect was reported by Lloyd *et.al.* (1993, 1996), Cadogan *et.al.* (1997), Nowson *et.al.* (1997), Renner *et.al.* (1999), Bonjour *et.al.* (2001), Rosen *et.al.* (2003), Stear *et.al.* (2003), and Matkovic *et.al.* (2004). Additional suggestive, but less compelling evidence was provided by Chan *et.al.* (1995) and Merrilees *et.al.* (2000). The only dietary intervention study that did not report a positive effect of calcium supplementation on BMD during adolescence was Johnston *et.al.* (1992). Nevertheless, this study did find an effect in prepubertal subjects. It is possible that a relatively high mean calcium intake of subjects at baseline (894 mg/d) diminished the effect of calcium supplementation in this study. The study did not report the baseline calcium intake of the subjects who underwent puberty during the experimental period.

In conclusion, as noted earlier, the studies reviewed in this section provide strong evidence in support of the proposed claim.

C. ADULTS

The available data discussed below provide credible support for the proposed claim in the adult population. The majority of prospective cohort studies that measured BMD found a positive association with calcium intake, as did a large majority of the cross-sectional studies. The dietary intervention trials reviewed in this section did not find a consistent effect of vitamin D or calcium on bone health, but the nutritionally replete nature of the subjects in these studies limits their applicability to the proposed claim.

1. Observational studies

Mazess *et al.* (1985) reported that post-winter mean serum 25(OH)D was only 16.6 ng/ml (41.5 nmol/L) in a cross-sectional study of 88 (45 female, 43 male) Alaskan Aleutian Islanders age 40-70. BMC of the forearm was less than the 85th percentile for U.S. whites in 30% of the sample as determined by photon absorptiometry with a radionuclide source (¹²⁵I). The authors categorized the concentration of 25(OH)D as “adequate”, but data that are more recent show that PTH concentrations remain elevated at 25(OH)D concentrations ≥ 50 nmol/L (Holick, 2002). Dietary data were not reported for this sample, but the authors noted that calcium intakes in Eskimo populations are often low, and could contribute to suboptimal BMC. This paper provides further evidence that vitamin D status is compromised in northern latitudes in the winter when exposure to sunlight is minimal, and that vitamin D insufficiency may be a factor associated with poor bone mass. [Study score = 4]

Vitamin D status and BMD of the mid- and distal radius and lumbar spine was determined in a cross-sectional investigation of 122 white women (aged 33-94) living in Rochester MN (Tsai *et.al.*, 1987). BMD was negatively correlated with age at all three sites as was serum 25(OH)D ($r = -0.29$; $p < 0.001$). However, multiple regression analysis showed that serum 25(OH)D was not associated with BMD after correcting for age. The authors concluded that reduced bioavailability of vitamin D was not shown to be a factor in reduced BMD of this population. The authors did not provide a mean value for serum 25(OH)D, but inspection of a plot of this parameter vs. age revealed that most values were >20 ng/ml (50 nmol/L) and therefore in the adequate range. It may be that vitamin D status would have been correlated with BMD had more of the subjects been deficient. The paper did not report what season of the year the blood samples were obtained. Furthermore, the paper did not report dietary intake data for vitamin D or calcium. This paper does not provide support for the premise that vitamin D is associated with osteoporosis, but interpretation of the results is limited by methodological constraints.

[Study score = 4]

A prospective study of 156 young adult (mean age = 21.4 years at baseline) women with an average follow-up period 3.2 years was conducted by Recker *et.al.* (1992). Baseline mean total calcium intake (diet plus supplements) was 820 mg/d and did not change significantly during the study. BMD increased during the study ($p < 0.0001$) at the forearm, lumbar spine and total body, which demonstrated that BMD can increase during the third decade of life. BMD was also positively associated ($p = 0.017$) with calcium intake adjusted for protein. Physical activity was also positively associated with BMD

($p=0.036$). The authors concluded that young women of college age might be able to reduce the risk of fractures in their elderly years with relatively minor changes in lifestyle. [Study score = 2+]

Metz *et.al.* (1993) conducted a cross-sectional study of 38 white women aged 24-28 years living in Chapel Hill, NC. Mean calcium intake was relatively high in this group (mean 1,000 mg/d) and was positively correlated with BMC of the distal- ($p=0.0143$) and mid-radius ($p=0.0025$), and with BMD of the distal- ($p=0.318$) and mid-radius ($p=0.0486$). Physical activity was also correlated to bone mass in this small cross-sectional study. The authors concluded that adequate calcium intake and physical activity had a positive impact on radial bone measurements in this sample of young women. [Study score = 4]

A cross-sectional study of 56 healthy, premenopausal women aged 21-47 (mean 31.5 years) living in the U.K. (Ramsdale *et.al.*, 1994) found that calcium intake was positively correlated with BMD at the lumbar spine ($p<0.05$) and three femoral sites (neck, $p<0.01$; Ward's triangle, $p<0.01$; trochanter $p<0.001$) but not at two radial sites. Mean calcium intake was 782.9 mg/d with a range of 200 to 1,550 (excluding one individual with a very high intake of 5,500 mg/d). Twenty-seven percent of the subjects had calcium intakes of <500 gm/d. No data on vitamin D intake or status were reported. The authors concluded that the low calcium intake found in a substantial proportion of this group of young women is a cause for concern. [Study score = 4]

Nieves *et.al.* (1995) conducted a cross-sectional study among 139 white women 30-39 years of age (mean age = 35.2 years) living in Rockland county, New York. Calcium intake was assessed with the Block FFQ modified to include additional foods thought to provide significant amounts of calcium to the subjects. Mean calcium intake of the current diet was 1,112 mg/d from foods and 1,159 mg/d from foods and supplements. Similar data for the diet during the teen years was 1,509 and 1,608 mg/d, respectively. Multiple regression analysis showed that total calcium intake during the teen years was significantly ($p<0.05$) associated with BMD at the femoral neck and trochanter. Dietary calcium during the teen years was associated with BMD of the femoral neck only. There were no significant associations at the Wards triangle, spine or radius. In addition, current dietary calcium intake was significantly associated with BMD at the trochanter ($p<0.05$), but not at the other sites examined. Addition of fiber to the multivariate model strengthened the association between calcium intake at the femoral neck and trochanter ($p<0.05$). The authors concluded that although the association between calcium intake and hip bone density is modest, if it were possible to increase the mean calcium intake of a population of teenagers from 800 mg/d to 1,200 mg/d, there would be a potential to increase bone density in the hip by an average of six percent. This potential increase, if maintained, may offer some protection against future hip fractures. [Study score = 4]

Davis *et.al.* (1996) studied a group of 421 women aged 25-34 living in Hawaii using a cross-sectional design. The following ethnic groups comprised the cohort: Hawaiian (n=66), Filipino (n=74), Japanese (n=144) and Caucasian (n=137). Information on frequency of milk consumption at during three periods (10-14, 15-19 and 20-24 years of

age) was obtained by questionnaire and used to classify the subjects into one of three categories. Univariate analysis found that milk consumption at ages 10-24 of the two highest categories was positively ($p < 0.05$) associated with BMD of the proximal radius compared to the lower consumption level. Similar results ($p < 0.05$) were seen in multivariate analysis after correcting for a variety of potentially confounding variables. Ethnicity was not statistically significant in multivariate models at any of the four bone sides measured. The authors concluded that peak bone mass is influenced by many factors. [Study score = 4]

Salamone *et al.* (1996) studied the interplay of genetic and lifestyle factors on BMD in a cross-sectional sample of 470 healthy, premenopausal women (mean age = 47 years). The women were assigned to one of two categories according to vitamin D receptor (VDR) genotype (i.e. subjects with a BB or Bb genotype vs. those with the bb genotype). Calcium intake was measured by a FFQ and did not differ between the genotypes (513 mg/d for BB + Bb and 538 mg/d for bb; $p = 0.23$). Univariate analysis showed that dietary calcium intakes $\geq 1,036$ mg/d were positively associated ($p < 0.05$) with BMD of the lumbar spine and femoral neck among the entire sample. Multivariate analysis including VDR genotype (BB + Bb), body weight ≥ 160 lb, physical activity $\geq 1,789$ kcal/wk and dietary calcium $\geq 1,036$ mg/d were independently associated ($p < 0.05$) with BMD at the spine and femoral neck. There was also a significant interaction between VDR genotype and dietary calcium at the femoral neck. Low or high dietary calcium (above or below 1,036 mg/d) was not associated with BMD in the bb genotype, but subjects with high calcium intake in the BB + Bb group had significantly ($p < 0.01$) greater BMD than those

with lower calcium intake. The authors concluded that prophylactic interventions aimed at achieving and maintaining optimal BMD, such as greater calcium intake or physical activity, may be important in maximizing one's genetic potential for BMD⁷. [Study score = 4]

A cross-sectional study designed to examine the relation between exercise, milk consumption and calcium supplement use on BMD of 25 adult women (mean age = 41 years) and their elderly mothers (mean age = 72 years) was reported by Ulrich *et al.* (1996). Milk and calcium supplement use was determined by a retrospective questionnaire and used to assign a "lifetime calcium intake" (LCI) score. The absolute amount of calcium consumed was not reported although mean intake from supplements ranged from 108 mg/day in early adulthood to 300 mg/d in mid-adulthood for the daughters and from 207 mg/d in mid-adulthood to 388 mg/d in late-adulthood for the mothers. Total and peripheral BMD were positively associated with supplemental calcium intake after age 60 in the mothers, but there were no associations between LCI score and BMD among the daughters. The authors speculated that calcium intake may have exceeded the threshold for additional effects on bone mass. [Study score = 4]

Feskanich *et al.* (1997) reported prospective data (12 year average follow-up period) on milk and calcium intake and fracture incidence among 77,761 members of the Nurses' Health Study cohort aged 34 – 59 at baseline. Self-reported fracture of the proximal

⁷ Three cross-sectional observational studies (Salamone *et al.*, 1996; Kiel *et al.*, 1997; Rubin *et al.*, 1999) and one randomized clinical trial (Graafmans *et al.*, 1997) that examined VDR genotype as a parameter of vitamin D/calcium metabolism are summarized in this petition. These studies are cited as part of the totality of scientific evidence, but their inclusion does not imply that this factor should be considered in determining applicability of the proposed claim.

femur and distal radius that occurred after low or moderate trauma were defined as endpoints of the study. Calcium intake ranged from a mean of 425 mg/d in the lowest quartile to 1,202 mg/d in the upper quartile. Analogous data for vitamin D intake were 156 and 448 IU/d, respectively. There were no significant associations between milk consumption or total dietary calcium intake from food and fracture incidence at either site after correcting for age or age and a variety of potentially confounding variables including BMI, menopausal status, hormone replacement therapy (HT), cigarette smoking, physical activity thyroid hormone medication, total energy and use of caffeine and alcohol. Calcium from dietary supplements was not considered in this study because data on the duration of supplement use before 1982 was unavailable and because of the possibility that supplement users would not reflect the population as a whole. This study did not provide measurements of BMD, so it is not possible to determine whether calcium intake was related to osteoporosis, but it does not support the premise that dietary calcium reduces the risk of fractures. In addition, it is not known whether including data on calcium supplementation would have affected the results. [Study score = 2+]

Similar data on the association between milk and calcium intake on hip and forearm fracture incidence from the male Health Professionals Follow-up Study was reported by Owusu *et al.* (1997). The incidence of 201 forearm and 56 hip fractures due to mild or moderate trauma were reported during an 8-year follow-up period among 43,063 men aged 40-75 years at baseline. Total calcium intake ranged from <512 mg/d in the lowest quintile to >1,227 mg/d in the upper quintile. There were no significant associations

between milk or calcium intake and incidence of fracture at either site after correction for age or age and a variety of potential confounding variables including alcohol consumption, smoking, BMI, physical activity and total energy intake. This study considered supplemental as well as food-based sources of calcium. Once again, this study does not provide support for the contention that calcium reduces the risk of fracture in adult men, but BMD data were not provided so it is not possible to determine whether there was an association with osteoporosis. [Study score = 2+]

A case-control study of 63 female, young (mean age = 21.4 years) stress fracture victims and 78 controls matched for age, gender, height, weight and BMI was reported by Cline *et.al.* (1998). The subjects were members of the military. There were no differences in bone density at five sites between the cases and controls, nor were there differences in calcium intake (cases = 1,154 mg/d; controls = 944 mg/d). Furthermore, there were no differences in fracture rates between subjects who consumed >800 mg calcium per day compared to those who had consumed <799 mg/d. This study does not provide evidence that dietary calcium is associated with the risk of osteoporosis or fracture in young Army recruits. The small size of the study and relatively high calcium intake of these young, healthy subjects may partially explain this negative finding. The study did not provide information on the intake or status of vitamin D. [Study score = 3-]

Teegarden *et.al.* (1998) conducted a cross-sectional study of 215 women aged 18-31 years. Mean calcium intake was 948 mg/d (range 251 – 2,552 mg/d) and was correlated with BMD of the radius ($p < 0.05$) and spine ($p < 0.05$) and with BMC of the spine

($p < 0.05$). Multivariate analysis that controlled for postmenarcheal age as well as lean and fat mass revealed that models including protein, calcium and the calcium-protein ratio, or phosphorous, calcium and the calcium-phosphorus ratio best explained the diet-bone mass associations. This paper did not report data on vitamin D intake or status of the subjects. The authors concluded that calcium intake of $\geq 1,200$ mg/d (and optimally 1,400 – 1,500 mg/d) would offset urinary losses or fecal losses associated with the current protein or phosphorus intakes observed in this population. [Study score = 4]

A cross-sectional study of 422 women (Uusi-Rasi *et al.*, 1998) in three age groups (25-30; 40-45; and 60-65) was conducted to examine the association between calcium intake and physical activity on bone mass. The subjects were divided into four groups based on level of physical activity (high or low) and calcium intake (high or low). Calcium intake in the low groups ranged from 596 mg/d in middle-aged subjects to 692 in the oldest age group. Analogous values for the high calcium group were 1,298 mg/d in the young subjects and 1,530 in the oldest group. After correcting for body weight, both high physical activity and high calcium intake were associated with total bone mineral content (TBBMC) compared to subjects in the low groups. In addition, both physical activity and dietary calcium were related to larger and mechanically more competent bones in the femoral and radial shafts. There were no data provided on vitamin D intake or status. The authors concluded that a relatively moderate level of physical activity or calcium intake can result in considerable long-term benefits with respect to mechanical competence of the skeleton if maintained from childhood. [Study score = 4]

Rubin *et al.* (1999) conducted a cross-sectional study among 677 healthy, white Canadian women aged 18-35 years. Calcium intake was positively correlated with BMD of the femoral neck ($p < 0.01$), but not the lumbar spine ($p = 0.08$). The distribution of calcium intakes were ≤ 250 mg/d, $n = 134$; 251-500 mg/d, $n = 228$; 501-750 mg/d, $n = 182$; > 750 mg/d, $n = 133$. The relationship between calcium intake and BMD was no longer significant after controlling for weight, height, age, physical activity, family history of osteoporosis and vitamin D receptor (VDR) genotype. However, there was a significant interaction between BMD genotype and calcium intake effects on BMD at the femoral neck, which suggests that BMD may play a role in modulating calcium's effect on BMD. [Study score = 4]

The cross-sectional study reported by Oliveri *et al.* (2000) discussed in the section on pre-pubertal children did not find a significant association between calcium intake and BMC or BMD of the ultra distal and distal radius among 234 young adults (133 women and 101 men) living in Argentina. However, there was a significant association between BMD/BMC values and dietary calcium in a subset of this cohort with intakes below 800 mg/d. [Study score = 4]

A prospective study of 224 Australian-born women aged 45-56 was conducted to examine the association between anthropometric, dietary and lifestyle factors on BMD over a four year period (Guthrie *et al.*, 2000). Mean calcium intake at baseline was 890 mg/d and increased by 245 mg/d during the study. Fifty-two percent of the women reported a calcium intake of less than 800 mg/d. BMD was not associated with calcium

intake at baseline. Change in BMD during the study was associated with time in relation to the final menstrual period, but not with any other variable. The authors speculated that the range of calcium intake in the study population was not sufficient to override the effect of ovarian changes on bone metabolism. [Study score = 20]

Lamberg-Allardt *et.al.* (2001) studied a cross-sectional sample of 328 adult women (n=202) and men (n=126) living in northern Europe. The purpose of the study was to determine vitamin D status at the end of winter and to investigate a possible association with forearm BMD. Mean vitamin D intake exceeded recommended amounts of 5 µg/d in men (5.6 µg/d) and approached this value in women (4.7 µg/d), but low concentrations of serum 25(OH)D (<25 nmol/L) were found in 26.2% of the women and 28.6% of the men. In addition, serum iPTH started to increase at 25(OH)D concentrations below 80 nmol/L in women and 40 nmol/L in men. BMD of the radius was lower in men with serum iPTH concentrations lower than 23 nmol/L (p=0.01) compared to those above this value, but no such relationship was evident (p=0.14) for women with serum iPTH values above or below 26 nmol/L. Mean calcium intake was reasonably high (962 mg/d in women and 1,037 mg/d in men) and was not correlated with forearm BMD in either gender. This paper suggests that adequate vitamin D status may be important to maintain bone health (especially in men) and that recommended intakes may not be adequate for people living in northern Europe. [Study score = 4]

A case-control study of 451 men and 725 women with hip fractures compared to an equal number of age-and gender matched controls was reported by Lau *et.al.* (2001). The

subjects were residents of Singapore, Malaysia, Thailand or the Philippines. Multiple regression analysis showed that calcium intake of <498 mg/d resulted in a significant Relative Risk (RR) of fracture of 2.0 (95% CI, 1.5-2.8) for women and 1.5 (95% CI, 1.0-2.2) for men. Mean calcium intake of the male fracture victims was 371.5 mg/d compared to 421.1 mg/d among cases ($p>0.05$) while female cases consumed a mean of 360.6 mg/d vs. 462.9 mg/d for controls ($p<0.01$). The authors concluded that calcium supplementation should result in a considerable reduction of fracture risk given the low intakes in Asian countries. [Study score = 30]

Filner *et.al.* (2002) studied a cross-sectional sample of 452 women 20 years or older living in Alaska. Calcium intake was very low in this population (mean intake = 306 mg/d) and was not associated with BMD of the calcaneus. The authors concluded that the native Alaskan population has a high incidence of risk factors for osteoporosis. The low calcium intake of the participants in this study may partially explain the negative findings. Information on the intake or status of vitamin D was not provided, and could also have impacted the results. [Study score = 4]

Holm *et.al.* (2002) conducted a two-year prospective study of 386 healthy black and white U.S. women aged 35-60 to determine the association between diet, physical activity and other factors on BMD of the lumbar spine. Mean calcium intake was 723 mg/d at baseline (755.8 for whites and 609.4 for blacks) and 726 mg/d (784 for whites and 660 for blacks) at the end of the study. Calcium intake was not associated with BMD at baseline, but it became significant at 24 months. The authors speculated that calcium

may become more important as women age (e.g. reach menopause) or there is a threshold effect that was exceeded at the end of the study. Multiple regression analysis revealed that race, age, weight, follicle stimulation hormone (FSH), calcium and years of tobacco intake formed the best model for BMD at baseline and at 24 months. Although black women had significantly higher BMD than their white counterparts, age-related reductions in BMD were evident in both races. [Study score = 2+]

Uusi-Rasi *et al.* (2002) reported four-year follow-up data from a previous cross-sectional study on the effect of calcium intake and physical activity on BMD in premenopausal women (Uusi-Rasi *et al.*, 1998) (see discussion earlier in this section). Ninety-two of the original sample of 132 young (aged 25-30 years) women participated in the study. Bone loss occurred in all three sites measured (femoral neck, trochanter and distal radius). Higher calcium intake was positively associated with a lower rate of bone loss from the proximal femur and distal radius after correcting for age, body weight, muscle strength and numerous other factors. [Study score = 2+]

Bischoff-Ferrari *et al.* (2004a) examined the association between BMD of the total hip and serum 25(OH)D among younger (20-49 years) and older (≥ 50 years) adult subjects in the NHANES III database. This cross-sectional analysis consisted of 13,432 subjects including 8,477 whites, 3,683 Mexican Americans and 3,754 blacks. A positive association between these parameters was observed in younger ($p < 0.001$) and older ($p < 0.001$) whites, younger ($p < 0.004$) and older ($p < 0.01$) Mexican Americans and older blacks ($p < 0.03$). BMD for the highest, compared to the lowest quintile of serum

25(OH)D concentration was 4.1% greater in younger whites, 4.8% greater in older whites, 1.8% greater in younger Mexican Americans, 3.6% greater in older Mexican Americans, 1.2% greater in younger blacks and 2.5% greater in older blacks. The authors concluded, "This study adds support to the concept that 25-hydroxy vitamin D levels at the upper end of the reference range are preferable to lower levels for better bone mineral density." [Study score = 4]

2. Intervention studies

The effect of supplementation with a combination of calcium (1,000 mg/d from calcium carbonate) and vitamin D₃ (25 µg/d) for three years was studied among 77 healthy, adult (mean age = 58 years) men using a randomized, placebo-controlled, double-blind protocol (Orwoll *et al.*, 1990). The placebo group was significantly younger ($p < 0.05$) than the experimental group, but there were no other significant differences in any of the variables measured at baseline. Mean calcium intake was relatively high (1,159 mg/d) at baseline and remained stable throughout the study. Dietary vitamin D was not reported, but serum 25(OH)D concentrations were in the normal range (60 nmol/L for the treatment group and 52 nmol/L for controls; $p > 0.05$). Radial and vertebral BMD declined significantly ($p < 0.05$) during the study and was unaffected by supplementation. The authors concluded that normal men experience substantial bone loss that is not prevented by calcium and vitamin D supplementation in a well-nourished population. The lack of an effect seen in this study is not surprising given the calcium and vitamin D replete status of the subjects. [Study score = 1+]

Patel *et.al.* (2001) studied the effect of vitamin D supplementation on biochemical parameters and BMD in a group of 70 healthy female subjects (mean age = 47.2 years) using a two-year randomized, double-blind, placebo-controlled cross-over design. Subjects were randomized to receive a daily oral supplement containing 20 µg (800 IU) cholecalciferol or a placebo for one year. The groups were reversed during the second year of the study. There were no differences between the groups at baseline for age, height, weight, BMI, calcium intake (553 mg/d vs. 586 mg/d), serum 25(OH)D, serum PTH, BMD of the total body, posteroanterior (PA) spine or total hip. There was a significantly greater ($p=0.02$) mean BMD of the femoral neck at the beginning of the study between the two groups (0.858 g/cm^2 vs. 0.788 g/cm^2 , respectively). Vitamin D supplementation resulted in a significant ($p<0.001$) increase in serum 25(OH)D compared to the non-treatment phase of the study, and serum PTH decreased ($p=0.011$) among subjects in the lowest quartile of baseline serum 25(OH)D. Vitamin D supplementation did not result in significant changes in BMD at any site. The authors concluded that the healthy, women in the study were too vitamin D replete to experience reciprocal changes in PTH, bone markers or BMD due to seasonal variations in serum 25(OH)D. They also speculated that “normal” calcium intake (which may have been underestimated by the instrument used to measure dairy intake) may have contributed to the absence of seasonal effects on BMD. [Study score = 1Ø]

A six-month, randomized, placebo-controlled, double-blind study of calcium supplementation (1,000 mg/d elemental calcium from calcium citrate) and weight loss among 38 healthy, obese (mean BMI = 35.0) premenopausal (mean age = 42.1 years)

women was conducted by Shapses *et.al.* (2001). The women were randomly assigned to one of two weight loss groups: calcium supplementation or a placebo. Women who did not lose >2.5% of their initial body weight at the completion of the study were included in a second control group that consisted of women who maintained their original body weight. Subjects in the weight loss groups were instructed to consume a reduced-calorie diet. There were no differences in nutrient intake (including vitamin D and calcium) at baseline between any of the groups. Mean calcium intake was significantly greater in the experimental group (1,835 mg/d) than the placebo group (459 mg/d) at the end of the study. There were no differences in vitamin D, phosphorus or magnesium intake between any group during the course of the study. The calcium-supplemented group gained 1.7% lumbar BMD compared to -0.2% in the placebo group and -0.8 in the weight stable group ($p < 0.05$). However, there were no other significant differences in the change of lumbar BMC, TBBMD or TBBMC. There was a seasonal change in serum 25(OH)D concentrations (higher in the summer months) but there was no seasonal variation in other parameters including bone turnover and mass. The concentration of serum PTH did not change with calcium supplementation, and the authors suggested that the relatively high baseline calcium intake (1,005 mg/d) stabilized serum PTH so that additional calcium did not have an effect. The calcium replete nature of these subjects may also explain why there was not a greater effect of calcium supplementation at sites other than the spine. [Study score = 1+]

Winters-Stone and Snow (2004) examined the effect of calcium supplementation (1,000 mg from calcium carbonate) for one year on BMD among 23 healthy, female runners

(mean age = 23.7 years) using a randomized, double-blind, placebo-controlled design. The subjects were randomized to the experimental (n=26) or control (n=25) groups and completed 4-day food diaries. BMD was measured at the lumbar spine, femoral neck, greater trochanter, total hip and femoral mid-shaft at baseline and at the conclusion of the experiment. Compliance was assessed by monthly logs of supplement consumption. There were no significant difference at baseline between the experimental and control subjects in any parameter measured. Mean dietary calcium intakes exceeded the AI for the supplemented (1,006 mg/d) and control (1,294 mg/d) groups. Twenty-eight subjects (55%) did not complete the study. Baseline characteristics of these subjects were not provided. Calcium supplementation resulted in significantly ($p < 0.05$) higher BMD at the femoral-mid shaft compared to placebo-treated controls. There were no differences in BMD at any other site. The results of this experiment are of particular interest because the physically active subjects were already consuming calcium-replete diets. However, the high dropout rate limits the conclusions that can be drawn from this otherwise well designed and executed study. [Study score = 10]

3. Summary and conclusions

The observational studies summarized in this section generally support the hypothesis that calcium intake during adulthood has a positive impact on skeletal health. The strongest evidence was provided by three prospective studies (Recker *et.al.*, 1992; Holm *et.al.*, 2002; Uusi-Rasi *et.al.*, 2002) that found calcium intake was associated with BMD in adult women. On the other hand, two other prospective studies did not find associations between dietary calcium intake and fracture incidence among female nurses

(Feskanich *et al.*, 1997) or male health professionals (Owusu *et al.*, 1997), but these studies did not measure BMD. One other prospective cohort study also did not find a protective effect of dietary calcium (Guthrie, *et al.*, 2000), but the authors speculated that the range of calcium intake was not wide enough to overcome the greater effect of HT in this cohort.

Two case-control studies had different findings. Lau *et al.* (2001) found that low calcium intake was a risk factor for fracture in men and women living in Asia, but total calcium intake was very low compared to the U.S. diet. Cline *et al.* (1998) did not find a difference in calcium intake between fracture victims and non-victims in a cohort of young women undergoing military training, however calcium intakes were relatively high in both patients and controls.

The cross-sectional studies consistently found that calcium intake was associated with bone mass in adult populations. Such associations were reported by Metz *et al.* (1993), Ramsdale *et al.* (1994), Nivens *et al.* (1995), Davis *et al.* (1996), Salamone *et al.* (1996), Teegarden *et al.* (1998) and Uusi-Rasi *et al.* (1998). Conditional evidence was provided by the studies of Oliveri *et al.* (2000) for subjects with low calcium intakes and Rubin *et al.* (1999) who reported an association between calcium intake and BMD in bivariate but not multivariate analysis. Lamberg-Allardt *et al.* (2001) did not find an association between calcium and bone health, but the subjects had relatively high calcium intakes that may have exceeded a threshold necessary to find a positive association.

Four cross-sectional studies provide evidence that vitamin D status is associated with bone health. Mazess *et.al.* (1985) showed that serum 25(OH)D concentrations were associated with bone mineral status in an Alaskan population. Tsai *et.al.* (1987) did not find such an association, but most of the subjects in this study appeared to have adequate vitamin D status, and Lamberg-Allardt *et.al.* (2001) reported a direct association between serum iPTH and BMD in men but not in women. The authors of this study concluded that vitamin D is important to maintain optimal bone health, but recommended intakes may not be adequate for people living in northern Europe. Finally, Bischoff-Ferrari *et.al.* (2004a) reported positive associations between total hip BMD and serum 25(OH)D among white, Mexican American and black participants in the NHANES III study.

Most of the observational studies with adult subjects were conducted in women, however two of the vitamin D studies (Mazess *et.al.*, 1985; Lamberg-Allardt *et.al.*, 2001) found similar results in men and women. The cross-sectional study in two cities in Argentina (Oliveri *et.al.*, 2000) included male subjects, but there was no association between calcium intake and bone health. One case-control study (Lau *et.al.*, 2001) found that low calcium intake was a risk factor for hip fracture in both men and women 50 years and older. These available data provide evidence that optimal bone health is dependent on adequate calcium and vitamin D status in both genders.

A large majority of randomized studies in the area of bone health has been done in children or older people because these periods of life are of greater interest for this parameter. Therefore, only four intervention studies that met the criteria for discussion in

this petition were found. Shapses *et.al.* (2001) reported that calcium supplementation increased BMD at one site among obese subjects on calorie-restricted diets, but there was no effect at other sites. Winters-Stone and Snow (2004) also reported that calcium supplementation increased BMD at one site among calcium-replete female runners. Two other studies did not find an effect of calcium (Orwoll *et.al.*, 1990) or vitamin D (Patel *et.al.*, 2001) supplementation, but the subjects in both studies were replete in these respective nutrients at baseline, which most likely explains the negative results.

In conclusion, as noted earlier, the studies reviewed in this section provide credible evidence in support of the proposed claim.

D. ELDERLY MEN AND POSTMENOPAUSAL WOMEN

The available data discussed below provide compelling support for the proposed claim among elderly men and postmenopausal women. With respect to vitamin D alone, the prospective observational studies yielded mixed results, but are compatible with the hypothesis that vitamin D is associated with bone mass. The case-control studies are very consistent in this regard as was the only cross-sectional study that was reviewed. The controlled intervention trials provide good evidence that vitamin D supplementation without calcium can improve bone mass – especially among subjects with marginal calcium intake and/or vitamin D status. With respect to calcium, with or without vitamin D, the results are highly consistent and convincing. The majority of prospective cohort studies that monitored intake of calcium reported positive associations with bone health. The available case-control studies also provided support for this conclusion, but the

studies were limited in number and of marginal quality. The cross-sectional studies in this area consistently found a positive association with calcium and bone mass in older subjects. The most compelling evidence, however, is provided by the randomized, controlled intervention trials that unanimously demonstrate the benefits of calcium supplementation (especially calcium supplementation combined with vitamin D) on reducing bone loss or fracture incidence in elderly men and postmenopausal women.

1. Observational studies

A case-control study (von Knorring *et.al.*, 1981) compared 58 elderly (mean age = 77 years) Finish femoral neck fracture victims (11 men and 47 women) with 41 (9 men and 32 women) age-matched controls during summer and winter months. The expected seasonal variation in serum 25(OH)D was seen in both patients and controls, but fracture victims had significantly lower concentrations of this compound than controls during the winter ($p<0.02$) and spring ($p<0.01$). In addition, patients were more likely to have decreased concentrations of 25(OH)D (<25 nmol/L) accompanied by increased PTH concentrations than controls (18% vs. 3%, respectively; $p<0.05$). This small study did not provide dietary information and had other methodological constraints, but provides suggestive evidence that vitamin D deficiency may be associated with hip fractures in elderly subjects living in areas with limited sunlight. [Study score = 3-]

Ulivieri *et.al.* (1986) also found evidence of reduced serum 25(OH)D concentrations in a group of female hip fracture victims living in Milan. Femoral neck fracture victims ($n=12$) were compared with 26 women with vertebral fractures. Although the study was

poorly controlled, the hip fracture victims were significantly older (mean age 76.7 years vs. 67.8 years, respectively; $p < 0.01$) than the vertebral fracture patients and had significantly lower concentrations of 25(OH)D ($p < 0.01$). [Study score = 3-]

Elderly (mean age = 75.9 years) hip fracture patients (84 women and 41 men) were compared with 74 (54 women and 20 men) age-matched community-based controls (Lips *et al.*, 1987). Calcium and vitamin D intake did not differ between the two groups, but serum 25(OH)D concentrations varied with exposure to sunlight, and were significantly lower ($p < 0.001$) in patients than in controls (18.5 nmol/L and 32.9 nmol/L, respectively). Serum PTH concentrations did not differ between the two groups. The authors concluded that 300 IU of vitamin D per day would be necessary to maintain normal concentrations of 25(OH)D in this population. [Study score = 3Ø]

Lamberg-Allardt *et al.* (1989) reported that vitamin D intake among 37 elderly hip fracture patients (30 women and 7 men) was significantly lower than in 24 age-matched controls, but there was no difference in serum 25(OH)D concentrations (22.3 nmol/L for patients vs. 27.5 nmol/L for controls). However, the low concentrations of 25(OH)D suggested vitamin D deficiency in both groups. [Study score = 3Ø]

Lau *et al.* (1989) also reported lower concentrations of serum 25(OH)D in 200 elderly men and women with hip fracture (aged 49-90 years) compared to 427 controls living in Hong Kong. Although dietary data were not reported, the authors noted that rich sources

of vitamin D are consumed infrequently by Chinese elderly and suggested that measures be taken to increase exposure of this population to sunshine. [Study score = 3-]

In contrast to these results, Rudman *et al.* (1989) reported that serum 25(OH)D was positively correlated with fracture at any site among 153 elderly (aged 48-96 years), male nursing home residents. However, serum 1,25(OH)₂D was inversely correlated with fracture incidence, and the authors speculated that conversion of 25(OH)D in the kidney might have been a factor. Serum PTH concentrations were not reported, and the significance of circulating 1,25(OH)₂D as an indicator of susceptibility to has been questioned (Holick, 2002). [Study score = 3-]

van Beresteijn *et al.* (1990) conducted an 8-year longitudinal study of 154 healthy perimenopausal (mean age = 53 years) women living in the Netherlands. All women were postmenopausal by year five of the study. The women were divided into three groups according to calcium intake: < 800 mg/d (n=28), 800-1350 mg/d (n=95) and >1,350 mg/d (n=31) at the beginning of the study. There were no significant differences at baseline in age, years after menopause, body height or BMC or the radius adjusted for bone width (BMC/BW). Women in the lowest calcium group were significantly (p=0.004) heavier (72.4 kg) compared to the high calcium group (63.8 kg). Mean calcium intake in each of the groups remained remarkably constant during the course of the study. BMC/BW of the radius decreased for all three groups, but there were no significant differences according to category of calcium intake. The authors concluded that BMI had a protective effect on bone mass while calcium intake was not related to

cortical bone density or the rate of bone loss in perimenopausal women. The study did not report data on the relationship between dietary calcium and radial BMC on a body weight-adjusted basis. [Study score = 20]

A cross-sectional study of 843 Chinese women 35-75 years of age (mean age ~52 years) living on one of five rural counties with a wide range of calcium intake was conducted by Hu *et.al.* (1993). There was no difference in age in subjects living in the different counties, but there were significant differences ($p < 0.05$) in weight, height, BMI and several other parameters. Calcium intake ranged from 724 mg/d in the county with highest intake, to 230 mg/d in the county with the lowest. Similar data for calcium intake from dairy foods ranged from 516 mg/d to 0 mg/d. Analysis by individual for all counties combined showed that BMC and BMD were correlated positively with total calcium ($r = 0.27-0.38$; $p < 0.0001$) and dairy calcium ($r = 0.34-0.40$; $p < 0.0001$) after adjustment for age and/or body weight. The authors concluded that dietary calcium, especially from dairy sources, increased bone mass in middle-aged and elderly women. [Study score = 4]

Looker *et.al.* (1993) prospectively analyzed data from the NHANES I Epidemiologic Follow-Up Study cohort from 4,342 white men and postmenopausal women ages 50 -- 74 with a follow-up period of up to 16 years. Calcium intake was estimated from a 24-hour recall that was administered at the beginning of the survey combined with data on the frequency of milk and cheese consumption determined at the same time. Hip fracture incidence during the follow-up period was 122 for females and 44 for men. There was no

difference in energy adjusted calcium intake between fracture victims and non-victims for either gender. In addition, although the RR of fracture for the upper quartile of calcium consumption compared to the lowest was low for males (RR=0.52) and postmenopausal women (RR=0.53) neither value was statistically significant ($p>0.05$). There was also no association between fracture incidence and calcium intake when adjusted for a variety of potentially confounding factors (i.e. age, alcohol use, smoking, physical activity, BMI and HT), or when expressed as selected cutpoints (i.e. ≤ 400 mg/d vs. ≥ 600 mg/d). The authors concluded that the data were not consistent with a dose-response effect of calcium, but that calcium may lower hip fracture risk in late menopausal women. The authors noted that the result of a single 24-hour food recall survey may not reflect usual intake. [Study score = 20]

A case-control study (Stracke *et.al.*, 1992) compared 65 elderly (mean age = 57 years) osteoporotic men (n=17) and women (n=48) with 76 age- and gender-matched healthy controls. Dietary calcium was estimated by a FFQ that compared three periods of life (childhood and adolescence, 20-30 years ago and current intakes) and expressed as the calcium intake index (CII). The CII is proportional to calcium intake weighted for frequency of consumption and prevalence of calcium-containing foods in the diet. The paper did not provide data on calcium intake expressed in absolute amounts. The CII for the period of childhood and adolescence was lower in patients than controls for both genders. The CIIs for the period 20-30 years before the study and for the current diet were associated with BMD of the proximal radius ($p<0.05$) for female subjects but not for their male counterparts. The authors concluded that an adequate calcium intake is a

prerequisite for optimal bone formation and enhanced bone resorption. [Study score = 30]

Cumming and Klineberg (1994) conducted a case-control study among 209 male and female hip fracture victims and 207 community-based controls (age range = 65-100 years). Calcium intake was estimated by a questionnaire pertaining to consumption of dairy products at ages 13, 20, 50 and the present. The questionnaire was completed by proxy for 84 cases and 30 controls who were unable to provide the information directly. Intake of dairy products was found to be a risk factor for hip fracture (RR=3.2; 95% CI, 1.37, 7.7 for highest vs. lowest quintile) in this study. The authors noted that the quality of their data on use of dairy products and physical activity is questionable and that the results, "should be interpreted with great caution". The use of proxy data, and the fact that the patients tended to be older and in poorer health than the controls, may have biased the results. The poor quality of this study limits the conclusions that can be drawn from it. [Study score = 3-]

A cross-sectional study of 284 women aged 44-74 years living in Cambridge was conducted by Murphy *et.al.* (1994). The group was divided into tertiles according to usual milk consumption (<1 glass/wk; <1 glass/d; and \geq 1 glass/d) based on data provided in a survey. Calcium intake as measured by 24-hour recall did not differ ($p=0.26$) among the three groups (758, 908, and 852 mg/d, respectively). Typical milk consumption before age 25 was associated with increased BMD of the total hip based on crude analysis ($p=0.23$) and after correcting for age, BMI, menopausal status, smoking, HT,

parity, oral contraceptive use, physical activity, alcohol use and years since menopause ($p=0.39$). There was no such association for BMD of the spine ($p=0.087$ for the crude analysis and $p=0.216$ for the multiple linear regression analysis). Milk consumption between age 25 and 44 years was associated with increased BMD of Ward's triangle ($p<0.05$ for both crude and adjusted analysis), but not at other sites. There were no significant associations for recent milk consumption and BMD in this sample. This study suggests that calcium intake (especially during young adulthood) is associated with bone mass in later life. [Study score = 4]

Soroko *et.al.* (1994) conducted a cross-sectional study of 581 white, elderly (mean age = 70.6 years) women who had been postmenopausal for an average of 24 years.

Participants quantified daily milk consumption during adolescence (12 – 19 years), midlife (20 – 50 years) and older adulthood (>50 years) as “rarely or never”, “about every week but not every day”, “1-2 glasses/d, about every day”, or “3 or more glasses per day, about every meal”. Positive, significant, graded associations between milk consumption in older adulthood and BMD at the spine ($p=0.002$), total hip ($p=0.03$) and midradius ($p=0.02$), but not the wrist ($p=0.98$) or femoral neck ($p=0.17$) were observed. Adolescent milk consumption showed similar associations at the spine ($p=0.06$) and midradius ($p=0.02$). [Study score = 4]

A longitudinal (2-year) study of 18 elderly (mean age = 77 years) women living in northern Maine was conducted by Rosen *et.al.* (1994). Mean initial intake of calcium and vitamin D were 700 mg/d and 6.2 $\mu\text{g/d}$, respectively. During the second year of the

study, calcium intake was unchanged (820 mg/d, $p < 0.10$) but vitamin D intake increased significantly ($p = 0.03$) to 8.2 $\mu\text{g}/\text{d}$. BMD at the hip and lumbar spine dropped significantly during the fall and winter of the first year. These changes were paralleled by a 13% decrease in serum 25(OH)D ($p = 0.06$) and a 27% increase in PTH ($p = 0.01$). Increased vitamin D intake during the second year of the study was associated with improved BMD of the hip ($p = 0.02$) and increased concentrations of 25(OH)D compared to the first winter. The study concluded that it may be prudent to consider supplementation of elderly women with and without osteoporosis with calcium and vitamin D. [Study score = 2Ø]

A four-year prospective study of 9,516 white women aged ≥ 65 years was conducted by Cummings *et.al.* (1995). There were 192 incidences of hip fracture not due to motor vehicle accidents during the study. Calcium intake was not related to the risk of hip fracture in this cohort. The RR for women who consumed ≤ 400 mg calcium per day (11% of the cohort) was 1.1 (95% CI, 0.5, 2.3) after eliminating women taking calcium supplements or estrogen. BMD of the proximal femur was associated with increased fracture risk, but no data on whether calcium intake was associated with BMD were reported. This study does not demonstrate a protective effect of calcium intake in elderly women of fractures. [Study score = 2+]

Davis *et.al.* (1995) conducted a longitudinal study of 1,027 elderly (mean age at baseline = 63.6 years) women living on Oahu, Hawaii with a mean follow-up period of 8.4 years. Mean calcium intake was low (384 mg/d) at baseline. The use calcium of supplements at

baseline (mean intake 355 mg/d) in combination with HT was associated with significantly less bone loss than for non-supplement users at the calcaneus ($p=0.008$) and the distal radius ($p<0.001$) but not at the proximal radius ($p=0.07$). However, diet records were not obtained at subsequent examinations so the influence of calcium supplementation is uncertain. [Study score = 2-]

Devine *et.al.* (1995) studied the effect of sodium and calcium intake on BMD among 168 postmenopausal women during a two year study. The subjects in this observational study were participants in a calcium supplementation trial (Prince *et.al.*, 1995) who had been randomized to receive a calcium supplement (1,000 mg/d in the form of calcium lactate-gluconate) with or without exercise (4-hours weight bearing exercise per week), a calcium supplement from skim milk powder (containing 1,087 mg calcium) or a placebo. The sodium content of the diet was not manipulated in the treatment or placebo groups, and the results, therefore, are observational. The results were combined for all analyses and presented as correlations between calcium and/or sodium intake and BMD at several sites. Calcium intake was estimated by 4-day dietary records and sodium intake was estimated by analysis of 24-hour urine samples. The mean intake of these two nutrients at baseline was 805 and 2,783 mg/d, respectively. Average dietary calcium intake was positively ($p<0.05$) associated with the change in BMD during the two-year study at the total hip, intertrochanter, femoral neck and ultra distal ankle. There were no correlations at the trochanter or lumbar spine. Sodium intake was negatively associated ($p<0.05$) with BMD at the total hip and intertrochanter. The authors concluded that a reduction in

sodium-rich foods may enhance the positive effect of calcium intake on BMD. [Study score = 20]

A case-control study of 2,086 female hip fracture victims (mean age = 78.1) and 3,532 age- and gender-matched controls was reported by Johnell *et.al.* (1995). Subjects were recruited from 14 centers in six European countries from all female fracture victims during a one-year period. Cases were taller ($p=0.004$), lighter ($p=0.0001$), had a lower BMI ($p=0.0001$) and had earlier menopause ($p=0.001$), but not age of menarche than controls. Calcium intake was estimated from a questionnaire that solicited current and past frequency of milk consumption. Information on sunlight exposure was also queried. Calcium intake was expressed as a weighted score that reflects lifetime exposure (maximum score = 12). The mean calcium intake score was 6 (equivalent to 1-2 glasses of milk per day or 240-480 mg/d). Higher intakes of calcium from milk were associated with lower risk of hip fracture regardless of the age range studied (i.e. milk consumption in childhood, adulthood or recent past). The association between dairy consumption and risk of hip fracture became significant ($p<0.05$) at consumption score of 4 and reached a plateau thereafter. This association was seen in all countries except Portugal. Direct exposure to sunlight was also associated with a significant risk of hip fracture. The highest exposure score (6) was associated with the lowest RR for each age range of exposure (RR=0.57, $p<0.05$; RR=0.44, $p<0.0001$; and RR=0.49, $p<0.0001$ for the recent past, young adulthood and childhood, respectively). Once again, this association was observed in all countries. The authors concluded that lifestyle factors are associated with significant differences in the risk of hip fracture. [Study score = 30]

Ranstam and Kanis (1995) conducted a case-control study of 1,634 women (≥ 50 years of age) with low-energy hip fractures and 3,532 age- and community matched controls to determine whether there was an association with the use of vitamin D supplements. The study did not estimate dietary intake of vitamin D or calcium, but did collect data on supplement use (for vitamin D), physical activity and exposure to sunlight. There was no difference in vitamin D supplement use between cases and controls. However, multivariate analysis showed that vitamin D supplement use was associated with reduced risk of hip fracture for subjects with BMIs < 20 (RR=0.45; 95% CI, 0.24, 0.84) and for subjects ≥ 80 years of age (RR=0.63; 95% CI, 0.40, 0.98). The authors concluded that vitamin D supplementation might be usefully targeted to the frail and elderly. The lack of dietary data collected in this study and the low percentage of subjects who used vitamin D supplements limits the conclusions that can be drawn. [Study score = 30]

A case-control study of 241 Italian postmenopausal hip fracture victims (mean age 64 years) and 719 hospital-based age- and gender-matched controls was reported by Tavani *et.al.* (1995). There was no difference in calcium intake or milk consumption between the cases and controls after correcting for age, education, BMI, smoking, alcohol consumption and estrogen replacement therapy. There were no data reported on vitamin D intake or status. The authors cite the difficulty of measuring nutrient intake, the use of hospital-based controls and the possibility of individual differences in the absorption and elimination of calcium as possible explanations for their negative results. [Study score = 30]

A case-control study conducted by Chan *et.al.* (1996) compared 144 vertebral “definite” fracture patients and 174 “doubtful” fracture victims with 163 age-matched controls. All subjects were elderly (mean age = 75 years) women living in China. Calcium intake was estimated using a 12-item FFQ representing the major sources of this nutrient in the Chinese diet. Mean calcium intake among all subjects was 318 mg/d. A comparison between “definite” cases and normal controls showed that subjects in the lowest quartile of calcium intake (<247 mg/d) were significantly more likely to have experienced vertebral fracture than participants in the highest quartile (≥ 382 mg/d) after adjusting for age (RR=2.1; 95% CI, 1.1, 3.9). Low calcium intake was not a risk factor for vertebral fracture among “doubtful” cases. [Study score 3Ø].

Hoover *et.al.* (1996) conducted a cross-sectional study of 62 healthy women two or more years post menopause (mean age = 62.9 years) who were not taking HT. Mean dietary calcium intake was 981 mg/d as determined by FFQ and the total mean calcium intake (including supplements) was 1,392 gm/d. Neither calcium from diet or total dietary calcium was associated with BMD of the femur or lumbar spine after adjustment for years since menopause, body weight or lean body mass. However, the product of dietary calcium and fractional absorption was positively associated with femoral BMD ($p < 0.05$). The authors concluded that a positive association between dietary calcium and BMD was detected only by taking intestinal absorptive efficiency into account. The small sample size of this cross-sectional study may have limited its ability to detect small, but significant associations. [Study score = 4]

Stone *et al.* (1996) examined the association between milk intake and BMD at three sites among a cross-sectional sample of 965 healthy Japanese men (mean age = 53.4; range = 27-83 years). Milk consumption was estimated from a survey using a three-point scale (0 = none, 3 = consumption of at least 180 ml every day). BMD decreased with age at all three sites. Multivariate analysis showed that milk intake was positively associated with BMD at the lumbar spine ($p=0.004$) among 853 subjects who did not exhibit degenerative vertebral changes. There were no such associations at the radius or femoral neck. Interpretation of this study is limited by the crude instrument used to assess calcium (milk) intake. [Study score = 4].

As discussed in the previous section on adult women, a cross-sectional study of 25 mother-daughter pairs (Ulrich *et al.*, 1996) found that calcium supplementation after the age of 60 was positively associated with total- and peripheral BMD among postmenopausal women (mean age = 72 years). The authors concluded that women may successfully enhance their genetically determined bone mass through weight-bearing exercise, post-menopausal HT and adequate calcium intake. [Study score = 4]

Cumming *et al.* (1997) conducted a prospective study (mean follow-up period = 6.6 years) among 9,704 postmenopausal women ≥ 65 years of age to investigate the association between calcium and vitamin D intake and fracture incidence. Mean dietary calcium intake at baseline was 714 mg/d. Thirteen percent of the subjects consumed $\geq 1,200$ mg calcium per day while 25% consumed < 400 mg/d. Fifty-one percent were current or past calcium supplement users, 21% were current or past users of Tums antacid

tablets and 53% fell into this category for vitamin D supplements. There were no associations between dietary calcium or milk intake and fracture incidence at any site after adjusting for numerous potentially confounding variables. Calcium supplementation was significantly associated with hip fractures (RR=1.5; 95% CI, 1.1, 2.0) and vertebral fractures (RR=1.4; 95% CI, 1.1, 1.9). Antacid use was also associated with proximal humeral fractures (RR=1.7; 95% CI, 1.2, 2.4). Vitamin D was not associated with fracture incidence at any site. The authors noted that their results do not rule out a large calcium effect in populations with a much lower mean intake of calcium. In addition, the authors concluded that the positive associations between supplement use and fracture incidence were most likely due to inadequately controlled confounding variables. [Study score = 2+]

A prospective study of 4,573 men and women (mean age = 58.5 years) living in Japan was conducted to determine the risk factors associated with non-violent hip fractures during a 12-14 year follow-up period (Fujiwara *et.al.*, 1997). There were six hip fractures not due to automobile accidents among male subjects and 49 among women during the follow-up period. Detailed dietary information was not reported, but the paper stated that mean calcium intake of the cohort at baseline was about 550 mg/d. A multivariate analysis showed that age, gender, BMI, milk intake, alcohol intake and prevalent vertebral fracture history were independently related to risk of hip fracture. High intake of milk (≥ 5 times per week) was marginally associated with fracture incidence compared to low intake (≤ 1 time/wk) in men (RR=0.54; 95% CI, 0.25, 1.07) and women (RR=0.58; 95% CI, 0.27, 1.18). The small number of cases and the lack of

detailed dietary information limit the conclusions that can be drawn from this study.

[Study score = 20]

A cross-sectional sample of elderly (aged 69-90 years) participants from the Framingham cohort (n=328) and a separate group of younger (aged 18-68) residents of the community (n=94) were examined to determine the association between calcium intake and BMD according to the vitamin D receptor (VDR) genotype (Kiel *et.al.*, 1997). The VDR genotype with respect to the *BsmI* restriction fragment length polymorphism was determined. Absence of the restriction site is indicated by "B" and presence by "b".

Dietary data were only provided in detail for the Framingham cohort members. Among these subjects there were significant associations between calcium intake and BMD only in the "bb" genotype. Subjects with calcium intakes greater than 800 mg/d had significantly higher BMDs at five of six skeletal sites than participants with intakes <500 mg/d. The data also suggested that BMD was higher in persons with the bb genotype only in the group with calcium intakes above 800 mg/d. [Study score = 4]

Michaëlsson *et.al.* (1997) conducted a cross-sectional study among 115 postmenopausal (mean age = 60 years) Swedish women who were classified by calcium intake as high (>1,400 mg/d), intermediate (800-1,200 mg/d) or low (400-550 mg/d) consumers.

Dietary assessment of usual intakes during the past six months was obtained using a 60-item FFQ. Multivariate analysis showed that the highest calcium group had greater BMD at the lumbar spine, femoral neck and total body after adjusting for energy intake as well as age, BMI, physical activity, menopausal age, estrogen use, smoking and former

athletic activity. The positive association between calcium intake and BMD was less pronounced in age-adjusted univariate analysis or in multivariate analysis that excluded adjustment for energy intake. [Study score = 4]

A cross-sectional study (Suleiman *et al.*, 1997) of 124 healthy women aged 52-62 was conducted to examine the effect of calcium intake, physical activity and other lifestyle factors on BMD of the spine, hip, oscarlis and of total body mineral content (TBMC). All subjects were 5-12 years postmenopausal and had not received HT or taken calcium or vitamin D supplements. The subjects were divided into four groups based on calcium intake and physical activity. Mean calcium intake of the two high groups was 910 and 870 mg/d while the low-calcium groups consumed a mean of 480 and 470 mg/d.

Calcium intake was correlated with BMD ($p < 0.01$ for the spine and femur; $p < 0.05$ for the oscarlis). Stepwise-multiple regression analysis for calcium, physical activity, age and weight as predictors of BMD and TBMC showed that calcium was significantly associated with BMD at the spine ($p = 0.0001$), hip ($p = 0.016$), oscarlis ($p = 0.0001$) and TBMC ($p = 0.0001$). Age and body weight were also associated with BMD at several sites. The authors conclude that lifestyle factors (including calcium intake and physical activity) in the past and present can modify the predisposition to osteoporotic fractures. [Study score = 4]

Turner *et al.* (1998) reported an analysis of cross-sectional data from the Third National Health and Nutritional Examination Survey (NHANES III) that was collected between 1988 and 1991. The sample consisted of 953 women aged ≥ 50 years who resided in the

southern region of the United States. Univariate analysis showed that fracture victims (self-reported data) were more likely to report consuming dairy products two or more times per day than non-fracture victims ($p=0.04$). However, the data were not corrected for potentially confounding variables, and limited dietary information was provided. The authors observed that analysis of this cross-sectional data could result in inappropriate conclusions if fracture victims increased milk consumption in an effort to prevent further incidence. [Study score = 4]

Burger *et.al.* (1998) reported data from The Rotterdam Study of 1,856 men and 2,452 women aged ≥ 55 years who were studied longitudinally with an average follow-up period of 2 years. BMD of the femoral neck decreased significantly in both men and women after adjustment for age and BMI. Calcium intake was inversely related to the rate of bone loss in men ($p<0.04$) but not in women ($p=0.65$). However, mean calcium intake was high in both genders (1,156 mg/d in men and 1,116 mg/d in women), and the authors speculated that a threshold for the effect of calcium on BMD was exceeded in women but not in the men. Vitamin D intake or status was not measured. The importance of consuming adequate calcium (i.e. 1,500 mg/d) in this age group was emphasized. [Study score = 2+]

The cross-sectional study by Uusi-Rasi *et.al.* (1998) described above in the section on adults found that calcium intake was most strongly correlated with TBBMC ($p=0.023$) among the oldest group of subjects (60- 65 years) examined. [Study score = 4]

A 24-month prospective study among 394 women (mean age at baseline = 53 years) who were members of the control group of the EPIC study (designed to assess the role of alendronate on the prevention of early postmenopausal bone loss) was conducted by Hosking *et.al.* (1998). Women whose baseline calcium intake was below 500 mg/d were advised to increase consumption of this nutrient but calcium supplements were not provided. Mean calcium intake increased from 876 mg/d at baseline to 976 mg/d at 24 months. Women in the lowest tertile were more likely to increase calcium intake (+167 mg/d) compared to subjects in the upper tertile (-36 mg/d). The entire cohort experienced a decline in BMD at the spine, forearm, hip and total body. Dietary calcium was not related to BMD loss at any site, nor did increased calcium intake protect against subsequent bone loss. The investigators noted that the non-randomized design of this study may have biased the results, but that its relatively long duration (24 months) would eliminate transient effects of increased calcium that may occur in short term randomized intervention trials. Many of the women in this study were perimenopausal (mean years after menopause = 5.6 years at baseline). The effect of menopause on bone loss is greater in the early postmenopausal years than later, and it is possible that such effects overshadowed those of dietary calcium in this cohort. [Study score = 20]

A cross-sectional study of 4,434 elderly (mean age = 80.7 years) women living in France was conducted to study the association between calcium intake from food and water with BMD at several sites (Aptel *et.al.*, 1999). Women who were taking calcium or vitamin D supplements or those using HT were excluded from the study. Calcium intake ranged from ≤ 400 mg/d in 354 subjects to $>2,000$ mg/d in 46 participants. Total mean calcium

intake was 834 mg/d and mean calcium from water was 126 mg/d. Univariate analysis showed that incremental calcium intake of 100 mg/d from both water and total diet was positively associated ($p < 0.05$) with BMD at the femoral neck, Ward's triangle and trochanter. Similar results for multivariate analysis found significant associations for calcium from water at all sites but the association for dietary calcium was significant ($p < 0.05$) at the femoral neck only. The authors concluded that women should be encouraged to consume more calcium and suggested that water may be a good source of this nutrient for individuals with limited intake of dairy products. [Study score = 4]

Pines *et al.* (1999) retrospectively studied the effect of calcium supplement use in early postmenopausal women ($n = 315$) who were or were not using HT during a 22-month follow-up period. Calcium supplementation was associated with improved BMD for all groups. HT subjects who took calcium supplements experienced an increase in BMD of 4.5% compared to an increase of 1.5% for those who did not ($p < 0.001$). Analogous results for non-HT subjects were -1.4% loss for calcium users and -3.7% for non-users ($p < 0.001$). Quantitative data on calcium intake were not provided. [Study score = 20]

Kanis *et al.* (1999) conducted a case-control study of 730 elderly (mean age = 73.9 years) male hip fracture patients and 1,132 age-matched (mean age = 74.1) community-based controls. The subjects were residents of Portugal, Spain, France, Italy, Greece or Turkey. Age- and BMI adjusted intake of cheese (RR for lowest compared to the highest quintile = 0.4; 95% CI, 0.25, 0.63) but not milk (RR = 0.94; 95% CI, 0.46, 1.57) was related to fracture risk. Although vitamin D status or dietary intake data were not provided, low

exposure to sunlight was also a risk factor for hip fracture (RR = 0.77; 95% CI, 0.61, 0.97). Multivariate analysis showed that exposure to sunlight and BMI were the largest “modifiable” risk factors associated with hip fracture in this group of subjects and accounted for 14% and 11% of the attributable risk, respectively. This study provides suggestive evidence that vitamin D status (as measured by exposure to sunlight) protects against hip fracture in elderly men. [Study score = 3Ø]

Aguado *et.al.* (2000) examined the association between serum 25(OH)D and PTH in a cross-sectional study of 171 postmenopausal women (mean age 56 years) with and without osteoporosis living in Madrid. Vitamin D deficiency was prevalent in this cohort based on serum 25(OH)D (samples collected between October and June) with 84% of subjects having concentrations <50nmol/L; 64% <37 nmol/L; and 36% <25 nmol/L. Dietary data were not provided. Serum 25(OH)D was correlated with hip BMD ($p<0.05$), but not spine BMD when concentrations were <37 nmol/L. Serum PTH was negatively associated with BMD at the spine ($p<0.01$) and hip ($p<0.01$) for subjects with serum 25(OH)D concentrations <50 nmol/L, and at the hip only ($p<0.05$) for subjects with serum 25(OH)D concentrations <37 nmol/L. Multiple regression analysis revealed that serum PTH was the only significant factor ($p=0.007$) associated with hip BMD in subjects with serum 25(OH)D concentrations >37 nmol/L, and 25(OH)D was the only significant factor ($p=0.028$) for hip BMD in women with concentrations of this compound < 37 nmol/L. The authors concluded that vitamin D supplementation should be provided to postmenopausal women in geographical areas similar to Madrid in the winter to prevent loss of bone mass. [Study score = 4]

Ensrud *et.al.* (2000) conducted a multi-center prospective cohort study of 5,453 women (mean age = 76.5) to determine whether low fractional calcium absorption in women with low calcium intake increases the risk of subsequent hip and other nonspine fractures. During an average follow-up period of 4.8 years, women with low fractional calcium absorption were at increased risk of hip fracture (RR per 7.7% decrease in fractional calcium absorption = 1.24 (95% CI, 1.05, 1.48)). Vitamin D supplement use was not clearly related to risk of hip fracture in subjects with low fractional calcium absorption and low (<400 mg/d) or higher (\geq 400 mg/d) calcium intakes. The authors suggest that low-dose supplemental vitamin D (i.e. 400 IU) may not be effective in preventing hip fracture in women with decreased absorption and low calcium intake, and concluded, "...our results suggest that supplementation with calcium and vitamin D or interventions to increase dietary calcium intake are most likely to be effective in reducing risk for hip fracture in elderly persons with low calcium intake". [Study score = 2+]

A four-year longitudinal analysis of 800 elderly (mean age at baseline = 74.5) men and women (63.2 % female) from the Framingham cohort was reported by Hannan *et.al.* (2000). Women had greater bone loss during the follow-up period than men at all sites, however, BMD decreased for both men and women. Multivariate analysis showed that bone loss as not associated with physical activity, serum 25(OH)D or calcium intake. The authors speculated that non-dietary risk factors may have larger effects on bone loss in this age group, or that such effects may be longer-term than could be detected during the 4-year follow-up period of the study. [Study score = 2+]

Nguyen *et.al.* (2000) conducted a cross-sectional study of 1,075 women and 690 men with a mean age of 69 years. Dietary calcium (median intake 580 mg/d) was positively associated with BMD of the lumbar spine ($p<0.001$) and femoral neck ($p<0.001$) in males after adjustment for age, BMI, physical activity and other factors. Similar results were seen in the femoral neck for women ($p<0.05$), but not in the spine. The authors suggested that an adequate diet (including calcium) and other lifestyle factors may be important to reduce the risk of osteoporosis and improve the quality of life among elderly people.

[Study score =4]

A 4-year, randomized, controlled study (Huuskonen, *et.al.*, 2001) on the effect of physical activity on BMD in 140 men aged 53-62 years reported data on the association between calcium intake and this parameter. Energy adjusted calcium intake (mean 112.7 mg/MJ/d in controls and 115.6 mg/MJ/d in the exercise group) was associated with BMD of the femoral neck. Subjects in the upper tertile of calcium intake maintained BMD during the study while those in the lowest tertile lost approximately 3% ($p=0.03$).

Increased aerobic exercise had no effect on femoral BMD. The authors concluded, "Since higher calcium intake was associated with decreased bone loss, we emphasize the importance of adequate calcium intake in the preservation of bone mass". [Study score = 2+]

A case-control study conducted in São Paulo, Brazil with 73 male and female proximal femur fracture patients aged >65 years and 50 age-matched hospital controls was conducted by Ramalho *et.al.* (2001). There was no difference in calcium intake (as

measured by intake of milk and other dairy products) between fracture patients and controls. Conclusions that can be drawn from this study are limited by design constraints including cursory dietary intake data and a small number of non-community-based control subjects. [Study score = 3-]

A two-year prospective study of 139 women (mean age = 58 years) living in Napoli, Italy was conducted by del Puente *et al.* (2002). Mean calcium intake (which included users of calcium and/or vitamin D supplements) ranged from 983 mg/d in the youngest (45-49 years) age group to 667 mg/d in the oldest (70-79 years). The percentage of subjects who failed to receive the recommended amounts of dietary calcium ranged from 63% in the youngest group to 95% for women aged 60-69 years. Vitamin D intake ranged from 5.0 µg/d in the youngest group to 2.9 µg/d among the oldest participants. The percentage of subjects with serum 25(OH)D concentrations below 37.5 nmol/L ranged from 9.1% in the youngest age range to 27.5% for women aged 60-69 years. Multiple regression analysis found that calcium intake ($p=0.04$) and serum 25(OH)D ($p=0.04$) were associated with BMD at the lumbar spine as was serum 25(OH)D at the femoral neck ($p=0.04$). Stepwise discrimination analysis found that calcium intake ($p=0.02$) and serum calcium ($p=0.03$) were the only independent determinants of BMD at the spine and serum 25(OH)D was the only independent determinant at the femoral neck ($p=0.04$). The authors conclude that adequate dietary calcium and vitamin D may play an important public health role in the prevention of osteoporosis. [Study score = 2+]

Ilich *et.al.* (2003) conducted a cross-sectional examination of 136 healthy, white postmenopausal women (mean age = 68.7 years) living in eastern Connecticut. Mean calcium intake (food and supplements) was 1,378 mg/d, and was positively related to BMD of the femur ($p=0.0158$) and of the spine ($p=0.0192$). Multiple regression analysis revealed that calcium intake was associated with BMD of the total body ($p=0.0111$), Ward's triangle ($p=0.0224$), femoral shaft ($p=0.0262$) and the hand ($p=0.0051$) after adjusting for age, lean body mass, total body fat, height, past physical activity, present mode of walking and energy intake. Serum 25(OH)D and PTH were not associated with bone mass. This lack of association may have been because calcium intakes were adequate. [Study score = 4]

An 18-year prospective study of hip fracture among 72,337 participants in the Nurses' Health study was reported by Feskanich *et.al.* (2003). There were 603 hip fractures resulting from low or moderate trauma during the follow-up period. Mean age of the fracture victims was 65 years. The age-adjusted incidence of hip fracture was significantly lower (RR=0.70; 95% CI, 0.52,0.92) for women in the upper quintile of total calcium intake ($\geq 1,200$ mg/d) compared to the lowest quintile (< 600 mg/d), however, the relationship was no longer significant when controlled for BMI, postmenopausal hormone use, physical activity, protein intake, vitamin D intake and other factors. Total vitamin D intake (i.e. from food and dietary supplements) was associated with reduced incidence of hip fracture (RR=0.63; 95% CI, 0.42, 0.94) for the upper quintile (≥ 12.5 $\mu\text{g/d}$) compared to the lowest (< 3.5 $\mu\text{g/d}$) quintile of intake after adjustment for numerous potentially confounding variables. The authors concluded that