Dietary Factors and Bone Health in Postmenopausal Women

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Institute of Medical Science
University of Toronto

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2012

Abstract

Introduction: About 80% of those affected by osteoporosis are postmenopausal women. Therefore, identifying beneficial or harmful dietary factors for postmenopausal osteoporosis may have a significant public health impact.

Objectives: The overall objective of this thesis was to examine the relations between various dietary factors and bone health in postmenopausal women aged ≥ 45 years using different analytical approaches.

Methods: First, the associations between fruit and vegetables (F&V) intake and indicators of bone health were assessed using a systematic review approach. Electronic databases were searched and peer-reviewed observational and interventional studies published in English with F&V intake as a main dietary exposure were included. Data selection, extraction and evaluation of risk of bias were performed independently by two reviewers. Second, the associations between an overall diet quality index (HEI-2005) and its components with bone turnover markers (BTMs) were examined. Third, the relationships between alpha-tocopherol intake, serum alpha- and gamma-tocopherol, two concentration biomarkers of vitamin E intake, and their ratio and BTMs were assessed. For the second and third studies, cross-sectional data from the National Health and Nutrition Examination Survey 1999-2002 were used. Weighted
multiple regression models with adjustments for relevant confounders were used to examine the relationship between exposures and serum bone-specific alkaline phosphatase (BAP), a biomarker of bone formation, and urinary N-Telopeptides/Creatinine (uNTx/Cr), a biomarker of bone resorption.

**Results:** There was significant between-study heterogeneity in design, definition and amount of F&V intake, outcomes, analyses and reporting of results in the eight included studies. Overall, cross-sectional and case-control analyses reported protective associations between F&V intake and bone health, whereas interventional and prospective cohort analyses did not.

There were no associations between total HEI-2005 scores and BTMs. However, the Milk Group component of HEI-2005 had a significant inverse relationship with uNTx/Cr.

Higher serum gamma-tocopherol and lower ratio of serum alpha- to gamma-tocopherol were associated with higher BAP concentrations but had no associations with NTx/Cr concentrations.

**Conclusions:** The results confirm the existing knowledge that a diet with adequate intake of dairy may reduce bone loss. Further research is needed to examine the potential anabolic effects of gamma-tocopherol on bone in postmenopausal women.
Acknowledgments

In memory of my grandfather, Dr. Davud Shafaie, I would like to dedicate this work to my thesis supervisor, Dr. Angela Cheung, and my parents, Mrs. Farideh Shafaie and Mr. Feraydoon Hamidi, words cannot express my appreciation and gratitude. I want to express my gratitude to my thesis advisory committee members, Dr. Valerie Tarasuk and Dr. Paul Corey, my senior collaborators, Dr. Prakash Shah and Ms. Beatrice Boucher, my internal examiners, Dr. Wendy Ward and Dr. Anthony Hanley, as well as my external examiner Dr. Katherine Tucker.

I am very grateful to Dr. Olga Gajic-Veljanoski, Dr. Luba Slatkovska, Mr. Edward Vidgen, Ms. Hajera Khaja, Ms. Hanxian Hu and Ms. Judy Scher, Ms. Suzanne Cohen, Dr. Marta Erlandson, Dr. Lianne Tile, Ms. Mandy Lau and Ms. Corrine Keogh for their friendship, encouragement, support, and professional guidance and advice. I also wish to thank my brother Kiavash Mark Hamidi, all my friends and family for their support, encouragement, and patience during my PhD training.

I am sincerely grateful to my mindfulness teacher, Ms. Sarah Greenwood, at the Mindfulness-Based Stress Reduction Programs at University Health Network, for teaching me and many others skills to use our innate resources and abilities to cultivate compassion, build resilience and respond more effectively to stress and the challenges of life.

Contributions

For all three manuscripts, I was responsible for conducting the studies, project conception, development of study design, data extraction and management, statistical analysis and interpretation of results as well as writing the initial draft and preparation of the manuscripts.
Dr. Cheung was responsible for the final content and approval of the three papers for submission.

For the first study Ms. Boucher, Drs. Shah and Cheung were all involved in the design and execution of the review and made substantial revisions to the manuscript. Dr. Cheung provided advice and interpreted findings on bone health, Ms. Boucher on nutrition, and Drs. Shah and Beyene on conducting systematic reviews and meta-analysis. Dr. Shah and I reviewed the selected full-text articles and determined the risk of bias independently. Ms. Boucher reviewed the selected articles and extracted data to ensure no information was missed. The risk of bias tool was designed by Dr. Shah and modified by me for studies in the field of nutrition and bone health.

For the second study, Drs. Cheung, Tarasuk and Corey were involved in the design of the study, statistical analysis, interpretation of the results and critical reviewing and editing the manuscript.

For the third study, Drs. Cheung and Corey were involved in the design of the study, statistical analysis and interpretation of the results; and critical reviewing and editing the manuscript.
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Figure 5.1. Selection of study population
List of Abbreviations

AHRQ: Agency for Healthcare Research and Quality

ALQ: Alcohol Use Questionnaire

BMC: Bone Mineral Content

BMD: Bone Mineral Density

BAP: Serum Bone-specific Alkaline Phosphatase

BTMs: Bone Turnover Markers

β: Regression coefficient

CDC: Centers for Disease Control and Prevention

CI: Confidence Intervals

Cr: creatinine

CTx: C-telopeptide

DRI: Dietary Reference Intakes

DXA: Dual-emission X-ray absorptiometry

Equiv: equivalent

EAR: Estimated Average Requirements (EARs)
F&V: Fruit and Vegetables

FAO: Food and Agriculture Organization

FSH: Follicle Stimulating Hormone

Healthy Eating Index: HEI

IGF-1: Insulin Growth Factor-1

HRT: Hormone Replacement Therapy

LCI & UCI: Lower and Upper 95% Confidence Intervals

M: mol

NCHS: National Center for Health Statistics

NHANES: National Health and Nutrition Examination Survey

uNTx: Urinary N-Telopeptides

OC: osteocalcin

oz: ounce

P: p-value

PTH: Parathyroid Hormone

P1NP: procollagen type I N-terminal propeptide

R2: R-square
SE: Standard Error

RCT: Randomized Controlled trials

RDA: Recommended Daily Allowance

RHQ: Reproductive Health Questionnaire

SMQ: Smoking and Tobacco Use Questionnaire

uNTx: urinary N-Telopeptides of collagen cross-links

US: United States

USDA: United States Department of Agriculture

WHO: World Health Organization
1 Literature Review

1.1 Postmenopausal Osteoporosis

Osteoporosis is an asymptomatic chronic bone disease characterized by low bone mass, micro-architectural deterioration of bone matrix and decreased bone strength, leading to increased risk of fractures (1). Osteoporotic fractures result in significant personal, social and economic burden and dramatically increase morbidity and mortality (2-4). Diagnosis of osteoporosis is based on its main outcome, a low-impact or fragility fracture, or its intermediate outcome, low bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the current standard for measuring BMD (5) which is expressed as grams of mineral per area scanned. A T-score is used to express BMD and is calculated by comparing current BMD to the mean peak BMD of a normal, young adult population, often matched for sex and race. T-score is the number of standard deviations from the mean peak BMD for a given population. In 1994 the World Health Organization (WHO) defined osteoporosis as having a T-score ≤ -2.5, low bone mass (previously known as osteopenia) as -2.5 < T-score < -1 and normal as T-score ≥ -1 (6). The lowest DXA T-score of the lumbar spine, femoral neck, total hip, or one-third (33%) radius (when the hip and spine BMD cannot be measured) is used to diagnose osteoporosis.

1.1.1 Prevalence

«Worldwide, the lifetime risk of any osteoporotic fracture for a person over the age of 50 is about 40-50% for women and 13-22% for men (7). About 80% of those affected by osteoporosis
are women, most of whom are postmenopausal women (8). The risk of osteoporotic fractures is much higher in women than men because of the physiological differences such as hormonal changes at the time of menopause, lower peak bone mineral density (BMD), skeletal size and bone geometry (9, 10). It has been reported that among women 45 years and older, osteoporotic fractures account for more days spent in hospital than other major diseases such as diabetes, heart attack and breast cancer (11). The term postmenopausal osteoporosis is used to identify osteoporosis caused by various factors (including loss of estrogen) associated with menopause in women.

1.1.2 Risk Factors

Bone mineral density is not the only determinant of fracture risk, and assessment of fracture risk is improved by combining BMD with information about other clinical risk factors (12). Just recently, WHO developed the FRAX algorithm for estimating the 10-year probability of a major osteoporotic fracture including fractures of the hip, shoulder, wrist, and vertebrae for postmenopausal women and men aged 40 to 90 years (13). The major risk factors associated with osteoporotic fractures that are included in FRAX are: age, sex, ethnicity, BMI (height and weight), low femoral neck BMD, previous fracture as an adult, family history of hip fracture, current cigarette smoking, ≥ 3 alcoholic drinks per day, rheumatoid arthritis, use of glucocorticoids medications, and secondary osteoporosis due to presence of certain medical conditions such as hyperparathyroidism or inflammatory bowel disease (13).

The models used in FRAX have been developed from studying population-based cohorts from Europe, North America, Asia and Australia. This model includes the femoral neck BMD as one of the risk factors. Femoral neck BMD, measured with DXA, was chosen because this site has been the most extensively validated, and provides a gradient of fracture risk (14-16). Based on
FRAX the prevalence of osteoporosis risk factors is as high as 20% for men and 40% for women age 50 years and older in the US (17, 18).

1.1.3 Pathophysiology

Bone is composed of a mineral phase, an organic phase, and a ground substance. The mineral phase consists of crystals of hydroxyapatite and other minerals; the organic phase (osteoid) is made of collagen fibres, 90% of which is type 1 collagen; and the ground substance is formed by glycoproteins and proteoglycans. Three cell types produce and maintain bone: 1) osteoblasts that are bone forming cells that work at bone surfaces and secrete osteoid, control the crystallization of hydroxyapatite and influence the activity of bone-resorbing cells; 2) osteoclasts that are bone-resorbing cells responsible for the resorption of bone which is the first step in the repair of bone surfaces and the remodelling of bone; and, 3) osteocytes that are osteoblasts that have become embedded within the mineralized matrix and connective tissue of bone regions.

Bone is a metabolically active tissue and undergoes continuous remodelling. Bone remodeling adjusts bone architecture to meet changing mechanical needs, repairs microdamage in bone matrix, prevents the accumulation of old bone, and helps maintain serum calcium homeostasis. Bone remodeling happens in four consecutive phases: 1) resorption phase, during which osteoclasts digest old bone; 2) reversal phase, when mononuclear cells appear on the bone surface; 3) formation phase, when osteoblasts lay down new bone to replace resorbed bone; and 4) resting phase, when mineralization of osteocytes is completed. Bone resorption and bone formation happen in a sequence, first by osteoclasts, followed by osteoblasts in a unit called 'basic multicellular unit' (BMU). During resorption phase, osteoclasts create resorption cavities with low pH microenvironments, which dissolve the inorganic matrix and expose the organic
matrix. Next, bone-reabsorbing enzymes digest the organic bone matrix and release breakdown products of type-I collagen including terminal peptide fragments from both ends of the type I collagen such as N-terminal telopeptides (NTx), C-terminal telopeptides (CTx) and ring structures (pyridinium crosslinks) (19). These collagen breakdown products can be measured in blood or urine samples. In healthy bone, the resorption cavity created by osteoclasts is then filled with new osteoid material secreted by osteoblasts during the reversal phase. In this phase, the mesenchymal stem cells are attracted to BMU sites, to cover the newly exposed bone surface and differentiate into active osteoblasts. The active osteoblasts secrete osteocalcin (OC), type-I pro-collagen peptides (N-terminal-PINP and C-terminal-PICP), and bone-specific alkaline phosphatase (BAP). Bone formation is then completed by mineralization in two phases. The formation phase, occurs immediately after the formation of osteoid, when hydroxyapatite crystals are deposited between organic matrixes. A slower secondary mineralization process happens in the resting phase. This phase continues over months when more mineral is gradually added.

The regulation of bone remodeling is both systemic and local. Parathyroid hormone (PTH), thyroid hormone, growth hormone, sex hormones (e.g. estrogens, androgens), calcitriol (vitamin D) and glucocorticoids are among the major systemic regulators. Other factors such as insulin-like growth factors (IGFs), prostaglandins, tumor growth factor-beta (TGF-beta), bone morphogenetic proteins (BMP), and other cytokines are also involved in systematic regulation of bone remodeling. The local regulation of bone remodeling involves a large number of growth factors and cytokines such as tumor necrosis factor (TNF), interferon gamma, and interleukins (20).
In adult years, bone resorption and formation are coupled. This means that they happen at the same rate, allowing a cycle of bone formation to follow each cycle of bone resorption which helps to maintain bone strength, structure and integrity. Physiological changes due to aging, metabolic bone diseases, lack of physical activity, poor dietary patterns, or medications that affect bone metabolism may cause pronounced imbalances in bone turnover and in the long run can affect bone structure, strength and mass. At the time of menopause, around the age of 45, the estrogen levels start to decline (21-23). Reduced estrogen levels results in reduced intestinal calcium absorption and renal calcium reabsorption (24). As a result, there is a marked increase in the rate of bone turnover, with a higher rate of bone resorption compared to that of bone formation (25, 26). This imbalance between bone resorption and formation leads to trabecular and cortical bone thinning, disappearance and loss of tissue connectivity and increased intracortical porosity (27-33). There is emerging evidence that increased oxidative stress in bone due to low estrogen levels in postmenopausal years, may decrease bone formation and increase bone resorption (34-36). Biomarkers of oxidative stress, such as reactive oxygen species (ROS) reduce osteoblastogenesis and the lifespan of osteoblasts and osteocytes (34).

1.1.4 Bone Turnover Markers

Independent of age and bone mineral density (BMD) and in the absence of osteoporosis drug therapy, an increased rate of bone remodeling in postmenopausal women has a detrimental effect on bone microarchitecture and is associated with an increased risk of osteoporotic fractures (31, 37, 38). Biochemical markers of bone turnover, or bone turnover markers (BTMs), represent bone matrix components or enzymes that are released into the circulation during bone formation or resorption (39). Bone turnover markers may predict fracture in two ways: first high bone turnover is associated with low BMD (40, 41), second, independently of
BMD, since increased bone turnover has a detrimental effect on bone microarchitecture and fragility (41, 42).

The BTMs are divided into biomarkers of bone formation and resorption. Biomarkers of bone formation reflect osteoblast activity and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes (37). Some examples are bone-specific alkaline phosphatase (BAP), procollagen type I N-terminal propeptide (P1NP), osteocalcin (OC). Biomarkers of bone resorption reflect osteoclast activity and are mostly degradation products of type I collagen (37). Some examples of breakdown products of type I collagen in bone are N-telopeptides (NTx), C-telopeptides (CTx), deoxypyridinoline (37, 43).

It has been shown that markers of bone resorption are significantly elevated in post-menopausal women with high risk of fracture, but the markers of bone formation are much less elevated and may be even decreased (23, 44-50). Decreased bone formation and increased bone resorption in postmenopausal women is associated with increased loss of bone mineral and matrix (51, 52) and increased risk of fractures (23, 51-54). A study has suggested that NTX can discriminate between post-menopausal women with normal BMD, women with osteopenia and women with osteoporosis, as defined by the WHO criteria (55). Increased bone resorption may also predict fracture risk independently of BMD as increased bone turnover can result in impairment of the trabecular bone architecture, without significantly affecting BMD (37, 46, 47). Although a single measurement of BTMs cannot predict fracture risk in an individual woman, there is evidence that increased bone resorption markers are associated with increased fractures in prospective and cross-sectional studies (23, 48, 55, 56).

Major factors that affect bone turnover in women are menopausal status, rheumatoid arthritis, liver and kidney disease, cancer, drugs such as estrogen, hormone replacement therapy (HRT)
and corticosteroids, immobility and fractures in previous year (57, 58). Bone remodeling processes have a circadian pattern, with less bone resorption during the day and more at nighttime (52). Therefore, the timing of sample collection is important in reducing variability (59). Measurement of urinary bone biomarkers is usually performed either as a single urine collection other than a first morning void, or in 2 to 24 hour urine collections. In each case, values need to be corrected for urinary creatinine which introduces additional pre-analytical and analytical variability because of its correlation with lean body mass (52). It has been suggested that food intake is the major cause of the observed variation in the biomarkers of bone resorption in a day (52, 60-63). The values of BTMs can also be influenced by food composition (52, 62, 63). However, fasting attenuates the circadian rhythm of bone turnover (64, 65) and reduces the biological variability of measurements (57).

1.1.5 Prevention and Management of Postmenopausal Osteoporosis

Osteoporotic fractures result in significant loss of independence and place a substantial burden on women, their social supports and the health care system. Therefore, understanding factors that contribute to fractures, strategies to prevent falls, prevention and treatment of osteoporosis are very important. The primary clinical goal of osteoporosis management is to reduce risk of fracture. This can be achieved by slowing the rate of bone loss, increasing the rate of bone formation, improving bone architecture, maintaining or increasing bone strength, and minimizing factors that contribute to falls. Management strategies include lifestyle modifications and pharmacologic interventions.
1.1.5.1 Pharmacologic Interventions

The primary goal of osteoporosis pharmacological interventions is to reduce the risk of future fracture, not just increase BMD. There are currently two classes of medications that are used for prevention or treatment of osteoporosis (66). The first includes antiresorptive medications that inhibit bone resorption in order to prevent bone loss. There is often an associated reduction in bone formation because bone formation and resorption are coupled processes. However, these medications reduce bone resorption more than increasing bone formation and thereby suppress bone turnover and loss. Some examples of such medications are bisphosphonates, some selective estrogen receptor modulators, estrogen, hormone therapy (estrogen in combination with progesterone), calcitonin and a monoclonal antibody against receptor activator of nuclear factor kappa B ligand (RANKL). These medications increase BMD modestly by increasing secondary mineralization (mineralization of old bone) as a result of lowered bone resorption (67). Currently there is no evidence of new bone formation with antiresorptive medications (68). Bisphosphonates are among the most commonly used medications to treat osteoporosis and work by inhibiting osteoclast activity and reducing bone resorption (69). Bisphosphonates are generally well tolerated; however, some adverse effects may limit their use in some people (69). Recent studies suggest that there is a relation between long-term use of bisphosphonates and the development of atypical femoral fractures (70). It has been proposed that the marked suppression of bone turnover results in micro-damage of the bone structure which can lead to fractures (70).

Estrogen and hormone therapy have been mostly used for the prevention, not treatment, of postmenopausal osteoporosis (69). There is evidence that estrogen and hormone therapy can reduce bone turnover, bone loss, and risk of fractures but may increase risk of stroke and venous
thromboembolism (71, 72). Therefore, other medications should first be considered for prevention of osteoporosis (69). Calcitonin is another antiresorptive agent that inhibits bone resorption by osteoclasts, thereby preventing bone loss (69). It has been shown that calcitonin reduces the risk of vertebral fractures, but not the risk non-vertebral or hip fractures, for this reason calcitonin is not considered first-line treatment for osteoporosis (69). Selective estrogen receptor modulators, such as Raloxifene, have an estrogen agonist effect in areas such as bone and lipid metabolism, while exert an estrogen antagonist effect in other areas such as breast and uterus (69). Denosumab is a human monoclonal antibody against RANKL, which may reduce osteoclastic activity (73). Studies have shown that it is effective in improving BMD, and preventing bone loss and fractures in women with or without cancer (73).

The second class includes medications that stimulate bone formation which may reverse bone loss. Recombinant human PTH analogues are members of this category that act as effective bone anabolic agents. These medications increase bone turnover, with bone formation more than resorption, thus resulting in an increase in BMD and reduction of vertebral and non-vertebral fractures (69, 74). Teriparatide, a recombinant form of PTH, is used for treatment of osteoporosis in men or postmenopausal women with severe bone loss and previous osteoporotic fractures (69). However, teriparatide should not be used in patients with a history of bone malignancy, Paget bone disease, hypercalcaemia, or skeletal exposure to radiation or in those younger than 18 years (69).

The search continues for treatment options that both inhibit bone resorption and stimulate bone formation that have minimal side effects. More recently strontium ranelate and nitric oxide (NO) are being studied for their potential effect of uncoupling of bone resorption and formation activities to increase bone formation and reduce bone resorption (68, 75).
Randomized controlled trials (RCTs) have shown that pharmacological interventions can decrease risk of vertebral fractures by about 50% (76). Despite this, patient compliance with pharmacotherapy for osteoporosis is typically poor in clinical practice resulting in higher fracture rates than observed compared to when compliance and persistence are in accordance with prescribed frequency and dosage (76-78). Some determinants of low adherence to drug therapy are side-effects, not having a prior fracture, old age and high price of medications (76, 78).

1.1.5.2 Lifestyle Interventions

The importance of lifestyle interventions for prevention of osteoporotic fractures increases with age (79). Current lifestyle recommendations in the 2010 position statement of The North American Menopause Society recommends that postmenopausal women participate in appropriate exercise, avoid cigarette smoke and excessive alcohol consumption, reduce risk of falling, maintain a healthy weight, have adequate calcium and vitamin D intake, and eat a balanced diet (80).

1.1.5.2.1 Physical Activity

Regular weight-bearing physical activity may improve BMD and reduce the rate of bone loss in postmenopausal years (81-84). In addition to its direct effect on BMD, weight-bearing and strength-training activities can also improve muscle strength and balance and therefore reduce the risk of falls that lead to fractures (83, 85). However, the reported adherence to physical activity is low in postmenopausal women partly because of fear of falling or getting injured (83, 85).
1.1.5.2.2 Smoking

There is evidence that tobacco exposure, actively or passively, is associated with decreased bone formation and BMD, and increased risk of fractures (86). Smoking in postmenopausal years significantly increases the lifetime risk of hip and vertebral fractures (87, 88). Serum cotinine is a marker for active and passive tobacco exposure. It has been shown that increased serum cotinine level is a risk factor for decreased bone mineral content in men and women (86). Possible mechanisms whereby smoking increases postmenopausal bone loss include: direct toxic effect on osteoblasts resulting in reduced bone formation, reduced calcium absorption and increased oxidative stress (87, 88). Interestingly, smoking has been shown to be associated with reduced bone formation without any effect on bone resorption (89).

1.1.5.2.3 Alcohol Intake

There is evidence of an association between small and moderate alcohol intakes (0.5-1 drinks/day or approximately 5-10 grams (0.18-0.36 ounces) alcohol/day) and increased BMD, reduced bone resorption; as well as a U-shaped relationship between alcohol intake and risk of fracture in postmenopausal women (89-93). One standard drink is 12-ounces of beer, 4 ounces of wine, or 1 ounce of liquor. The beneficial effect of alcohol on bone could be related to its ability to increase endogenous estrogen concentrations (94, 95). Also, alcohol stimulates the secretion of calcitonin, which is an inhibitor of bone resorption (96). On the other hand, excessive alcohol intake decreases bone formation and increases urinary calcium loss and falls (80, 96, 97).
1.1.5.2.4 Fall Prevention

Falls are the most common cause of fractures (98). The risk of falling increases with age often due to impaired eye sight, poor balance, difficulty with movements, side effect of some medications, dementia and sarcopenia (80). Every year about 30% of individuals over the age of 65 years fall (99). Exercises that improve balance and muscle strength, adjusting medications that affect balance, and reducing fall hazards in the home are some strategies than can be used to reduce the risk of falling (100).

1.1.5.2.5 Diet and Supplement Intake

Diet plays an important role in maintaining BMD and reducing the rate of bone loss during perimenopausal and postmenopausal years. Several studies have shown that dietary modifications can favorably affect bone remodelling during this period (101). Furthermore, the optimal use of osteoporosis medications requires adequate calcium and vitamin D intake before and during therapy (69). In vivo and in vitro studies have shown that dietary factors can affect osteoblasts at different stages including proliferation, differentiation and mineralization (102). In humans, changes in bone remodeling can begin immediately after nutritional interventions (61, 103-108). The current main focus of dietary interventions for osteoporosis is increasing calcium and vitamin D intakes through diet or diet and supplements (109). Postmenopausal women have lower energy and higher calcium requirements compared to other population groups, which makes it difficult for them to meet the recommended intakes of calcium without significantly increasing energy intakes (110, 111). In clinical practice, supplementation is advocated as the easiest, fastest, most economical way to increase calcium and vitamin D intakes without increasing energy intake (112). However, there are major drawbacks to
supplementation, including the low long-term adherence and adverse events such as kidney stone formation, stroke or cardiovascular events (78, 112, 113, 114, 115). Some reasons for low adherence to supplement intake, especially calcium supplements, are difficulty swallowing tablets and the side effects such as abdominal pain, bloating, constipation or indigestion (116, 117). In the next section, the effects of diet on bone health will be further described.

1.2 Dietary Factors and Bone Health in Postmenopausal women

1.2.1 Nutrients and Other Dietary Components

Multiple macro and micronutrients influence bone health (101, 118, 119). Deficiencies or excesses of individual nutrients have been shown to affect bone status. Calcium, phosphorus, magnesium and zinc are the primary bone-forming minerals of which approximately 99%, 80%, 60% and 30% respectively are in the bones and teeth (120). Vitamin D and fluoride also play a key role in the development and maintenance of bone and other calcified tissues (118). Calcium and vitamin D are the two most studied nutrients in relation to bone health. However the findings of studies are inconsistent partly because the majority of existing studies administered a combination of calcium and vitamin D (121). This reduces the opportunity to examine the independent effects of each of these nutrients (121).

1.2.1.1 Calcium

The primary role of calcium in the skeleton and teeth is to provide structural rigidity. Ninety nine percent of the body's calcium is present in bones and 1% in body fluids. Calcium is involved in muscle contraction, nerve transmission, hormonal secretion and blood clotting. It also operates as a signal transmitter and protein activator within cells. Serum calcium
concentrations are kept within a narrow range by a process mediated by parathyroid hormone (PTH), vitamin D, and calcitonin. PTH is released from the parathyroid gland when blood calcium concentrations drop. This increases phosphate excretion and calcium reabsorption in the kidney, stimulates bone resorption by osteoclasts and activates vitamin D in the kidneys which increases calcium absorption in the gut. Calcitonin is a hormone released from the clear cells of the thyroid gland in response to high serum calcium concentrations that inhibits osteoclastic bone resorption thus reducing the amount of calcium moving from bone into the bloodstream.

Dairy products, green leafy vegetables and some legumes (e.g. white beans, soy beans), are good sources of dietary calcium (122-124). The bioavailability of calcium from some green vegetables (e.g. broccoli, bok choy) is more than 50%, compared to about 32% for milk (125-127). However, the calcium content and amount of commonly consumed servings of vegetables are low in North America (123, 128). For example to achieve the amount of bioavailable calcium in a cup of milk (6 mg) 4.5 cups of broccoli should be consumed (127). Dairy products and more recently calcium-fortified beverages and calcium-fortified juices are the main dietary sources of calcium in North America, providing approximately 80% of total calcium intake of postmenopausal women (80).

The dietary requirements set by the U.S. Institute of Medicine (IOM), for calcium are based on existing evidence related to BMD and fracture risk (121). The calcium Recommended Dietary Allowance (RDA) for women 19-50 years is 1000 mg/day and for women >50 years is 1200 mg/day (121). There is evidence that adequate calcium intake can maintain bone mass and reduce risk of fracture (129, 130). Low calcium intake in postmenopausal women is associated with high bone resorption, and increasing calcium intakes may suppress PTH secretion and reduce bone resorption (131, 132). Calcium intervention trials report that changes in BMD and
bone resorption are more pronounced in individuals with low habitual calcium intakes than those consuming recommended concentrations of calcium (107, 112, 133). There is evidence that calcium supplements may slightly reduce the rate of bone loss in people aged 50 years and older (129) but the effects on fracture prevention is not clear (134). There is evidence that calcium retention, and therefore BMD, improves as calcium intake rises, up to some threshold intake value, above which no further increase in intake will alter retention (135). Based on the IOM’s recent report, total calcium intake from foods and supplements should remain below the level of the Tolerable Upper Intake Level (UL) of 2000 mg, to avoid possible adverse effects (121). The IOM report indicated that people in the US did not exceed the UL for calcium intake from foods sources alone. However, when calcium supplement use was taken into account, women at the 95th percentile of calcium intake were at risk for exceeding the UL (121). This suggests that a high calcium intake from supplements may be concerning.

There is evidence that calcium supplements are associated with an increased risk of myocardial infarction and urinary tract stone formation (112, 114, 115). One proposed mechanism is that ingestion of calcium supplements result in an acute increase in serum calcium concentrations which in long-term may lead to increased vascular calcification and urinary tract stone formation (114, 115). It has been shown that ingestion of equivalent doses of calcium from dairy products gives a smaller rise to serum calcium concentrations than from calcium supplements (62). Observational studies do not show increased risk of urinary tract stones or cardiovascular disease with higher calcium intake from food sources (115, 136). Therefore, calcium intake from food sources is recommended. Additionally, high-calcium foods contain other essential components such as protein, vitamin D, potassium, magnesium that are also beneficial to bones (137, 138).
1.2.1.2 Vitamin D

Vitamin D is essential for the intestinal absorption of calcium. Low serum vitamin D, due to inadequate intake or little skin exposure to sunlight, leads to reduced calcium absorption, elevated serum concentrations of PTH, and increased rates of bone resorption that may lead to fractures. Vitamin D can be produced in the human body through the interaction of sunlight with the skin.

The recent U.S. Institute of Medicine (IOM) report states that there is no additional health benefit associated with vitamin D intakes above the level of the new Recommended Dietary Allowance (RDA) and that intakes above the Tolerable Upper Intake Level (UL) should be avoided (121). Based on this report, the vitamin D RDAs for adults ≤ 70 years is 600 IU (15 mcg) and for adults >70 years is 800 IU (20 mcg). The UL for all adults is 4000 IU (100 mcg).

Dietary sources of vitamin D are limited to fortified milk and margarine products, fatty fish, and fish oils. One cup of milk, 3 ounces of salmon and 1 teaspoon of cod liver oil contain about 120, 450 and 450 IU of vitamin D respectively (139). Therefore, vitamin D supplements are often advocated for postmenopausal women to avoid vitamin D insufficiency (80).

Although, some studies have shown that vitamin D supplementation alone, reduces the risk of falls and fractures(140-142), a meta-analysis that included 45 trials with 84,585 participants found that taking vitamin D supplement alone, either as vitamin D2 (830 to 1100 IU daily) or vitamin D3 (approximately 830 IU daily), is unlikely to prevent fracture in community living postmenopausal women (143). The results of this meta-analysis suggest that women receiving vitamin D supplements, with or without calcium supplements had a higher risk of hypercalcaemia, gastrointestinal symptoms and renal disease (143). Another recent meta-analysis concluded that combined calcium (500 to 1200 mg/d) and vitamin D (300 to 1100 IU/d)
supplementation, but not vitamin D supplementation alone, can reduce the fracture risk in older adults. Therefore, it may be that the combination of calcium and vitamin D may be beneficial for prevention and management of osteoporosis in postmenopausal women (129, 144).

1.2.1.3 Vitamin E

There is growing evidence that oxidative stress and inflammation, as a result of aging and hormonal changes in postmenopausal women, may have detrimental effects on bone (35, 145-148). There is great interest to identify food components that are able to reduce the rate of bone resorption and increase the rate of bone formation. Because of antioxidant and anti-inflammatory properties of vitamin E, there are plausible hypotheses for its involvement in skeletal health. Although vitamin E has been extensively studied in the areas of cardiovascular disease and cancer prevention, few studies have examined its effect on bone in postmenopausal women (149-152).

Vitamin E has eight naturally occurring forms: alpha-, beta-, gamma- and delta- tocopherols and tocotrienols. Alpha and gamma-tocopherol are the predominant forms of vitamin E in the human body and diet respectively. These forms of vitamin E differ in their degrees of biologic and antioxidant activities. The major circulating form of vitamin E, also the most frequently studied and used in dietary supplements, is alpha-tocopherol. Gamma-tocopherol is the predominant form in the US, representing about 70% of the vitamin E consumed in the typical US diet (153). Nuts, seeds, and sunflower and safflower oils, green leafy vegetables and fortified cereals are among the best sources of alpha-tocopherol while soybean, canola and corn oils are among the sources of gamma-tocopherol (154).
Alpha and gamma-tocopherols are absorbed without preference by the intestinal cells, transported via the lymph system into the circulation, and taken up by the liver. Hepatic tocopherols bind to the alpha-tocopherol transfer protein for incorporation into VLDL and enter the circulation (155). However, the alpha-tocopherol transfer protein has a higher affinity for alpha-tocopherol, and high intakes of alpha-tocopherol, by using vitamin E supplements, saturate the alpha-tocopherol transfer protein and result in suppressed serum gamma-tocopherol concentrations (156). The alpha-tocopherol transfer protein keeps serum alpha-tocopherol concentrations within a narrow range. For this reason, consuming large doses of vitamin E supplements does not increase serum alpha-tocopherol concentrations more than 2- to 4-fold (157). Plasma alpha-tocopherol concentrations are about 4 to 10 times higher than of gamma-tocopherol (158-161). Although, gamma tocopherol has about half of the antioxidant and about one tenth of the biologic activities of alpha tocopherol, it is superior in its anti-inflammatory properties and in neutralizing electrophiles, such as reactive nitrogen oxide species, that cause inflammation (157, 158, 162). Suppressed concentrations of serum gamma-tocopherol may result in unfavorable health outcomes (159, 162). The serum ratio of alpha-tocopherol to gamma-tocopherol is another measure that is used to indicate the degree to which alpha-tocopherol transfer protein is saturated (163, 164). This ratio can serve as an index of alpha-tocopherol ingestion, as it is elevated by modest levels of vitamin E supplementation that do not markedly raise plasma alpha-tocopherol concentrations (164, 165).

It has been proposed that tocopherols may reduce lipid peroxidation and increase the synthesis of antioxidant proteins (150) which may be beneficial to bones. As an antioxidant, alpha-tocopherol functions as a free radical scavenger that can potentially suppress bone resorption by reducing the effects of oxidative stress associated with bone loss due to cellular lipid peroxidation in cartilage and bone cells (166-168). It can also prevent the accumulation of bone
resorbing cytokines (169), or increase osteoprotegerin (OPG), a protein which protects bone from being degraded (170). As an antioxidant, gamma-tocopherol neutralizes nitrogen dioxide and produces nitric oxide which has the ability to reduce bone resorption and stimulate bone formation (68, 171). As an anti-inflammatory agent, gamma-tocopherol can reduce the deleterious effects of pro-inflammatory cytokines and reduce bone resorption (150). The associations between vitamin E on BTMs will be further evaluated and discussed in chapter 5.

1.2.1.4 Other Dietary Components

Many other dietary components may be important for bone health and the prevention of osteoporotic fractures (172). Boron may enhance calcium absorption and estrogen metabolism (120, 173). Copper, manganese, zinc, iron and vitamins C and K may help enzymes and local regulators of bone matrix function properly and may enhance bone strength (172, 174, 175). Fluoride may stimulate bone formation and may increase spine and hip BMD (176).

Magnesium may enhance bone quality and improve bone mineral density (120, 177-179).

Potassium may prevent calcium loss from bones and reduce urinary calcium excretion (120, 179, 180). Caffeine may interfere with calcium absorption, especially when calcium intake is inadequate (181). The phytates, phosphorous and oxalates in some plant foods may combine with the calcium in the intestines and decrease calcium absorption (138, 182). High intakes of sodium and sulfate are associated with higher urinary calcium excretion (172). Excessive intake of the vitamin A in form of retinol (> 1000 IU) may increase bone resorption, decrease bone formation or interfere with vitamin D metabolism and increase risk of fractures (172, 175). Phenolic compounds and flavonoids in plant foods may reduce bone resorption (66, 102, 119).
1.2.2 Food Groups

Most studies on dietary intake and skeletal health have been based on individual nutrients. Although this approach has helped identify the effect of specific nutrients such as calcium and vitamin D on bone health, the findings are inconsistent. Foods contain matrices of biologically active components, consisting of both nutrients and non-nutrients. The positive associations between nutrient intakes from foods and bone health in epidemiological studies may be attributable to other components contained in foods or be a consequence of intake of other nutrients. An examination of the health effects of foods or food groups rather than nutrients may therefore be more informative and also more meaningful to the target populations. Many studies have reported low adherence to supplementation (78, 113) or increased adverse events associated with high dosage of dietary supplements (136, 143, 183). In addition, translation of nutrient recommendations to food intake requirements is difficult for the general public and may lead to increased consumption of dietary supplements. Therefore, the focus of nutrition research is shifting towards examining the relations between foods and health outcome to facilitate development of food-based dietary guidelines. Although different countries use different dietary guidelines, essentially five food groups exist: 1) grains, 2) fruit and vegetables, 3) dairy, 4) protein foods, and 5) other foods such as snacks and sweets. The existing evidence for effect of these food groups on bone health is discussed below.

1.2.2.1 Grains

Only one study has reviewed grains in relation to bone (184). This review reports grains as deleterious to bones because grains, 1) may promote an acidic pH, which may cause bone loss (184, 185), and 2) contain phytic acid and gliadin which may reduce calcium absorption from
the intestines. Phytate content reduces intestinal calcium absorption by forming insoluble complexes with calcium and gliadin that may promote gut inflammation resulting in reduced calcium absorption (184). The effect of different types of grains on bone status should be further studied.

1.2.2.2 Fruit and vegetables

A number of studies have shown that diets rich in fruit and vegetables (F&V) are associated with increased BMD and improvements in bone remodeling rates as well as bone microarchitecture (149, 186-191). Several mechanisms are proposed by which F&V improve bone health. Fruit and vegetables provide magnesium, boron, vitamins K, A, and C, β-carotene, carotenoids, flavonoids, and other phytochemicals all of which may promote bone health (120, 174, 178, 192). Fruit and vegetables also promote an alkaline environment, which may reduce calcium loss (179, 188, 193, 194). Polyphenols, a type of antioxidant, mainly found in berries, plums and pomegranate have shown to promote bone formation in animal studies and in one clinical trial (102, 195). It has been shown that the flavonoids in F&V can inhibit bone resorption (66). Fruit and vegetables also contain certain types of fibre (e.g. soluble fibre, inulin) that can stimulate higher calcium absorption from dairy products (196, 197).

There is evidence that dried plums and onions may have beneficial effects on bone health in postmenopausal women (198, 199). Arjmandi et al showed that compared to dried apples, dried plums at 100g/day increased bone formation and BMD in postmenopausal women (199). This beneficial effect was attributed to dried plum’s high antioxidant capacity, high levels of phenolic compounds and trace minerals such as boron and selenium (199). Another study, using
NHANES data 2003-4, showed that postmenopausal women who consumed onions at least once a day had higher BMD than of those who consumed onions at most once a month (198). Here, three compounds in onions were named, gamma-l-glutamyl-trans-S-1-propenyl-l-cysteine sulfoxide, quercetin and kaempferol that may increase bone formation or reduce bone resorption (198).

Although the WHO dietary guidelines recommend increasing fruit and vegetables to promote healthy bones (200) so far there has been no systematic review or meta-analysis to assess the effect of fruits and vegetables on bone health, in particular among women aged 45 and over. As the number of studies on F&V intake and bone health continues to grow, it becomes more difficult to draw a single conclusion from divergent research findings, especially when contradictory results are considered in isolation from each other. In chapter 4, the association between F&V intake and bone health will be further examined.

1.2.2.3 Dairy

Dairy products have a major contribution to daily intakes of calcium, vitamin D, magnesium, phosphorus, potassium, riboflavin, protein, and carbohydrates in North America (201-203). In addition to the beneficial effects of several components of milk such as calcium, vitamin D and protein, it has been proposed that dairy products may generate a positive estrogenic environment in the body, which may result in greater BMD values in women who consume significant amounts of dairy (202, 204). Milk basic protein (MBP) is a specific protein fraction in milk that has been postulated to suppress bone resorption (205). Low intake of dairy products is associated with low intakes of calcium, vitamin D, potassium, magnesium, protein and B vitamins (203, 206) and diets low in dairy may be deleterious to bones (207). Studies that have specifically evaluated dairy sources of calcium reported that a higher intake of calcium from
dairy foods has more favorable effects on BMD and bone resorption than calcium supplements alone (132, 202, 204, 208, 209). Besides the amount of calcium in the diet, absorption of dietary calcium also plays an important role in maintaining bone health. Several milk components such as lactose, lactulose and casein phosphopeptides are potential enhancers of calcium absorption (210).

1.2.2.4 Protein Foods

Protein provides the structural matrix of bone (205, 211). Therefore, adequate intakes of the amino acids required for collagen matrix formation is necessary to maintain bone health. Very high intake of protein in conjunction with inadequate calcium intake is associated with increased acid load in the blood. It has been hypothesized that high acid load decreases reabsorption of calcium by kidneys, or increases bone resorption to release calcium from bones in order to buffer the acidity (212, 213). As a result hypercalciuria (urinary calcium loss) and bone loss may happen (214). However, there is evidence that the intestinal absorption of calcium increases with increased protein intake (205, 211) and, except for women with very low calcium intakes (400 mg/d), a higher protein intake is beneficial to bones (212, 215). With adequate calcium intakes, increasing intake of dietary protein increases insulin-like growth factor I (IGF-1), calcium absorption, and muscle strength and mass, which are all beneficial to bones (205, 216, 217). Insulin-like growth factor I is a growth factor that may increase bone formation (212) and higher serum levels of IGF-1 is associated with increased bone mineralization and decreased risk of fractures (211). Additionally, the effects of dietary protein on urinary calcium and bone metabolism are modified by other nutrients found in that food. For example, the high calcium or vitamin D content of dairy products compensate for the urinary calcium loss as a result of their protein content; high potassium or isoflavones levels of legumes will decrease
urinary calcium loss; or in a calcium-replete diet, the high phosphate content of meat products has hypocalciuric effects and may partially offset the hypercalciuric effect of the protein (218). Some studies have shown that high-protein calcium-replete diets may protect against bone loss during weight reduction programs (219, 220).

A recent meta-analysis showed that protein intake had a positive association with BMD and BMC and a negative association with bone resorption markers (221). The same study also evaluated the relationships between protein from plants, milk, and meat sources and bone health and found no superiority of one source over another. It has been proposed that the soluble fibre in legumes may enhance calcium and magnesium absorption (66) and that the saturated fat in animal products may bind to calcium in the intestines and form insoluble compounds which lead to reduced calcium absorption and increased calcium excretion (66). Despite these, at this time there is not enough evidence to support that plant protein should be favoured over animal protein (205, 221).

1.2.2.5 Other Foods

Several studies have shown that BMD is positively associated with tea consumption, possibly due to bioactive components present in tea (222-226). Conversely, studies have shown a negative association between coffee consumption and BMD among people with very low calcium intakes (227, 228). Several studies have shown that high intakes of long chain poly-unsaturated fatty acids (PUFA) in fish or flaxseed oils can reduce bone resorption and increase BMD by suppressing the production of pro-inflammatory cytokines involved in bone resorption (229-232) and may be beneficial to bones.
Animal studies have shown that a high sucrose diet decreased the mechanical strength of bones (233) and that glucose has more deleterious effects on mineral balance rather than fructose (234). There are no human studies that have specifically examined the relation between sugar intake and bone status. However, a few studies have reported a negative association between dietary patterns high in sugar and bone status (190, 235, 236). It should be noted that a dietary pattern high in sugar intake is often an indicator of an overall nutrient-poor diet (235, 237) which may be detrimental to bone. The direct effect of different types of sugar on bone health needs to be further studied in humans. Regular consumption of cola is also associated with lower BMD in postmenopausal women possibly due to high caffeine or phosphoric acid content which may increase urinary calcium loss or reduced calcium absorption in the intestines (238).

1.2.3 Overall Diet

The small effects of single foods and food components on bone health and the high correlation between their intakes, makes it difficult to examine their independent effects on bone. Moreover, there are numerous biologically active non-nutrient food components (e.g. phytochemicals) that affect bone for which complete food composition data are not available. Also, the contributions of single nutrients to BMD are often small. For example, it has been shown that calcium intake contributes to about 1% of the population’s BMD variance (239). Similarly, a recent meta-analysis showed that protein intake accounts for 1–2% variability in BMD (221). On the other hand, it has been proposed that about 8-16% of the variance in the BMDs of adults is linked to variations in total diet composition (116, 240, 241). The small effect of individual food components on bone status may be partly responsible for the failure of clinical trials replicating the results of observational studies by using isolated nutrients. The effect of each individual nutrient although small, can be independent and additive, so that in
combination they may be sizable and important in altering the rate of bone loss (101, 241). For example, diets high in calcium tend to be high in dairy and consequently high in vitamin D, protein, magnesium, potassium, riboflavin, vitamin B6, vitamin B12, and thiamine, all of which have been shown to be beneficial for bones (206, 242). Certain types of fibre (e.g. soluble fibre, inulin) in F&V can improve intestinal calcium absorption from dairy products (196, 197). For these reasons it is of great interest to examine the relations between overall diet quality and bone status.

1.2.3.1 Studies of Overall Diet and Bone Health

1.2.3.1.1 Observational Studies

Currently there are three different methods of assessing overall diet quality in relation to diseases in observational studies (243, 244, 245). 1) Theoretically-driven (or hypothesis-oriented approach) overall diet quality scores or indexes, that are based on existing hypotheses about the role of dietary factors in disease prevention. Such indexes often consist of nutrients and foods that are considered to be healthful or detrimental based on current nutrition knowledge. These scores are the total sum of single food or nutrient effects. 2) Data-driven (or exploratory approach) dietary patterns rely on statistical methods such as factor and cluster analysis to derive patterns from collected dietary data. 3) A hybrid of the theoretically- and data-driven methods predicts food combinations related to nutrients or biomarkers with hypothesized associations with particular health outcomes (e.g. trans fatty acids or Low-density lipoprotein). The most common statistical method used in this category is reduced rank regression (RRR), which identifies linear functions of predictors (for example, food groups) that explain as much variation as possible in a set of outcome variables (for example disease
biomarkers) (246). The majority of studies that have examined the associations between overall diet quality and bone health in postmenopausal women have used the data-driven method as described below.

Tucker et al were the first to examine the associations between overall dietary patterns and BMD in 2002 by using cluster analysis. This study included the elderly cohort of the Framingham Osteoporosis Study population (mean age 75±4 years) (235). The six identified dietary patterns include: 1) meat, dairy, and bread; 2) meat and sweet baked products; 3) sweet baked products; 4) alcohol, 5) candy, and 6) fruit, vegetables, and cereal. Bone mineral density was measured at the right femoral neck, trochanter, Ward’s area and at the 33% radial shaft. In this study, women in the candy group had significantly lower radius BMD compared to women in other dietary pattern groups, with the exception of sweet baked products group. There was no significant difference in the radius BMD of women the candy group compared to the sweet based products group. Women with diets high in fruit, vegetables and cereal group as well as diets that contained alcohol tended to have higher BMD than other groups. Women in the alcohol group had a significantly higher radius BMD than the women in the candy group (p<0.05). Interestingly, the protective effect of alcohol on bone was not found in men. Although, intakes of calcium and vitamin D were greatest in the meat, dairy, and bread group, they were also high in the fruit, vegetables, and cereal group (for calcium: 933 ±20 versus 873±33 mg respectively, p< 0.05; for vitamin D: 384 ± 142 versus 375 ± 242 IU, p> 0.05).

Another study examined the relationship between dietary patterns, using factor analysis, and BMD in pre-menopausal Japanese farmwomen aged 40–55 years (247). In this study, women in the highest quintile of the healthy pattern had significantly higher BMD compared with those in the lowest quintile, whereas those in the highest quintile of the western pattern had significantly
lower BMD than those in the lowest quintile. The ‘healthy’ pattern was defined as a diet high in green and white vegetables, mushrooms, fish and shellfish, fruit, processed fish, seaweed and soy products; and the ‘western’ pattern was defined as a diet high in fats and oils, meat and processed meats.

In a sample of adult Greek women (mean age 48 ± 12 years), a dietary pattern, identified by factor analysis and characterized by high consumption of fish and olive oil and low red meat intake was associated with significantly higher lumbar spine and total body BMD (248). In this study, the associations between a dietary index, the Mediterranean diet score (249), and bone outcomes were also examined. A Mediterranean diet was defined as a diet with high intake of fruits, vegetables, legumes, non-refined cereals and olive oil, low intake of red meat and full-fat dairy products and moderate consumption of poultry, fish and alcoholic beverages (249). Surprisingly, there were no significant associations between the Mediterranean diet score and lumbar spine or total body BMD in this population (248). Authors suggest that the discrepancy between findings of factor analysis and the Mediterranean diet index could be because of inclusion of acid-producing food groups such as non-refined cereals and legumes as healthful, and full-fat dairy products as a food group as detrimental to overall health and that such assumptions may not hold true for bone health (248).

The Canadian Multicentre Osteoporosis Study (CaMos) examined the associations between dietary patterns, using factor analysis, and BMD and risk of fractures among Canadian women and men aged 25 and over in a randomly selected population-based longitudinal cohort (250). Among women aged 50 and over (mean age 61 ±12 years), a negative association was found between BMD and an energy dense dietary pattern that was high in intake of soft drinks, potato chips and French fries, hamburger, hot dog, lunch meat, bacon, and sausage, doughnuts,
chocolate and ice cream. There was no association between a nutrient-dense diet, defined by high intakes of fruits, vegetables, and whole grains, and BMD. On the other hand, the nutrient-dense diet was associated with low risk of fracture whereas the energy-dense diet was not associated with risk of fracture (251).

Several limitations apply to data-driven approaches to define dietary patterns such as arbitrary choices of the number of food groups to be included, number of patterns to retain in the final solution, or naming of the patterns (252). These approaches are by nature population-specific and the results of such studies may not be reproducible in other populations. For example the definition and components of a Japanese healthy diet may be different from a healthy Mediterranean or North American diet. The derived patterns are also based on the eating practices of the sample which may be different from the eating habits of the source population. Another weakness of exploratory methods is that the identified patterns only reveal existing dietary practices in the population under study and do not represent optimal diets and therefore, may not be related to potential pathways by which diet may influence health outcomes. Similar to index based dietary scores, categorizing foods into food groups in dietary pattern analysis are often done based on culinary usage, nutrient or phytochemical content, or the current knowledge of the relations between diet and health outcomes, which may affect the patterns derived. A major weakness of diet quality index based methods is the exclusive focus on selected aspects of diet (e.g. intake of sodium, saturated fat, fibre) that are based on the current knowledge of the relations between diet and health (253).

1.2.3.1.2 Nutritional Interventions

In 2003, an ancillary study to the Dietary Approaches to Stop Hypertension (DASH) -Sodium dietary intervention study was the first clinical trial that examined the effects of dietary patterns
on bone and calcium metabolism in a randomized dietary intervention trial (187). The DASH-Sodium study examined the effects on blood pressure of three levels of sodium intake and two dietary patterns among adults with higher than optimal blood pressure (>120/80 mm Hg). The control diet was similar to a typical American diet, whereas the DASH diet emphasized higher intakes of fruits, vegetables, low fat dairy products, whole grains, poultry, fish and nuts, and lower intakes of fats, red meat, sweets and sugar-containing beverages. In terms of micro- and macro- nutrients, the DASH diet contains reduced amounts of total fat, saturated fat and cholesterol, and increased amounts of potassium, calcium, magnesium, dietary fiber and protein. A total of 186 women and men, aged 23-76 years, with a blood pressure of 120-159 mm Hg systolic and 80-95 mm Hg diastolic, ate their assigned dietary pattern at three sodium levels (lower:50, intermediate: 100 and higher: 150 mmol/day), each for 30 days in random order in a crossover design. In the DASH diet group, the serum bone formation marker (osteocalcin) was reduced by 8-11% and bone resorption marker (C-terminal telopeptide of type I collagen, CTx) by 16-18% (both P < 0.001) at each sodium level. Serum PTH and urinary calcium did not differ between the DASH and control groups. Low sodium intake reduced calcium excretion in the DASH diet and control groups, and serum OC in the DASH group. It is notable that the decrease in bone resorption and formation markers in this study did not happen concurrently. In the DASH diet group, the bone resorption concentrations decreased rapidly by the end of the first feeding period, whereas bone formation concentrations had the greatest fall at the end of the second period. Biomarkers of bone formation and resorption increased in the control diet (187).

More recently, a 14 week randomised, parallel-design dietary intervention study compared the effects on BTMs of a low sodium DASH-type diet with a high-carbohydrate low fat and protein diet in women aged 45-75 years with prehypertension or stage 1 hypertension. Both diets contained more than 800 mg of dietary calcium per day (185). The interventions did not affect
serum 25-hydroxyvitamin D, PTH and osteocalcin concentrations. After the intervention period, the low sodium DASH-type diet had greater reduction in urinary calcium excretion; however both groups had a similar increase of 23% in bone resorption marker CTx (P < 0.0001). Women in both groups had a small but significant weight loss during the study, which may have resulted in increased bone resorption in both treatment groups (185). The high-carbohydrate low-fat diet group also had an 11% increase in the bone formation marker N-terminal propeptide, type I procollagen (PINP, P = 0.003). The increase of both bone formation and resorption in the high-carbohydrate low-fat diet could indicate an overall increased rate of bone remodeling (185).

The Women’s Health Initiative (WHI) Dietary Modification intervention examined the associations between a low-fat dietary pattern group and incident osteoporosis-related fractures, falls, and BMD in a subset of women aged 50–79 years at the time of enrollment in the trial over 8 years (254). Women were assigned to a low-fat dietary pattern group with total fat <20% of kcal; saturated fat 7% of kcal; fruit and vegetables > five servings/day; grain > six servings/day or control diet (the habitual diet of participants) (254). The change in F&V was about 1 serving/day and for grains was 0.9 serving/day greater in the intervention group than in the control group. The change in percentage of energy from fat was at least 8% greater in the intervention group than in the control group. Between baseline and first year, the mean daily intake of omega-3 fatty acids and omega-6 fatty acids was decreased in the intervention group by 0.65 and 6.0 grams/day, respectively, whereas the respective mean decreases in the control group were 0.22 and 2.2 grams/day. After approximately 8 years of follow-up, there were no differences in osteoporotic fractures between the groups. The BMD at the total hip was 0.4–0.5% lower (P = 0.003) in the dietary intervention group than in the comparison group at years 3, 6 and 9. The positive finding of this study was that dietary modification group had a lower
risk of falling (falling two times or more during the follow-up period) than the comparison group (HR: 0.92; 95% CI: 0.89–0.96; P < 0.01). Given that the intervention goals were reducing all dietary fats and increasing intake of all vegetables, fruit, and grains, it was not clear which of these factors was causally related to the reduction in total hip BMD in the intervention group.

1.2.4 Biomarkers of Dietary Intake

Dietary intake assessment methods such as food records, 24-hour dietary recalls or food frequency questionnaires have limitations in accurately measuring the food and nutrient intakes. Some examples of measurement errors are under or over-reporting of intake, incomplete or inaccurate food composition databases, variations in nutrient content due to fortification or food additives or geographical area, and bio-availability based on the food source (255, 256). Biomarkers of dietary intake are used where measurements of dietary intake using traditional dietary intake assessment methods have major limitations. Serum concentrations of selenium, vitamin E (tocopherols) (256, 257), vitamins A (retinol), B group, C, D, and K, folate, and the carotenoids are also used for assessing nutritional status and examining the relations between diet and disease (258). Several observational studies have explored the relations between various biomarkers of dietary intake and bone health. Different studies have shown that high serum concentrations of retinol and low serum concentrations of vitamins B12, C, D and K and carotenoids are associated with lower BMD and higher risk of fractures (259-264). The associations between serum alpha and gamma-tocopherol forms of vitamin E on BTMs will be further evaluated and discussed in chapter 5.
1.3 National Health and Nutrition Examination Survey

The National Health and Nutrition Examination Survey (NHANES), performed by the National Center for Health Statistics (NCHS) part of the Centers for Disease Control and Prevention (CDC), is a series of studies designed to assess the health and nutritional status of adults and children in the United States (265). Some of the research and public health goals of NHANES survey are to estimate the prevalence of diseases and risk factors, estimate disease incidence, develop population reference distributions of health parameters, monitor secular changes in disease and risk factors, identify causes of secular changes in health status, and identify new risk factors for diseases and their contribution to disease etiology (265).

Since the early 1960s, the NHANES has been conducted as a series of surveys focusing on different population groups or health topics. In 1999, the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements. Every year, approximately 7,000 individuals, of all ages, are interviewed in their homes and examined in mobile examination centers (MECs). Approximately 5,000 out of the 7,000 interviewed persons complete the health examination component of the survey. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The health examinations in MECs collect blood and urine samples, dietary intake data, medical data, and physiological measurements. The NHANES survey design is a stratified, multistage probability sample of the civilian non-institutionalized U.S. population. The stages of sample selection are: 1) selection of Primary Sampling Units (PSUs) such as counties or small groups of neighboring counties; 2) segments within PSUs (for example a block or group of blocks that contain a cluster of households); 3) households within segments; and 4) one or more participants within each household.
The NHANES over-samples low-income persons, adolescents 12-19 years, persons 60+ years of age, and African Americans and Mexican Americans. Sample weights are created in NHANES to account for the complex survey design, over-sampling, survey non-response, and post-stratification. A weighted sample in NHANES is representative of the U.S. civilian non-institutionalized census population.

NHANES is unique in that it contains information on socio-demographic, lifestyle factors, laboratory data and physical examinations. It is representative of the U.S. civilian non-institutionalized census population. All tests are done by highly trained medical personnel in a standardized environment. Furthermore, NHANES survey data are available on World Wide Web at no cost. For these reasons NHANES data are often used to identify new risk factors for various diseases and their contribution to the understanding of disease etiology.

1.4 The Healthy Eating Index

The Healthy Eating Index (HEI) is the most commonly used diet quality assessment tool in the US, combining dietary guidelines for nutrients and food groups into one summary measure. The original HEI is a theoretically derived dietary index that was developed in 1995 by the United States Department of Agriculture (USDA). Since its release, it has been used to examine the association between several health outcomes and dietary patterns (258, 266-269).

The original HEI has 10 components with scores ranging from 0 to 10 and a total possible score of 100 (270). The component scores are weighted equally and are based on the consumed number of servings of each of the five food groups (grain, fruit, vegetable, dairy and meat), the percentage of calories from total and saturated fat, the amounts of cholesterol and sodium, and a measure of dietary variety. A maximum score of 10 is assigned to each of the five food group
components when the intake levels meet or exceed the recommended number of servings for that food group based on the 1992 Food Guide Pyramid (gender and age group specific). A score of zero is assigned to no intake and intermediate scores are computed proportionately to the number of servings consumed (270).

After release of the 2005 dietary guidelines for Americans, HEI was revised to reflect the changes (271). The HEI-2005 with a score also ranging from zero to 100, differs from the original HEI in that: 1) the contribution of each component to the total score varies (0-5, 0-10 and 0-20), 2) assessment of the diet quality is based on energy density (per 1000 calories or as a percent of energy) and, 3) it has 12 components with some new components such as oils (found in food mixtures), whole fruits, dark green and orange vegetables and legumes, whole grains, calories from solid fats, alcoholic beverages, and added sugars. Unlike the original HEI, the HEI-2005 emphasises on consumption of whole fruits, whole grains, and orange and dark green vegetables as well as legumes (271). For nine components including total grains, total fruit, total vegetables, whole grains, whole fruit, dark green and orange vegetables and legumes (5 points each), milk, meat and beans, and oils (10 points each), a maximum score is given when the intake levels of the lowest recommended amount per 1000 calories of these components are met, maximum scores are achieved. Scores for lesser intake levels are prorated linearly. Minimum and maximum scores for three components (saturated fat (10 points), sodium (10 points), and calories from solid fats, alcoholic beverages, and added sugars (a proxy for discretionary calories (20 points)) are based on population probability densities and expressed per 1000 calories for sodium and as percentages of total calories for saturated fat and discretionary calories. Scores for intakes between the maximum and minimum levels are prorated (271). The association between the HEI-2005 and BTMs will be examined in chapter 5.
1.5 Summary and Rationale

Osteoporotic fractures are associated with significant chronic pain, reduced mobility and increased mortality in postmenopausal women. At this time, long-term drug therapy is used to reduce the risk of fractures; however, the observed adherence to such therapy is poor due to reasons such as absence of bone-loss symptoms, costs and adverse effects (77, 272-274). It has been proposed that the marked suppression of bone turnover associated with long term use of bisphosphonates may result in microdamage of the bone structure and lead to fractures (70). Therefore, identifying non-pharmaceutical interventions, such as dietary factors that can stimulate bone formation and reduce bone resorption is of great interest (102, 119). Bone is a complex living tissue and a wide spectrum of food components are involved in its metabolism. The results of many studies suggest that diet plays a critical role in in changing the rate of bone loss in postmenopausal women (210).

Among food groups, the focus of nutrition research has been mainly on dairy products, however fruit and vegetables are also rich sources of vitamins, minerals and phytochemicals which are involved in bone metabolism and may be beneficial to bones (101, 186, 275, 276). Given our limited understanding of the number, types and functions of various compounds in the complex composition of F&V, instead of examining individual components, it is more meaningful to examine the associations between F&V intake as a food group with bone health outcomes. Although several studies have assessed the effects of F&V on bone health, the results are inconsistent. Therefore, a systematic review of the association between the consumption of fruit and vegetables and bone health outcomes will assist to summarize the existing evidence and identify potential research gaps.
Diets high in dairy products, F&V, fish, nuts and seeds have been associated with better bone status, whereas diets high in intake of sugars, sodium and saturated fatty acids have been associated with poorer bone status (187, 235, 251, 277-280). Despite this, it is not clear whether diet as a whole or as individual dietary components is a better predictor of bone metabolism among aging women. Because the relations between various dietary factors and bone health may not be linear (281), the use of index-based diet quality assessment-tools that focus on meeting the recommendations of dietary guidelines may be more appropriate. In these index-based tools, once someone meets the recommended intake amount for a food group, then a complete score is achieved for that component of the index, and a higher intake does not result in a higher score. The Healthy Eating Index, developed by the USDA is an overall dietary assessment tool that has been developed to assess the diet quality of Americans based on the US dietary guidelines. The HEI can be used as a practical standard for assessing dietary quality. The advantage of using an overall diet quality index to dietary pattern analysis is that it allows examination of its components. It would help to determine if specific components of the diet drive the possible association. Although several studies have assessed the effect of dietary patterns on bone health the associations between HEI and bone health has not been explored.

Increased oxidative stress and inflammation resulting from aging and declining estrogen levels is one of the proposed causes of increased bone loss in postmenopausal women (34-36, 282-284). Alpha- and gamma-tocopherol, the two predominant isomers of vitamin E in nature, may be beneficial to bone due to their antioxidant and anti-inflammatory properties. The effects of dietary alpha- and gamma-tocopherol on bone metabolism have not been well studied in humans. The estimates of dietary intake of alpha- and gamma-tocopherol are affected by incomplete or inaccurate values in food composition databases (256, 285) or under-reporting of dietary fat which is one of the main sources of tocopherol intake (286-289). Biomarkers of
nutritional intake, such as alpha- and gamma-tocopherol, are often used when the reported
intake of a nutrient is a poor measure of its true intake (256, 290, 291). Serum tocopherols are
affected by short-term food intake (292, 293) which makes them suitable for examining the
relationship between tocopherols and BTMs.

NHANES is suitable for examining the relationships between dietary factors and BTMs because
it provides detailed data on BTMs, daily nutrient and food group intake, HEI-2005 scores,
biomarkers of nutritional intake, medical history, laboratory data, use of prescribed medications,
use of dietary supplements and various lifestyle characteristics such as physical activity,
smoking and alcohol intake as well as socio-demographic and body measures. The 1999-2000
and 2001-2002 cycles of NHANES have BTMs data for all adult women. The two BTMs
measured in NHANES, BAP and uNTx, are among the most sensitive BTMs (294). BAP is an
osteoblast-related matrix protein that helps in the mineralization of bone matrix and is involved
in skeletal calcification by increasing local concentrations of inorganic phosphate (295). BAP
has low biological, diurnal and intra-individual variability and has a circulatory half-life of 1 to
2 days (295). There is evidence that high uNTx concentrations are associated with lower BMD
and higher risk of fracture (31, 55, 296-300) independently of BMD and age (38). Because of
day to day variation of uNTx it has limited value for predicting fractures in individuals.
However, in population-based studies stratifying subjects according to uNTx concentrations can
identify those with greater risk of osteoporotic fractures (38, 43, 48). High concentrations of
both BAP and uNTx are risk factors for osteoporotic fractures and low BMD among healthy
postmenopausal women who are not on osteoporosis medications (23, 56, 296). Furthermore,
these two BTMs can be used in cross-sectional assessment of dietary effects on bone health
because they respond quickly to dietary changes (60-63, 105, 177, 187, 301).
The focus of nutrition research in the area of bone health has been mainly on the effects of dairy products, calcium and vitamin D intake, and much less in known about the effects of other nutrients and food groups on bone. In the past decade, the role of fruit and vegetables, overall diet quality and vitamin E on bone health have been emerging in the literature. Studying the relationships between these, less studied but potentially beneficial, dietary factors for bone health using different research approaches has the potential to significantly contribute to our understanding of the unique contributions of diet to bone metabolism in postmenopausal women and to help in identifying research gaps and nutritional strategies to minimize bone loss.
Chapter 2

2 Overall Thesis Objectives

The overall objective of this thesis was to gain a better understanding of the relationship between various dietary factors that have been less studied and bone health in community living postmenopausal women aged 45 years and over using different approaches.

1) The objectives of the first study were to investigate the independent relations between F&V intake and incidence of osteoporotic fractures, BMD, and BTMs and to identify potential research gaps using a systematic review approach.

Hypothesis: Increased intakes of fruit and vegetables are inversely associated with the risk of fractures, low BMD and high bone turnover.

2) The objectives of the second study were to assess the associations between overall diet quality and BTMs using the HEI-2005, and to explore the relations between the components of the HEI-2005 and, the MyPyramid food groups and BTMs.

Hypothesis: Overall diet quality, expressed by total HEI-2005 score, is associated with BTMs.

3) The objectives of the third study were to examine the associations between dietary and total (from diet and supplements) alpha-tocopherol intake, serum alpha- and gamma-tocopherol concentrations and their ratio and BTMs.

Hypotheses: Dietary and total alpha-tocopherol intake as well as serum alpha and gamma-tocopherol and their ratio are associated with BTMs.
Chapter 3

3 Study 1: Fruit and vegetable intake and bone health in women aged 45 years and over: a systematic review

Maryam Hamidi, Beatrice A Boucher, Angela M Cheung, Joseph Beyene, Prakash S Shah

3.1 Introduction

Osteoporosis is a chronic and often asymptomatic disease that involves bone loss leading to fractures and significant personal and societal burden (302). About 80% of those affected by osteoporosis are women (303). Due to hormonal changes around the age of 45 years, an increased rate of bone loss places women at higher risk for osteoporotic fractures (304). Among women 45 years and older, osteoporosis accounts for more days spent in hospital compared to other major diseases such as diabetes, heart attack and breast cancer (11). To prevent osteoporotic fractures, long term drug therapy is required; however, the observed adherence to such therapy is poor due to reasons such as absence of bone-loss symptoms, costs and adverse effects (77, 272-274). Therefore, identifying non-pharmaceutical interventions, such as dietary factors that can reduce the rate of bone loss in this population, is of great interest (102, 119).

Diet is a modifiable lifestyle factor in the prevention and management of osteoporosis (101, 210, 305). Dairy products are well established as an important food group for bone health, but less is known about other food groups (186, 202). Several studies have investigated the possible beneficial effect of fruit and vegetable (F&V) intake on bone health and the results have been conflicting. Fruit and vegetables contain many components such as minerals (e.g. magnesium,
potassium, and calcium), vitamins (e.g. vitamins C and K), antioxidants (e.g. polyphenols), and phytochemicals (e.g. phytoestrogens) that are involved in bone metabolism and can synergistically affect bone health (101, 102, 196, 306, 307). Due to their high potassium and magnesium content, diets high in F&V have a lower dietary acid load and may promote a positive calcium balance (275, 276, 308). Additionally, high acid load may inhibit osteoblast function and increase osteoclast activity resulting in reduced bone formation and increased bone resorption (309, 310). Fruit and vegetables are also high in vitamin K and vitamin C which, along with magnesium, are involved in the synthesis of bone matrix (101, 306). Additionally, phytochemicals and other antioxidants present in F&V could protect bone by increasing bone formation or reducing bone resorption (101, 102, 197, 311).

The number of studies on F&V intake and bone health continues to grow with often contradictory results. Determining the effect of F&V intake on bone health among aging women is clearly an important objective with implications for setting nutritional guidelines for the prevention and management of postmenopausal osteoporosis. Thus, the objective of this study was to assess the independent effects of F&V on fragility fractures, BMD or BTMs among women aged ≥ 45 years and to identify potential research gaps by performing a systematic review of the literature.

3.2 Methods

3.2.1 Eligibility Criteria

A protocol was developed prior to conducting the review. No attempt was made to publish the review protocol. Interventional (randomized or nonrandomized controlled trials) and
observational studies (cohort, case-control or cross-sectional) that specifically reported F&V intake as a main dietary exposure among community living women 45 years and older, at baseline or at the time of interview, were included. Published peer-reviewed articles and abstracts, and book chapters reporting studies in humans, written in English were eligible for inclusion.

We excluded studies limited to 1) individual fruits (e.g. plums), vegetables (e.g. onions), or select F&V categories based on specific nutrient content (e.g. high in vitamin C), 2) biomarkers of F&V intake (e.g. serum carotenoids, net acid excretion), 3) nutrients or food components (e.g. potassium, magnesium, vitamin C, phytochemicals, vegetable protein), 4) specific eating patterns such as Western, Mediterranean, vegetarian, and 5) where the independent effect of F&V could not be separated from that of other foods or food groups.

We included fractures, BMD and BTM as outcomes of bone health. Clinically, osteoporosis manifests itself by the occurrence of fragility fractures of the hip, vertebrae, and forearm. Fragility fractures are defined as those resulting from no apparent or minimal trauma such as falls from standing height or less, and verified by X-Ray or other imaging reports (e.g. magnetic resonance imaging) (312). Since few studies directly link F&V intake with fractures, BMD at lumbar spine, total hip, femoral neck or forearm (radius), measured primarily by dual energy X-ray absorptiometry (DXA), was chosen as an intermediate outcome since it is often used as a surrogate for fracture risk (313, 314). Cohort studies and randomized controlled trials (RCTs) with a follow-up of at least 6 months for BMD were included as, in the absence of disease and medications, the estimated bone remodeling cycle is 6 months and it is unlikely that any intervention-related BMD changes happen prior to this period (315). Bone turnover markers include serum or urinary bone resorption and/or bone formation biomarkers, such as
carboxy-terminal telopeptides of collagen cross-links (CTx) (resorption markers) and BAP, procollagen extension peptides or osteocalcin (formation markers). Independent of BMD, in the absence of drug therapies, increased BTMs indicate detrimental effects on bone microarchitecture and fragility (37, 316).

3.2.2 Information sources and search strategy

We searched electronic databases to July 31st 2010: MEDLINE (from 1950), EMBASE (from 1980), CAB (from 1973), PUBMED, the Cochrane Library (Issue 2, 2010) and Google Scholar (first 100 hits). Reference lists of reviews and retrieved full-text articles were examined for other relevant publications. We also reviewed trial registries (clinicaltrials.gov/ and www.controlled-trials.com/mrct/). The search strategy included both truncated free-text and exploded MeSH terms and was modified according to each database (Box 3.1).
Box 3.1: Search Strategy

**MEDLINE:**
exp "Bone and Bones"/ or bone mineral density.mp. or (bone mineral density).ti,ab. or exp Bone Density/ or bone remodeling/ or bone regeneration/ or bone resorption/ or (Biological Markers/ and "Bone and Bones")/ or exp Osteoporosis, Postmenopausal/ or exp Osteoporosis/ or exp Fractures, Bone/ or (bone adj2 turnover).ti,ab. or (bone adj2 marker*).ti,ab. or (bone adj2 biomarker*).ti,ab. or (bone adj2 formation).ti,ab.
exp fruit/ or exp vegetables/ or (Fruit* and Vegetable*).mp. or fruit*.mp. or vegetable*.mp. or (fruit* adj2 vegetable*).ti,ab.
(clinical trial, all or clinical trial, phase i or clinical trial, phase ii or clinical trial, phase iii or clinical trial, phase iv or clinical trial or controlled clinical trial or meta analysis or multicenter study or randomized controlled trial).pt. or exp Clinical Trials as Topic/ or exp cross-sectional studies/ or exp clinical trial/ or exp clinical trials as topic/ or exp clinical trials, phase i as topic/ or exp clinical trials, phase ii as topic/ or exp clinical trials, phase iii as topic/ or exp clinical trials, phase iv as topic/ or exp controlled clinical trials as topic/ or exp randomized controlled trials as topic/ or exp multicenter studies as topic/ or exp intervention studies/ or exp twin studies as topic/or epidemiologic studies/ or exp study characteristics/ or exp epidemiologic study characteristics as topic/or exp epidemiologic research design/ or exp case-control studies/ or exp retrospective studies/ or exp cohort studies/ or exp longitudinal studies/ or exp follow-up studies/ or exp prospective studies/ or observational study.mp.

**EMBASE**
1 exp bone/ or exp bone demineralization/ or exp bone mineralization/ or exp bone density/ or exp fracture/ or exp bone fragility/ or exp osteoporosis/ or exp fragility fracture/ or exp bone mass/ or bone metabolism/ or exp bone mineral/ or exp bone remodeling/ or exp bone regeneration/ or exp bone turnover/ or exp osteoporosis/ or exp postmenopause osteoporosis/ or exp primary osteoporosis/ or exp secondary osteoporosis/ or exp bone regeneration/ or exp bone remodeling/ or exp bone density/ or exp bone turnover/ or exp bone/ or exp ossification/ or (bone adj2 turnover).ti,ab. or (bone adj2 marker*).ti,ab. or (bone adj2 biomarker*).ti,ab. or (bone adj2 formation).ti,ab.
2 fruit/ or vegetable/ or (fruit* and vegetable*).mp. or fruit*.mp. or vegetable*.mp. or (fruit* adj2 vegetable*).ti,ab.
3 exp clinical study/ or exp intervention study/ or exp longitudinal study/ or exp prospective study/ or exp retrospective study/ or exp case control study/ or exp hospital based case control study/ or exp population based case control study/ or exp clinical trial/ or exp multicenter study/ or exp phase 1 clinical trial/ or exp phase 2 clinical trial/ or exp phase 3 clinical trial/ or exp phase 4 clinical trial/ or exp controlled clinical trial/ or exp randomized controlled trial/ or exp clinical trials/ or ct.fs. or clinical trial/ or phase 1 clinical trial/ or phase 2 clinical trial/ or phase 3 clinical trial/ or phase 4 clinical trial/ or controlled clinical trial/ or randomized controlled trial/ or multicenter study/ or meta analysis/ or (random: or (doubl: adj2 dummy) or ((Singl: or double: or trebl:) adj25 (blind: or mask:)) or RCT or RCTs or (control: adj25 trial:) or multicent: or placebo: or metaanalysis: or (meta adj5 analys:) or observational stud:);ti,ab.
4 1 and 3 and 2

**CAB**
1 exp fruit/ or fruits/ or exp vegetables/ or fruit*.mp. or vegetable*.mp. or (fruit* and vegetable*).mp. or (fruit* adj2 vegetable*).ti,ab.
2 clinical trials/ or (random: or (doubl: adj2 dummy) or ((Singl: or double: or trebl:) adj25 (blind: or mask:)) or RCT or RCTs or (control: adj25 trial:) or multicent: or placebo: or metaanalysis: or (meta adj5 analys:) or sham or effectiveness or efficacy or comp.ar):ti,ab. or exp trials/ or exp randomized controlled trials/ or exp clinical trials/ or exp trials/ or exp randomized controlled trials/ or exp epidemiological surveys/ or (observational study or case-control study or cohort study or cross-sectional study.mp.
3 osteoporosis/ or osteoporosis, postmenopausal/ or exp bone formation/ or exp bone resorption/ or exp bones/ or exp bone mineralization/ or exp bone density/ or exp bone fractures/ or exp demineralization/ or (bone adj2 turnover).ti,ab. or (bone adj2 marker*).ti,ab. or (bone adj2 biomarker*).ti,ab. or (bone adj2 formation).ti,ab.
4 1 and 3 and 2

**PUBMED**
3.2.3 Study selection

Titles and abstracts were screened by one reviewer (MH). Full-text articles were retrieved for further assessment if initial screen indicated potential eligibility. All potentially relevant articles were reviewed independently by two reviewers (MH & PSS). Discrepancies were resolved by consensus and, when necessary, arbitrated by a third independent person (BAB for nutrition and AMC for bone health related topics).

3.2.4 Data extraction and assessment of risk of bias

Data from eligible studies were extracted by two authors (MH and PSS) independently and discrepancies were resolved by consensus and, when necessary, in consultation with a third reviewer (BAB). For observational studies, two reviewers (MH and PSS) developed a tool to assess the risk of bias, incorporating guidelines from the Agency for Healthcare Research and Quality for the field of nutrition (317-319). The tool (Form A) consists of seven domains including sample selection, F&V assessment, outcome assessment, confounding factors, analytical factors, selective reporting and attrition biases. The risk of bias in clinical trials was assessed using the Cochrane Collaboration’s tool (320) slightly modified to reflect dietary studies (318, 321) (Form B). This tool also consists of seven domains. Risk of bias assessment was performed independently by the reviewers, and any disagreement was discussed and resolved. The assignment of low, moderate or high risk depended on the number of domains considered not to be biased.
### Form A: Risk of Bias for observational studies- developed by authors

<table>
<thead>
<tr>
<th>Bias</th>
<th>No</th>
<th>Yes</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Selection</strong></td>
<td>Consecutive unselected population</td>
<td>Sample ambiguous &amp; likely not representative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample selected from general population rather than a select group</td>
<td>A select group of population (based on race, ethnicity, residence etc.) studied</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eligibility criteria explained</td>
<td>Eligibility criteria not explained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Criteria for case &amp; control selection explained</td>
<td>Criteria for case &amp; controls not explained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Follow up or assessment time explained (longitudinal studies)</td>
<td>Follow up or assessment time not explained (longitudinal studies)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excluded those with diseases or medications that affect bone metabolism (e.g. kidney or liver disease, use of osteoporosis treatment medications, corticosteroids, estrogen or HRT)</td>
<td>Included those with diseases or medications that affect bone metabolism</td>
<td></td>
</tr>
<tr>
<td><strong>Exposure (F&amp;V) assessment</strong></td>
<td>F&amp;V described (types of F&amp;V described)</td>
<td>F&amp;V subgroups or categories not described</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assessment of usual intake using FFQ, several 24 hour recalls, or food records</td>
<td>In longitudinal studies: single dietary assessment &gt; 2 years prior to outcomes assessment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In RCTs &amp; longitudinal studies: reporting baseline F&amp;V intake as well as final visit F&amp;V intake (or change from baseline)</td>
<td>Quantity of F&amp;V intake not assessed, only frequency</td>
<td></td>
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<tr>
<td></td>
<td>Reporting the amount of F&amp;V used as intervention</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Reporting ranges or distributions of F&amp;V intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methods or instruments for assessing dietary intake were reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dietary assessment method was validated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Measurement errors of the dietary assessments were reported or discussed</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outcome assessment</strong></td>
<td>Fractures: Using x-rays, MRI or radiology reports verified by a physician</td>
<td>Self-reported, no clinical evidence</td>
<td>Assessment from non-validated sources</td>
</tr>
<tr>
<td></td>
<td>BMD: Measured by DXA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Confounding factors</strong></td>
<td>Adjusted for calcium intake and possible common confounders such as intake of other food groups, age, BMI, physical activity, smoking, alcohol use, vitamin D intake, season of BMD measurement</td>
<td>No adjustments for confounders</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Evaluations of potential confounding or interactions of the F&amp;V-outcome association</td>
<td>Not adjusted for calcium and vitamin D intake</td>
<td></td>
</tr>
<tr>
<td><strong>Analytical</strong></td>
<td>Analyses appropriate for the type of sample</td>
<td>Analyses not accounting for common statistical adjustment (e.g. multiple analyses) when appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analytical method accounted for sampling strategy in cross-sectional study</td>
<td>Sample size calculation not performed, but all available eligible patients studied</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample size calculation performed &amp; adequate sample size included</td>
<td>Sample size estimation unclear or only sub-sample of eligible patients studied</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potential impacts of the errors from assessing the food exposures on the food-outcome association were reported or discussed</td>
<td>Analyses inappropriate for the type of sample/study</td>
<td></td>
</tr>
<tr>
<td><strong>Selective Reporting Bias</strong></td>
<td>Outcomes listed in the methods same as those in the results section</td>
<td>Non-significant results are mentioned but not reported</td>
<td></td>
</tr>
<tr>
<td><strong>Attrition</strong></td>
<td>0-10% attrition</td>
<td>&gt;10% attrition, reasons for loss of follow up not explained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-20% attrition &amp; reasons for loss of follow up explained</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All subjects from initiation of study to the final outcome assessment were accounted for</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall assessment of bias</strong></td>
<td>Low: if no (risk of bias) in at least 5 domains, Moderate: if no in 3 or 4 domains, High: if no in 2 or less domains.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HRT: Hormone Replacement Therapy, F&V: Fruit & Vegetables, RCT: Randomized Controlled Trial, FFQ: Food Frequency Questionnaire, BMD: Bone Mineral Density, DXA: Dual energy X-ray absorptiometry. a. Some items were incorporated from AHRQ guidelines (317-319, 321), b. Low: if no (risk of bias) in at least 5 domains, Moderate: if no in 3 or 4 domains, High: if no in 2 or less domains.
### Form B: Risk of Bias for Intervention Trials (Modified Cochrane Collaboration’s tool)

<table>
<thead>
<tr>
<th>Domain</th>
<th>Description</th>
<th>Review authors’ judgment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sequence generation</strong></td>
<td>Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.</td>
<td>Was the allocation sequence adequately generated?</td>
</tr>
<tr>
<td><strong>Allocation concealment</strong></td>
<td>Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.</td>
<td>Was allocation adequately concealed?</td>
</tr>
<tr>
<td><strong>Blinding of participants, personnel and outcome assessors</strong></td>
<td>Assessments should be made for each main outcome (or class of outcomes) Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective</td>
<td>Was knowledge of the allocated intervention adequately prevented during the study?</td>
</tr>
<tr>
<td><strong>Reporting Baseline Dietary Exposure</strong></td>
<td>Reporting the baseline dietary intake using validated dietary assessment tools</td>
<td>Were the baseline intakes of dietary exposures reported?</td>
</tr>
<tr>
<td><strong>Incomplete outcome data</strong></td>
<td>Assessments should be made for each main outcome (or class of outcomes) Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors</td>
<td>Were incomplete outcome data adequately addressed?</td>
</tr>
<tr>
<td><strong>Selective outcome reporting</strong></td>
<td>State how the possibility of selective outcome reporting was examined by the review authors, and what was found</td>
<td>Are reports of the study free of selective outcome reporting?</td>
</tr>
<tr>
<td><strong>Other sources of bias</strong></td>
<td>State any important concerns about bias not addressed in the other domains in the tool</td>
<td>Was the study apparently free of other problems that could put it at a high risk of bias?</td>
</tr>
<tr>
<td><strong>Overall assessment of bias</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Low**: if no (risk of bias) in at least 5 domains, **Moderate**: if no in 3 or 4 domains, **High**: if no in 2 or less domains

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a. The Cochrane Collaboration’s tool for assessing risk of bias (http://www.cochrane-handbook.org/), with the addition of “Reporting of Baseline F&V Intake” domain
b. Low: if no (risk of bias) in at least 5 domains, Moderate: if no in 3 or 4 domains, High: if no in 2 or less domains
3.2.5 Synthesis of results

After detailed review of all the studies, a systematic review was performed without meta-analysis because of the heterogeneity in F&V classification (e.g. inclusion of potatoes or pulses as vegetables), amount of intake (e.g. various amounts reported in servings, grams, tertiles or quintiles), methods of assessing intake (food frequency questionnaire (FFQ), 24-hr dietary recalls or food records (FR)), research design (e.g. study type, inclusion criteria, type of comparison group), analyses (adjusted or unadjusted for covariates) and reporting of outcome variables (e.g. various BMD sites, not reporting non-significant values). The results of the studies, as presented in the articles, are summarized below.

3.3 Results

3.3.1 Study Selection and characteristics

The results of the literature search and article selection process are outlined in Figure 3.1. Eight studies were included for this systematic review. Two studies were RCTs (322, 323), three were cross-sectional studies (324-326), one was a prospective cohort study (327), one a case-control study (328) and one cohort study reported cross-sectional as well as longitudinal data (179). The reasons for exclusion of studies are also outlined in Figure 3.1.
Figure 3.1. Summary of study selection

Records identified through database searching (n = 1095)

Additional records identified through other sources (n = 2)
  Reference list of reviews: 2
  Reference list of retrieved full-text articles: 0
  Trial registries: 0

Records after duplicates removed (n = 781)

Records screened (n = 781)

Records excluded (n = 738)
  Irrelevant content (not directly related to bone and/or F&V): 679
  Not written in English: 2
  Not relevant outcome data (e.g. bone stiffness): 1
  Animal studies: 30
  Reviews: 16
  Children, youth, women aged < 45 y: 10

Full-text articles assessed for eligibility (n = 43)

Full-text articles excluded, with reasons (n = 35)
  Not relevant outcome data (e.g. heel BMD): 2
  The effect of F&V could not be separated from other food groups or dietary patterns (e.g. high F&V & seafood, dairy or grain products): 17
  Individual fruits or vegetables (e.g. onions, plums, tomatoes): 4
  Surrogates of F&V intake (e.g. potassium, vitamin C, serum carotenoids, net acid excretion): 12

Studies included in qualitative synthesis (n = 8)

Studies included in quantitative synthesis (meta-analysis) (n = 0)
The characteristics of included studies are reported in Table 3.1. All women were postmenopausal. Of the eight included studies, six were published in the past five years. The intake levels and assessment of F&V intake as well as follow up time varied among studies. Four studies reported outcomes for F&V combined (179, 322, 323A, 324), all others reported fruits separate from vegetables as well as combined (325-328). Only one study reported examining the effect of subcategories of fruits or vegetables (citrus fruits, green leafy vegetables, etc.) on bone health but no data were shown (325). Three studies did not describe the F&V group (179, 322, 324); among those that did, one study included potatoes, tubers (325) and one included pulses (326) as vegetables. Two studies did not count the vegetables used in mixed dishes (326, 327). One study specifically mentioned the inclusion of nuts and fruit juices as fruits (326) whereas two studies assessed nuts and seeds as non-F&V items (327, 328).
Table 3.1. Description of observational studies and randomized controlled trials included in the systematic review

<table>
<thead>
<tr>
<th>Reference Location</th>
<th>Number of women Follow up</th>
<th>Age (years)</th>
<th>Dietary assessment method Intake reference period</th>
<th>Intake level</th>
<th>Relevant outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-Control Study</strong></td>
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<tr>
<td><strong>Cross-sectional Studies</strong></td>
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</tr>
<tr>
<td>Tucker et al 1999 (179) USA</td>
<td>562 NA</td>
<td>69-97</td>
<td>126-item FFQ/ previous year (baseline)</td>
<td>Mean ± SD servings/d(^b): F&amp;V: 5.30 ± 2.65</td>
<td>Femoral neck &amp; forearm (radius) BMD</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Methodology</td>
<td>Dietary Assessment</td>
<td>Nutritional Intake</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Chen et al 2006 China-Hong Kong</td>
<td>670</td>
<td>48-63</td>
<td>60-item FFQ/ previous 12 months</td>
<td>Mean ± SD; Median (range) g/d: F&amp;V: 470 ± 200; 434 (117, 1090) F: 175 ± 102; 160 (0, 640) V: 295 ± 150; 259 (22, 894)</td>
<td>Lumbar spine &amp; total hip BMD</td>
</tr>
<tr>
<td>Ebrahimof et al 2006 Iran</td>
<td>51</td>
<td>45-60</td>
<td>Two 24-hour recalls</td>
<td>Mean ± SD g/d: F: 456 ± 270 V: 192 ± 115</td>
<td>Lumbar spine &amp; total hip BMD BTM: Serum osteocalcin &amp; crosslaps</td>
</tr>
<tr>
<td><strong>Prospective Cohort Studies</strong></td>
<td></td>
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</tr>
<tr>
<td>Tucker et al 1999 USA</td>
<td>399</td>
<td>69-97</td>
<td>126-item FFQ/ previous year (baseline)</td>
<td>Mean ± SD servings/d(^bc): F&amp;V: 5.3 ± 2.7</td>
<td>4-year change in femoral neck &amp; forearm (radius) BMD (baseline-year 4)</td>
</tr>
<tr>
<td>Kaptoge et al 2003 UK</td>
<td>474</td>
<td>65-74</td>
<td>7-day FR/ baseline</td>
<td>Median (5(^{th}), 95(^{th}) percentile) g/d: F&amp;V: 258 (88, 568) F: 166 (22, 408) V: 94 (27, 218)</td>
<td>Hip BMD at baseline &amp; on average 3 years later. Mean rate of total hip BMD loss (% per annum)</td>
</tr>
<tr>
<td><strong>Randomized Controlled Trials</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Study</td>
<td>Intervention/Control</td>
<td>Age</td>
<td>Study Design</td>
<td>Baseline/12-weeks</td>
<td>Summary</td>
</tr>
<tr>
<td>------------------------------</td>
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</tr>
<tr>
<td>Macdonald et al 2008 (322)</td>
<td>Intervention: 66</td>
<td>55-65</td>
<td>4-day FR/ before randomization &amp; at 1 year</td>
<td>Difference at year 1 from baseline</td>
<td>Lumbar spine &amp; total hip BMD at baseline &amp; year 2</td>
</tr>
<tr>
<td>Placebo: 70</td>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD (95% CI) g/d:</td>
<td>BTM: P1NP, CTx &amp; fDPD/Cr</td>
</tr>
<tr>
<td>Scotland-UK</td>
<td></td>
<td></td>
<td></td>
<td>Intervention: F: 291 ± 271 (223, 359)</td>
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<tr>
<td></td>
<td></td>
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<td>V: 39 ± 94 (15.63)</td>
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<td></td>
<td></td>
<td>Placebo: F: -17 ± 184 (-66,32)</td>
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<td></td>
<td>V: -18 ± 98 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Ebrahimof et al 2009 (323A)</td>
<td>Control: 22</td>
<td>50-60</td>
<td>2 x 24-hour recalls/ at baseline &amp; 12-weeks</td>
<td>Mean ± SD servings/d:&lt;sup&gt;a&lt;/sup&gt;:</td>
<td>BTM: Serum osteocalcin &amp; crosslaps</td>
</tr>
<tr>
<td>Iran</td>
<td>Intervention: 23</td>
<td></td>
<td></td>
<td>Intervention: F: Baseline: 2.3 ± 1.6</td>
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<td></td>
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<td>Final: 6.4 ± 1.9</td>
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<td>V: Baseline: 2.7 ± 1.5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Final: 5.7 ± 2.4</td>
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<td></td>
<td></td>
<td>Control: F: Baseline: 2.6 ± 1.8</td>
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<tr>
<td></td>
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<td>Final: 3.2 ± 2.3</td>
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<td></td>
<td>V: Baseline: 2.2 ± 1.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Final: 2.0 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>


a. This study analyzed data cross-sectionally as well as longitudinally
b. The average weight of one F&V serving is about 80 grams (329, 330)
3.3.2 Risk of Bias

Two studies had low (322, 325), two had moderate (179, 327, 328) and four had high risk of bias (323A, 324, 326) (Tables 3.2 and 3.3). Six studies failed to adjust for calcium and vitamin D intake (322, 323A, 324, 327, 328). In both cohort studies, dietary assessment was performed only at baseline (179, 327). All studies with BMD measurements used DXA except for Tucker et al (179), where dual-photon absorptiometry (DPA) was used for baseline hip (DXA was used for final visit) and single photon absorptiometry (SPA) was used for baseline and final radius BMD. The hip BMD measurements were corrected for differences between DPA and DXA.
<table>
<thead>
<tr>
<th>Reference Type of study</th>
<th>Selection</th>
<th>Exposure assessment</th>
<th>Outcome assessment</th>
<th>Confounding factors</th>
<th>Analytical reporting bias</th>
<th>Selective reporting bias</th>
<th>Attrition</th>
<th>Overall risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tucker et al 1999</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>Prospective cohort</td>
<td>Cross-sectional</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>Moderate</td>
</tr>
<tr>
<td>Kaptoge et al 2003</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>Prospective cohort</td>
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</tr>
<tr>
<td>Prynne et al 2006</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>High</td>
</tr>
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<td>(326) Cross-sectional</td>
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</tr>
<tr>
<td>Chen et al 2006</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>Low</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Ebrahimof et al 2006</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td>Cross-sectional</td>
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</tr>
<tr>
<td>Xu et al 2009(328)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td>Case-control</td>
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</tr>
</tbody>
</table>

N/A: Not applicable for cross-sectional and case-control studies

a. Low: if no (risk of bias) in at least 5 domains, Moderate: if no in 3 or 4 domains, High: if no in 2 or less domains
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sequence generation</th>
<th>Allocation concealment</th>
<th>Blinding of outcome assessors</th>
<th>Reporting baseline F&amp;V intake</th>
<th>Addressing incomplete outcome data</th>
<th>Selective reporting bias</th>
<th>Other bias</th>
<th>Overall risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macdonald et al 2008 (322)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes: Did not provide calcium and vitamin D supplementation</td>
<td>Low</td>
</tr>
<tr>
<td>Ebrahimof et al 2009</td>
<td>No</td>
<td>Unclear</td>
<td>Unclear</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes: All subjects were women with osteopenia Adjusted confounders unclear</td>
<td>High</td>
</tr>
</tbody>
</table>

F&V: Fruit and vegetables.

a. Low: if no (risk of bias) in at least 5 domains, Moderate: if no in 3 or 4 domains, High: if no in 2 or less domains
3.3.3 Outcomes

3.3.3.1 Fragility fractures

One study reported data on fragility fractures. In their case-control study, Xu et al (328) reported that meeting World Health Organization (WHO) recommendations for F&V intake (described as 370 grams/day) was associated with a 74% lower odds of forearm fractures in Chinese postmenopausal women, and that for each quintile increase in vegetable intake, the estimated risk for fractures was reduced (Table 3.4). Compared to the first quintile (0-104 grams/day), the Odds Ratio (OR) and 95% Confidence Interval (CI) for those in the 4th quintile (254-397 grams/day) of vegetable intake was 0.17 (0.07-0.46) and for those in the 5th quintile (≥398 grams/day) was 0.11 (0.04-0.35). The association with fruit intake was not separately reported.

3.3.3.2 Bone Mineral Density

Three cross-sectional analyses reported positive associations between F&V intake and BMD, whereas one cross-sectional (where all subjects had osteopenia), two cohort studies and one RCT reported no associations (Table 3.4).

In their cross-sectional analysis of baseline data, Tucker et al (179) reported a positive association between a daily increase of one serving of F&V and BMD at the forearm (radius) but not at the femoral neck (Table 3.4). It is noteworthy to mention that unlike hip BMD, the radius BMD was not measured using DXA. In their longitudinal analysis, no significant
association was found between baseline F&V intake and subsequent 4-year changes in BMD at the femoral neck or radius.

Chen et al (325) also found a positive association between a daily increase of 100 grams of F&V intake and BMD at the lumbar spine and total hip. The positive relationship between F&V intake was linear for lumbar spine BMD (trend $P=0.003$) but not for total hip (trend $P=0.108$). The authors also reported that associations between subcategories of fruits or vegetables and BMD were lower than those for total fruits or total vegetables, but these data were not shown.

Prynne et al analyzed their data using two different models (Table 4.4) (326). In the main model no significant associations were found. Using an alternate model, similar to Tucker et al (235), they reported a significant increase (5.6%) in lumbar spine BMD with doubling of fruit intake ($P \leq 0.05$).

In a study of 51 women with osteopenia (low BMD), Ebrahimof el al reported that total hip or lumbar spine BMD were not significantly different between those who consumed greater or less than 400 g/d of F&V, but data were not shown (324).

Kaptoge et al (327) reported that baseline F&V intake (both combined and separately) had no significant effect on the annual rate of total hip BMD loss over an average of three years.

In an RCT, Macdonald et al (322) assessed the effect on BMD of F&V, as well as two doses of potassium citrate, against placebo given for two years. They reported no significant differences in mean percentage change in lumbar spine or total hip BMD between the F&V and placebo groups (Table 4.4).
3.3.3.3 Bone Turnover Markers

Two RCTs reported that adding F&V to baseline diet had no effect on BTM (Table 3.4) (322, 323A). Macdonald et al reported no significant differences in BMT changes from baseline (at 3, 6, 12, 18 or 24 months) between the F&V and placebo groups (322). Ebrahimof et al reported that 12 servings of F&V per day lowered BTM concentrations in the intervention group (P-value not reported) but only among those with significantly higher mean osteocalcin (bone formation marker) and CTx (bone resorption marker) at baseline (323A). In an earlier cross-sectional study (324), the same researchers had reported that those with F&V intakes > 400 g/d had significantly lower concentrations of osteocalcin (Table 3.4). Crosslaps concentrations were also lower but the difference was not statistically significant.
Table 3.4. Fruits and/or vegetables intake and bone health outcomes (fractures, BMD or BTM)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Reported outcome</th>
<th>Adjusted confounders</th>
<th>Association with fruits</th>
<th>Association with vegetables</th>
<th>Association with F&amp;V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FRACTURES</strong></td>
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<tr>
<td><strong>Case-Control Study</strong></td>
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<td></td>
</tr>
<tr>
<td>Xu et al 2009</td>
<td>Decreased risk of forearm fractures for quintiles of intake or intakes &gt;370 g/d</td>
<td>Final model adjustments were not specified, but considered confounders were variables with P&lt;0.05 in univariate analysis such as: other food groups, height, income, family history of fracture, physical activity, smoking, energy intake Cases &amp; controls matched for age &amp; urban district</td>
<td>Statistics not reported</td>
<td>Yes: For each quintile increase Trend test OR (95% CI): 0.53 (0.42-0.67)</td>
<td>Yes: For Intakes &gt;370g/d OR (95% CI): 0.26 (0.14-0.48)</td>
</tr>
<tr>
<td><strong>BONE MINERAL DENSITY</strong></td>
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<tr>
<td><strong>Randomized Controlled Trials</strong></td>
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</tr>
<tr>
<td>Macdonald et al 2008</td>
<td>2-year mean % change in BMD (g/cm²) for 300 g/d increase in F&amp;V</td>
<td>Age, weight, height, social deprivation category</td>
<td>Not analyzed separately</td>
<td>Not analyzed separately</td>
<td>No: Lumbar spine&lt;sup&gt;a,b&lt;/sup&gt;: F&amp;V: -2.1± 0.3 % Placebo: -1.8± 0.5 % Total hip&lt;sup&gt;a,b&lt;/sup&gt;: F&amp;V: -1.5± 0.3 % Placebo: -1.3± 0.3 %</td>
</tr>
<tr>
<td><strong>Cross-sectional Studies</strong></td>
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</tr>
<tr>
<td>Tucker et al 1999 (179)</td>
<td>Change in BMD (g/cm²) per one serving difference in F&amp;V intake</td>
<td>Age, BMI, physical activity, smoking, alcohol use, calcium and, vitamin D supplement use, estrogen use, season of BMD measurement, energy &amp; other food groups intake (milk, other dairy, bread &amp; cereal, meat, poultry &amp;</td>
<td>Not analyzed separately</td>
<td>Not analyzed separately</td>
<td>Yes: Forearm&lt;sup&gt;c&lt;/sup&gt;: 0.0049, (SE not reported) , P&lt;0.01 No: Femoral neck&lt;sup&gt;c&lt;/sup&gt;: 0.0024, SE not reported, P= NS</td>
</tr>
<tr>
<td>Study</td>
<td>Outcome Measure</td>
<td>Predictors Added to the Model</td>
<td>Analysis Results</td>
<td></td>
<td></td>
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<tr>
<td>--------------------</td>
<td>------------------------------------------------------</td>
<td>------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Chen et al 2006    | Change in BMD (g/cm²) per 100 g difference in F &/or V intake | Age, years since menopause, height, weight, physical activity, dietary energy, protein & calcium intake | Yes: Lumbar spine: 0.019±0.0055, P<0.001  
No: Total hip: 0.005±0.0046, P=0.214  
Yes: Total hip (without calcium in the model): 0.006±0.0033, P=0.074  
No: Lumbar spine: 0.058, SE=0.037, P= 0.117  
No: Total hip: 0.003±0.0023, P=0.025 |
| Prynne et al 2006  | % Change in BMD (g/cm²) per 100% change in F &/or V intake (g/d) | Age, energy, calcium & vitamin K intake, time spent in recreational activities, smoking, supplement use, age at menopause & HRT use  
*Alternate model height, BMI, energy intake, time spent in recreational activities, smoking, supplement use, age at menopause & HRT use | No: Lumbar spine: %β=3.9, SE=2.0, P=0.06  
Yes (alternate model): Lumbar spine: %β=5.6, SE= not reported , P≤ 0.05  
No: Total hip: %β =2.2, SE=1.9, P=NS  
No: Femoral Neck: %β =2.5, SE=1.8, P=NS |
| Ebrahimof et al 2006 | Mean BMD (g/cm²) in a group with >400 g/d of F&V compared to a group with ≤400 g/d | No adjustments | Not analyzed separately  
Not analyzed separately  
Statistics not reported  
All women had osteopenia |
### Prospective Cohort Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Outcome Measure</th>
<th>Covariates</th>
<th>Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaptoge et al 2003</td>
<td>Difference in annual mean rates of BMD loss (% per year) among tertiles of F&amp;/or V intake</td>
<td>Weight change, stair climbing, past physical activity levels &amp; activities of daily living score</td>
<td>No Statistics not reported</td>
<td>No: Total hip:&lt;br&gt;Lowest tertile: 0.53%&lt;br&gt;Middle tertile: 0.40%&lt;br&gt;Upper tertile: 0.39%&lt;br&gt;P=0.528</td>
</tr>
<tr>
<td>Tucker et al 1999</td>
<td>4-year change in BMD (g/cm²) per one serving difference in F&amp;V intake</td>
<td>Age, BMI, physical activity, smoking, alcohol use, calcium and, vitamin D supplement use, estrogen use, season of BMD measurement, energy &amp; other food groups (milk, other dairy, bread &amp; cereal, meat, poultry &amp; fish) intake, baseline BMD</td>
<td>Not analyzed separately</td>
<td>No: Forearm:&lt;br&gt;0.000 g/cm², (SE not reported), P=NS&lt;br&gt; No: Femoral neck:&lt;br&gt;0.0005 g/cm², (SE not reported), P=NS</td>
</tr>
</tbody>
</table>

### Bone Turnover Markers

### Randomized Controlled Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Outcome Measure</th>
<th>Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macdonald et al 2008</td>
<td>2-year change in BTM from baseline for 300g/d increase in F&amp;V</td>
<td>Not specified/ unclear</td>
<td>Not analyzed separately</td>
</tr>
</tbody>
</table>
Ebrahimof et al 2009

| 12-week change\(^1\) in BTM from baseline for 400 g/d increase in F&V | Final model adjustments were not specified, but considered | Not analyzed separately | Not analyzed separately | No: Osteocalcin\(^d\) (µg/l): 
Intervention: -3.1±4.5 
Control: -0.41±5.5 
P=0.2 
Crosslaps\(^d\) (µg/l): 
Intervention: -0.05±0.17 
Control: 0.01±0.1 
P=0.3 |

Cross-sectional Studies

Ebrahimof et al 2006

| Means of BTM in a group with >400 g/d of F&V compared to a group with ≤400 g/d | No adjustments | Not analyzed separately | Not analyzed separately | Yes: Osteocalcin\(^d\) (µg/l): >400 g:18±6.5 
≤400g: 30±13.7 
P<0.05 
No: Crosslaps\(^d\) (µg/l): >400 g: 0.65±0.27 
≤ 400g: 0.74±0.41 
P=NS |

F&V: Fruit & Vegetables, g: grams, d: day, OR: Odds Ratio, CI: Confidence Intervals, BMD: Bone Mineral Density, SE: Standard Error, β: regression coefficient, P: p-value, NS: not significant (at P=0.05), R\(^2\): R-square, SD: Standard Deviation, HRT: Hormone Replacement Therapy BTM: Bone Turnover Markers, P1NP: Serum procollagen type 1 amino-terminal propeptide, CTx: carboxy-terminal collagen crosslinks, fDPD: free deoxypyridinoline,

a. Values are estimated from published figure where exact values were unavailable
b. Mean ± SE
c. Regression coefficient for F&V intake ± SE
d. Mean ± SD
3.4 Discussion

In this systematic review of eight studies that examined the associations between F&V intake and bone health considerable between-study heterogeneity was identified, and four of the eight studies were considered to have high risk of bias. Considering only those studies with low or moderate risk of bias, little evidence was found to support the association of F&V and bone health. Fruit and/or vegetable intakes were associated with higher BMD in two cross-sectional analyses but not in the two cohort studies or one RCT. The association of F&V intake with BMD sites varied between studies. Only one RCT examined the association between F&V and BTM and found no association. Among studies with high risk of bias, F&V intake as well as vegetables alone were associated with reduced incidence of forearm fractures in one case-control study. One cross-sectional study found a positive association between F&V intake and BMD, one did not. One cross-sectional study found a negative association between F&V intake and BTM and no associations were found in one RCT.

This is the first systematic review that specifically assessed the effect of F&V intake as a main exposure on outcomes of bone health. Although earlier reviews have reported protective effects of F&V on bone health (101, 186, 276), these findings were based on studies that assessed potential markers of F&V intake (e.g. high intake of potassium, magnesium, vitamins C or K, or low net acid excretion) or dietary patterns that, in addition to being high in F&V are also high in other food groups (such as dairy products) that are beneficial for bone. We did not include studies that used markers of F&V intake. Although F&V are high in magnesium and potassium, because of the generally low consumption of F&V in Western populations, dairy products are the common dietary sources of these nutrients (202, 206, 331). We also excluded studies of
dietary patterns such as Dietary Approaches to Stop Hypertension (DASH), or Mediterranean diet that are high in F&V but are also high in low-fat dairy and/or whole grains, poultry, fish and nuts and lower in saturated fat, red meat, sweets, sugar containing beverages and sodium (187, 248, 332). Given that such dietary patterns include a wide range of food groups, it is not possible to assess the independent effect of F&V on bone health. This review excluded studies that assessed a single fruit or vegetable such as plums or onions, or select F&V based on high amounts of a single nutrient such as vitamin C. There were several reasons for this decision. First, it would be difficult to identify any single fruit, vegetable, or nutrient as superior to others, and consumption of one item in large quantities may result in unpleasant side effects, or omission of other healthful foods. Second, dietary guidelines promote consuming a variety of F&V and do not identify or recommend any single item to achieve public health outcomes. Finally, hypotheses based on single foods or nutrients underestimate the synergistic effects of foods and their numerous components.

Intake levels of ≥ 400 g/day (five servings) of F&V (excluding potatoes and other starchy tubers) have been advocated by the WHO and International Osteoporosis Foundation to reduce the incidence of osteoporosis (200, 333). However, given the importance of dairy products for bone health, and that augmentation of one food type in the diet may lead to the reduction of another, F&V should be advised as part of a healthy diet that also includes at least three servings of dairy per day (202, 334, 335).

In this review we identified the variable impact of F&V intake on specific indices of bone health in women aged ≥ 45 years: forearm fracture; BMD of forearm radius, femoral neck, lumbar spine, and total hip; and BTM (serum osteocalcin, N-terminal propeptide of type 1 collagen, CTx and free urinary deoxypyridinoline cross-links). Differences in association between fruit
and/or vegetables (as individual groups or in combination) with various BMD sites may be due to different F&V intake levels, types and usage among these populations. The definitions of F&V vary considerably between studies and the reported intake would be affected by such differences (329, 336). Based on recommendations from a joint WHO and Food and Agriculture Organization (FAO) workshop in 2004, the vegetables group includes botanical fruits (such as tomatoes, peppers, cucumbers, eggplants), mushrooms and seaweed and excludes potatoes, tubers and dry pulses (329). Nuts can be included in the fruit group, although their intake levels are in general very low. The common approach of excluding vegetables in mixed dishes may lead to underestimation of intake while including potatoes and tubers may lead to overestimation, resulting in dilution bias and reduced ability to detect associations. Some suggest that positive findings of cross-sectional studies reflect measurements that are more representative of long-term dietary patterns and accumulated bone status while longitudinal changes in BMD are time dependent (change from baseline) and represent the effect of recent diet on bone status (179, 322). Under this assumption, it may be that long-term (particularly premenopausal) F&V intake is beneficial to bones and that the deleterious effects of hormonal changes outweigh the beneficial effects of F&V in later years.

3.4.1 Limitations

Possible limitations of this review include the heterogeneity of included studies and the presence of low risk of bias in only two of eight studies. Additionally, in all observational studies high F&V intake may be a surrogate measure of multiple healthy lifestyle characteristics that are beneficial for bones. Studies have reported that high F&V intakes are associated with dietary supplement intake, physical activity, moderate alcohol intake and not smoking (325, 337-339), however most included studies adjusted for at least some of these confounding variables.
Finally, dietary measurement in the two cohort studies (179, 327) were only performed at baseline, therefore changes in BMD from baseline were not linked to dietary changes over the term of the studies (3-5 years).

3.5 Conclusions

Based on the paucity of studies identified, further research is needed to explore the effects of F&V, as the main intervention, on bone health while controlling for consumption of other foods, nutrients, and confounding lifestyle characteristics, and to compare the independent effects in premenopausal and postmenopausal women. Furthermore, dietary interventions can identify the effective amount of F&V (total, as well as subcategories) for bone health and support development of clinical and public health practice guidelines for osteoporosis prevention and management. Future cohort studies would be strengthened by measuring dietary intake at baseline as well as final visit, to account for changes over time. There is a need for researchers to assess dietary intake using comparable and standardized methods across populations. A WHO report provides useful guidelines for the assessment of F&V intake (329). To improve the utility of future publications and outcome reporting, the CONSORT (340) and STROBE (341) statements should also be used. Complying with these guidelines will result in more comparable literature, facilitate future systematic reviews and meta-analyses and ultimately lead to the development of evidence based dietary recommendations.

Based on our review it remains unclear whether F&V intake in postmenopausal women can prevent osteoporotic fractures, improve BMD or slow the rate of bone loss as few studies were found that examined this, and inadequate reporting of study characteristics, compliance, attrition, effect estimates and confounding factors made interpretation of results and assessment
of potential biases difficult. Given that cross-sectional studies, although limited, suggest
positive results, there is a particular need for long-term F&V intervention trials or cohort studies
to confirm these findings.
Chapter 4

4 Study 2: The association between Healthy Eating Index and bone turnover markers among US postmenopausal women aged 45 years and older

Maryam Hamidi, Valerie Tarasuk, Paul Corey, Angela M. Cheung


4.1 Introduction

Diet is a major modifiable lifestyle factor in the prevention and management of osteoporosis (101, 305). In the past decade, the focus of nutrition research has shifted from examining single nutrients, such as calcium or vitamin D, to food groups such as dairy or fruit and vegetables as well as overall dietary patterns (175, 186, 276, 342). There is some evidence that in addition to dairy products, adequate intake of other food groups such as fruits, vegetables, whole grains and meat and beans group may improve bone status (184, 342). Each of these dietary factors is involved in certain aspects of bone health and can explain a small portion of overall bone status (241). Therefore, the overall diet may better predict bone status than does a single food group (101, 175). However, study results are inconsistent; some studies report healthier dietary patterns improve bone status (187, 235, 250, 251, 343) while others show no association (185, 254).

Currently, it is not clear whether diet as a whole or as individual dietary components is a better predictor of bone metabolism among aging women. The main objective of this study was to assess the relation between overall diet quality and BTMs, using the Healthy Eating Index 2005 (HEI-2005) (271) in US postmenopausal women aged ≥ 45 years. We also explored the
associations between the components of the HEI-2005 and the MyPyramid food groups (344) and BTMs.

4.2 Methods

4.2.1 Study design and setting

The cross-sectional data from the NHANES cycles 1999-2000 and 2001-2, that was conducted by the NCHS/CDC was used. This survey, is a series of cross-sectional studies with a complex, stratified multistage probability sample design, and is representative of the civilian, non-institutionalized United States population. Detailed descriptions of the protocols, data collection methods and response rates are documented elsewhere (345).

The NHANES protocol was reviewed and approved by the National Center for Health Statistic’s Institutional Review Board (346). Informed consent was obtained from all participants. The University Health Network Research Ethics Board approved this study protocol.

4.2.2 Subjects

Our study included postmenopausal women 45 years and older who, at the time of interview or examination, were not on steroids, estrogen or hormone replacement therapy, osteoporosis treatment medications, had no cancer (except for basal cell carcinoma), liver or kidney disease, or rheumatoid arthritis. Women were considered postmenopausal if they were 45 years or older with at least 12 months of amenorrhea, or in case of surgical menopause, they had had hysterectomy with bilateral oophorectomy, or were at least 50 years of age with FSH
concentrations greater than 45 (IU/L) (347). Kidney disease was assigned if women had reported weak or failing kidneys or had serum creatinine concentrations >2.74 (mg/dL) (348). Liver disease was assigned if women reported a liver condition or had serum concentrations of alkaline phosphatase>283 (U/L) or aspartate transaminase>83 (U/L) or alanine transaminase>75(U/L) (349). Those with missing data for inclusion criteria, dietary intake data, and both outcome variables were also excluded from the analysis (less than 10% of total eligible sample).

4.2.3 Exposures

The HEI, developed in 1995 by the USDA, is the most commonly used diet quality-assessment tool in the US, combining dietary guidelines for nutrients and food groups into one summary measure (350). In 2006, the HEI was revised to reflect changes in the 2005 Dietary Guidelines for Americans (271). The HEI-2005 has 12 components and its total score ranges from 0 to 100. When the intake levels of lowest recommended amount per 1000 calories are met for the 9 components (total grains, total fruit, total vegetables, whole grains, whole fruit, dark green and orange vegetables and legumes (5 points each), milk, meat and beans, and oils (10 points each)), the maximum scores are achieved and scores for lesser intake levels are prorated linearly. Minimum and maximum scores for 3 components - saturated fat, sodium (10 points each), and “calories from solid fats, alcoholic beverages, and added sugars” (a proxy for discretionary calories) (20 points)- are based on population probability densities and expressed per 1000 calories for sodium and as percentages of total calories for saturated fat and discretionary calories. Scores for intakes between the maximum and minimum levels are prorated.
A 24-hour dietary recall interview was conducted by trained personnel to record all foods and beverages consumed from midnight to midnight the day before the examination (351, 352). Data from the 24-hour dietary recalls were considered “Reliable” by NCHS if they had less than 15% foods with missing amounts, and less than 25% foods with missing descriptive information and that all reported meals had at least one known food (351, 352). Data files for calories and MyPyramid food groups, based on the 24-hour dietary recall, were downloaded from the USDA Center for Nutrition Policy and Promotion website (344). SAS codes, provided by the USDA, were used to calculate the HEI-2005 scores (353), from the 24-hour dietary recall data.

4.2.4 Outcomes

We chose to examine BTMs as our primary outcomes because BTMs are independent predictors of fracture and low BMD (37, 56). In addition, BTMs change quickly with interventions and are more appropriate for this cross-sectional study with a 24-hour dietary recall than are BMDs, which may respond to longer-term interventions. The 2 BTMs measured in NHANES 1999-2002 are bone-specific alkaline phosphatase (BAP, in µg/L), a marker of bone formation, and urinary N-terminal cross-linked telopeptide of type I collagen (uNTx, in nmol of bone collagen equivalents (nM BCE/L)), a marker of bone resorption. Both of these markers are among the most sensitive and specific markers of bone turnover (37). High concentrations of BAP and uNTx are independent risk factors for osteoporotic fractures and low BMD in healthy postmenopausal women who are not taking osteoporosis treatment medications (38, 56). For years 1999-2001 and 2002, the Hybritech Tandem-MP Ostase Immuno Enzymetric (Hybritech Inc., SanDiego, CA) and the Beckman Access Ostase (Beckman Coulter Inc., Fullerton, CA) assays were used for the quantitative measurement of BAP, respectively (354). The coefficient
of variation for BAP in the NHANES 1999-2000 sample was 7.2 -9.8 % and for the 2001-2 sample was 4.7-9.1% (354). A regression equation, provided by NCHS, was used to convert years 1999-2001 values to NHANES 2002 values (354). In NHANES 1999-2001 and 2002, the Osteomark (Ostex International Inc, Seattle, WA) and the Vitros ECI (Ortho-Clinical Diagnostics, Rochester, NY) instruments were used to measure uNTx, respectively (354). A 24 hour urine collection or single urine collection, other than a first morning void, was used for measurement of uNTx. The coefficient of variation for uNTx in the NHANES 1999-2000 sample was 11.4- 12.5 % and for the 2001-2 sample was 7.9-10.9 % (354). The reported values of uNTx were already adjusted for the change of laboratory methods. In this study sample, the 6 values outside of plausible reportable range for uNTx were deemed unreliable and treated as missing data.

Some evidence indicates that fasting reduces the effect of food intake, intestinal alkaline phosphatase and circadian rhythm on BTMs (60, 61, 65). Therefore only those who fasted for at least six hours were included in our analysis. We adjusted uNTx concentrations for urinary creatinine (Cr) excretion to control for urine dilution and the results (uNTx/Cr) are expressed as nmol Bone Collagen Equivalents per mmol creatinine (nM BCE/mM Cr).

4.2.5 Confounders

All confounders in the linear regression models were chosen a priori based on their reported relationships with bone health in the existing literature. The following variables were considered as confounders in the regression models: age group (45-65, > 65 years) (355); ethnicity ( non-Hispanic white, non-Hispanic black, Mexican American, and other Hispanic and other ethnicities, including multiracial (356, 357); education (high school education or less, or
some college/graduate school); income (<$20,000 or ≥ $20,000) (358); physical activity (yes or no) (359); nicotine exposure (yes or no) (86); alcohol intake (yes or no) (360); daily supplement use (yes or no); calcium and/or vitamin D supplement use (yes or no) (89); caffeine (<300 mg or ≥ 300 mg / day) (361); total calorie intake (calories) (241); season of examination (November 1st through April 30th or May 1st through October 31st) (362); osteoporosis (yes or no); fractures (yes or no); session (morning, afternoon or evening) (363); use of prescribed medications in past month (yes or no) (364) and central adiposity (yes or no) (365, 366).

In this study, women who reported regular walking, climbing stairs or hills, heavy work, carrying heavy loads or doing moderate or vigorous activity in the past month, in the Physical Activity Questionnaire, were considered to be physically active. Nicotine exposure was defined as currently smoking or serum cotinine concentrations > 3 ng/mL (367). Alcohol intake was based on the reported number of alcoholic drinks on the 24-hour recall. According to CDC a standard drink is equal to 13.7 grams of pure alcohol (368). In this study, women who reported using calcium and/or vitamin D supplements at least 15 days within the past 30 days, on the Dietary Supplements Questionnaire, and whose average calculated daily intakes of calcium and/or vitamin D supplements were not zero were considered calcium and/or vitamin D supplement users. Central obesity was defined as waist circumference ≥ 88 cm (267).

4.2.6 Statistical methods

The analyses were performed by using Statistical Analysis Software (SAS) version 9.2 (SAS Institute, Cary, NC) (369). As recommended by the National Center for Health Statistics survey procedures, 4-year dietary weights, stratum and primary sampling unit variables were used in all analyses to account for complex sampling design and non-response of the NHANES survey.
The domain option in SAS survey procedures was used to identify the study population. Separate analyses were performed for each BTM in relation to each dietary variable. Sample characteristics were summarized and presented as frequencies and percentages for categorical variables and as mean ± SE and confidence limits for continuous variables. Similar to HEI-2005, the energy density method was used to take into account the energy intake; therefore all MyPyramid food group variables are presented and used in the models, per 1000 calories. The variations in food group intakes are often due to variations in total energy intake, which is dependent on physical activity and/or body size, and may be unrelated to health outcomes and confound the associations (370). Therefore, density standards allow a common standard to be used which is independent of an individual’s energy intake.

In the analytic sample, women with missing confounders, other than waist circumference, were excluded. In this study, for the 14 women who had missing waist circumference, the values were imputed as such: if the body mass index (BMI, in Kg/m2) was <25, then waist circumference was considered < 88 cm. If the BMI> 25 or missing (4 cases), the most common category (waist circumference ≥ 88 cm) was assigned (267).

Regression analyses were performed to examine the linear associations between each of BTMs and dietary variables, also to examine the correlations between total HEI-2005 score and MyPyramid food groups. Additionally, the means of each biomarker for tertiles of dietary variables, adjusted for relevant confounders, were compared using multivariate regression models. Wald’s F-Test was used to test the associations between BTMs and tertiles of dietary variables in one-way analysis of variance models. To build multiple regression models, first bivariate regressions of each bone biomarker on the main dietary variables and individual non-diet variables were conducted. Covariates that changed the dietary variable’s β estimate by ≥10
% were selected for inclusion in the full multiple regression model. As a result, age, nicotine exposure, calcium or vitamin D supplements, physical activity, income and alcohol intake were considered as potential confounders in the BAP models, and age, nicotine exposure, central obesity, income and ethnicity in the NTx/Cr models. Finally, these potential confounders were removed from the full model one at a time and those variables whose removal altered the main dietary variable’s $\beta$ estimate by $\geq 10\%$ were considered confounders and retained in the final model. This was done to minimize the number of potential confounders in the final model (371).

Presence of effect modification was examined by creating cross-product terms of dietary variables and potential modifiers, including age group, income, calcium or vitamin D supplement intake, nicotine exposure, physical activity, and ethnicity. Presence of interactions between dietary factors and confounders were tested one at a time and as combined in the model. All tests were 2-tailed, and significance was set at a P-value $< 0.05$. Analyses were also performed with natural logarithmic transformation of BTMs.

4.3 Results

4.3.1 Study population

Our study population included 827 postmenopausal women aged $\geq 45$ years, details of the sample selection are presented in Figure 4.1. Mean ($\pm$SE) age was 64.8$\pm$0.5 years, and 53% of women were between 45 and 65 years of age. In the NHANES survey, an age of 85 years was assigned to those who were $>85$ years of age. Most women were white, not exposed to nicotine and had abdominal obesity (Table 4.1).
The mean (±SE) score on the HEI-2005 was 54.82 ±0.79. The summary statistics for dietary variables are shown in Table 4.2. The mean (±SE) values for BAP and uNTx/Cr, were 16.28 ± 0.36 µg/L and 45.51±1.14 nM BCE/mM Cr respectively. Of the 827 women, 28 had missing BAP and 15 had missing uNTx/Cr values.
Figure 4.1. Selection of study population

Non-pregnant, not Lactating Women Aged ≥ 45 years (n = 2955)
Examined (n = 2621)
Postmenopausal (n = 2174)
Ambulatory (not on wheelchair, no amputations) (n = 2118)
Reliable Dietary Data (n = 2029)

Not on Steroids (n = 1961)
  On Steroids (n = 64)
  Missing (no data in Prescription Medication) (n = 4)

No Cancer Treatment Medications (n = 1933)
  Cancer Treatment Medications (n = 28)

No Bone Medications (n = 1809)
  Bone Medications (n = 124)

No Estrogen or HRT (n = 1337)
  Reported using estrogen or HRT in Prescription Medication file (n = 410)
  Reported using HRT, estrogen pills or patches in Reproductive Health Questionnaire (n = 62)

No Cancer (Except non-Melanoma Skin Cancer) (n = 1197)
  Cancer (n = 139)
  Missing (n = 1)

No Liver Disease (n = 1175)
  Liver Disease (n = 22)

No Kidney Disease (n = 1163)
  Kidney Disease (n = 12)

No Rheumatoid Arthritis (n = 1019)
  Rheumatoid Arthritis (n = 143)
  Missing (n = 1)

Final Sample (n = 827)
  Missing income, fasting, education & physical activity data (n = 66)
  Fasting < 6 hours (n = 121)
  Missing both BAP & NTx/Cr (n = 5)
| Table 4.1. Demographic and lifestyle characteristics of postmenopausal women aged ≥45 years in the NHANES 1999-2002 |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
|                                                  | Unweighted                                      | Weighted                                       | SE     |
|                                                  | n                                              | %                                              | %      |
| Age (y)                                          |                                                 |                                                 |        |
| 45-65                                            | 385                                            | 52.4                                          | 2.0    |
| > 65                                             | 442                                            | 47.6                                          | 2.0    |
| Race/Ethnicity                                   |                                                 |                                                 |        |
| Non-Hispanic White                               | 414                                            | 73.10                                         | 3.33   |
| Non-Hispanic Black                               | 139                                            | 9.13                                          | 1.62   |
| Mexican American                                | 190                                            | 4.03                                          | 0.75   |
| Other Hispanic/ Other races including multiracial| 84                                             | 13.74                                         | 3.07   |
| Abdominal Obesity                                |                                                 |                                                 |        |
| Waist Circumference < 88                         | 200                                            | 23.76                                         | 2.46   |
| Waist Circumference ≥ 88                         | 627                                            | 76.24                                         | 2.46   |
| Education                                        |                                                 |                                                 |        |
| High School or Less                              | 580                                            | 63.88                                         | 2.20   |
| More Than High School                            | 247                                            | 36.12                                         | 2.20   |
| Household CPS Family Income                      |                                                 |                                                 |        |
| < $20,000                                        | 390                                            | 43.01                                         | 2.68   |
| ≥ $20,000                                        | 437                                            | 56.99                                         | 2.68   |
| Physical Activity                                |                                                 |                                                 |        |
| No (none)                                        | 153                                            | 16.96                                         | 1.71   |
| Yes                                              | 674                                            | 83.04                                         | 1.71   |
| Use of Calcium or Vitamin D Supplements in Past Month |                                 |                                                 |        |
| No                                               | 437                                            | 47.53                                         | 2.59   |
| Yes                                              | 390                                            | 52.47                                         | 2.59   |
| Use of Prescribed Medications in Past Month      |                                                 |                                                 |        |
| No                                               | 258                                            | 31.76                                         | 2.68   |
| Yes                                              | 569                                            | 68.24                                         | 2.68   |
| Nicotine Exposure                                |                                                 |                                                 |        |
| No                                               | 692                                            | 81.46                                         | 1.75   |
| Yes                                              | 135                                            | 18.54                                         | 1.75   |
| Alcohol Intake ( based on 24-hour recall)        |                                                 |                                                 |        |
| No                                               | 705                                            | 82.26                                         | 2.38   |
| Yes                                              | 122                                            | 17.74                                         | 2.38   |
| Caffeine Intake ( based on 24-hour recall)       |                                                 |                                                 |        |
| < 300 mg                                         | 717                                            | 80.93                                         | 2.07   |
| ≥300 mg                                          | 110                                            | 19.07                                         | 2.07   |
| Sodium Intake ( based on 24-hour recall)         |                                                 |                                                 |        |
| < 2300 mg                                        | 461                                            | 49.47                                         | 2.80   |
| ≥2300 mg                                         | 366                                            | 50.53                                         | 2.80   |

Table 4.2. Dietary characteristics of postmenopausal women (n=827) aged ≥45 years in the NHANES 1999-2002

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total HEI-2005 Score</td>
<td>19.2 - 88.8</td>
<td>54.8</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>HEI-2005 Components (standard for maximum score)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Fruit† (≥0.8 cup equiv./1000 calories)</td>
<td>0 - 5</td>
<td>2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Whole Fruit† (≥0.4 cup equiv./1000 calories)</td>
<td>0 - 5</td>
<td>2.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Total Vegetables‡ (≥1.1 cup equiv./1000 calories)</td>
<td>0 - 5</td>
<td>3.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Dark Green &amp; Orange Vegetables &amp; Legumes (≥0.4 cup equiv./1000 calories)</td>
<td>0 - 5</td>
<td>1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Total Grains (≥3.0 oz equiv./1000 calories)</td>
<td>0 - 5</td>
<td>4.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Whole Grains (≥1.5 oz equiv./1000 calories)</td>
<td>0 - 5</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Milk Group§ (≥1.3 cup equiv./1000 calories)</td>
<td>0 - 10</td>
<td>5.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Meat &amp; Beans Group§ (≥2.5 oz equiv./1000 calories)</td>
<td>0 - 10</td>
<td>7.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Oils§ (≥12 grams/1000 calories)</td>
<td>0 - 10</td>
<td>5.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Saturated Fat (≤7% of energy)</td>
<td>0 - 10</td>
<td>6.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Sodium (≤0.7 gram/1000 calories)</td>
<td>0 - 10</td>
<td>4.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Calories from Solid Fat, Alcohol &amp; Added Sugars (≤20% of energy)</td>
<td>0 - 20</td>
<td>10.7</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Food Group Servings (MyPyramid Servings)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Grains (oz equiv.)</td>
<td>0 - 18.73</td>
<td>5.25</td>
<td>0.18</td>
</tr>
<tr>
<td>Total Grains (oz equiv./1000 calories)</td>
<td>0 - 9.69</td>
<td>3.35</td>
<td>0.07</td>
</tr>
<tr>
<td>Total Vegetables (cup equiv.)</td>
<td>0 - 9.69</td>
<td>1.45</td>
<td>0.07</td>
</tr>
<tr>
<td>Total Vegetables (cup equiv./1000 calories)</td>
<td>0 - 7.58</td>
<td>0.96</td>
<td>0.04</td>
</tr>
<tr>
<td>Vegetables excluding potatoes (cup equiv.)</td>
<td>0 - 9.69</td>
<td>1.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Vegetables excluding potatoes (cup equiv./1000 calories)</td>
<td>0 - 7.36</td>
<td>0.76</td>
<td>0.04</td>
</tr>
<tr>
<td>Potatoes (cup equiv.)</td>
<td>0 - 4.31</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Potatoes (cup equiv./1000 calories)</td>
<td>0 - 3.12</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Total Fruits (cup equiv.)</td>
<td>0 - 12.37</td>
<td>1.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Total Fruits (cup equiv./1000 calories)</td>
<td>0 - 8.09</td>
<td>0.77</td>
<td>0.04</td>
</tr>
<tr>
<td>Milk Group§ (cup equiv.)</td>
<td>0 - 13.75</td>
<td>1.31</td>
<td>0.09</td>
</tr>
<tr>
<td>Milk Group§ (cup equiv./1000 calories)</td>
<td>0 - 5.82</td>
<td>0.80</td>
<td>0.05</td>
</tr>
<tr>
<td>Meat &amp; Beans Group (oz equiv.)</td>
<td>0-21.04</td>
<td>4.40</td>
<td>0.14</td>
</tr>
<tr>
<td>Meat &amp; Beans Group (oz equiv./1000 calories)</td>
<td>0-12.66</td>
<td>2.78</td>
<td>0.07</td>
</tr>
<tr>
<td>Soy, Nuts, Seeds &amp; Legumes (oz equiv.)</td>
<td>0 - 14.02</td>
<td>0.87</td>
<td>0.10</td>
</tr>
<tr>
<td>Soy, Nuts, Seeds &amp; Legumes (oz equiv./1000 calories)</td>
<td>0 - 7.91</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>Animal Protein-Meat, Poultry, Fish, Eggs (oz equiv.)</td>
<td>0 - 21.04</td>
<td>3.54</td>
<td>0.13</td>
</tr>
<tr>
<td>Animal Protein-Meat, Poultry, Fish, Eggs (oz equiv./1000 calories)</td>
<td>0 - 12.66</td>
<td>2.30</td>
<td>0.07</td>
</tr>
<tr>
<td>Discretionary Oil§ (grams)</td>
<td>0 - 115.49</td>
<td>13.42</td>
<td>0.83</td>
</tr>
<tr>
<td>Discretionary Oil§ (grams/1000 calories)</td>
<td>0 - 71.47</td>
<td>7.80</td>
<td>0.35</td>
</tr>
<tr>
<td>Discretionary Solid Fat (grams)</td>
<td>0 - 145.67</td>
<td>34.93</td>
<td>1.23</td>
</tr>
<tr>
<td>Discretionary Solid Fat (grams/1000 calories)</td>
<td>0 - 49.26</td>
<td>20.95</td>
<td>0.42</td>
</tr>
<tr>
<td>Added sugars (teaspoon equiv.)</td>
<td>0 - 98.01</td>
<td>14.26</td>
<td>0.70</td>
</tr>
<tr>
<td>Added sugars (teaspoon equiv./1000 calories)</td>
<td>0 - 29.48</td>
<td>8.52</td>
<td>0.32</td>
</tr>
<tr>
<td>Alcohol (drinks)</td>
<td>0 - 9.33</td>
<td>0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Alcohol (drinks/1000 calories)</td>
<td>0 - 6.00</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Soy, Nuts, Seeds &amp; Legumes (oz equiv.)</td>
<td>0 - 14.02</td>
<td>0.87</td>
<td>0.10</td>
</tr>
<tr>
<td>Soy, Nuts, Seeds &amp; Legumes (oz equiv./1000 calories)</td>
<td>0 - 7.91</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>Animal Protein-Meat, Poultry, Fish, Eggs (oz equiv.)</td>
<td>0 - 21.04</td>
<td>3.54</td>
<td>0.13</td>
</tr>
<tr>
<td>Animal Protein-Meat, Poultry, Fish, Eggs (oz equiv./1000 calories)</td>
<td>0 - 12.66</td>
<td>2.30</td>
<td>0.07</td>
</tr>
</tbody>
</table>

equiv: equivalents, oz: ounce, † Includes 100% juice, ‡ Does not include juice.  † Includes any vegetable or legume (e.g. white potatoes) that is not counted as a serving of meat and beans. § Based on intake of all milk products, such as fluid milk, yogurt, and cheese, and soy beverages. 5. Includes legume intake only if the meat and beans standard is otherwise not met. 6. Includes non-hydrogenated vegetable oils and oils in fish, nuts, and seeds.
4.3.2 Relationships between age and time since menopause and BTMs

No associations between age and BAP or NTx/Cr or between the time since menopause (≤5 years compared with > 5 years, and ≤10 years compared with > 10 years) and both BAP and NTx/Cr were found. In addition, no effect modification between HEI-2005 and all the above mentioned age and time since menopause variables for both BAP and NTx/Cr was found. Moreover, running separate analyses for women aged 45-65 years and >65 years to examine the association between each BTM and HEI-2005 did not change our overall results. Thus, we report our results for the whole cohort of postmenopausal women.

4.3.3 Relationships between dietary factors and BTMs

Overall, few significant linear relations between dietary factors and BTMs were found. On the basis of the unadjusted analyses, no associations were found between absolute intake of food groups and BTMs (Table 4.3). BAP had a significant positive association with energy-adjusted added sugars (per 1000 calories) (Table 4.3), and the milk group component of HEI-2005 had a significant negative association with uNTx/Cr. After adjusting for potential confounding factors, no significant linear association was found between BAP and energy-adjusted added sugars (β=0.11, SE=0.05, p =0.057) (mcg/L), however the association between uNTx/Cr and the milk group component of HEI-2005 remained significant. Each point increase in the milk group score was associated with a difference of -0.71 (SE: 0.30, P = 0.022) (nmol BCE NTx/mM Cr) in uNTx/Cr. Adjustment for potential confounders did not change the results for the associations between BTMs and other dietary variables in the linear multiple regression models.
### Table 4.3. Unadjusted associations between dietary variables and BTMs among postmenopausal women (n=827) aged ≥45 years in the NHANES 1999-2002

<table>
<thead>
<tr>
<th>HEI-2005 Component Scores</th>
<th>BAP (mcg/L)</th>
<th>uNTx/Cr (nM BCE/mM Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Total HEI-2005 Score</td>
<td>-0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Total Fruit</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>Whole Fruit</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Total Vegetables</td>
<td>-0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Dark Green &amp; Orange Vegetables &amp; Legumes</td>
<td>-0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Total Grains</td>
<td>0.11</td>
<td>0.23</td>
</tr>
<tr>
<td>Whole Grains</td>
<td>-0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>Milk Group</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Calories from Solid Fat, Alcohol &amp; Added Sugar</td>
<td>-0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Food Groups-Absolute Intakes (MyPyramid Servings)**

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>LCI</th>
<th>UCI</th>
<th>P</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>0.00</td>
<td>0.12</td>
<td>-0.24</td>
<td>0.24</td>
<td>0.997</td>
<td>0.000</td>
</tr>
<tr>
<td>Vegetables</td>
<td>-0.30</td>
<td>0.23</td>
<td>-0.78</td>
<td>0.18</td>
<td>0.207</td>
<td>0.003</td>
</tr>
<tr>
<td>Vegetables excluding potatoes</td>
<td>-0.30</td>
<td>0.34</td>
<td>-1.00</td>
<td>0.40</td>
<td>0.386</td>
<td>0.002</td>
</tr>
<tr>
<td>Fruit</td>
<td>-0.24</td>
<td>0.19</td>
<td>-0.64</td>
<td>0.15</td>
<td>0.214</td>
<td>0.002</td>
</tr>
<tr>
<td>Milk Group</td>
<td>0.04</td>
<td>0.11</td>
<td>-0.18</td>
<td>0.27</td>
<td>0.691</td>
<td>0.000</td>
</tr>
<tr>
<td>Meat &amp; Beans</td>
<td>0.09</td>
<td>0.18</td>
<td>-0.28</td>
<td>0.47</td>
<td>0.609</td>
<td>0.002</td>
</tr>
<tr>
<td>Meat, Poultry, Fish &amp;Eggs</td>
<td>0.20</td>
<td>0.22</td>
<td>-0.25</td>
<td>0.65</td>
<td>0.371</td>
<td>0.007</td>
</tr>
<tr>
<td>Discretionary Solid fat</td>
<td>0.12</td>
<td>0.02</td>
<td>-0.04</td>
<td>0.04</td>
<td>0.871</td>
<td>0.000</td>
</tr>
<tr>
<td>Discretionary Oil</td>
<td>-0.03</td>
<td>0.02</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.121</td>
<td>0.005</td>
</tr>
<tr>
<td>Added sugars</td>
<td>0.04</td>
<td>0.04</td>
<td>-0.05</td>
<td>0.13</td>
<td>0.342</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Food Group Intakes (MyPyramid Servings /1000 kcal)**

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>LCI</th>
<th>UCI</th>
<th>P</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>0.08</td>
<td>0.19</td>
<td>-0.31</td>
<td>0.47</td>
<td>0.684</td>
<td>0.000</td>
</tr>
<tr>
<td>Vegetables</td>
<td>-0.36</td>
<td>0.35</td>
<td>-1.08</td>
<td>0.37</td>
<td>0.322</td>
<td>0.002</td>
</tr>
<tr>
<td>Vegetables excluding potatoes</td>
<td>-0.32</td>
<td>0.43</td>
<td>-1.19</td>
<td>0.56</td>
<td>0.463</td>
<td>0.001</td>
</tr>
<tr>
<td>Fruits</td>
<td>-0.01</td>
<td>0.30</td>
<td>-0.62</td>
<td>0.61</td>
<td>0.981</td>
<td>0.000</td>
</tr>
<tr>
<td>Milk group</td>
<td>-0.01</td>
<td>0.32</td>
<td>-0.67</td>
<td>0.65</td>
<td>0.976</td>
<td>0.000</td>
</tr>
<tr>
<td>Meat &amp; Beans</td>
<td>0.12</td>
<td>0.16</td>
<td>-0.22</td>
<td>0.45</td>
<td>0.478</td>
<td>0.001</td>
</tr>
<tr>
<td>Meat, Poultry, Fish &amp;Eggs</td>
<td>0.20</td>
<td>0.15</td>
<td>-0.11</td>
<td>0.51</td>
<td>0.192</td>
<td>0.002</td>
</tr>
<tr>
<td>Discretionary Solid fat</td>
<td>0.01</td>
<td>0.32</td>
<td>-0.07</td>
<td>0.04</td>
<td>0.616</td>
<td>0.000</td>
</tr>
<tr>
<td>Discretionary Oil</td>
<td>-0.07</td>
<td>0.04</td>
<td>-0.15</td>
<td>0.00</td>
<td>0.063</td>
<td>0.006</td>
</tr>
<tr>
<td>Added sugars</td>
<td>0.13</td>
<td>0.05</td>
<td>0.02</td>
<td>0.24</td>
<td>0.022</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**BAP**: Bone Alkaline Phosphatase; **uNTx**: Urinary N-telopeptides; **Cr**: Creatinine; **β**: regression coefficients; **SE**: Standard Error; **LCI & UCI**: Lower & Upper 95% Confidence Intervals; **P**: P-Value; **Adj R²**: Adjusted R². Because of the high number of zero values for potatoes, beans and legumes as well as alcohol, linear relationships were not assessed for these items.
No significant associations were found between tertiles of HEI-2005, energy adjusted total grains, fruit, vegetables, discretionary oil or fat, or meat and beans group and BAP or uNTx/Cr (Table 4.4). A significant association (P= 0.046) was found between tertiles of energy-adjusted MyPyramid servings of milk group and uNTx/Cr. Women in the lowest tertile of milk group had significantly greater uNTx/Cr than did the middle tertile (Table 4.4). Similar results were found for absolute intake of milk group (data not shown). A significant overall association between MyPyramid servings of energy-adjusted added sugars (P=0.046) and BAP was found. Those in the highest tertile had significantly higher BAP concentrations than did those in the lowest tertile (P=0.015) (Table 4.4). However, the tertiles of the absolute intake of added sugars were not associated with BAP (data not shown).

After adjusting for central obesity, ethnicity, age and nicotine exposure the association between tertiles of energy-adjusted MyPyramid servings of milk group and uNTx/Cr remained significant. Women in the lowest tertile of milk group had a significantly higher uNTx/Cr (53.10 ± 2.08 (nM BCE NTx/mM Cr)), than did those in the middle (46.90±2.13 (nM BCE NTx/mM Cr)) and highest (47.54±2.79 (nM BCE NTx/mM Cr) tertiles. Inclusion of potential confounders in the final models examining the associations between BTMs and other dietary variables did not change the results.

No correlation between total HEI-2005 score and energy-adjusted MyPyramid servings of milk group (r=0.04, P=0.334) was found, whereas the relation between energy-adjusted MyPyramid servings of added sugars and HEI-2005 was moderate (r=-0.41, P=0.0001).
Table 4.4. Unadjusted associations between tertiles of dietary predictors and BAP and uNTx/Cr among postmenopausal women (n=827) aged ≥45 years in the NHANES 1999-2002

<table>
<thead>
<tr>
<th></th>
<th>BAP (mcg/L)</th>
<th>uNTx/Cr (nmol BCE NTx/mM creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSM</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Tertiles of HEI-2005</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.2 ≤ HEI-2005 ≤ 48.7</td>
<td>16.73</td>
<td>0.57</td>
</tr>
<tr>
<td>48.7 &lt; HEI-2005 ≤ 61.8</td>
<td>16.03</td>
<td>0.61</td>
</tr>
<tr>
<td>61.8 &lt; HEI-2005 ≤ 88.8</td>
<td>15.78</td>
<td>0.39</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.004</td>
<td>0.022</td>
</tr>
<tr>
<td>P</td>
<td>0.344</td>
<td>0.487</td>
</tr>
<tr>
<td><strong>Tertiles of MyPyramid Food Groups/1000 calories</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ≤ Total Grains ≤ 2.6</td>
<td>16.22</td>
<td>0.64</td>
</tr>
<tr>
<td>2.6 &lt; Total Grains ≤ 4.0</td>
<td>15.96</td>
<td>0.47</td>
</tr>
<tr>
<td>4.0 &lt; Total Grains ≤ 9.7</td>
<td>16.36</td>
<td>0.48</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>P</td>
<td>0.827</td>
<td>0.935</td>
</tr>
<tr>
<td>0 ≤ Total Vegetables ≤ 0.46</td>
<td>17.10</td>
<td>0.81</td>
</tr>
<tr>
<td>0.46 &lt; Total Vegetables ≤ 1.07</td>
<td>15.51</td>
<td>0.46</td>
</tr>
<tr>
<td>1.07 &lt; Total Vegetables ≤ 7.6</td>
<td>15.92</td>
<td>0.53</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.011</td>
<td>0.004</td>
</tr>
<tr>
<td>P</td>
<td>0.153</td>
<td>0.161</td>
</tr>
<tr>
<td>0 ≤ Total Fruits ≤ 0.2</td>
<td>16.01</td>
<td>0.55</td>
</tr>
<tr>
<td>0.2 &lt; Total Fruits ≤ 0.9</td>
<td>16.62</td>
<td>0.78</td>
</tr>
<tr>
<td>0.9 &lt; Total Fruits ≤ 8.1</td>
<td>15.90</td>
<td>0.41</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>P</td>
<td>0.703</td>
<td>0.543</td>
</tr>
<tr>
<td>0 ≤ Milk Group ≤ 0.4</td>
<td>16.64</td>
<td>0.50</td>
</tr>
<tr>
<td>0.4 &lt; Milk Group ≤ 0.9</td>
<td>15.50</td>
<td>0.75</td>
</tr>
<tr>
<td>0.9 &lt; Milk Group ≤ 5.8</td>
<td>16.40</td>
<td>0.38</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.006</td>
<td>0.014</td>
</tr>
<tr>
<td>P</td>
<td>0.332</td>
<td>0.046</td>
</tr>
<tr>
<td>0 ≤ Meat &amp; Beans Group ≤ 1.9</td>
<td>15.90</td>
<td>0.45</td>
</tr>
<tr>
<td>1.9 &lt; Meat &amp; Beans Group ≤ 3.2</td>
<td>15.93</td>
<td>0.62</td>
</tr>
<tr>
<td>3.2 &lt; Meat &amp; Beans Group ≤ 5.8</td>
<td>16.70</td>
<td>0.69</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>P</td>
<td>0.490</td>
<td>0.762</td>
</tr>
<tr>
<td>0 ≤ Oil ≤ 2.9</td>
<td>16.59</td>
<td>0.50</td>
</tr>
<tr>
<td>2.9 &lt; Oil ≤ 8.9</td>
<td>16.24</td>
<td>0.66</td>
</tr>
<tr>
<td>8.9 &lt; Oil ≤ 71.5</td>
<td>15.70</td>
<td>0.54</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.003</td>
<td>0.000</td>
</tr>
<tr>
<td>P</td>
<td>0.431</td>
<td>0.891</td>
</tr>
<tr>
<td>0 ≤ Solid Fat ≤ 17.0</td>
<td>16.34</td>
<td>0.60</td>
</tr>
<tr>
<td>17.0 &lt; Solid Fat ≤ 24.8</td>
<td>15.46</td>
<td>0.49</td>
</tr>
<tr>
<td>24.8 &lt; Solid Fat ≤ 49.3</td>
<td>16.74</td>
<td>0.47</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>P</td>
<td>0.107</td>
<td>0.598</td>
</tr>
<tr>
<td>0 ≤ Added Sugars ≤ 5.3</td>
<td>15.18$^a$</td>
<td>0.33</td>
</tr>
<tr>
<td>5.3 &lt; Added Sugars ≤ 10.3</td>
<td>16.07$^{ab}$</td>
<td>0.46</td>
</tr>
<tr>
<td>10.3 &lt; Added Sugars ≤ 29.5</td>
<td>17.27$^b$</td>
<td>0.76</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.017</td>
<td>0.005</td>
</tr>
<tr>
<td>P</td>
<td>0.046</td>
<td>0.262</td>
</tr>
</tbody>
</table>

BAP: Bone Alkaline Phosphatase, uNTx: Urinary N-telopeptides, Cr: Creatinine, LSM: Least Square Means, SE: Standard Error, P-Value (F-test). Values with different superscript letters are significantly different ($P_{t-test}<0.02$).
No significant interactions were found when tested one at a time or combined in the model. No quadratic relations were found between BTMs and dietary variables. Analyses using natural logarithmic transformation of BTMs produced similar results; therefore data are only shown for non-transformed BTMs. We also examined the associations between the original HEI and BTMs in a similar fashion and the results were similar (data not shown).

4.3.4 Discussion

This is the first population-based study that specifically assessed the associations between overall diet and BTMs. The results indicate that, although no relations between BTMs and HEI-2005 scores were found, a significant association was found between BAP and energy-adjusted added sugars intake and between uNTx/Cr and milk group score of HEI-2005, as well as tertiles of energy-adjusted MyPyramid milk group intake. The findings suggest that the associations between dietary factors and BTMs are small and perhaps not linear for milk group.

4.3.4.1 No relationships between HEI-2005 and BTMs

Several reasons are possible for the lack of association between total HEI-2005 score and BTMs. First, we found no relation between milk group intake and HEI-2005. Despite a high dairy intake, one can attain a lower HEI-2005 score due to high fat or sodium intake. Previous studies have shown that high intake of dairy products is associated with high saturated fat, calories and sodium intakes (110, 111). Second, a similar score of HEI-2005 does not reflect similar diets. There are no upper limits on intakes of grains and meat and alternatives food servings in the HEI-2005; thus, high consumption of these food groups results in maximum component scores. Grains and meat and alternatives are considered acid-producing foods which may not be beneficial to bones (342). Third, because most of the US population does not meet
recommendations for dairy, fruit, and vegetables, the effects of a healthy diet may not be readily
discovered because of the small number of subjects at the higher end of the diet quality scores
(372, 373). Fourth, those with a low HEI score tend to have greater abdominal obesity or a
larger BMI (258, 374). The potential negative effect of a diet of low quality might be offset by
a greater waist circumference (375) because some studies suggest those with a higher BMI or
waist circumference have lower BTMs (376, 377). Finally, although few observational studies
have reported protective effects of healthy dietary patterns on bone health in postmenopausal
women (179, 248, 250, 251, 328), these findings were based on BMD or fracture data which
may indicate long-term effect of diet on bone health. Dietary interventions in postmenopausal
women have failed to support the beneficial effect of healthy eating on bone status in the
postmenopausal years (185, 254, 322, 378). It is possible that lifelong healthy eating is
beneficial to bones and the deleterious effects of hormonal changes outweigh the beneficial
effects of healthy eating in postmenopausal years (179).

4.3.4.2 Relationships between milk group and added sugars and BTMs

The beneficial relation between dairy intake and BTMs in postmenopausal women has been
shown in previous studies (379, 380). In the Western diet, dairy products are the major source
of calcium, protein, magnesium and potassium; with intakes less than three servings, it would be
difficult to achieve adequate amounts of these nutrients through other dietary sources (202,
335). Although we found a significant association between milk group intake and NTx/Cr, no
dose-response relation was found. It is possible that when sufficient nutrient intake is achieved,
no further improvements in bone status occur.

In our study, a high intake of sugar per 1000 calories was associated with higher BAP.
However, we found no relation between absolute sugar intake and BAP. Energy-adjusted high
sugar intake may be indicative of a poor-quality diet. In the absence of osteoporosis drug therapy; the markers of bone formation and resorption are coupled and an increase in BTMs is associated with increased risk of fractures and lower BMD (37, 381). Tucker et al (235) reported that high candy intake, which indicates a nutrient-poor diet, was associated with lower bone mineral density in older women. A previous study reports that the fruit, vegetable, milk, and grain intakes of individuals with high sugar intake were lower than others consuming the same relative amount of energy but a lower sugar intake (237).

4.3.4.2.1 Potential limitations of the study

One limitation of our study was its cross-sectional design, which cannot provide evidence of a causal relationship between HEI-2005, its components or food groups and BTMs. However, BTMs are dynamic and respond to immediate changes and cross-sectional design may be more appropriate than a longitudinal design. Also, 24-hour recalls do not reflect usual intakes and under-reporting of total energy by subjects is a common shortfall of this method. Currently, there is no standard adjustment for correcting the underreporting bias. Also it is not clear which underreported foods, result in energy underreporting and how this would affect our results. The high frequency of zero values for subcategories of food groups such as milk, yogurt, cheese, berries, citrus fruits, dark green vegetables, fish and plant protein sources, limited the examination of the effect of these groups on bone health. Nonetheless, the use of 24-hour recalls in large population-based surveys is currently the best method available (382). Another limitation was the low between-subject variability in the scores of HEI5s and their components and food group intakes in this population. Analyses and assessment of BTMs are also subject to day-to-day variation and measurement errors. Despite these limitations, studies show that BTMs are independent variables of fracture risk in postmenopausal women (37, 295). Given
that BTMs respond quickly to dietary changes (61, Eastell, 2008 #207), the cross-sectional
design of NHANES 1999-2002 and the use of a 24-hour dietary recall, BTMs are more suitable
outcomes compared to BMD or other long-term measures of bone health. Finally, because of
the exploratory nature of this study, the multiple comparisons were made without a Bonferroni
adjustment (383); therefore these findings should be viewed as trends that need to be further
investigated.

4.4 Conclusions

Our results confirm the role of well-established dietary risk factors for bone loss and support the
ability of a diet with adequate dairy intake to promote bone health in aging women. However,
we found that HEI-2005 has limitations in assessing diet in relation to bone health. Given the
synergy between various dietary components (196, 384), the effect of overall diet quality on
bone health is worthy of further investigation. In order to ascertain diet and bone health
associations, future dietary intervention trials or assessment of populations with large between-
subject variation in the dietary intake of food groups are required. Diet is a major modifiable
lifestyle factor and a properly designed and tested index-based diet quality assessment tool can
be used to address the complexity of diet and the interaction between multiple dietary
components in relation to bone health. Research is needed to design and validate an overall diet
quality-assessment tool in relation to bone health in postmenopausal women.
Chapter 5

5 Study 3: Effects of vitamin E on bone turnover markers among US postmenopausal women

Maryam S Hamidi, Paul N Corey, Angela M. Cheung

5.1 Introduction

About 80% of those affected by osteoporosis are women, most of whom are postmenopausal women (8). Therefore, identifying beneficial or harmful nutrients for postmenopausal osteoporosis could have a significant public health impact. It has been shown that increased oxidative stress and production of pro-inflammatory cytokines, due to decline in estrogen levels after menopause, are associated with increased bone loss (145, 146, 385). Since vitamin E has antioxidant and anti-inflammatory properties (386), it may be beneficial to bones. However, the association between vitamin E and bone metabolism has not been well studied in humans.

Alpha- and gamma-tocopherol are two predominant isomers of vitamin E in the human body and diet respectively. Alpha-tocopherol has higher antioxidant activities than gamma-tocopherol and is the form used in most vitamin E supplements (159, 160). Sources of dietary alpha-tocopherol include nuts, seeds, olive, sunflower and safflower oils, whole grains and dark green leafy vegetables, vitamin E-fortified, ready-to-eat breakfast cereals and processed foods preserved with vitamin E (153, 387-391). Gamma-tocopherol is the major form of vitamin E in the United States (US) diet (153, 154), and is superior to alpha-tocopherol in its anti-inflammatory properties (159, 392). Soybean, corn and canola oils, seeds and nuts are foods with high gamma-tocopherol content (154, 159). It has been reported that most of the alpha and
gamma-tocopherol in the US diet come from fats and oils added to foods (154, 162, 256, 389-392) of which 80% is comprised of soybean oil (154).

There are several limitations to the assessment of dietary intake of tocopherols. First, it is not possible to accurately capture the amount of fats and oils added during food preparation (256, 393). Second, different oils have different concentrations of tocopherols, and the exact proportions of specific fats or oils used in processed foods are often not reported (256). Third, dietary fat and oil intakes are often under-reported (394, 395). Fourth, there are variations in bio-availability of tocopherols based on food source, and tocopherol content of foods due to fortification and food preservation practices or geographical area (255, 256, 387). Finally, for fortified foods and dietary supplements, the reported dosage on the label is the minimum content present in the product (396, 397). For these reasons, true intakes of tocopherols may be different from reported intakes. Because of these limitations, it has been proposed that serum alpha- and gamma-tocopherol and their ratio are better indicators of dietary intake of tocopherols (256, 291, 292, 398).

Both alpha- and gamma-tocopherol have been extensively studied in the prevention of cancer, cardiovascular and Alzheimer’s diseases (162, 399, 400). Some studies have reported that intake of vitamin E from food sources, but not from vitamin supplements, is associated with reduced risk of these diseases (401-404). Recent data suggest that high-dose vitamin E supplementation (>180 mg) may increase all-cause mortality (405). Only one study, a sub-study of the Women's Health Initiative (WHI), has examined the relationships between vitamin E intake, serum alpha- and gamma-tocopherol and bone health in postmenopausal women (150), and found no associations between tocopherols and BMD. A limitation of the WHI study could be that serum tocopherols reflect short-term dietary intake (292, 293) whereas BMD is affected
by long-term dietary intake. Bone turnover markers (BTMs) have been demonstrated to better reflect dietary effects on bone metabolism than BMD in the short term (37). Increased bone resorption and decreased bone formation in the absence of osteoporosis drug therapy has a detrimental effect on bone microarchitecture and is associated with an increased risk of osteoporotic fractures in postmenopausal women (23, 42). This relationship between BTMs and fracture risk in postmenopausal women is independent of age, years since menopause and BMD (31, 38, 355, 357).

The objective of our study was to explore the associations between serum alpha- and gamma-tocopherol, and the ratio of serum alpha- to gamma-tocopherol and BTMs in postmenopausal women age 45 years and older in the US. We also examined the relationships between dietary and total (diet and supplements) alpha-tocopherol intake and BTMs.

5.2 Methods

We used data from the National Health And Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (NCHS) at Centers for Disease Control and Prevention (CDC). Detailed descriptions of the protocols and data collection methods for interview and examination components are fully documented elsewhere (406). The NHANES protocol was reviewed and approved by the NCHS Review Board and informed consent was obtained from all participants. The University Health Network Research Ethics Board approved our study protocol.

5.2.1 Design and Setting

The NHANES is a series of cross-sectional studies with a complex, stratified, multistage probability sample design, and represents the civilian, non-institutionalized United States
We combined data from two NHANES cycles (1999-2000 and 2001-2002) as these cycles have data on BTMs for postmenopausal women. Response rates for NHANES 1999-2002 range from 71% for age group 50-60 years to 52% for age group over 80 years (407).

### 5.2.2 Population and Eligibility Criteria

Our study sample included postmenopausal women who were not on steroids, estrogen therapy, or osteoporosis medications, were free from liver and kidney disease, rheumatoid arthritis, all cancers except for basal cell carcinoma and, were fasting for more than 9 hours prior to examination.

The Reproductive Health Questionnaire (RHQ) was used to identify eligible postmenopausal women age 45 years and over. We included women who had follicle-stimulating hormone (FSH) concentrations >45 (IU/L) (347, 408) and did not have a period in the previous 12 months or had surgical menopause (hysterectomy and bilateral oophorectomy). Women who had missing RHQ data or reported hysterectomy only, were included if they were over age of 50 with follicle-stimulating hormone (FSH) concentrations >45 (IU/L) (347, 408). We excluded those who reported a liver condition, had serum concentrations of alkaline phosphatase >283 (U/L), aspartate aminotransferase >83 (U/L), alanine transaminase >75 (U/L) (349), reported weak or failing kidneys, or had serum creatinine concentrations >2.74 (mg/dL) (348). Women with missing data for inclusion or exclusion criteria, or both outcome variables were excluded from our study sample. This represented less than 10% of total eligible sample. Figure 5.1 displays the selection of study population for this study.
Figure 5.1. Selection of study population

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Pregnant, not lactating Women aged 45 and over (n = 2955)</td>
<td></td>
</tr>
<tr>
<td>Examined (n = 2621)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal (n = 1954)</td>
<td></td>
</tr>
<tr>
<td>Ambulatory (not on wheelchair, no amputations) (n = 1900)</td>
<td></td>
</tr>
<tr>
<td>No Steroids (n = 1837)</td>
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</tr>
<tr>
<td>On Steroids (n = 60)</td>
<td></td>
</tr>
<tr>
<td>Missing (no data in Prescription Medication) (n = 3)</td>
<td></td>
</tr>
<tr>
<td>No Cancer Treatment Medications (n = 1810)</td>
<td></td>
</tr>
<tr>
<td>Cancer Treatment Medications (n = 27)</td>
<td></td>
</tr>
<tr>
<td>No Osteoporosis Medications (n = 1686)</td>
<td></td>
</tr>
<tr>
<td>Bone Medications (n = 124)</td>
<td></td>
</tr>
<tr>
<td>No Estrogen or HRT (n = 1238)</td>
<td></td>
</tr>
<tr>
<td>Reported using estrogen or HRT in Prescription Medication file (n = 328)</td>
<td></td>
</tr>
<tr>
<td>Reported using HRT, estrogen pills or patches in Reproductive Health Questionnaire (n = 46)</td>
<td></td>
</tr>
<tr>
<td>No Cancer (Except non-Melanoma Skin Cancer) (n = 1169)</td>
<td></td>
</tr>
<tr>
<td>Cancer (n = 142)</td>
<td></td>
</tr>
<tr>
<td>Missing (n = 1)</td>
<td></td>
</tr>
<tr>
<td>No Liver Disease (n = 1150)</td>
<td></td>
</tr>
<tr>
<td>Liver Disease: (n = 19)</td>
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<tr>
<td>No Kidney Disease (n = 1138)</td>
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</tr>
<tr>
<td>Kidney Disease: (n = 12)</td>
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</tr>
<tr>
<td>No Rheumatoid Arthritis (n = 997)</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis (n = 140)</td>
<td></td>
</tr>
<tr>
<td>Missing (n = 1)</td>
<td></td>
</tr>
<tr>
<td>Fasted for more than 9 hours (n = 497)</td>
<td></td>
</tr>
<tr>
<td>Fasting ≤ 9 hours (n = 480)</td>
<td></td>
</tr>
<tr>
<td>Missing (n = 20)</td>
<td></td>
</tr>
<tr>
<td>Had at least one bone marker data (n = 497)</td>
<td></td>
</tr>
<tr>
<td>Had at least one exposure data (i.e. dietary or serum tocopherols) (n = 497)</td>
<td></td>
</tr>
<tr>
<td>Only dietary data only (n = 21)</td>
<td></td>
</tr>
<tr>
<td>Only serum alpha- or gamma-tocopherol (n = 16)</td>
<td></td>
</tr>
<tr>
<td>Dietary and serum-tocopherol data (n = 460)</td>
<td></td>
</tr>
<tr>
<td>Final Sample (n = 497)</td>
<td></td>
</tr>
</tbody>
</table>
5.2.3  Exposures

5.2.3.1 Dietary and Total Alpha-Tocopherol Intake

On the examination day, a 24-hour dietary recall was performed by trained personnel to record all foods and beverages consumed from midnight to midnight the day before the examination (409, 410). This recall was used to determine the energy and dietary alpha-tocopherol intake using the Food and Nutrient Database for Dietary Studies for NHANES 1999-2000 and 2001-2002 (411, 412). The average daily intake of alpha-tocopherol from vitamin E supplements was calculated using the Dietary Supplements Questionnaire (DSQ) which contains self-reported intake data in the past 30 days (413). Total alpha-tocopherol intake was calculated by adding the intake from the 24-hour dietary recall to the average daily intake of vitamin E supplements (in the form of alpha-tocopherol). In our study, respondents were classified as vitamin E supplement users if they reported using supplements at least 15 times in the past 30 days and the average calculated daily intake of vitamin E in the past month was not zero. Data for gamma-tocopherol intake was not available.

5.2.3.2 Serum Tocopherols

Fasting serum samples were collected on the examination day. Alpha- and gamma-tocopherols were measured using high performance liquid chromatography (HPLC) with photodiode array detection (Waters Chromatography Division, Milford, MA) (414). Reportable range of results for alpha-tocopherol is 100–6000 μg/dL and the normal range for 98-99% of US adult population based on NAHNES III is 512-2875 μg/dL. None of the values in this study sample
were outside the plausible reportable range. The coefficients of variation for serum alpha-tocopherol and gamma-tocopherol ranged from 1.9 to 3.9% and 2.1 to 5.4%, respectively (414).

The intestinal absorption of alpha- and gamma-tocopherol and their secretion into chylomicrons is similar, but the serum concentrations of alpha-tocopherol are much higher than of gamma-tocopherol (415-417). One reason is that the transfer of absorbed tocopherols in the liver into the circulation is mediated by alpha-tocopherol transfer protein, which preferentially transfers alpha-tocopherol into the circulation and has lower affinity for gamma-tocopherol (155, 159). Another proposed mechanism for lower serum gamma-tocopherol concentrations is the preferential cellular uptake of gamma-tocopherol over alpha-tocopherol (418). High intake of alpha-tocopherol, especially from vitamin E supplements, results in decreased circulating gamma-tocopherol because of saturation of alpha-tocopherol transfer protein (155, 419). The ratio of serum alpha-tocopherol to gamma-tocopherol is often used as an indicator of alpha- and gamma-tocopherol balance in the body (420). The concentrations increase by modest increase in intake of alpha-tocopherol supplementation (163). It has been reported that this ratio is elevated by modest levels of vitamin E supplementation that do not notably raise plasma α-tocopherol concentrations (163).

5.2.4 Outcomes

The two BTMs measured in NHANES 1999-2002 are bone specific alkaline phosphatase (BAP) (µg/L), a marker of bone formation, and urinary N-terminal cross-linked telopeptide of type I collagen (uNTx) (nanomoles of bone collagen equivalents (nM/BCE)), a marker of bone resorption (354). Both of these markers are among the most sensitive and specific markers of bone turnover (37).
In years 1999-2001, BAP was measured using the Hybritech Tandem-MP Ostase Immuno Enzymetric assay (Hybritech Inc., SanDiego, CA) and in 2002, using the Beckman Access Ostase assay (Beckman Coulter Inc., Fullerton, CA). Adjustments were made to correct for this change of methods (354). The coefficient of variation for BAP in the NHANES 1999-2000 sample was 7.2-9.8 % and for the 2001-2 sample was 4.7-9.1 % (354). All BAP values in our study sample were within plausible reportable range (354). In years 1999 to 2001, uNTx was measured using the Osteomark method (Ostex International Inc, Seattle, WA) and in 2002, using the Vitros ECI method (Ortho-Clinical Diagnostics, Rochester, NY). The reported values of uNTx were adjusted by NCHS for the change in laboratory methods (354). A 24-hour urine collection or single urine collection, other than a first morning void, was used for the measurement of uNTx (354). In our study sample, the 4 values outside of plausible reportable range for uNTx (< 20 nmoL BCE NTx/mM creatinine) (354) were deemed unreliable and treated as missing data. The coefficient of variation for uNTx in for the total NHANES 1999-2000 sample was 11.4-12.5 % and for the total 2001-2 sample was 7.9-10.9 % (354). The uNTx concentrations are adjusted for urinary creatinine (Cr) excretion to control for urine dilution and the results (uNTx/Cr) are expressed as nM BCE per mmol creatinine (nM BCE/mM Cr). Creatinine was measured in urine with a Beckman Synchron CX3 clinical analyzer (Beckman Instruments, Inc., Brea, CA) (421).

Because of the change in laboratory methods for BAP and uNTx, NCHS performed t-tests after regression equations were applied to the new BAP and NTx values (422). Based on NCHS report, there were no significant differences in BAP or uNTx test means between the two methods after conversion (422).
5.2.5 Covariate Information

All covariates in the linear regression models were decided a priori based on their reported associations with tocopherols and BTMs in the existing literature. Age groups (45-65, 65 and over), ethnicity (non-Hispanic white, non-Hispanic black, Mexican Americans, other Hispanics and all other ethnicities including multiracial), nicotine exposure (yes/no), central adiposity (waist circumference (WC) ≥ 88 cm/less) and dietary supplements use in the past month (yes/no) were examined as potential confounders. Nicotine exposure was defined using the following categories: currently smoking some days, every day or having serum cotinine concentrations greater than 3 ng/mL (the cotinine cutoff point used to distinguish smokers from nonsmokers) (367). In our study, dietary supplement use (yes/no) was defined as using supplements containing minerals, vitamins, or both in the past month based on DSQ data. Respondents were classified as dietary supplement users if they reported using supplements at least 15 times in the past 30 days. The variations in nutrient intakes are often due to variations in total energy intake, which is dependent on age, physical activity and/or body size which can confound the associations (370). Therefore, intake of alpha-tocopherol was adjusted for total energy intake. Since alpha- and gamma-tocopherols are transported in the lipoprotein fractions in blood, total cholesterol and triglycerides were also considered as potential confounders (156). Serum cholesterol was measured enzymatically on a Hitachi 704 Analyzer (Roche Diagnostics, Indianapolis, IN) using commercial reagents (423), and serum triglyceride was measured on a Hitachi 917 multichannel analyzer (Roche Diagnostics, Indianapolis, IN) (424).
5.2.6 Statistical Analyses

Separate multiple linear regression models were used to examine the association between each BTM and each vitamin E variable. As recommended by the NCHS survey procedures, four-year dietary weights or four-year mobile examination center weights, as well as stratum and primary sampling unit variables were used in all analyses to account for the complex sampling design and non-response of the NHANES survey. Natural logarithmic transformation of all biomarkers and dietary data were performed to reduce the within sample variability and to avoid inappropriate exclusion of outliers (425). Therefore, the size of the regression coefficient represents the proportional change in BAP or uNTx/Cr associated with a unit change in the predictor variable. Adjusting for total serum cholesterol instead of serum triglycerides gave similar results to the unadjusted analysis, therefore only serum triglyceride was used.

Analyses were performed with Statistical Analysis Software (SAS) version 9.22 (SAS Institute, Cary, NC, USA) (369). All tests were 2-tailed, and significance stated for P-value < 0.05. Presence of effect modification was examined by creating cross-product terms of vitamin E variables and all covariates, tested one at a time as well as combined in the model. To examine for linear trends, least-squares means of BTMs were calculated for the quintiles of total, dietary and serum tocopherols. Tests for linear trend were performed by using orthogonal polynomial contrasts. We ran the sensitivity analysis for those who fasted for > 9 hours and attended morning examination session.
5.3 Results

5.3.1 Study Population

Our study population includes 497 postmenopausal women with a mean age of 65.5 (SE 0.6) years. Table 5.1 summarizes the socio-demographic, dietary alpha-tocopherol intake, serum alpha- and gamma-tocopherol and BTM concentrations of our sample. Among those who used dietary supplements, 81.4% consumed a supplement that contained alpha-tocopherol. Of those women who took alpha-tocopherol (vitamin E) supplements, 39.4% had intakes > 180 mg (400 IU) of alpha-tocopherol. Over 80% of vitamin E supplement users also used supplements that contained calcium or vitamins A, C or D. The average daily intake of alpha-tocopherol from vitamin E supplements, based on self-reported usage in the past month, ranged from 3 to 540 mg/day (to convert to IU multiply by 2.22) with the median intake of 41.2 mg/day. The geometric mean (95% CI) of dietary alpha-tocopherol intake among users and non-users of vitamin E supplements were 4.9 (4.2-5.7) and 4.1(3.7-4.6) mg/day respectively. These intakes were not significantly different from each other (p=0.08).

5.3.2 Relations between Dietary Vitamin E Intake and Serum Alpha-And Gamma-Tocopherols

Vitamin E supplement users had significantly higher serum alpha-tocopherol concentrations compared to non-users (mean (95% CI): 1905(1783-2035) versus 1219 (1152-1291) mcg/dL for serum alpha-tocopherol. On the other hand, the serum gamma-tocopherol concentrations were significantly lower in the vitamin E supplement users compared to non-users (130(112-152) versus 266 (240-295) mcg/dL).
There were significant positive correlations between total alpha-tocopherol intake and serum alpha-tocopherol concentrations in the total sample and in vitamin E supplement users and non-users. In the total sample and vitamin E supplement users group, total alpha-tocopherol had significant negative associations with serum gamma-tocopherol concentrations and significant positive associations with the ratio of serum alpha- to gamma-tocopherol (Table 5.2).
Table 5.1. Characteristics of study sample: postmenopausal women aged ≥45 years in the NHANES 1999-2002

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<th>Unweighted n</th>
<th>Weighted %b</th>
<th>SE</th>
</tr>
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<tbody>
<tr>
<td>45-54</td>
<td>78</td>
<td>23.8</td>
<td>2.5</td>
</tr>
<tr>
<td>55-64</td>
<td>153</td>
<td>28.7</td>
<td>3.0</td>
</tr>
<tr>
<td>65-74</td>
<td>144</td>
<td>25.5</td>
<td>2.4</td>
</tr>
<tr>
<td>75-85</td>
<td>122</td>
<td>22.0</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Unweighted n</th>
<th>Geometric Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Hispanic or Other Race including Multi-Racial</td>
<td>52</td>
<td>15.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Hispanic/Mexican American</td>
<td>125</td>
<td>4.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>92</td>
<td>11.00</td>
<td>2.0</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>228</td>
<td>69.6</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abdominal Obesity</th>
<th>Unweighted n</th>
<th>Weighted %b</th>
<th>SE</th>
</tr>
</thead>
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<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Waist Circumference &lt; 88 cm</td>
<td>134</td>
<td>25.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Waist Circumference ≥ 88 cm</td>
<td>352</td>
<td>72.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary Supplement Use in Past Month</th>
<th>Unweighted n</th>
<th>Weighted %b</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>238</td>
<td>44.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Yes</td>
<td>259</td>
<td>55.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin E Supplement Use in Past Month</th>
<th>Unweighted n</th>
<th>Weighted %b</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>293</td>
<td>54.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Yes</td>
<td>204</td>
<td>45.4</td>
<td>2.9</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Nicotine Exposure</th>
<th>Unweighted n</th>
<th>Weighted %b</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>416</td>
<td>80.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>19.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analysis Variable</th>
<th>Unweighted n</th>
<th>Geometric Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Bone Alkaline Phosphatase (mcg/L)</td>
<td>475</td>
<td>15.7</td>
<td>14.8-16.6</td>
</tr>
<tr>
<td>Urinary N-Telopeptides (nmol BCE NTx/mM creatinine)</td>
<td>485</td>
<td>43.4</td>
<td>40.6-46.4</td>
</tr>
<tr>
<td>Serum alpha-tocopherol c (mcg/dL)</td>
<td>476</td>
<td>1500.4</td>
<td>1444.9-1558.0</td>
</tr>
<tr>
<td>Serum gamma-tocopherol d (mcg/dL)</td>
<td>431</td>
<td>192.2</td>
<td>175.6-210.4</td>
</tr>
<tr>
<td>Serum alpha: gamma tocopherol ratio</td>
<td>431</td>
<td>7.8</td>
<td>7.0-8.7</td>
</tr>
<tr>
<td>Dietary alpha-tocopherol (mg/day)</td>
<td>481</td>
<td>4.4</td>
<td>4.1-4.8</td>
</tr>
<tr>
<td>Total alpha-tocopherol intake e (mg/day)</td>
<td>481</td>
<td>14.7</td>
<td>11.5-18.8</td>
</tr>
<tr>
<td>Energy (calories)</td>
<td>481</td>
<td>1462.5</td>
<td>1389.6-1539.3</td>
</tr>
</tbody>
</table>

SE= Standard Error, CI = Confidence Interval.
a n= 497
b Sample weighted percentages.
c To convert to µmol/L multiply by 0.0232.
d To convert to µmol/L multiply by 0.0240.
e Intake from diet and supplements.
**Table 5.2.** Correlations between total alpha-tocopherol intake (mg/d) (intake from diet and supplements) and serum alpha-, gamma- and the ratio of alpha- to gamma-tocopherols for the total study population and vitamin E supplement users and non-users subgroups

<table>
<thead>
<tr>
<th>Total alpha-tocopherol intake (mg/d)</th>
<th>Total</th>
<th>Vitamin E Supplement Users</th>
<th>Vitamin E Supplement Non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=481</td>
<td>n=199</td>
<td>n=282</td>
</tr>
<tr>
<td>Serum alpha-tocopherol (mcg/dL)</td>
<td>0.60</td>
<td>0.39</td>
<td>0.17</td>
</tr>
<tr>
<td>Serum gamma-tocopherol (mcg/dL)</td>
<td>-0.57</td>
<td>-0.44</td>
<td>-0.07</td>
</tr>
<tr>
<td>Serum alpha: gamma tocopherol ratio</td>
<td>0.66</td>
<td>0.50</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*r* = Pearson correlation coefficient derived from weighted linear regression. All variables are log transformed.
5.3.3 Relations between Vitamin E Variables and BTMs

Vitamin E supplement users had significantly lower BAP concentrations compared to non-users in the unadjusted and adjusted analyses (Table 5.3). In the total sample, high total alpha-tocopherol intake and serum alpha-tocopherol were associated with decreased BAP concentrations in the unadjusted analysis, but not in the analyses adjusted for potential confounders. High alpha-tocopherol status, defined by the ratio of serum alpha- to gamma-tocopherol, had a significant inverse relation with BAP concentrations in unadjusted and adjusted analyses. Conversely, high serum gamma-tocopherol concentrations were associated with high BAP concentrations in both unadjusted and adjusted models. There were no associations between vitamin E variables and uNTx/Cr (Table 5.3). No interactions were found between serum tocopherols and dietary supplement use in any of the analyses, therefore we did not stratify the data based on supplement use. The results were similar when we ran the sensitivity analysis for those who fasted for >9 hours and attended morning examination session (n= 414).

The unadjusted results for the associations between quintiles of vitamin E variables and BTMs are presented in Table 5.4. We found significant trends toward decreasing concentrations of BAP with increasing quintiles of total alpha-tocopherol intake, serum alpha-tocopherol and the serum ratio of alpha- to gamma-tocopherol. Conversely, we found a significant trend toward increasing concentrations of BAP with increasing quintiles of serum gamma-tocopherol. There were no statistically significant trends across the quintiles of any of the vitamin E variables and uNTx/Cr.
<table>
<thead>
<tr>
<th></th>
<th>BAP</th>
<th>uNTx/Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Vitamin E supplement use (n=497)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.10</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Dietary alpha-tocopherol (mg/day) (n =481&lt;sup&gt;b&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.01</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Total alpha-tocopherol intake (mg/day) (n =481&lt;sup&gt;c&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.02</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Serum Tocopherol Levels (n=497)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-tocopherol (mcg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Gamma-tocopherol (mcg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Alpha:Gamma-tocopherol ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-0.08</td>
<td>0.03</td>
</tr>
</tbody>
</table>

BAP=bone-specific alkaline phosphatase, uNTx/Cr=urinary N-Telopeptide/Creatinine, \( \beta \)= regression coefficients, SE= Standard Error, \( p \)= p-Value, LCI & UCI= Lower & Upper 95% Confidence Intervals, Adj \( R^2 \)= Adjusted \( R^2 \).

All continuous variables are log transformed.

<sup>a</sup> Adjusted for age, ethnicity, nicotine exposure and central obesity.

<sup>b</sup> 16 women did not have dietary alpha-tocopherol intake data.

<sup>c</sup> Adjusted for energy intake, age, ethnicity, nicotine exposure and central obesity.

<sup>d</sup> Adjusted for age, ethnicity, nicotine exposure, central obesity, dietary supplement use and serum triglycerides.

<sup>e</sup> Adjusted for age, ethnicity, nicotine exposure, central obesity, and dietary supplement use.
# Table 5.4: Unadjusted associations between quintiles of vitamin E variables and BAP (mcg/L) and uNTx/Cr (nmol BCE Ntx/mM creatinine)

<table>
<thead>
<tr>
<th>Quintiles of dietary alpha-tocopherol (mg/day)</th>
<th>Range</th>
<th>BAP</th>
<th>uNTx/Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quintiles</td>
<td>LSM</td>
<td>LCI</td>
<td>UCI</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>≤ 2.4</td>
<td>16.1</td>
<td>14.6</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>2.4 &lt;to≤ 3.8</td>
<td>17.5</td>
<td>15.2</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>3.8 &lt;to≤ 5.3</td>
<td>14.1</td>
<td>12.6</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.3 &lt;to≤ 7.7</td>
<td>16.1</td>
<td>14.6</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>&gt; 7.7</td>
<td>17.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Quintiles of total alpha-tocopherol (mg/day)</td>
<td>Range</td>
<td>BAP</td>
<td>uNTx/Cr</td>
</tr>
<tr>
<td>Quintiles</td>
<td>LSM</td>
<td>LCI</td>
<td>UCI</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>≤ 3.1</td>
<td>17.6</td>
<td>15.7</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>3.1 &lt;to≤ 6.1</td>
<td>15.7</td>
<td>14.3</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>6.1 &lt;to≤ 19.3</td>
<td>15.4</td>
<td>13.2</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>19.3 &lt;to≤ 99.5</td>
<td>17.6</td>
<td>15.7</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>&gt; 99.5</td>
<td>15.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Quintiles of serum alpha-tocopherol (mg/dl)</td>
<td>Range</td>
<td>BAP</td>
<td>uNTx/Cr</td>
</tr>
<tr>
<td>Quintiles</td>
<td>LSM</td>
<td>LCI</td>
<td>UCI</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>≤ 1046</td>
<td>16.6</td>
<td>14.9</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>1046 &lt;to≤ 1297</td>
<td>17.6</td>
<td>15.1</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>1297 &lt;to≤ 1645</td>
<td>15.1</td>
<td>13.5</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1645 &lt;to≤ 2123</td>
<td>15.6</td>
<td>14.2</td>
</tr>
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<td>&gt; 2123</td>
<td>13.9</td>
<td>12.5</td>
</tr>
<tr>
<td>Quintiles of serum gamma-tocopherol (mg/dl)</td>
<td>Range</td>
<td>BAP</td>
<td>uNTx/Cr</td>
</tr>
<tr>
<td>Quintiles</td>
<td>LSM</td>
<td>LCI</td>
<td>UCI</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>≤ 103</td>
<td>13.6</td>
<td>12.2</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>103 &lt;to≤ 167</td>
<td>15.7</td>
<td>14.0</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>167 &lt;to≤ 252</td>
<td>15.4</td>
<td>14.0</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>252 &lt;to≤ 356</td>
<td>16.8</td>
<td>15.3</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>&gt; 356</td>
<td>16.9</td>
<td>15.0</td>
</tr>
<tr>
<td>Quintiles of serum alpha:gamma-tocopherol ratio</td>
<td>Range</td>
<td>BAP</td>
<td>uNTx/Cr</td>
</tr>
<tr>
<td>Quintiles</td>
<td>LSM</td>
<td>LCI</td>
<td>UCI</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>≤ 3.2</td>
<td>16.8</td>
<td>14.2</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>3.2 &lt;to≤ 4.9</td>
<td>16.8</td>
<td>15.5</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>4.9 &lt;to≤ 9.2</td>
<td>16.4</td>
<td>14.9</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>9.2 &lt;to≤ 18.5</td>
<td>14.7</td>
<td>12.8</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>&gt; 18.5</td>
<td>13.7</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Adj R²: Adjusted R²; BAP = Bone alkaline phosphatase; uNTx/Cr = Urinary N-telopeptides/Creatinine; LSM = Least square means (geometric means); LCI & UCI = Lower & Upper 95% Confidence intervals; p = p-Value (linear trend).
5.4 Discussion

This is the first population-based study that examined the relationships between serum alpha- and gamma-tocopherol concentrations and BTMs in postmenopausal women. We found a significant positive association between serum gamma-tocopherol, and a significant negative association between serum ratio of alpha- to gamma-tocopherol and BAP concentrations. There were no associations between any of the vitamin E variables and uNTx/Cr concentrations. These results may be explained by possible uncoupling of bone formation and resorption by gamma-tocopherol.

In vivo studies have shown that gamma-tocopherol is able to neutralize nitric dioxide (426, 427), a compound that inhibits collagen synthesis (420). As a result of this reaction, nitric oxide (NO) is produced (426) which can uncouple bone resorption and formation, reduce bone resorption and stimulate bone formation (68, 171). On the other hand, high NO levels in presence of pro-inflammatory cytokines may inhibit osteoblast growth and differentiation (428). It has been shown that gamma-tocopherol can decrease high levels of reactive nitrogen species (429) and therefore can improve osteoblastic activity. Additionally, gamma-tocopherol and its metabolite gamma- 2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman (gamma-CEHC), but not alpha-tocopherol, may inhibit cyclooxygenase-2 (COX-2) activity and the resulting over-production of prostaglandin (PG)E2 (158, 159, 430). Reducing PGE2 synthesis has been associated with increased bone formation in growing rats, minimized bone loss in ovariectomized adult rats and improved osteoblast function in vitro (431, 432). Furthermore, gamma tocopherol and its metabolites may inhibit pro-inflammatory cytokines that induce osteoclast differentiation (433). Therefore, it is possible that gamma-tocopherol may stimulate bone formation without increasing bone resorption.
Few animal studies have examined the effects of alpha-tocopherol supplementation on bone health; those that have, their results are conflicting. Some studies suggest that alpha-tocopherol may improve bone quality and strength (166, 311), maintain BMD (434), decrease bone resorption (435), and increase bone formation (436). Hypothesized mechanisms include preventing the accumulation of bone resorbing cytokines (437), reducing the number of osteoclasts (436) and increasing the number of osteoblasts (436). Conversely, other studies suggest that alpha-tocopherol supplementation did not improve bone quality, BMD or BTMs (438, 439), and in fact one study suggests that a high dose of vitamin E may have a detrimental effect on bone quality (438). The hypothesized mechanisms for the negative and null effects of alpha-tocopherol on bone include the excessive suppression of local mediators which may reduce osteoblastic activity and bone formation (438). It is also possible that antioxidants become pro-oxidants when intake exceeds the need (438, 440). A recent in-vitro study showed that alpha-tocopherol inhibited differentiation of osteoblasts (441). Given that BAP reflects the differentiation stage of osteoblastogenesis (442), it is possible that excess alpha-tocopherol intake may reduce BAP concentrations. Although we found significant inverse associations between total alpha-tocopherol intake and serum alpha-tocopherol and BAP concentrations in the unadjusted analysis, these relationships disappeared after adjusting for confounders.

Our finding, that vitamin E supplement users had significantly lower serum gamma-tocopherol concentrations than non-users, is consistent with previous studies which showed that high doses of vitamin E supplements suppressed serum gamma-tocopherol concentrations (419, 443). We found that high serum alpha- to gamma-tocopherol ratio was associated with low BAP concentrations. The ratio of serum alpha- to gamma-tocopherol is a sensitive indicator of alpha-tocopherol intake and can be elevated by modest concentrations of supplementation that do not raise plasma alpha-tocopherol concentrations (163, 164). A high ratio therefore indicates a high
intake of alpha-tocopherol from dietary supplements (163, 291). Miller et al has suggested that depletion of serum gamma-tocopherol is a potential explanation for the discrepancy between the health-protective effects of alpha-tocopherol in observational studies and null or negative findings of vitamin E interventions, which mostly used high doses of alpha-tocopherol supplements (405). A recent study showed that dietary supplementation with vitamin E significantly increased the risk of prostate cancer among healthy men (281). It was proposed that for most naturally occurring dietary constituents, including alpha-tocopherol, a U-shaped-dose response curve may exist where either deficiency or intakes beyond needs may be harmful (281).

Although certain foods are naturally high in alpha- or gamma-tocopherol, in general foods contain a mixture of tocopherols and, therefore, do not result in an imbalance between serum alpha- and gamma-tocopherol concentrations. Moreover, it has been proposed that a mixture of tocopherols may act synergistically and result in a greater reduction in oxidative stress, inflammation and lipid peroxidation, and greater increase in synthesis of antioxidant proteins than either alpha- or gamma-tocopherol alone (404, 429, 444). A number of studies have shown that vitamin E intake from food sources is associated with beneficial outcomes in heart disease (401), colon cancer (402), and Alzheimer disease (403). It is noteworthy that over 80% of fats and oils used in the US are comprised of soybean oil (154). High consumption of soybean oil is associated with increased serum gamma-tocopherol concentrations (392, 445). In addition to gamma-tocopherol, soybean oil is also a dietary source of alpha-tocopherol (392), vitamin K (446), and omega-3 fatty acids (154), which may also have beneficial effects on bone (447, 448). To our knowledge, no studies have examined the association between soybean oil intake and bone metabolism. Future studies can examine the effect of soybean oil on bone metabolism in postmenopausal women.
Other than our current study, a sub-study of the Women's Health Initiative has examined the associations between serum alpha and gamma-tocopherol concentrations, dietary and total vitamin E and BMD among postmenopausal women and found no associations between dietary or total intake of vitamin E, or serum concentrations of tocopherols and BMD (150). The lack of association between serum tocopherol concentrations and BMD in the Women's Health Initiative study may be because serum tocopherol concentrations reflect short-term dietary intake and may not be suitable for the examination of longer-term surrogates of bone status such as BMD. A study by Macdonald et al (149) found a significant negative association between dietary vitamin E, but not total vitamin E (diet and supplements), and femoral neck BMD. The authors’ explanation for this negative finding was that dietary vitamin E intake may be a surrogate marker of poly-unsaturated fatty acids (PUFA) intake and that PUFA may result in reduced intestinal absorption of calcium by forming insoluble calcium–fatty acid soaps (149). A limitation of this study is that it is not clear whether dietary vitamin E intake was in the form of alpha- or gamma-tocopherol or both.

5.4.1 Limitations and strengths

Our study has several limitations. First, due to the cross-sectional design of this study, a causal relationship between vitamin E and BTM concentrations could not be determined. Second, people consume foods and often multiple dietary supplements, not single nutrients. Due to considerable multi-collinearity in intake and serum concentrations of nutrients and other biologically active components (e.g. phyttochemicals), conclusions about the association of any single nutrient or select number of nutrients with disease outcomes may be incorrect because of residual confounding. Third, the dietary intake of gamma-tocopherol was not available for NHANES 1999–2002; therefore, it was not possible to examine the relations between dietary gamma-tocopherol intake and BTMs. However, there is evidence that plasma gamma-
tocopherol reflect dietary intakes of gamma-tocopherol (445). Fourth, the findings of this study may not be generalizable to other populations as we only included postmenopausal women age 45 and above who were not on hormone replacement therapy or osteoporosis medications. Furthermore, other populations may have different food and supplement consumption patterns than US postmenopausal women. For example, diets in most countries, other than the US and Canada, provide less gamma-tocopherol due to the lower consumption of soybean oil (154).

Fifth, as is the case in the assessment of dietary intake measures, biomarkers are also subjected to measurement errors and biological and laboratory variability. However, using serum tocopherols as biomarkers of alpha and gamma-tocopherol intake provides more accurate and objective measures of dietary exposure than tocopherol intake data. Also, estimates of intake are highly dependent on respondents’ memory and ability to accurately report food and supplement intake, and the accuracy of the databases used to estimate tocopherol intake from food and supplements. To reduce the biological variation of serum tocopherols in our sample, only women who reported fasting for > 9 hours were included and adjustments were made for serum triglycerides. Sixth, BAP and uNTx concentrations follow circadian rhythms (59). However, fasting has been shown to reduce biological variability due to the circadian rhythm of BTMs (61, 65). We limited our study sample to those who reported fasting for > 9 hours prior to examination. In addition, our sensitivity analyses indicate that the findings are similar regardless of fasting status and examination session. Another limitation of our study is the change in the NHANES laboratory methods for analysis of BTMs in year 2002. However, adjustments were made, as discussed in the methods section, to correct for this issue. Lastly, because of the exploratory nature of this study, the multiple testing was done without Bonferroni adjustments (383); therefore, these findings should be viewed as trends that need to be further investigated.
Our study has several strengths. Data from NHANES is appropriate for use in epidemiological studies examining the relations between nutrition and various diseases as it contains detailed demographic, lifestyle, socioeconomic, dietary, reproductive health and medical information, prescription medication and dietary supplement use as well as physiological measurements and laboratory tests (411). These allow for detailed inclusion and exclusion criteria of the study sample and adjustments for potential confounders. Unlike RCTs where health-conscious volunteers are more likely to participate (449), NHANES data is a representative sample of the US population. The two NHANES BTMs that we used are suitable for examining the associations between dietary factors and BTMs in cross-sectional study design. Several studies have shown that baseline elevated BAP and NTx, or decreased BAP and increased NTx are predictors of BMD loss and increased fracture risk in postmenopausal women (23, 37, 56, 296). The NHANES 24-hour dietary recall represents food intake the day prior to the blood and urine collection day for analysis of BTMs, and may reflect the short term effect of diet on bone metabolism. Serum tocopherol concentrations reflect short-term dietary intake (164, 445) and are also suitable for the examination of the relations between short-term surrogates of bone status such as BTMs.

5.5 Conclusions

In this cross-sectional population-based study, we found that postmenopausal women who used vitamin E supplements had lower serum gamma-tocopherol, higher serum alpha-tocopherol concentrations and a higher ratio of serum alpha- to gamma-tocopherol than nonusers. We also found that high serum gamma-tocopherol concentrations and low ratio of serum alpha- to gamma-tocopherol were associated with increased BAP concentrations but not increased uNTX/Cr concentrations. Based on our data and findings of in vitro and animals studies, we
hypothesize that gamma-tocopherol may uncouple bone turnover, resulting in increased bone formation without affecting bone resorption. Vitamin E supplements in the form of alpha-tocopherol suppress serum gamma-tocopherol concentrations and may have negative impact on bone formation. Further research is needed to investigate the potential anabolic effect of gamma-tocopherol from food sources on bone.
Chapter 6

6  General Discussion

As the number of aging women increases, the prevalence of osteoporotic fractures also increases. At this time, around 30% of older women are considered eligible for osteoporosis treatment with pharmacological agents (450). On the other hand, there are an increasing number of studies that report adverse effects of pharmacological agents and dietary supplements on health (70, 115, 143, 281, 451-453). For this reason, there is great interest in identifying lifestyle strategies that reduce the risk of osteoporotic fractures.

Numerous studies have examined the relationships between various food components, food groups and overall diet on bone health, in search of an ideal diet for prevention and management of osteoporosis, but the results have been inconclusive. The overall aim of this work was to examine the associations between various dietary factors and bone health in postmenopausal women aged ≥45 years using different analytical approaches. First, a systematic review of observational and interventional studies was performed to investigate the relationships between F&V intake and incidence of osteoporotic fractures, BMD and BTMs. Second, the associations between an overall diet quality index, its components, and various food groups and BTMs were assessed using data from NHANES. Finally, the associations between alpha-tocopherol intake from food and supplements, biomarkers of alpha- and gamma-tocopherol intake, namely serum alpha- and gamma-tocopherol, and their ratio in the serum and BTMs were explored. The results of each study was discussed in previous chapters; in this chapter, a summary of key findings and a discussion of the whole body of work are presented.
6.1 Comment on key findings

6.1.1 Study 1: Fruit and vegetable intake and bone health in women aged 45 years and over: a systematic review

The first study was a systematic review of eight previously published studies that examined the effect of F&V on bone health. There was considerable between-study heterogeneity due to differences in: F&V classification (e.g. inclusion of potatoes as vegetables, nuts as fruits), amount of intake (dosage), methods of assessing intake (FFQs, 24-hr dietary recalls or FRs), research design (e.g. inclusion criteria, type of comparison group), analyses (e.g. adjustments for covariates) and reporting of outcome variables (e.g. various BMD sites, not reporting non-significant values). Due to these limitations a meta-analysis was not performed. Out of the eight included studies, only two were determined to have a low risk of bias. Overall, RCTs and prospective cohort studies found no beneficial effects, while the case-control study and cross-sectional analyses reported a positive association between F&V intake and bone health. The discrepant findings of cross-sectional studies versus RCTs and cohort studies are not unusual in the field of nutrition (454). The positive findings of cross-sectional studies and null results of RCTs and longitudinal studies may be due to residual confounding effects of other healthful lifestyle behaviours that are associated with high F&V intake that may not be accounted for in cross-sectional analyses (325, 337-339). It has been proposed that the positive findings of cross-sectional studies may also reflect measurements that are more representative of long-term dietary patterns and accumulated bone (179). With this assumption, it may be that lifelong F&V intake is beneficial to bones and that the deleterious effects of hormonal changes after menopause may outweigh the beneficial effects of F&V in later years. Overall, based on this
systematic review, it is unlikely that F&V intake affect bone health in postmenopausal women aged ≥ 45 years. However, this study was limited by the small number of studies with low risk of bias and considerable between-study heterogeneity in design, measurements, analyses and the reporting of the results.

Earlier narrative reviews that reported protective effects of F&V on bone health (101, 186, 276), were based on studies that found associations between high intake of calcium, potassium, magnesium and protein, and bone status (149, 179, 190, 306). However, in North America and Europe, high intake of calcium, potassium, magnesium is often an indication of high dairy intake and not a high F&V intake (202, 331, 455, 456). Other reports also indicate that most of the US population does not meet recommendations for dark green, orange vegetables, legumes, and whole grains (128, 372, 373, 457). In fact, it has been shown that diets high in F&V but low in protein, calcium or fat are not associated with better bone health (248, 254, 322, 458, 459). This could be because an increase in intake of a certain food group usually leads to compensatory changes in intake of other food groups (243). For example, RCTs, that enforced reduction of dietary fat and increased F&V among free living people, have shown that the intake of some bone protective foods such as dairy products (460, 461), n-3 fatty acids (254), calcium and protein (207, 462) decreased, which resulted in increased bone resorption (207), decreased BMD (254, 461) or increased incidence of fractures (461). On the other hand, diets with high intakes of dairy, protein group (e.g. legumes, nuts, fish) and F&V have been associated with reduced risk of fractures (247, 251), low bone resorption concentrations (343) or high BMD of the total hip, femoral neck or lumbar spine or total BMC (248, 279). It could be that the benefits of high intake of F&V might be achieved in combination with sufficient intake of other food groups such as dairy (196) and that it is the overall diet quality that is associated with bone health and not just one food group. To further explore this, the second study examined the
associations between an overall healthy diet and BTMs, using cross-sectional NHANES 1999-2002 data. The HEI 2005 was used as a measure of overall diet quality and associations between components of HEI-2005 and MyPyramid food groups and BTMs were also assessed.

6.1.2 Study 2: The association between Healthy Eating Index and bone turnover markers among US postmenopausal women aged 45 years and older

In this study, no association was found between the total HEI-2005 score and any of the BTMs. However, there was a significant relationship between the Milk Group component of HEI-2005 as well as tertiles of energy-adjusted dairy intake and bone resorption. The negative association between intake of dairy and NTx/Cr is consistent with other studies (379, 380, 463-466). The possible antiresorptive properties of milk have been attributed to its calcium, protein, phosphorous, magnesium, potassium, zinc and vitamin D content (174, 221, 467-469). Further examination of the results showed that there were no independent associations between calcium or protein intake from dairy and BTMs. Studies that have specifically evaluated the effect of dietary sources of calcium and protein on bone report that consumption of dairy foods has more favorable effects on BMD and bone resorption than individuals nutrients alone (132, 202, 204, 208, 209, 465). It has been suggested that the effect of dairy products, as a whole food, on bone health is greater than any of single milk ingredients (464, 470). It is possible that several milk components such as lactose, lactulose and casein phosphopeptides enhance the absorption of minerals in dairy foods (210, 471). The synergistic effects between minerals, protein and vitamin D present in dairy may also play a part (464).
Despite plausible mechanisms of associations between overall diet quality and bone health, there were no associations between total HEI-2005 scores and BTMs in this study. The HEI-2005 measures diet quality in terms of compliance with the US Federal dietary guidelines (271, 472). These guidelines focus on the reduction of total energy, fat, saturated fat, cholesterol and sodium intakes, maintaining calcium and protein intake, and increase in intake of F&V and whole grains to prevent obesity, coronary heart disease, stroke, diabetes and certain types of cancer (271, 472). To achieve a high HEI-2005 score one needs to consume a diet adequate in calcium and protein, high in fibre and, low in sodium and fat (271). It has been shown that high intake of dairy products is associated with high saturated fat, calories and sodium intake (111, 271). Therefore, even with a high dairy intake, one can attain a lower HEI-2005 score possibly due to high fat or sodium intakes. The ability of a diet quality score to predict bone health depends on how well it measures dietary factors involved in bone metabolism. The HEI-2005 does not include non-caloric foods such as diet sodas, carbonated water, coffee, and tea. It has been suggested that intake of these foods can affect bone health (224, 361, 473). The recommended food patterns of the 2005 dietary guidelines do not meet the Recommended Dietary Allowances (RDAs) for vitamin E and adequate intake (AI) for potassium (271). Therefore, another limitation of HEI-2005 is that because it is based on the 2005 dietary guidelines, a perfect HEI-2005 score does not mean sufficient intake of vitamin E and potassium (271). Both of these nutrients may be beneficial to bone (188, 474). All these factors limit the HEI-2005’s ability to predict bone health.

The lack of associations between overall diet quality and BTMs could also be because of poor diet quality of majority of US population and insufficient variability in HEI-2005 scores. Recent reports indicate that the diet of nearly the entire US population is not in accordance with the US federal dietary guidelines (128, 457, 475). Over 80% of persons age 71 y and over and
90% of all other sex-age groups had intakes of empty calories that exceeded the discretionary calorie allowances (128). The overall HEI-2005 score in the second study, was about 55 out of 100, and the two HEI-2005 components with highest mean scores were the total grains and protein groups, whereas, dark green, orange vegetables, legumes, and whole grains had lowest mean scores. This is in accordance to another report that found the majority of the US population only meets the recommendations for total grains and meat and beans food groups (128).

Although RCTs have shown that high intake of sodium chloride is associated with high NTx (bone resorption) (476, 477), we found no associations between sodium (absolute or energy-adjusted) intake and the sodium component of the HEI-2005 and BTMs. This could be because of poor measurement of dietary sodium intake, as the 24-hour dietary recall did not include sodium intake from medications, dietary supplements, drinking water and added salt at the table or during cooking. A recent report has shown that among US adults aged 18 and over, 99.4% consume >1,500 mg and 95.0% consume ≥ 2,300 mg on a usual daily basis (478). The overall high intake of sodium in the US population and little between-subject variation in intakes of sodium may also result in null findings of associations between sodium intake and bone health.

Interestingly, a significant linear association was found between energy-adjusted added sugar intake and bone formation. Although bone resorption concentrations followed the trend as BAP across increasing tertiles of energy-adjusted added sugars, the association with BAP was not significant. This may suggest that there was an overall increase in bone turnover. It is noteworthy, that there were no associations between absolute intake of added sugars and any of BTMs. It has been shown that energy-adjusted high sugar intake is an indicator of a poor-quality diet defined by low intakes of fruit, vegetables, milk group, and grains (237), nutrients
intakes below Estimated Average Requirements (EARs) levels (479) or low nutrient-density (235). Therefore, energy-adjusted sugar intake may be an indicator of diet quality, with higher values reflecting poorer diets which may be detrimental to bones.

Only one other study, by Kontogianni et al (248), has examined the associations between an overall diet index and BMD. This index measures adherence to a traditional Mediterranean diet (249). Kontogianni et al, also did not find any associations between the overall diet score and spine or total body BMD (248). Using factor analysis, the same study found that a dietary pattern high in fish and olive oil and low in red meat was positively associated with lumbar spine BMD and total body BMC (248). The authors suggested that the protective associations between a diet high in fish and olive oil and indices of bone mass may be due to the bone protective properties of omega-3 fatty acids or vitamin E that are found in high concentrations in fish and olive oil (248). Similar to HEI, the Mediterranean diet score considers full-fat dairy products as a food group with a negative impact on the overall health. Therefore, it is possible that dietary indexes that consider full-fat dairy products as a food group with a negative impact on health may not be a good measure of diet quality in relation to bone health.

6.1.3 Study 3: The Associations between Vitamin E and Bone Turnover Markers in US Postmenopausal Women

Although overall dietary patterns are important in determining risk of osteoporosis, identifying associations between individual nutrients can be the first step for several reasons. It may be that there are specific compounds or groups of compounds that are fundamentally related to the pathophysiology of osteoporosis. Dietary intake measurement and assessment methods have limitations in accurately measuring the dietary intakes of nutrients (255). Several studies have
shown that people tend to over-report intake of foods considered healthy such as F&V and under-report intake of fat, oil and sweets (393, 395, 480). For dietary supplements and fortified foods, by law, the listing of ingredient amounts on the label is the minimum content (396) and therefore, true intakes may be higher than reported intakes. Furthermore, many nutrients have inaccurate or incomplete nutrient values in food composition databases (258, 481). Therefore, biomarkers of nutrient intake can be used as surrogates of intake (256). In the case of vitamin E, serum or plasma alpha- and gamma-tocopherol are used as biomarkers of their intake (256, 291, 445). In the third study, the associations between serum alpha- and gamma-tocopherol and the ratio of serum alpha- and gamma-tocopherol, and BTMs were examined using cross-sectional NHANES 1999-2002 data. The relationships between dietary alpha-tocopherol intake from food and supplements and BTMs were also explored. In this study, although there were no independent associations between intake of alpha-tocopherol and its serum biomarker of intake (serum alpha-tocopherol) and BTMs, there was an inverse relationship between serum ratio of alpha- to gamma-tocopherol, an indicator of alpha-tocopherol intake from vitamin E supplements, and bone formation. In addition, increased serum gamma-tocopherol intake, a biomarker of its dietary intake, was associated with increased bone formation. There were no associations between any of the serum tocopherols and bone resorption concentrations.

Gamma-tocopherol has anti-inflammatory properties that may improve osteoblastic activity and inhibit osteoclast differentiation which could result in increased bone formation (433). Thus, gamma-tocopherol may stimulate bone formation without increasing bone resorption. Further examination of data in the third study showed that the intake of gamma-tocopherol had a significant negative association with the score of the Milk Group component of HEI-2005 (data not shown). As discussed earlier, an increased score of Milk Group component of HEI-2005 is
associated with decreased uNTx/Cr (bone resorption) concentrations. This may explain the reason for a lack of negative association between gamma-tocopherol intake and bone resorption.

In this study sample, similar to the findings of other studies (155, 419), the intake of vitamin E supplements was associated with reduced serum gamma-tocopherol concentrations which in return may be harmful to bones. Many interventions that used supplemental vitamins A, C, D, E and K, beta-carotene, folate or calcium for the prevention of cardiovascular disease, cancer or osteoporosis have failed to demonstrate a consistent significant effect on incidence of disease outcomes (482, 483). In fact, many of these studies report increased risk of severe adverse events such as increased mortality, risk of myocardial infarction, urinary tract stones or cancer associated with the high-dose nutrient intervention (405, 482-486). Although certain foods are naturally high in alpha- or gamma-tocopherol, in general, foods contain a mixture of tocopherols, and therefore may not result in an imbalance between serum alpha- and gamma-tocopherol concentrations. In the US diet, vegetable oils are the main source of omega-3 fatty acids, vitamin E or K (154, 446) all of which have been shown to be beneficial to bones (229, 232). It is possible that the roles and serum concentrations of different dietary fat components are interdependent and may be complementary (487, 488). Two clinical studies that examined the effects of high carbohydrate and low fat diets with adequate intakes of calcium on bone health outcomes, have shown decreased BMD (254) and increased bone formation and resorption (185). Together with the results of the third thesis study, this suggests that dietary fat may have an important role in bone metabolism. Vegetable oils may contain dietary components that may be beneficial to bones. For this reason, dietary advice to limit the intake of dietary fat may have adverse effects on bone.
6.2 Strengths

The first study of this thesis was the first to systematically assess the effect of F&V on bone health. The second and third thesis studies are the first population-based studies that examined the relationships between HEI-2005 and its components, dietary alpha-tocopherol, serum alpha- and gamma-tocopherol concentrations (as biomarkers of tocopherol intake) and BTMs in a population based sample of postmenopausal women. Use of cross-sectional data from the NHANES has several advantages. Data from NHANES is appropriate for use in epidemiological studies examining the associations between nutrition and various diseases (265). Unlike RCTs where health-conscious volunteers are more likely to participate (449), NHANES data is a representative of the US population. In addition, NHANES data, which are available on World Wide Web and free of charge, include detailed demographic, lifestyle (e.g. smoking, alcohol use, physical activity), socioeconomic, dietary, weight, reproductive health, prescription medication or dietary supplements and medical information as well as physiological measurements and laboratory tests. These allowed for defining detailed inclusion and exclusion criteria of the study sample and multivariable adjustment of potential confounders.

The two BTMs in the NHANES data are suitable for examining the associations between dietary factors and BTMs in a cross-sectional study design. Several studies have shown that baseline elevated BAP and NTx are predictors of BMD loss and increased fracture risk in postmenopausal women (37, 43, 51, 295, 489-493). Longitudinal studies have shown that high baseline NTx concentrations are associated high BMD loss in postmenopausal women who are not on osteoporosis treatment medications (494, 495). It has been shown that the effect of dietary factors on BTM could be as short as few hours to 2 days (61, 103, 104, 229, 295, 496-502). The NHANES 24-hour dietary recalls represent the food intake the day prior to blood and
urine collection day for analysis of BTMs and may reflect the short term effect of diet on bone metabolism. Serum tocopherol concentrations reflect short-term dietary intake (164) and are also suitable for the examination of the relations between short-term surrogates of bone status such as BTMs.

6.3 Limitations

Systematic reviews and meta-analyses are necessary for the development of evidence-based dietary guidelines and scientific consensus statements. However, it was not possible to perform a meta-analysis in the first study because of inconsistencies in study design, measurement of dietary intake, inadequate reporting of study characteristics, compliance, attrition, effect estimates, confounding factors and their possible biases of the existing studies of F&V and bone health. These factors also made interpretation of the results of the studies, and drawing a final conclusion difficult.

The major limitation of studies two and three are their cross-sectional design, for which a causal relationship between the examined dietary factors and BTM concentrations could not be determined. Although the selected sample in these two studies, represent the US postmenopausal population, the findings may not be applicable to other populations (e.g. men, premenopausal women) in the US or other parts of the world.

In the second and third studies, efforts were made to adjust for the effects of potential confounders, there may still be residual confounders that were not accounted or adjusted for. Twenty four-hour recalls do not reflect usual intakes, which result in high frequency of zero values for foods that are not consumed on a daily basis. For this reason, we could not examine the associations between subcategories of food groups such as legumes, milk, yogurt, cheese,
berries, citrus fruits, dark green vegetables, fish and soy, and BTMs. Analyses and assessment of serum concentrations of BAP, alpha and gamma-tocopherol as well as uNTx/Cr are subject to measurement errors and biological and laboratory variability. Despite these limitations, studies show that BTMs are independent variables of fracture risk in postmenopausal women (37, 295). In an effort to reduce the biological variability, the study sample was limited to those who fasted for at least 6 hours and corrections were made for urinary creatinine for NTx and for serum triglycerides for serum alpha- and gamma tocopherol.

6.4 Research challenges in nutrition and osteoporosis

There may be many reasons why the protective role of diet against postmenopausal osteoporosis remains inconclusive, despite all plausible mechanisms. Nutrition research in the area of bone health is very challenging. There are at least 30 food components that have been attributed to bone health (120, 164, 186, 210, 305, 342, 503). It is possible that rather than a single and universal nutrient requirement, there are requirements linked to the intake of other nutrients (173, 205, 504, 505). For example it has been shown that high protein and low calcium diets are detrimental to bones, whereas high calcium and high protein diets are protective against osteoporotic fractures (141, 220, 506-508). Moreover, the amount of multiple nutrients or food groups required to prevent osteoporosis may vary from individual to individual, and there may be no single, universal dietary regimen that is osteogenentic (202).

There is a high degree of correlation and interaction between these various food components that cannot be accounted for in statistical analysis. Adjusting for confounding variables in multivariable analysis may not remove all the confounding effects since many of these factors likely interact with each other in numerous ways. Overall diet quality indexes and dietary
pattern analyses are often used to overcome some of these limitations. However, overall diet and dietary patterns are highly correlated with other socio-demographic and lifestyle characteristics that also affect bone health.

It is often unclear what the important exposure from a food source is: a specific compound, combinations of food compounds, a metabolite or other unknown constituents in the food source. If a single dietary component is driving the relationship between diet and bone, then data reduction techniques such as grouping different foods and nutrients together would result in null findings. Grouping foods into different categories are based on arbitrary assumptions and often food preparation methods, foods consumed together as meals or mixed dishes, source, type and form of food, time of meals and snacks, ingredients, and the size of individual portions are lost in food grouping (513). In the case of F&V, different F&V may have different effects on bone metabolism (198, 199, 509-512). Therefore, future studies can examine the associations between specific fruits (e.g. plums) or vegetables (e.g. onions) on bone health. Furthermore, breaking down the mixed foods and recipes to single food items and quantifying to food group servings does not take in to account the synergistic or antagonistic effects of foods mixtures nor does it considers the ingredients added during cooking (e.g. spices).

Randomized nutritional prevention trials provide the most robust estimate of causal effects of diet on bone, as randomly assigning individuals to a prescribed diet ensures comparability between groups, and minimizes the effects of confounding factors. However RCTs in the field of nutrition are challenging. In a long-term RCT of osteoporosis prevention, double blinding of the treatment is not possible and feeding subjects controlled and prescribed diets is very costly. The experience from many nutritional RCTs has revealed that changing a group’s diet is a difficult task, and the adherence to prescribed diets diminish over time (514, 515).
challenges for nutritional RCTs include the timing of the prescribed diet as a preventive agent in the bone loss process, the effective dose, and the baseline food and nutrient intakes in the study sample as many RCT volunteers are health conscious and consume healthier diets compared to the general population (449).

The relationship between dietary intake and bone health may have conflicting results in part due to differences in the dietary assessment methods (152). To overcome this limitation some scholars have suggested using biomarkers of dietary intake for a more accurate and objective measure of dietary exposure (256, 257). However, similar to dietary intake, biomarkers are also subjected to day to day variation and a single biomarker may not only reflect the status of that particular nutrient, but may be reflective of several nutrients, their interactions and metabolism (257). For example, serum alpha- and gamma-tocopherol concentrations are correlated with other serum nutrients such as vitamins A, C, D and K, or selenium. Furthermore, if an association is found between the concentrations of a biomarker and risk of a clinical outcome, for practical reasons, an estimate of the nutrient intake that corresponds with the clinical outcome should be determined. This task could be challenging as the concentrations may vary from one person to another. Nutritional biomarkers can also be affected by genetic factors, and disease effects (257). In addition, several serum nutrients are under physiologic control and unless their intakes are below minimum requirements or beyond tolerable intakes, they cannot be used as biomarkers of intake. For example, serum calcium and retinol are under homeostatic control and cannot be used as surrogates of dietary intake unless in severe cases of deficiency or toxicity (257). Analyses and assessment of bone and dietary biomarkers are subjected to biological (preanalytic) and laboratory (analytic) variability (321).
Some the issues mentioned above are inherent to the diet and bone research and there are no optimum methods at this time. In the future direction section, some strategies are proposed to deal with some of these problems.
Chapter 7

7 Conclusion

The overall objective of this thesis was to gain a better understanding of the relationships between various dietary factors that have been less studied and bone health in community living postmenopausal women aged 45 years and over using different analytical approaches.

1) The objectives of the first study were to investigate the independent relationships between F&V intake and incidence of osteoporotic fractures, BMD, and BTMs and to identify research gaps using a systematic review approach.

At this time there is insufficient high quality evidence to support an association between F&V intake and bone health in postmenopausal women. In this systematic review, case-control and cross-sectional studies suggest positive association between F&V intake and bone health among women aged >45, whereas trials and cohort studies do not. The protective associations between F&V intake and bone health in cross-sectional studies may reflect the associations between life-long F&V intake and accumulated bone status, whereas the null findings of RCT and longitudinal studies in postmenopausal women may reflect the effect of F&V on changes in BMD in postmenopausal years. Under this assumption, it may be that long-term (particularly premenopausal) F&V intake is beneficial to bones and that the deleterious effects of hormonal changes outweigh the beneficial effects of F&V in postmenopausal years. However, the findings of this study are greatly limited by the small number of high quality RCTs and prospective cohort studies that specifically examined the effects of F&V intake on bone health in postmenopausal women.
2) The objectives of the second study were to assess the associations between overall diet quality and BTMs using the HEI-2005, and to explore the relations between the components of the HEI-2005 and the MyPyramid food groups and BTMs.

In this study, there were no associations between total HEI-2005 score and any of the BTMs. However, there was a significant inverse relationship between the score of the Milk Group component of HEI-2005 as well as tertiles of energy-adjusted dairy intake and bone resorption. These results support the ability of a diet with adequate dairy intake to promote bone health in aging women. The ability of a diet quality score to predict bone health depends on how well it rates dietary factors involved in bone metabolism, and one of the limitations of HEI-2005 is that despite a high dairy intake, one can attain a low HEI-2005 score due to high fat or sodium intakes. Given that dairy is a major source of dietary minerals, vitamin D and protein in Western diets, it is not surprising that increased intake of this food group was associated with reduced bone resorption. The findings of this study are limited by its cross-sectional design, and dietary and BTMs measurement errors.

3) The objectives of the third study were to examine the associations between dietary and total (from diet and supplements) alpha-tocopherol intake, serum alpha- and gamma-tocopherol concentrations and their ratio and BTMs.

The results of this study suggest that gamma-tocopherol may uncouple bone turnover, resulting in increased bone formation without affecting bone resorption. On the other hand, intake of alpha-tocopherol from vitamin E supplements is associated with suppressed serum gamma-tocopherol concentrations and therefore, may be detrimental to bone health. The findings of this study are limited by its cross-sectional design and measurement errors of dietary and serum tocopherols as well as BTMs.
Together with the results of other studies, one can conclude that irrespective of the methods used to determine the relationships between diet and bone, a diet consistent with current notion of a bone healthy diet-namely a diet with adequate intake of dairy is associated with better bone health outcomes in postmenopausal women. Based on the results of this thesis there is no strong evidence of a link between intakes of food groups, with the exception of milk group, or overall diet quality measured by HEI-2005 and bone health in postmenopausal women. Dietary indices are useful because they provide a summary measure of the degree to which an individual's diet conforms to the existing dietary recommendations for optimal health. Because our current knowledge and understanding of the relations between diet and bone health is still limited, it is not possible to create a meaningful dietary assessment tool for bone health. Some major challenges are selection of individual components of the index and defining cut-off points for each component. Therefore, at this time assessment of dairy intake may be a fast and easy way to identify dietary risk factors for bone loss in postmenopausal women. Further research is needed to investigate the potential anabolic effect of gamma-tocopherol from food sources on bone.
Chapter 8

8 Future Directions

Various dietary factors may affect bone metabolism in postmenopausal women. With high prevalence of osteoporotic fractures in postmenopausal women (9), the effect of dietary factors on bone status, however small, may have a significant public health impact. A better understanding of the type of diet that is best for bone health will allow development of population-based dietary prevention and management approaches.

Based on the paucity of studies identified in the systematic review in this thesis, future dietary interventions should explore the effects of F&V, as the main intervention, on bone health, and also compare the independent effects in pre- and postmenopausal women. However, additional studies without significant improvements in methodology will not improve the state of our knowledge with respect to the health effects of F&V on bone. There is a need for researchers to assess dietary intake using comparable and standardized methods across populations. To improve the clinical impact of future publications and outcome reporting, The US Agency for Healthcare Research and Quality (AHRQ) (516), CONSORT (340) and STROBE (341) statements should be used. Complying with these guidelines will result in more comparable literature, facilitate future systematic reviews and meta-analyses and ultimately lead to the development of evidence-based dietary recommendations. In the case of F&V, a joint report of WHO and Food and Agriculture Organization (FAO) provides useful guidelines for assessment of F&V intake (329). Consistent use of these guidelines consistently in future studies that examine the relations between F&V intake and health allows meaningful interpretation of
results. In systematic reviews and meta-analyses, this standardization would ensure that F&V exposures quantitatively describe the same effect.

Some studies suggest that F&V intakes are overestimated by study subjects (517). Biomarkers of F&V intake such as serum carotenoids and vitamin C can be used to overcome the limitations of F&V intake assessment (518, 519). Since few studies that reported associations between biomarkers of F&V intake and bone health (260, 261, 520), future systematic reviews may help summarize the exiting evidence. Not all fruits and vegetables show the ability to suppress bone loss. Some animal studies have shown that be specific subtypes of F&V have beneficial effects on bone while others may be inert (198, 199, 509-512). Future studies could explore the effects of specific F&V and their effective dose on bone health.

It has been suggested that dietary behaviours may change during observational and intervention studies (521). Therefore, prospective cohort studies and RCTs need to measure baseline dietary intake as well as final visit intake. It would be more appropriate to relate dietary and BMD changes together during the follow-up period. There is a high frequency of zero values for legumes and subcategories of food groups in 24-hour recalls, as most people do not consume these foods on a daily basis. To overcome this limitation, since 2003, an additional FFQ (food propensity questionnaire) is included in the NHANES (522). This FFQ captures frequency of consumption of commonly but occasionally consumed foods over the past year (522). Researchers at the National Cancer Institute (NCI) have developed and validated a method to estimate the usual dietary intakes of foods and nutrients for individuals using the 24-hour recall data and this FFQ (523). Future observational studies could examine the relationship between bone health and overall diet quality using individual usual food intake calculated by this
method. This will allow examination of subgroups of food groups that are not consumed on a daily basis.

In observational studies reduced rank regression (RRR) (524) can be used to determine linear functions of usual intake of foods (predictors), that explain maximal variation in bone health outcomes (e.g. BMD at various sites, BTMs). Because this dietary pattern analysis method has the ability to include multiple outcomes and handle the multi-collinearity among the predictors as well as outcome variables, future studies can use RRR to identify combinations of foods that may affect bone health.

There is much work to be done on the topic of vitamin E and bone health. Future studies are needed to examine the potential bone-protective properties of gamma-tocopherol from food sources. Soybean oil is a good dietary source of both gamma- and alpha-tocopherol (392), omega-3 fatty acids and vitamin K (154, 446) all of which have been shown to be beneficial to bones (229, 232). Future studies can examine the effect of soybean oil on bone status. Future clinical trials can also examine the independent contributions of alpha and gamma-tocopherols from food and supplements to bone health.

Over and under-reporting of foods in self-reported dietary data(286-289), fortification of foods, and the accuracy of the nutrient databases limit accurate estimation of dietary intake (256). To gain a better understanding of the roles of food components in osteoporosis development, there is a need to discover biomarkers of dietary intake that can accurately reflect intake. Furthermore, there is a need to learn what other factors affect biomarker concentrations (e.g., genetic factors or disease effects) and, develop valid laboratory procedures that allow for comparisons among laboratories. Ultimately, biologic measures of the intake of food groups or combinations of biomarkers that describe dietary patterns would enhance diet and bone health research efforts.
Finally, in addition to its direct effects on bone status, diet also affects risk of fractures indirectly through modification of hormones, muscle mass and function, alertness, cognitive function and balance. For example, it has been shown that there is an association between serum retinol and PTH (525). Given that PTH is involved in bone metabolism, serum retinol may indirectly affect bone health. Future studies need to examine such indirect effects of diet on fractures.

Each marker of bone formation and resorption reflects a different stage of bone formation or resorption, and therefore, each may respond differently to a dietary intervention (102, 119). For example while osteocalcin may increase, BAP may decrease with the same dietary intervention (526). This makes interpretation of studies more difficult as studies do not consistently use the same BTMs. The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend one bone formation marker (i.e. s-PINP) and one bone resorption marker (i.e. s-CTx) as reference markers, that should be measured by standardized assays in observational and intervention studies (41).

The effect of overall diet quality on bone health in postmenopausal women is worthy of further investigation. There is much work to be done to identify dietary factors that might be considered a risk factor for osteoporotic fractures as well as factors that are osteogenic and may improve bone health. Further research is needed to identify combinations of food groups that result in maximal bone formation and minimal bone resorption. It has been proposed that because dietary components are part of normal physiology, a U-shaped-dose response curve may exist for majority of these components where either deficiency or excess beyond needs may be harmful (281). This makes identifying intake cut-offs or ranges for food components or food groups challenging. Although long-term RCTs are often used to fill the gap between knowledge and practice on the relationship between nutrition and chronic diseases, there are many...
challenges in running nutritional RCTs for prevention of osteoporosis. Some examples are, as
the high cost of running such long-term RCTs, timing, dosage and duration of the prescribed
diet as a preventive agent in the bone loss process and compliance to prescribed diet. For these
reasons, large food-based RCTs to prevent osteoporosis in postmenopausal women may not be a
good investment, as clear answers are unlikely. We first need to gain a better understanding of
nutrients, foods and food groups and dosages that are healthful or detrimental to bones. The best
information on prevention of osteoporotic fractures by diet may come from long-term
prospective studies and short term RCTs using BTMs and BMD as intermediate outcomes
BTMs provide dynamic information regarding bone status and are independent and
complementary to BMD measurements (295). Therefore, a combination of multiple BMD and
BTMs may provide an appropriate evaluation of bone status.

The next step is constructing a diet quality index for osteoporosis that includes dietary
components that are directly relevant to bone health. Such properly designed and tested index-
based diet quality assessment tool can be used by clinicians to evaluate diets and to educate
postmenopausal women about dietary patterns associated with better bone health.
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Author: Maryam Hamidi, Valerie Tarasuk, Paul Corey, Angela M Cheung

Publication: The American Journal of Clinical Nutrition

Publisher: American Society for Nutrition

Date: Jul 1, 2011

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