

**DIETARY INTAKE AND BONE MINERAL DENSITY  
IN YOUNG-ADULT FEMALES**

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The late second and early third decades of life are critical periods for bone health due to the attainment of peak bone mass during this time, yet little is known about relationships between lifestyle factors and bone health among young-adult females. Therefore, anthropometric, body composition, and nutritional variables were examined in relation to bone mineral density (BMD) and biochemical markers of bone turnover in a group of 60 healthy, young-adult females aged 18 to 25 years. Body weight, body mass index (BMI), fat-free soft tissue mass (FFST), and fat mass had statistically significant and positive associations with BMD. Mean daily dietary protein, magnesium, and iron intakes had statistically significant and negative associations with BMD. A second study compared dietary intake, BMD, and biochemical markers of bone turnover in young-adult females with chronic dieting habits to nondieters. Anthropometric and body composition variables between chronic dieters and nondieters were not statistically different; however, chronic dieters had statistically significantly lower average daily dietary intakes of energy, macronutrients, and selected micronutrients compared to nondieters. Chronic dieters had statistically significantly higher whole body (WB) BMD compared to nondieters. Moderate effects were observed for WB, lumbar spine, trochanter, and total proximal femur BMD such that chronic dieters possessed greater BMD compared to nondieters. It appears that among young-adult females, total body weight, particularly FFST mass, has an important association with BMD. Although nutritional inadequacies among young-adult females raise concerns, overconsumption of nutrients may increase the likelihood of nutrient-nutrient

interactions that may have a less than optimal impact on BMD. Future investigations of dietary intake and BMD among young-adult females are warranted.

INDEX WORDS: Body composition, Bone mineral density, Dietary intake, Energy restriction, Serum osteocalcin, Urinary N-telopeptide, Young-adult female

## DEDICATION

This Thesis is dedicated to my father, my hero, role model, and guardian angel who got me through this process.

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## CHAPTER I

### INTRODUCTION

Osteoporosis is a disease of the skeletal system characterized by low bone mass and a continued loss of bone tissue with an associated rise in fracture risk (Kleerekoper and Avioli 1996). Osteoporosis is the most prevalent metabolic bone disease in developed countries (Wasnich 1996). It has been estimated that among postmenopausal Caucasian women, as many as 30% have osteoporosis while an additional 54% have osteopenia (Melton 1995). With improvements in health care and an associated increase in life expectancy, the occurrence of osteoporosis is expected to triple by the year 2050 with an estimated 4.5 million fractures worldwide (“Consensus Development...” 1993). Nearly \$20 billion are spent annually in the United States on direct medical costs associated with osteoporosis (“Consensus Development...” 1993, Lindsay 1995, Packard and Heaney 1997). As the prevalence of osteoporosis increases, so will expenses.

Women are four times more likely than men to develop osteoporosis. An estimated 50% of women will suffer osteoporotic bone fractures during their lifetimes (“Position of the American...” 1999, Turner et al. 1998). Moreover, with each fracture, risk of sustaining another fracture increases greatly (Kotowicz et al. 1994). Within one year of a hip fracture, approximately 20% of individuals die from secondary complications (Drinkwater 1994). Survivors live with pain, disabilities, and deformities that lead to losses in independence and decreased quality of life (Galsworthy and Wilson 1996, Turner et al. 1998).

Pharmaceutical treatment options that help to maintain bone mass and prevent further losses in bone mineral are available. Increases in bone mineral content (BMC) and bone mineral density (BMD) from these treatments are not rapid, however. Additionally, side effects from

these medications, that are often expensive and require lifelong compliance, hinder the long-term effectiveness in reversing bone mineral losses (Eisman 1995). Thus, osteoporosis prevention continues to be the most desirable, reliable, and cost-effective strategy for managing osteoporosis.

Throughout life, bone tissue is continually added and removed in the process of bone remodeling. Although the incidence of osteoporosis is most prevalent in postmenopausal women, this disease begins early in life and takes years to manifest itself. The principle goal in prevention of osteoporosis, then, is to maximize peak BMD by the young-adult years and to maintain bone integrity throughout adulthood. Bone mineral is accumulated more rapidly than removed until around the third decade of life when peak bone mass is achieved (Matkovic 1991, Recker et al. 1992). At this time, bone remodeling equilibrates with an eventual favoring of bone resorption (Cassidy 1999, Matkovic 1991). By maximizing peak bone mass during the first three decades, an individual may better withstand natural periods of bone loss such as during aging and menopause. Strategies for osteoporosis prevention, targeted for young women and women in midlife, will ideally allow women to pass through menopause and into older age with high bone mineral reserves. Therefore, primary prevention of osteoporosis must be aimed at females in their late teen years and early twenties – the years prior to and around the age of peak bone mass (Dombrowski 2000).

Hormonal, medical, genetic, nutritional, and life-style factors influence bone metabolism and, ultimately, risk for osteoporosis (Dombrowski 2000). Because osteoporosis is a multifactorial disease, research that investigates the individual and interrelated factors, both endogenous and exogenous, that effect BMD must be conducted. Of particular interest to nutritional scientists, is the complex role of nutrition in bone health. Much research has focused

on the relationship between calcium and BMD. It is evident that calcium is essential to bone during both the formative years (Johnston et al. 1992, Lloyd et al. 1993) and throughout adulthood (Baran et al. 1989). Other nutritional components with implications for bone health include, but are not limited to, vitamin D, phosphorus, protein, sodium, magnesium, zinc, and fiber (Kleerekoper and Avioli 1996, “Position of the American...” 1999, Rubin et al. 1999). Due to a multitude of nutrient-nutrient interactions, it is difficult to accurately assess the benefit or detriment from a single nutrient (Rude et al. 1978, Spencer et al. 1997). It is clear, however, that nutritional factors other than calcium impact bone health and that more research must be conducted to further understand these relationships. Therefore, examination of dietary intake patterns is important as well as individual nutrient consumption.

It has been well established that severe undernutrition is associated with reduced BMD (Mazess et al. 1990). Of interest and with great implication for western societies are those studies that have observed negative effects on bone health from caloric restriction and weight reduction (Compston et al. 1992, Grinspoon et al. 1995, Ndiaye 1995). In societies in which low body weight and thinness are valued, weight loss diets are common, often without supervision from medical professionals. Weight loss efforts by an individual may be sporadic, frequent, acute, or chronic. Of particular concern are dieting practices designed to minimize weight that are continued for extended periods of time. Such practices are referred to as chronic dieting (Grunewald 1985). Chronic dieting is particularly common among young-adult females regardless of an actual need for weight loss (Biener and Heaton 1995). For this reason, it is necessary to investigate relationships between chronic dieting and bone health in young-adult females. The current study was designed to: (1) investigate relationships between anthropometric, soft tissue mass, and dietary intake variables and BMD measures, and markers

of bone turnover in young-adult females, and (2) examine differences in dietary intake, body composition variables including BMD, and markers of bone turnover between young-adult females with chronic dieting habits and nondieters of the same age. The first study found that body weight, particularly FFST mass, was associated with high BMD among young-adult females. In this group of young women, protein, magnesium, and iron intakes were all found to have significant and negative associations with BMD (Chapter 3). When investigating differences in variables of interest between young-adult female chronic dieters and nondieters, anthropometric differences between groups were not found, yet chronic dieters had significantly lower dietary intakes of most nutrients compared to controls. Unexpectedly, chronic dieters had significantly higher WB BMD than nondieters (Chapter 4). Findings from these studies are novel and raise questions regarding relationships between dietary intake and bone health in young-adult females; therefore, Chapter 5 provides suggestions for future research.

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## CHAPTER II

### REVIEW OF LITERATURE

Osteoporosis is the most prevalent metabolic bone disease in developed countries (Wasnich 1996). It is a systemic skeletal disease characterized by low bone mass and a continuous loss of bone tissue with an associated risk of fractures. Fracture risks increase with each standard deviation (SD) below average peak bone mass values (Wasnich 1996). Therefore, the World Health Organization (WHO) defined osteoporosis as a bone mineral density (BMD) value of greater than 2.5 SD below average peak BMD of young, healthy, gender-matched individuals (Kleerekoper and Avioli 1996). Osteopenia, another prevalent bone disorder that is characterized by a reduced bone mass, increases the risk of osteoporosis. According to the WHO, osteopenia is a BMD value between 1.0 and 2.5 SD below mean peak BMD of young, healthy, gender-matched individuals (Kleerekoper and Avioli 1996).

The estimated prevalence rate of osteoporosis among postmenopausal Caucasian women ranges from 13% (Looker et al. 1997) to 30% (Melton 1995); the estimated incidence of osteopenia is even higher than osteoporosis (Looker et al. 1997, Turner et al. 1998). Collectively, more than 30 million Americans currently have osteoporosis or osteopenia (“Position of the American...” 1999, Turner et al. 1998). Moreover, greater than 50% of women aged fifty years or older will experience an osteoporosis related bone fracture (Bellantoni 1996). Additionally, the risk of sustaining an additional fracture increases significantly with each occurring bone fracture (Kotowicz 1994).

Approximately 1.5 million fractures are sustained annually as a result of osteoporosis. With improvements in health care and associated increases in life expectancy, the occurrence of

osteoporosis is expected to triple by the year 2050 with an estimated 4.5 million fractures worldwide (“Consensus Development...” 1993). Within one year of experiencing a hip fracture, approximately 20% of these individuals die from secondary complications. The remaining 80% of individuals who survive often live with pain, disabilities, and deformities, all contributing to a loss of independence and a decreased quality of life (Drinkwater 1994, Galsworthy and Wilson 1996, Turner et al. 1998).

Osteoporosis has an associated health care cost of \$10 to \$20 billion (“Consensus Development...” 1993, Lindsay 1995, Packard and Heaney 1997). However, these cost estimates generally consider only direct medical costs from fractures of the hip, spine, and wrist, but do not account for indirect costs from disability or loss of life (Barrett-Connor 1995, Lindsay 1995). These direct health care costs, therefore, are a mere fraction of total expenses associated with osteoporosis. Calculating the full direct and indirect cost of osteoporosis is nearly impossible.

The pathology of osteoporosis is multifactorial. Several modifiable and nonmodifiable factors are associated with the development of osteoporosis. Table 1 presents identified risk factors related to low bone mass and osteoporosis (Kleerekoper and Avioli 1996, Rubin et al. 1999, Salamone et al. 1996, Turner et al. 1998).

Osteoporosis is difficult to reverse. Pharmacological therapies are available and are aimed at reducing rates of bone resorption, thereby increasing bone formation. Such treatment options help to maintain bone mineral density (BMD) and prevent further loss in BMD, but improvements in bone mineral from available medications are slow to develop. Additionally, side effects, expenses, and often committed lifelong compliance with these medications limit their use by individuals and hinder potential benefits (Eisman 1995). Therefore, prevention remains the most reliable and cost-effective strategy for managing osteoporosis.

Table 1. Comparative factors influencing bone health.

	Low Bone Mass	Normal or High Bone Mass
Age:	≥ 65 years old	18 to 64 years old
Gender:	Female	Male
Ethnicity:	Caucasian	African-American
Nutrition:	Inadequate dietary intake	Adequate dietary intake
Body Mass:	Low	Normal or High
Physical Activity:	Low or Sedentary	Moderate or High*
Tobacco use:	Regular	Abstinence
Alcohol use:	Regular	Abstinence
Chronic illnesses:	Diabetes Mellitus Cystic Fibrosis Malabsorptive disorders	None
Menopausal age:	Postmenopausal	Premenopausal
Exposure to Sex-steroid Hormones:	Amenorrhea Oligomenorrhea	Eumenorrhea
Medications:	Corticosteroids Laxatives Antacids	Oral contraceptives

\* Exercise-induced amenorrhea may be associated with reduced BMC and BMD.

Although osteoporosis is generally considered a disease among older individuals, osteoporosis manifests itself early in life, taking years to develop. Throughout life, bone tissue is constantly added and removed in the process of bone remodeling. In the first three decades of life, bone formation occurs more rapidly than does bone resorption, allowing attainment of peak bone mass (Matkovic 1991, Recker et al. 1992). Although peak bone mass is largely determined by genetics, other multiple factors influence accrual of bone mineral (Kleerekoper & Avioli 1996, Rubin et al. 1999, Salamone et al. 1999, Turner et al. 1998).

Within a few years of the onset of puberty, the growth plates of long bones fuse, long bone extension ceases and maximum height is achieved (Boot 1997). The rate of bone mineral accumulation continues beyond puberty with bone mineral deposition exceeding removal until the third decade of life when peak bone mass is achieved (Matkovic 1991, Recker et al. 1992). A longitudinal study by Recker and colleagues (1992) investigated changes in bone mass after cessation of linear growth. A group of 156 young-adult (18.5 to 26.0 years) Caucasian females were studied over a five year period. Bone density measurements by dual energy X-ray absorptiometry (DXA) examined the whole body, lumbar spine and forearm BMD at the beginning and end of the study period. At all body sites included, BMD increased significantly over the course of the study. Acquisition of bone mass was estimated to cease at nearly 29 years of age (Recker et al. 1992). By age 30, bone remodeling equilibrates, and shortly thereafter, bone mineral resorption is favored over formation, resulting in a gradual loss of BMC and BMD until menopause in women (Cassidy 1999, Matkovic 1991). By achieving her peak bone mass potential during the first three decades of life, a female can better withstand natural periods of gradual bone loss that occur during midlife, rapid loss of bone mass during menopause, and additional bone mass losses in the postmenopausal years. Because the ability or inability to

maximize peak bone mass “sets the stage” for risk of osteoporosis, prevention should be targeted toward premenopausal females who have yet to achieve peak bone mass. Prevention strategies must benefit the ultrastructure of bone so that the strength of individual bones and the entire skeleton are enhanced. Thus, an understanding of the architecture of bone is necessary.

### **Anatomy and ultrastructure of bone**

Bone has four main purposes for the body: (1) structural support and locomotion, (2) protection of internal organs, (3) site of red blood cell production, and (4) storage of metabolically active ions (Baron 1996). Bone can be divided into two specific types - cortical and trabecular. These bone types, like all living tissue, are comprised of cellular and extracellular components that are vital for maintenance of proper functions. Although the cellular and matrix constituents are the same for these two types of bone, cortical and trabecular bones differ structurally and functionally. As much as 90% of cortical bone, also referred to as compact bone, is calcified; conversely, only 15% to 25% of trabecular bone is calcified at any point in time (Baron 1996). While cortical bone serves in mostly a structural role, trabecular bone is considered the “metabolically active” type of bone. Trabecular, or spongy, bone is highly vascular, contains large amounts of connective tissue, and houses bone marrow (Baron 1996).

Approximately 90% of the protein matrix of bone is comprised of collagen fibers, more specifically, type I collagen (Knott and Bailey 1998). On and around collagen fibers are crystallized mineral structures referred to as hydroxyapatite with a chemical structure of  $3\text{Ca}_3(\text{PO}_4)_2 \cdot (\text{OH})_2$  (Baron 1996). Collagen fibers of normal, healthy, adult bone are organized in a lamellar structure or in alternating orientation of fibers to provide greater collagen density within bone (Baron 1996).

Embedded deep within the protein matrix of bone are osteocytes, or bone cells (Baron 1996). Osteocytes begin as osteoblasts, or the bone forming cells, but become trapped in newly synthesized bone matrix and are subsequently calcified (Baron 1996). Gap junctions provide communication lines from osteocytes to the bone surface and to other osteocytes (Baron 1996). Osteocytes remain within the ultrastructure of bone until osteoclastic bone resorption occurs. Although most bone turnover occurs in the internal bone surface, cell signaling allows osteocytes to play an important role in initiation of bone resorption (Baron 1996).

### **Bone resorption**

Osteoclasts are cells responsible for resorption of bone. Osteoclasts are large multinucleated cells that are found independently or in small groups at the bone surface and possess ruffled borders. This ruffled border of the osteoclast allows secretion of protons that are necessary to decrease the microenvironmental pH at the resorptive site, dissolve hydroxyapatite, and expose the protein matrix. Subsequently, lysosomal and non-lysosomal enzymes (i.e., collagenase) are secreted to resorb the bone matrix (Mundy 1996).

Following osteoclastic resorption, there is a reversal phase for bone. During this reversal phase, osteoblasts move into the newly exposed surface area and produce more bone (Baron 1996).

### **Bone formation**

Osteoblasts are mononucleated cells that cluster at the bone surface and produce both collagenous and non-collagenous organic products for the bone matrix (Puzas 1996). Twenty percent of the protein produced by osteoblasts is type I collagen (Puzas 1996). The most abundantly produced non-collagenous protein secreted by osteoblasts is osteocalcin, constituting approximately 1% of the bone matrix protein (Eriksen et al. 1995).

In addition to bone synthesis, osteoblasts serve important roles in bone resorption. Receptors for parathyroid hormone (PTH), calcitriol (vitamin D), prostaglandins, and interleukins (compounds that enhance bone resorption) have been identified on the surface of osteoblasts (Martin and Ng 1994). Once osteoblasts produce the bone matrix components, mineralization of the matrix may occur.

### **Calcium homeostasis**

Approximately 99% of the body's calcium stores are found in bone (Volpe 1999). Calcium is a major constituent of hydroxyapatite or the mineral matrix of bone that provides structure and support. This mineralization of bone occurs in response to a high level of calcium in the blood. The blood calcium concentration is maintained at 9 to 11mg/dL or 2.5 mmol/L (Bronner and Pansu 1999, Volpe 1999). When serum calcium concentration rises above normal, calcitonin is secreted by the C-cells of the thyroid gland. Calcitonin serves to inhibit osteoclastic resorption of bone so that deposition of calcium into the bone matrix is allowed. This withdrawal of calcium from the blood returns serum calcium to normal while allowing bone mineralization (Delftos 1996).

Just as readily as calcium can be deposited into bone, calcium can be withdrawn. Hydroxyapatite provides a source of calcium when the blood calcium level is low (Volpe 1999). The major hormone involved in releasing calcium from bone is PTH. When the extracellular calcium level falls by minute increments, PTH is secreted from the parathyroid gland into circulation. The role of PTH in controlling serum calcium concentration is threefold. This hormone allows: (1) bone resorption of calcium, (2) renal reabsorption of calcium, and (3) renal synthesis of vitamin D to its active form (1,25(OH)<sub>2</sub>D<sub>3</sub>) to enhance calcium absorption in the small intestine (Holick 1996, Kronenberg 1996).



Interestingly, PTH receptors exist in osteoblasts rather than osteoclasts, indicating a direct inhibition of osteoblast activity and bone matrix production (McSheehy and Chambers, 1986). Osteoblasts, when stimulated by PTH, secrete factors that trigger osteoclast activity, thereby increasing bone mineral breakdown and release of calcium into circulation (McSheehy and Chambers 1986).

When the blood calcium level falls below normal, PTH signals the kidney to increase renal tubular reabsorption of calcium so that calcium is routed back into circulation. Lastly, PTH enhances renal tubular conversion of vitamin D to its active form (Holick 1996, Kronenberg 1996). Dihydroxycholecalciferol, or calcitriol, enhances intestinal absorption of calcium and stimulates osteoclast production, which in turn releases calcium into circulation and mobilizes calcium stores from bone, respectively. The result of PTH activity is a return of serum calcium concentration to normal (Holick 1994).

Calcitriol, or the active form of vitamin D, plays a dual role in calcium homeostasis with a net action of maintaining bone integrity, thereby preventing osteoporosis (Holick 1996). Provitamin D<sub>3</sub>, located in the skin undergoes conversion to previtamin D in the presence of solar ultraviolet-B (UVB) photons during sun exposure. Previtamin D is then converted to inactive vitamin D. Dietary sources of vitamin D, along with UV-converted vitamin D, are hydroxylated in the liver and again in the kidneys to result in the active form, 1,25-dihydroxyvitamin D<sub>3</sub> (Holick 1996)

Although it appears that vitamin D does not have a direct role in the mineralization of bone, it is indirectly essential as vitamin D assists in maintenance of the serum calcium concentration and prevention of bone mineral disorders (Holick 1996). Expression of both calbindin (the intracellular calcium binding-protein) and the plasma membrane calcium pump are

dependent on vitamin D (Johnson and Kumar 1994). Therefore, active absorption of calcium requires vitamin D (Pansu et al. 1983). With insufficient calcitriol, either from low dietary intake or reduced subcutaneous conversion, intestinal absorption of calcium is reduced. A reduction in calcium absorption stimulates PTH release so that a normal serum calcium concentration, through osteoclastic bone resorption, is maintained (McSheehy and Chambers 1986). In healthy individuals, this circle of calcium homeostasis continues and adjusts as needed to fluctuations in nutrient intakes and blood calcium concentration changes.

### **Nutrition and bone**

Variations in dietary intakes of nutrients, specifically calcium and vitamin D, impact the integrity of bone through alterations in nutrient homeostasis in the body. Bone is a storage compartment for several minerals within the body (Broadus 1996) and dietary components are required for synthesis and function of enzymes (Czajka-Narins 1992), hormones (Seelig 1993, Zofkova and Kancheva 1995), and bone cells (Czajka-Narins 1992), all of which are necessary for maintenance of bone metabolism. Therefore, it is essential that relationships between nutrition and bone health be understood.

### **Calcium and bone**

Because 40% of the mineral found in bone is calcium, dietary intake of calcium has a strong association with BMD. Calcium is absorbed both actively and passively from the small intestine (Bronner and Pansu 1999). Calcium channels, located at the apical membrane of the duodenum allow for active transcellular transport of calcium ions. Within the cytoplasm, calcium ion concentrations are tightly regulated. It is, therefore, necessary for calcium entering the cell to quickly bind to calbindin. Calcium is then transported to the ATP-dependent calcium

pump of the basolateral membrane. Here, calcium is transported out of the cell against a concentration and electrochemical gradient (Bronner and Pansu 1999).

Active absorption of calcium is dependent on numerous factors. When dietary intake is low, transcellular transport is upregulated, and the active process is the predominant absorptive mechanism (Bronner and Pansu 1999). At dietary intakes above 800 mg/d, a larger proportion of calcium is absorbed via passive transport (Pansu et al. 1993). At low dietary intakes, bioavailability of calcium from the food source is of great importance (Heaney and Weaver 1990), but at higher intakes, bioavailability has less importance (Deroisy et al. 1997).

Paracellular transport of calcium occurs passively down a chemical gradient throughout the small intestine, predominantly in the jejunum and ileum (Bronner and Pansu 1999). With high calcium intake, there is a down regulation of the active transport process (Buckley and Bronner 1980); thus, passive diffusion is the primary calcium transport mechanism when calcium intake is adequate or high (Pansu et al. 1983).

In addition to the effect of dietary calcium intake levels on overall calcium absorption, vitamin D, glucose and lactose, intact digestive tract integrity, and increased dietary requirements (i.e., pregnancy) enhance calcium absorption (Volpe 1999). Fiber, phytates and oxalates, encountered naturally in some high calcium containing foods, bind calcium and, thereby, decrease calcium bioavailability (Volpe 1999). Diets that are high in protein and sodium increase urinary calcium excretion (Volpe 1999). High dietary intakes of phosphorus may lead to bone loss by lowering the ionized serum calcium level and inducing PTH action (Calvo and Park 1996).

Numerous studies provide evidence of a positive relationship between dietary calcium intake and BMD (Rubin et al. 1999, Salamone et al. 1996, Turner et al. 1998). The

recommendation for the Adequate Intake (AI) of calcium has recently been increased based on current reports of the strong link between high dietary calcium intake and high BMD (Food and Nutrition Board 1989).

Although an abundance of research has focused on the relationship between calcium and BMD, dietary factors important to bone metabolism are not limited to calcium. Several other nutrients affect bone directly or through effects on calcium economy (New et al. 2000, Rubin et al. 1999). Table 2 provides a list of nutrients and dietary components with identified effects on bone health (Kleerekoper and Avioli 1996, New et al. 2000, “Position of the American...” 1999, Rubin et al. 1999). Those nutrients and food components with widely recognized relationships to BMD are discussed.

#### Phosphorus and bone

Along with calcium, phosphorus is an essential component of hydroxyapatite. Approximately 85% of the body’s phosphorus is stored in hydroxyapatite crystals (Broadus 1996). Phosphorus is, therefore, necessary for maintaining bone integrity.

Phosphorus is easily absorbed by passive diffusion (Calvo and Park 1996). Low phosphorus consumption is not frequently encountered in the United States; thus, phosphorus deficiency is not of great concern for Americans (Czajka-Narins 1992). In contrast, excess phosphorus is consumed in the typical American diet through carbonated beverages and processed foods containing phosphates (Calvo and Park 1996). Moreover, these foods contain high amounts of phosphorus relative to their calcium contents (Calvo and Park 1996). Although phosphorus is an essential component of bone and phosphorus can reduce urinary calcium excretion, a ratio of calcium to phosphorus consumption greater than 1:1 may be detrimental to

bone. Excessive dietary phosphorus intakes may lead to bone loss by lowering the ionized serum calcium level

Table 2. Dietary components with relationships to bone mineral density.

Minerals	Vitamins	Food Components	Other
Calcium	Vitamin A	Fatty Acids	Alcohol
Copper	Vitamin C	Fiber	
Fluoride	Vitamin D	Oxalates	
Iron	Vitamin K	Phytates	
Magnesium		Phytoestrogens	
Sodium		Protein	
Zinc			

and inducing PTH action (Calvo and Park 1996). Although negative effects on BMD from high dietary intakes of phosphates are disputed, there is cause for concern because high intakes of phosphorus containing foods may alter calcium regulating hormones as well as displace good food sources of calcium such as fluid milk.

### Magnesium and bone

Bone houses approximately two-thirds of the body's magnesium content (Broadus 1996). Intestinal absorption of magnesium occurs via a common pathway with calcium, and metabolic functions of magnesium and calcium are interrelated (Seelig 1993, Alcock and MacIntyre 1962). Magnesium is essential for normal bone metabolism by maintaining adequate function of the calcium regulating hormones PTH, vitamin D, and calcitonin (Seelig 1993, Zofkova and Kancheva 1995). Thus, hypocalcemia and bone loss can result from inadequate intake, absorption, or retention of magnesium (Rude et al. 1978) and may, therefore, be a cause for osteoporosis in some individuals. New and colleagues (2000) provide evidence of an indirect relationship between magnesium intake and pyridinoline excretion, a marker of osteoclast activity or bone resorption.

High intakes of calcium inhibit magnesium absorption (Alcock and MacIntyre 1962). Because magnesium is necessary for proper maintenance of calcium homeostasis, the benefits of a high calcium diet may be undermined if magnesium is inadequately absorbed. Because calcium and magnesium are both essential for preserving bone health, adequate intakes at appropriate ratios of these minerals must be met. More research is necessary to determine an optimal calcium to magnesium ratio for maximum accrual and retention of BMD.

## Zinc and bone

Approximately 1.5 to 2.5 g of zinc is found in the human body, 29% of which is housed in bone (Volpe 1999). Zinc is absorbed in the small intestine via a carrier-mediated process as well as by diffusion (Czajka-Narins 1992). Zinc absorption is enhanced by the presence of amino acids which form zinc chelates (Czajka-Narins 1992, Lonnerdal 2000). Zinc absorption is decreased when fiber and phytates are present in a meal, and a high dietary copper intake inhibits zinc absorption. Because iron and zinc compete for absorption, a high dietary iron intake can inhibit zinc absorption and vice versa (Abdel-Mageed and Oehme 1991, Czajka-Narins 1992).

Zinc and calcium nutrition are interdependent. Zinc has been shown to stimulate osteoblast differentiation and bone protein synthesis (Chen et al. 1998, Hashizume and Yamaguchi 1993, Yamaguchi et al. 1988) while inhibiting resorptive osteoclast activity (Moonga and Dempster 1995) *in vitro*. *In vivo* studies show zinc-induced stimulation of bone synthesis, but at high intakes of zinc, this benefit is lost. At high dietary intakes of zinc, bone microarchitecture is weakened and overall bone strength is reduced (Kawamura et al. 2000). Additionally, high supplemental doses of zinc (140 mg/d) reduce intestinal calcium absorption when dietary calcium intake is low (Spencer et al. 1997). In animal models, addition of zinc to a low-calcium diet has the additive effect of reducing BMC and bone strength (Kenney and McCoy 1997). Thus, zinc is a necessary component of bone that assists in bone formation. Zinc is particularly beneficial to bone when dietary calcium intake is adequate; however, zinc may be detrimental to BMD and bone strength when consumed in high amounts concurrently with a low dietary calcium intake.



### Fat, protein, sodium and bone

Not only do nutrients have direct effects on BMD through storage of minerals in bone, but nutrients and dietary components also impact BMD through effects on calcium excretion (New et al. 2000, Rubin et al. 1999). The typical American diet exceeds recommended levels for fat, protein, and sodium (Kennedy et al. 1999). The effect of such diets on BMD have not been well characterized, but there may be need for concern. Complex relationships exist for nutrient-nutrient interactions between dietary fat and calcium, protein and calcium, and sodium and calcium (Heaney 1993). Dietary fat may bind calcium in the intestinal tract and prevent absorption (Bronner and Pansu 1999). The benefit of an adequate calcium intake in maximizing peak bone mass in young-adult women is offset when the calcium to protein ratio decreases (Teegarden et al. 1998). Both protein and sodium have been shown to stimulate renal excretion of calcium (Packard and Heaney 1997). In light of high dietary intakes of fat, protein, and sodium and accumulation of evidence indicating inverse relationships between these three nutrients and BMD, the recommendation for a higher AI level for calcium is further supported (Food and Nutrition Board 1999).

### Fiber and bone

Dietary fiber consumption in the United States is below the recommended intake for Americans (Hendricks and Herbold 1998). However, increased fiber consumption is commonly advised for health maintenance and disease prevention (Davidson et al. 1996). An inverse relationship between dietary fiber intake and BMD exists (Lloyd et al. 1987) with data suggesting that dietary fiber inhibits mineral absorption (Davidson et al. 1996). A year-long study by Hoffman and colleagues (1999) investigated effects of a fat plus fiber diet compared to a control diet of sugar plus starch on BMC of growing foals. After 12 months, foals that

consumed the fat plus fiber diet had significantly lower BMC compared to controls. The authors concluded that the fat and fiber contents of the experimental diet bound calcium, thereby decreasing calcium absorption availability (Hoffman et al. 1999).

In a recent study, premenopausal women who consumed high amounts of fruits and vegetables during childhood were found to have higher BMD in their fifth and sixth decades of life compared to women with low fruit and vegetable consumption during childhood (New et al. 2000). Fruits and vegetables are also good sources of zinc, potassium, magnesium, and vitamin C. Although fruits and vegetables contain fiber, the other nutrients that contribute to BMD may override the negative effect of fiber on BMD. Such a contention is supported by a study in which high dietary intakes of zinc, potassium, magnesium, and vitamin C were found to be associated with high bone mass (New et al. 1997).

Metabolism, absorption, and function of many nutrients are interrelated. Contradictions among epidemiological and experimental data imply that single micronutrients may have either positive or negative effects on bone health but that these effects are dependent on overall dietary status (Spencer et al. 1997). Therefore, it is important to examine overall dietary intakes of individuals when regarding the impact on BMD.

### **Dietary intakes of college women**

Little information has been reported regarding the overall dietary intakes of young-adult, American, college women. Dietary habits of college females are difficult to assess. Inconsistent eating patterns are common among college women and can occur for numerous reasons such as changes in daily schedules, social influences, and school-related stresses. In terms of bone health, the understanding and evaluation of dietary practices of traditional college-aged women is important as these dietary practices impact the attainment of peak BMD.

The National Health and Nutrition Evaluation Survey of 1988 to 1991 (NHANES III) calculated average daily intakes of females aged 20 to 29 years from 24-hour dietary recall data (Hendricks and Herbold 1998). It was found that for women of this age group, fat and saturated fat intakes were above recommendations while intakes of fiber, calcium, iron, zinc, and folate were less than adequate (Hendricks and Herbold 1998). For women in this age range, the most frequently reported sites for food consumption other than home or campus, were fast food restaurants, followed by vending machines and convenience stores (Sneed and Holdt 1991). Many of the foods available in such restaurants, stores, and machines are high in total fat and saturated fat contents.

Vickery and colleagues (1985) evaluated the dietary intakes of 335 college women from 2-day dietary records. Diets were analyzed for protein, carbohydrate, fat, calcium, iron, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid contents. It was found that more than 75% of the participants consumed greater than two-thirds of the 1980 Recommended Dietary Allowances (RDAs) for each nutrient except iron, vitamin A, and niacin (Vickery et al. 1985). The nutrient evaluations of this study are limited, and total dietary adequacy cannot be assessed from this information. Furthermore, since this report in 1985, AI for calcium for this age group has been increased from 800 mg/d to 1,000 mg/d (Food and Nutrition Board 1999). When re-evaluating these data according to updated recommendations, it appears that a much higher percentage of these females consumed less than two-thirds of the AI for calcium.

Social and psychological factors influence young women's food choices. Findings from a survey of 141 college females indicated that 54% were dissatisfied with body weight (Koszewski and Kio 1996). These women reported, on average, initiating food restriction at 15.2 years of age. Use of laxatives during the previous year was reported by 9%, purging by 10%,

and 40% of these women had lost and re-gained 10 pounds during the previous two years (Koszewski and Kuo 1996).

Dieting for weight loss is common among women in western societies. College women may be more prone to dieting behaviors because of strong social biases toward being thin. Grunewald (1985) studied weight control by dieting in 166 college women aged 18 to 24 years. More than 18% were classified as chronic dieters on the basis of their self-reporting spending greater than 50% of the time during the previous eight months dieting; 45.2% reported periodic dieting (dieting 50% or less of the time during the previous eight months), and 36.7% were nondieters (reporting not having dieted over the previous eight months; Grunewald 1985). This study also asked respondents to list previously attempted weight loss tactics. Of these 166 women, 58 (34.9%) reported fasting/starving, 56 (33.7%) reported use of diet pills or supplements, 9 (5.4%) reported self-induced vomiting, and 9 (5.4%) reported laxative use (Grunewald 1985). The top two sources for dieting information among these women were lay-magazines or newspapers (44.0%) and family or relatives (26.5%; Grunewald 1985). Such resources often provide misinformation or inappropriate interpretations of nutrition research findings.

Unhealthy body images plague many young women. Bailey and Goldberg (1989) reported that among a group of 59 college-age females, who averaged 95% of their ideal body weight, 85% perceived themselves as overweight. Among a group of sorority women asked to identify a most desirable body silhouette, 80% chose an underweight image as the most preferred body size (Schulken et al. 1997).

In addition to inadequate nutrient intakes and a high prevalence of food restriction and weight loss tactics among college-aged females, low awareness of risk factors of diseases that

affect primarily women, particularly osteoporosis, may add to the risk factors for this age group. Survey results showed that although a majority (90%) of a group of undergraduate females had heard of osteoporosis, fewer than 50% had received information from the school or health care system regarding this disease (Kasper et al. 1994). Most members of this group had knowledge of some risk factors, yet only 6.7% identified calcium intake and physical activity as relating to osteoporosis. The majority did not feel that they were at risk for osteoporosis and, furthermore, felt it was less serious than other diseases (Kasper et al. 1994). The food and nutrient intakes of college women do impact health, specifically BMD. Dieting habits in the young-adult years may predispose many females to osteoporosis in later life. Several lines of evidence support the impact of restricted diets on BMD.

### **Dieting and bone mineral density**

Diets designed to control body weight are highly prevalent in the United States and other Western cultures (Beales & Manore, 1999). Although dieting practices among females in non-western societies are not as widespread, disordered eating patterns are seen globally (Lake et al. 2000).

Weight loss diets are not restricted to overweight individuals. In fact, Biener and Heaton (1995) reported that 47% of Caucasian women dieters had body mass indexes (BMI;  $\text{kg}/\text{m}^2$ ) below 25, the upper limit of normal. Age is also not a limiting factor for dieting. There is an increased frequency of dieting among younger individuals. Results from the Minnesota Adolescent Health Survey (Story et al. 1998) revealed that 57% of adolescent girls reported trying to lose weight. Among these dieters, 5.5% reported use of vomiting or diet pills as a means of weight loss (Story et al. 1998). Such drastic dieting methods are not uncommon and may also include extreme caloric restriction, fasting, use of laxatives, and excessive physical

activity (Grunewald 1985, Story et al. 1991, 1998). As girls transform from adolescence into adulthood, dieting often becomes more common and more extreme. Hawkins and Clement (1980) reported that an astounding 22.1% of college-age women engaged in self-induced vomiting as a means of weight loss.

Although some males do engage in weight loss diets, women constitute the vast majority of disordered and non-disordered dieters (Story et al. 1998). Body mass is also protective of BMD; therefore, low BMD among males is less common. Due to the greater prevalence of dieting behaviors and osteoporosis among women, further investigations of the relationships between diet, weight, and bone health in females are warranted.

Body mass variables and body weight have positive associations with BMD due to the mechanical forces placed on bone from high mass and weight (Rubin et al. 1999, Salamone et al. 1996). Additionally, adipose tissue is capable of converting androgens to estrogens; thus, women with high body fat masses tend to have high BMD (Davidson et al. 1996). Overweight and obese individuals appear to have less risk for osteoporosis, but when these individuals engage in dietary behaviors to control or alter body mass and weight, nutrient intakes critical to BMD may be restricted.

Not only may anthropometric changes (i.e., weight loss) occur with dieting, but nutritional status may change as well. Dieting can induce alterations in energy balance and micronutrient status (Ramsdale and Bassey 1994) as well as acid-base balance (Grinspoon et al. 1995). Unsupervised weight loss diets vary greatly, making global assessment of nutrition while dieting difficult; nonetheless, the impact of self-initiated weight loss diets on BMD must be investigated.

Energy restriction and dietary fat reduction are associated with reduced micronutrient intakes (Horvath et al. 2000). Because calcium rich foods such as milk and cheese are often perceived to be high in fat and calories, they are frequently limited by women who diet (Shah et al. 1996). Without dairy and other high calcium food sources in the diet, calcium intake may be low; thus, the body may resorb bone mineral (i.e., calcium) from skeletal stores to meet functional demands. In addition to altered calcium balance, changes in other essential nutrients may be induced through dieting as well.

#### Evidence from animal studies

Ndiaye and colleagues (1995) investigated the effects of energy restriction on bone formation, as measured by serum osteocalcin, in post-weaning male rats. Four groups of rats were fed diets that were 0% (control group), 20%, 40%, or 60% below energy requirements for four weeks. Diets were isocaloric for fat and protein quantities but were reduced in Calories by limitations in dietary carbohydrate concentrations for the three energy-restricted groups. After four weeks, serum was collected, pooled according to group, and analyzed for osteocalcin, calcitriol, PTH, calcium, and phosphorus. Serum calcium, phosphorus, and osteocalcin were significantly lower in all three experimental groups compared to controls. Significant differences in serum PTH or calcitriol levels were not observed in any group after four weeks of receiving these dietary intakes. These data provide evidence of a relationship between energy restriction and a reduced bone formation rate as measured by osteocalcin. Furthermore, the reduction in bone formation appeared independent of vitamin D or PTH (Ndiaye et al. 1995).

In a separate study, three-month old and ten-month old female rats were used to investigate the effects of energy restriction, calcium restriction, or calcium plus energy restriction on bone turnover during a nine week period of time (Talbot et al. 1998). Control animals

consisted of one set of rats from each age group that were fed a diet adequate in both energy and calcium. Energy restriction was achieved by a reduction in the carbohydrate content of feed while dietary fat, protein, fiber, vitamin, and mineral levels were equal. Calcium restriction was induced by limiting calcium to 15 mg per day while calcium control diets received 78 mg daily. In order to maintain the same calcium to phosphorus ratio of control diets, phosphorus was reduced in proportion to calcium (Talbot et al. 1998).

Bone resorption (i.e., urinary tritiated tetracycline levels) was measured weekly throughout the study. Bone formation (i.e., serum osteocalcin) was measured at baseline and after the nine-week study period. Whole body BMD was measured by dual-energy x-ray absorptiometry (DXA) at the beginning and end of the nine-week period (Talbot et al. 1998).

Body weight was reduced among both 3-month-old and 10-month-old energy restricted rats at the end of the study. Urinary tritiated tetracycline levels were significantly higher among all energy restricted and calcium restricted groups compared to controls regardless of age, but after week six, bone resorption in all restricted groups decreased to levels similar to control levels. An additive effect on bone resorption was not observed in the animals receiving the calcium plus energy restricted diets. Energy restricted rats also had higher serum osteocalcin concentrations than calcium restricted and control rats regardless of age (Talbot et al. 1998).

Over the course of nine weeks, BMD increased in all 3-month-old rats. Following nine weeks of calcium restriction in the 3-month-old rats, BMD was significantly less compared to BMD of controls. Energy restricted 3-month-old rats had final BMD values similar to control rats, but body weight remained low. These data imply that among young rats, energy restriction alone will prevent an increase in body mass but will not have a negative impact on BMD if



calcium intake is adequate. However, when dietary calcium is restricted, attenuation of bone mineral acquisition among young rats will occur (Talbot et al. 1998).

Among older rats, final BMD increased in control animals but not in any energy or calcium restricted group. Significant differences in urinary excretion of tritiated tetracycline and BMD were observed between 10-month-old energy restricted rats and 10-month old controls. These results indicate that both younger and older female rats have higher rates of bone turnover in response to calcium and/or energy restriction but that the response to energy restriction on BMD may be age related (Talbot et al. 1998).

#### Evidence from human studies

Compston and colleagues (1992) investigated the effects of dietary induced weight loss with subsequent weight gain on whole body BMD in a group of 13 women aged 37 to 60 years. Obese women followed a very-low-calorie (405 kcal/d) diet for 10 weeks after which weight gain occurred over a 10-month period. Whole body BMD was measured by DXA at baseline, weeks 11, 23, and 57. Significant reductions in body weight were achieved by week 11. Subjects returned to their original, pre-diet weights within 10 months. Whole body BMD was significantly reduced by week 11 but returned to baseline following the weight gain. This study implies that weight loss induced by compliance with a very-low-calorie diet is associated with a rapid reduction in whole body BMD. However, with subsequent weight gain, whole body BMD returns to pre-diet level. While findings from this study were interesting, women within a wide age range were included (Compston et al. 1992). Additionally, dietary-induced weight loss may be accompanied by site-specific reductions in BMD, particularly of the hip (Salamone et al. 1999) and spine (Ramsdale and Bassey 1994). The use of whole body BMD analysis alone did not provide information regarding site specific BMD changes induced by weight cycling at

common osteoporotic fracture sites (Compston et al. 1992). Although BMD returned to baseline following post-diet weight gain (Compston et al. 1992), it was unknown if bone mass returned to specific areas from which it was lost.

Post-dieting return of site-specific BMD accompanying the return of lost body mass was not the result of a study conducted by Avenell and colleagues (1994). The effect of a 6-month high fiber, weight reduction diet on BMD in overweight postmenopausal women, followed by return to starting weight, was examined. Sixteen postmenopausal women were placed on a low-calorie (1200 kcal/d), low-fat (21% of energy), high fiber (19 g cereal fiber/d) diet for six months in order to lose 20% of excess body weight. Following the diet period, six months were allotted for participants to return to their starting weights. Lumbar spine and femoral neck BMD measures were conducted by DXA at the start of the study, and then at 3, 6, and 12 months for dieters. Forty-six age-matched non-dieting women were used as controls. Control women (nondieters) had lumbar spine and femoral neck BMD measured at baseline and 12 months. Both controls and dieters had reductions in BMD at both the lumbar spine and femoral neck over the course of this study; a significant difference between the two groups at the femoral neck was not observed at 12-months. Dieters sustained a two-fold greater reduction in spinal BMD than the estimated loss of spinal BMD for controls at month 6. Continued losses in spinal BMD during the 6-month weight regain period were observed in dieters so that by month 12, loss of spinal BMD of dieters was greater than twice that of controls. These data support other studies that have shown losses in BMD accompanied by weight reduction. Furthermore, with subsequent weight gain, bone mineral may not be restored to the body sites from which the bone mineral was resorbed (Avenell et al. 1994). This study has implications for the many “weight cyclers” in our society.

Ramsdale and Bassey (1994) investigated the effect of weight loss induced by long-term, moderate caloric restriction on BMD among premenopausal women. Forty-five women participated in this 6-month weight reduction program. Three-day weighted food records were completed prior to dietary reduction and again four months later. Participants were individually advised to reduce caloric intake by consuming low-fat, low-sugar diets in order to achieve an ideal BMI of 20 to 25 within 5%. Total body, lumbar spine, and proximal femur BMD were measured by DXA at baseline and after 3 and 6 months of dieting. Reductions in all macronutrients, as well as calcium, phosphorus, and vitamin D paralleled energy restriction. Total body and lumbar spine BMD were significantly lower at the 6-month measurement point, but femoral BMD did not decline significantly. It was also found that individuals who lost more than 5% of initial body weight ( $n = 23$ ) had nearly a two-fold greater loss of whole body and lumbar spine BMD compared to the rest of the group (Ramsdale and Bassey 1994).

In conjunction with dietary weight loss tactics, exercise has also been used as a method of weight loss (Anderson et al. 1997, Salamone et al. 1999, Svendsen et al. 1993). Weight-bearing exercise has been associated with an increased BMD among young individuals (Teegarden et al. 1996) and attenuation of bone loss in adulthood (Packard and Heaney 1997). Svendsen and colleagues (1993) studied the effects of diet or diet plus exercise on changes in BMD in overweight postmenopausal women. Women were randomly assigned to a control ( $n = 21$ ), a 4,200 kJ/d diet ( $n = 51$ ), or a 4,200 kJ/d diet plus aerobic and anaerobic exercise group ( $n = 49$ ). Intervention was maintained for 12 weeks. Body composition and BMD measurements of the whole body, lumbar spine, and forearm were made at baseline and after 12 weeks of intervention. Weight loss was significant in both diet groups compared to controls. A significant difference in total weight loss was not observed between intervention groups; however, women

in the diet-plus exercise group lost significantly more fat mass than non-exercising dieters (9.6 kg versus 7.8 kg, respectively). Among both diet groups (exercise and non-exercise), a pattern of a reduction in whole body BMD compared to controls was observed. Spinal BMD was significantly reduced among the diet plus exercise participants (Svendsen et al. 1993). This study contradicts other reports of the benefits of exercise on BMD by showing that bone loss accompanies weight reduction and that exercise may increase the rate of bone loss.

A similar study by Anderson and coworkers (1997) investigated the effects of diet alone or diet plus resistance exercise training on changes in BMC and BMD in obese postmenopausal women. Twenty-one obese women were placed on diets of 925 to 1,500 kcals for 24 weeks. Women in the diet alone group (n = 9) were instructed to refrain from engaging in any resistance exercise training and to limit physical activity. Women in the diet plus resistance exercise group (n = 12) participated in regular supervised resistance exercise training sessions. At the end of the study, both groups had significant reductions in body weight, fat-free mass, and fat mass without significant differences observed between the two groups. At the end of 24 weeks, there was a significant decrease in femoral neck BMD and BMC and trochanter BMC in the nonexercise group. Similar reductions were found in the exercise group who also experienced a significant reduction in trochanter BMD. Absolute reduction in BMD was not significantly different between the two groups, but when losses were calculated as a percentage of baseline measurements, the exercise group lost approximately twice the BMD at both the femoral neck and trochanter compared to the nonexercise group. These results indicate that in postmenopausal women, resistance training does not attenuate losses in bone mass from diet induced weight reduction and that the addition of resistance exercise training to an energy restricted diet may actually expedite losses of bone mass (Anderson et al. 1997).

Talbott and Shapses (1998) examined changes in biochemical markers of bone turnover in a group of male rowers. These 14 male subjects were placed on a 24-hour fast, including abstinence from vitamin and/or mineral supplements. Thirteen male control subjects were instructed to keep 24-hour food logs. Bone resorption was measured by total urinary pyridinium cross-links, and bone formation was measured by serum osteocalcin. Markers of bone formation and resorption were significantly reduced following this 24-hour fast indicating a quick metabolic response by bone cells to energy restriction. Among non-fasting controls, a wide range of energy was consumed during this 24-hour period resulting in negative energy balance for some control subjects. Through simple linear regression, a significant negative correlation was identified between energy intake and markers of bone resorption, and a significant positive association between energy intake and markers of bone formation, further indicating a relationship between energy consumption and bone metabolism (Talbot and Shapses 1998).

A 1995 study by Grinspoon and colleagues researched the effects of acute fasting and related acidosis on bone metabolism in young women. Fourteen women were placed on 4-day fasts. All subjects received a standard multivitamin containing vitamin D but not calcium. The group was divided into two subgroups. Test subjects received oral potassium bicarbonate to counteract acidosis. Controls were not provided with this neutralizing agent. Serum bicarbonate, calcium, ionized calcium, venous pH, and PTH were measured at baseline and at the end of the study period. From baseline to day five, bone turnover was assessed through changes in markers of bone formation [serum osteocalcin and type-I procollagen carboxy-terminal propeptide (PICP)] and markers of bone resorption [urinary pyridinoline (PYD) and deoxypyridinoline (DPD)]. Serum and total ionized calcium as well as urinary calcium excretion increased significantly in controls over the 4-day fast. Acidosis among controls was also related to a

significant reduction in PTH. A significant change in markers of bone resorption was not observed in either group. Interestingly, the short-term fasting caused a significant decline in markers of bone formation independent of acid-base balance. Results from this study indicate that acidosis may enhance mineral dissolution of bone, thereby causing changes in calcium balance that are not dependent on osteoclast activity or PTH. Also, regardless of acid-base balance, fasting reduces osteoblast activity (Grinspoon et al. 1995).

The majority of research investigating the impact of weight loss diets on BMD has utilized cohorts of overweight (Svendsen et al. 1993) or obese individuals (Anderson et al. 1997). Salamone and colleagues (1999) noted that such findings cannot appropriately be extrapolated to dieters of normal weight. Therefore, Salamone and coworkers (1999) examined changes in body weight through lifestyle intervention in normal weight ( $BMI \leq 24.44$ ), overweight ( $BMI = 24.45$  to  $26.44$ ) and obese ( $BMI \geq 26.45$ ) individuals. A group of 236 healthy, premenopausal women aged 44 to 50 years were randomly assigned to an intervention ( $n = 115$ ) or a control ( $n = 121$ ) group. Participants assigned to the intervention group were instructed to decrease dietary fat intake to less than 25% of total energy, as well as increase moderate aerobic activities (i.e., walking). Moderate weight loss goals were set according to initial BMI. Once weight loss was achieved, participants were instructed to gradually increase energy consumption in order to maintain weight. Supplemental calcium of 1,200 mg/d was encouraged. Whole body, lumbar spine, and total proximal femur (TPF) BMD measurements were performed by DXA at baseline and after 18 months. Serum osteocalcin and urinary N-telopeptides were measured at baseline and 18 months. Dieters significantly reduced dietary fat intake and increased physical activity and were, therefore, able to significantly decrease body weight (mean  $\pm$  SD;  $-3.2 \pm 4.7$  kg) in the form of fat mass ( $-3.5 \pm 4.2$  kg). The control group

maintained dietary intake and physical activity as well as weight throughout the 18 months. Over the course of this study, both groups had decreases in lumbar spine and TPF BMD. Among the dieting individuals, annual TPF BMD reduction was twice that of the control group ( $-0.81 \pm 1.3\%$  and  $-0.42 \pm 1.1\%$ , respectively). A similar, but non-significant pattern was noted for spinal BMD losses in the two groups ( $-0.70 \pm 1.4\%$  and  $-0.37 \pm 1.5\%$  in the weight-loss and control groups, respectively). Attenuation of spinal BMD losses were observed among individuals with the greatest increases in physical activity, but physical activity did not have a significant effect on loss of TPF BMD. Changes in biomarkers were not significantly different between the two groups, but among individuals who lost the greatest percentage of initial body weight ( $\geq 5.3\%$ ,  $n = 17$ ), changes in N-telopeptide were significantly greater than remaining intervention and control subjects. Significant differences for osteocalcin were not noted. Results from this study further support the negative association between weight loss and BMD. Moreover, these results may be better generalized to women who are not over weight or obese (Salamone et al. 1999).

Research has demonstrated negative effects of acute energy restriction on bone metabolism. It has been documented that chronically and severely undernourished individuals such as females with anorexia nervosa have markedly less bone mass than adequately nourished individuals (Mazess et al. 1990). Severe chronic undernutrition is known to decrease bone formation (Mazess et al. 1990). For example, females with anorexia nervosa have less BMD and BMC compared to age and gender-matched individuals with normal eating behaviors (Sundgot-Borgen et al. 1998). Mazess, Barden, and Ohlrich (1990) also found that the average total skeletal mineral content of a group of young-adult females with anorexia nervosa to be 25% less than that of young women without anorexia nervosa. One of the hallmark characteristics of

anorexia nervosa is loss of menstrual cycles or amenorrhea. With anorexia nervosa, estrogen levels drastically decline. This decrease in estrogen has a major impact on BMD.

### **Estrogen and bone**

A positive relationship between estrogen and BMD is well documented (“Position of the American...” 1999, Dombrowski 2000). Aside from natural aging, estrogen deficiency is probably the most recognized cause of bone loss among women (Dombrowski 2000). The largest gain in BMC and BMD occurs at puberty, coinciding with increases in estrogen (Bonjour et al. 1991, Dombrowski 2000). Estrogen is protective of bone throughout the gynecological life of a female (Dombrowski 2000). Loss of estrogen production during any stage of the female life cycle has devastating effects on bone (Wasnich 1996). With cessation of endogenous estrogen synthesis and a resultant lack of menstrual function, such as during lactation, menopause, or with anorexia nervosa, bone resorption increases while bone formation decreases (Gallagher et al. 1980). This results in a loss of BMC and BMD with an increased risk of fracture from osteoporosis. Amenorrhea, or extended loss of menstruation, simulates menopause in that there is a decrease in estrogen production and, like menopause, an increase in bone resorption leading to bone loss (Rigotti et al. 1991). Amenorrhea usually accompanies drastic weight loss and severe energy deficits (Cassidy 1999) but may also accompany weight loss or energy restriction in individuals who are maintaining a healthy weight (Rencken et al. 1996).

Estrogen replacement therapy in peri- and post-menopausal women has been shown beneficial in preventing bone loss and potentially increasing bone content (“Position of the American...” 1999, Prestwood et al. 1999). Oral contraceptive use enhances BMD among premenopausal females with amenorrhea, yet additive effects on BMD from oral contraceptive use in eumenorrheic females is controversial (MacDougall et al. 1999).



Estrogen is thought to both directly (Erikson et al. 1988) and indirectly (Gallagher et al. 1980) affect bone turnover. Concentration of biochemical markers of bone formation (Gorai et al. 1998) and calcium (Muneyyirci-Delale et al. 1998) fluctuate throughout the menstrual cycle. Direct effects of estrogen occur through the presence of estrogen receptors located on both osteoblasts (Erikson et al. 1988, Komm et al. 1988) and osteoclasts (Pensler et al. 1990). Receptor-mediated actions are likely responsible for changes in serum osteocalcin concentrations which correlate with serum estradiol (Nielsen et al. 1990).

Estrogen has an indirect effect on bone by augmenting calcitriol synthesis, thereby increasing intestinal absorption of calcium (Gallagher et al. 1980). Changes in serum concentrations of ionized magnesium occur throughout the menstrual cycle as well (Muneyyirci-Delale et al. 1998). This too is thought to occur in response to fluctuating estrogen concentrations (Muneyyirci-Delale et al. 1998). Estrogen helps regulate magnesium metabolism by enhancing cellular uptake and bone retention of magnesium (Seelig 1993). In postmenopausal osteoporosis, magnesium deficiencies are common due to low estrogen levels (Seelig 1990) and reduced intestinal absorption of magnesium (Cohen et al. 1983). Because magnesium is essential for proper functioning of the calcium regulating hormones, PTH and vitamin D (Seelig 1993), a magnesium deficiency may result in depletion of bone calcium content and decreased intestinal calcium absorption (Seelig 1993, Zofkova and Kancheva 1995). Therefore, it is necessary to ensure adequate magnesium intake among women receiving combined estrogen plus calcium therapy (Seelig 1993, Sojka 1995).

For many women, whether engaging in mild dieting behaviors or extremely restrictive practices such as in anorexia nervosa, high-fiber diets through increased fresh fruit and vegetable consumption with the exclusion of other foods are recommended. It has been reported that

individuals who consume high-fiber diets have increased fecal excretion of estrogens (Dorgan 1996, Goldin et al. 1981, 1982) and reduced circulating estrogen levels (Bagga 1995).

Considering the positive effects of estrogen on bone health (Rubin et al. 1999, Salamone et al. 1996), this is an area for further investigation.

In summary, dieting practices are common among young-adult females in the United States (Story et al. 1991, Grunewald 1985). Such dieting practices may have serious, negative consequences for short-term and long-term bone health. Better and more complete characterization of bone status of young-adult females who do or do not chronically diet is required before concrete recommendations can be provided for osteoporosis prevention among these females.

## **Methods of evaluating bone mineral and bone metabolism**

### General methods

Techniques used to study BMD and bone metabolism are useful for establishing baseline values for an individual or cross-sectional population as well as for monitoring changes in BMD or the rate of bone turnover with time. Investigative techniques are continually being developed and upgraded. Current methods frequently used to evaluate bone mineral and bone metabolism in research and clinical settings are described here.

### Bone mineral content and density

Dual energy X-ray absorptiometry is a highly validated tool for analyzing BMC and BMD (Shore and Posnanski 1996). Photons at two different energy levels, which are absorbed by body tissue, are admitted by DXA. Whole body and site-specific BMC and BMD, lean body mass, and fat mass can then be calculated. Compared to previous measurement tools (i.e., single photon absorptiometry, dual photon absorptiometry), DXA provides greater precision and

accuracy in a relatively short time with limited radiation exposure (Shore and Poznanski 1996). Bone scans from DXA technology are also useful for monitoring changes in BMC and BMD over a period of time or identifying individuals at risk of fracture (Cummings and Black 1995, Rizzoli et al. 1995).

Bone mineral content is a measurement of total body or site-specific bone mass, whereas BMD partially accounts for surface area of the site measured (Katzman et al. 1991). Bone mineral density measured by a single, two-dimensional DXA scan is referred to as areal BMD ( $\text{g}/\text{cm}^2$ ), because it is not a true volumetric ( $\text{g}/\text{cm}^3$ ) or three-dimensional measurement (Katzman et al. 1991). Volumetric BMD can be achieved through combination of anterior-posterior and lateral measurements, but these values are rarely found in published literature and make comparisons across studies difficult (Cassidy 1999).

#### Biomarkers of bone turnover

Bone turnover is the cycle of bone resorption and bone formation. The rates of formation and resorption can be measured by analyzing enzymatic activity of osteoblasts and osteoclasts or by quantifying components of bone matrix which appear in body fluids (Eyre 1996). Due to a wide range of normal values as well as limited sensitivity of measurement techniques, the usefulness of a single time point measurement in an individual remains in question (Delmas 1993). Measurements are valid for monitoring skeletal disease progression and adherence to treatment in an individual over time, as well as for population investigations (Beck-Jensen 1997)

Biomarkers of bone formation are metabolites of osteoblast activity measured in urine or serum. Osteoblasts are cells that produce the protein matrix of bone by forming a thin layer on an area of newly exposed bone surface (Puzas 1996). Receptors for PTH (McSheehy and Chambers 1986), vitamin D (Puzas 1996), and estrogen (Erikson et al. 1988) have been identified on the

surface and nuclei of osteoblasts. The most abundant non-collagenous bone matrix protein secreted by osteoblasts is osteocalcin, contributing 1% to 2% of total bone protein (Gundberg 1983). Osteocalcin is secreted by osteoblasts during bone formation and is subsequently incorporated into newly formed bone. During synthesis, a portion of newly formed osteocalcin spills into circulation (Erikson et al. 1995). Osteocalcin can be measured by radioimmunoassay (RIA; Delmas 1993). Because osteocalcin measured in serum is believed to be of *de novo* synthesis, rather than breakdown of bone matrix, and because osteocalcin is a useful marker of bone formation, it is perhaps the most highly validated marker of bone synthesis (Beck-Jensen 1997, Marcus 1996, Eriksen et al. 1995).

Biomarkers of bone resorption are metabolites of osteoclast activity measured in serum or urine. Osteoclasts are cells responsible for enzymatic breakdown of the protein matrix and dissolution of the crystalline mineral structure of bone (Baron 1996) with subsequent release of calcium. Receptors for calcitonin (Baron 1996) and estrogen (Erikson et al. 1988) have been located on the surface of osteoclasts which, when activated, inhibit osteoclast activity (Erikson et al. 1988). The lamellar structure of bone is formed by alternating alignment of collagen fibers (Baron 1996). These fibers are further strengthened by covalently crosslinked amino acid side chains formed from condensation reactions of lysyl and hydroxylysyl residues (Calvo et al. 1996). Two predominant crosslinks exist in type I collagen. N-telopeptide (NTx) is a crosslink of two aminopeptides located near residue 930; C-telopeptide (CTx) is a crosslink between two carboxytelopeptides near residue 87 (Calvo et al. 1996). Following osteoclastic bone resorption, degradation products are metabolized by the liver and kidney. Both NTx and CTx are small enough to be released into urine where they can be measured as markers of bone resorption (Calvo et al. 1996).

Enzyme-linked immunosorbent assay (ELISA) kits are available in which a monoclonal antibody is used to detect NTx in urine. This method has been shown useful in monitoring bone turnover. Compared to other markers of bone resorption, such as measurement of free or total pyridinolines by high-performance liquid chromatography, urinary NTx has shown greater increase at menopause and greater decrease in response to antiresorptive agents and estrogen therapy (Calvo et al. 1996). Commercial ELISA kits require less time than other measurement techniques because pretreatment or hydrolyses of samples are not needed (Calvo et al. 1996). Values are reported per mmol of creatinine (Calvo et al. 1996).

### **Summary**

Osteoporosis is a highly prevalent, devastating, and costly disease. Prevention of osteoporosis remains the most effective strategy for minimizing individual and global ramifications of osteoporosis. Ideally, proper prevention begins early in life, prior to achieving peak bone mass. By optimizing peak bone mass, an individual is better able to cope with natural periods of bone loss such as during menopause. Although it is thought that approximately 75% of the variability in peak BMD is genetically predetermined, additional factors are important for achieving a maximal bone mass level. It has been established that peak bone mass is achieved by the end of the third decade of life; however, few studies have investigated nutrition, lifestyle factors, and BMD of healthy females in the years just prior to peak bone mass.

Nutrition has both direct and indirect effects on bone. Many dietary minerals are housed in bone, lending to the structural support of bone. Nutrients are also necessary for regulation of bone cells and calcium-regulating hormones. Finally, energy and dietary components impact circulating estrogen levels, which in turn regulate bone metabolism. Most studies investigating the role of nutrition in bone health have utilized children or peri- and post-menopausal women.

Because bone metabolism changes throughout the life cycle, findings from one age group do not necessarily apply to another age group; therefore, the void of information regarding bone health in young-adult females must be filled through studies utilizing females from this particular age group. The need for additional investigations among young-adult females is supported by the abundance of information associating diet-induced weight reduction and losses in BMD. Such studies have utilized women of a wide age range with little focus to young-adult females. Due to the overwhelming prevalence of dieting among females, particularly college-aged females, there is a substantiated need for studies of nutrition, diet, and bone health directed toward young-adult females. The purpose of the present study, therefore, was to examine relationships between anthropometric, soft tissue mass, and dietary factors and BMD as well as biomarkers of bone turnover in a group of eumenorrheic, healthy, young-adult females who engaged in limited hours of moderate, hard, and very hard physical activity. A second study compared dietary intake, BMD, and biomarkers of bone turnover variables in young-adult females with chronic dieting habits to young-adult female nondieters.

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ANTHROPOMETRICS, BONE MINERAL DENSITY, AND DIETARY INTAKE IN A  
GROUP OF YOUNG-ADULT FEMALES<sup>1</sup>

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## Abstract

Associations between anthropometric, body composition, and dietary intake data and measures of bone mineral density (BMD) and biomarkers of bone turnover in 60 young-adult (age =  $20 \pm 1.9$  years) females were investigated. Fat-free soft tissue (FFST) mass, fat mass, total body (TB) and site-specific BMDs were measured by dual energy X-ray absorptiometry. Average daily dietary intakes of selected nutrients were estimated from a food frequency questionnaire. Serum osteocalcin and urinary N-telopeptide (NTx) were measured by radioimmunoassay and enzyme linked immunosorbent assay, respectively. Positive associations were found between: body weight and TB ( $p < 0.05$ ), lumbar spine (LS, L<sub>2</sub>-L<sub>4</sub>,  $p < 0.01$ ), and total proximal femur (TPF) BMD ( $p < 0.0001$ ); FFST mass and TB ( $p < 0.001$ ), LS ( $p < 0.01$ ), and TPF BMD ( $p < 0.0001$ ), and fat mass and LS ( $p < 0.05$ ), while LS BMD had a negative association with protein, magnesium, and iron intakes (all  $p < 0.05$ ). Vitamin D intakes had a positive association with ultradistal forearm BMD ( $p < 0.01$ ). Urinary NTx has a negative association with age ( $p < 0.001$ ). These data support the influence of body weight and soft tissue mass, particularly FFST mass, on BMD in young-adult females. Consumption of excess dietary protein may deter while dietary vitamin D contributes to site-specific BMD.

**KEY WORDS: BODY COMPOSITION, BONE MINERAL DENSITY, DIETARY INTAKE, SERUM OSTEOCALCIN, URINARY N-TELOPEPTIDES, YOUNG-ADULT FEMALES**

## Introduction

Osteoporosis has been well characterized as a prevalent and costly disease for older individuals. Involving low bone mass with increased risk for bone fractures, osteoporosis affects over 25 million Americans (1). Prevalence estimates for osteoporosis indicate that this disease will become even more widespread during the next few decades (2). While advances have been

made in pharmaceutical treatments for osteoporosis, these therapies are often expensive, require dedicated compliance, and result in adverse side-effects for many individuals (3). Prevention remains the most viable strategy for avoiding this disease and protecting the majority of women against osteoporosis-related bone fractures.

Attainment of peak bone mass is a primary goal for osteoporosis prevention (4,5). The rationale for maximizing bone mineral density (BMD) in the years up to the age of peak bone mass is to better allow a female to withstand natural periods of bone mineral losses that occur during midlife, menopause, and beyond menopause. Arriving in the postmenopausal years with more BMD will, theoretically, reduce the probability that a woman will suffer an osteoporotic bone fracture.

Current estimates suggest that peak bone mass is fully developed by the end of the third decade of life (4,6). Although genetics largely influence peak bone mass potential (7), roles for anthropometric variables (8,9) and lifestyle factors (4,10,11) in accrual of bone mass during the younger years have been shown. While a compelling body of evidence exists to support the positive relationships between body mass and BMD (8,9) and physical activity and BMD (4,11), dietary factors related to post-pubertal bone mass measures are less clear. Only a few studies have investigated dietary relationships with BMD among young-adult females (4,12-16). Moreover, these studies have focused only on a few selected nutrients (14,15) or ratios of nutrients (4,16), or have included young-adult females across a wide age range (12,14). Further investigation of relationships between dietary factors and bone mineral measurements are required to better characterize the role of nutrition during the final stage of bone mineral accretion.

Many females in the young-adult years are concerned with future health but are also susceptible to current dieting trends, body image concerns, and body weight dissatisfaction (17). Such trends and concerns may influence food consumption choices and patterns. Thus, further investigation of relationships between anthropometric measures, dietary intake, and bone mass at an age that is critical to the future health of the skeleton is essential. The current, cross-sectional study was designed, therefore, to identify anthropometric and dietary factors associated with BMD in a group of 18- to 25-year-old females.

## Materials and Methods

### Study participants

Caucasian females, aged 18 to 25 years, were recruited from the Virginia Polytechnic Institute and State University (VPI&SU; Blacksburg, Virginia, U.S.A.) campus and surrounding communities to participate in this cross-sectional investigation of nutrition and bone health in young adults. Flyers, electronic-mail announcements, and personal contacts were used as recruitment tools.

An investigator-designed general health questionnaire was used for initial screening of potential participants. Individuals were excluded if, during the previous year, they: (1) engaged in greater than five total hours of moderate, hard, and very hard physical activities per week, (2) experienced irregular menstrual cycles, (3) used oral contraceptives or other medications or substances known to effect bone metabolism, or (4) sustained a bone fracture. Additionally, females with metabolic disorders were excluded.

This research project was approved by the Institutional Review Board for Research Involving Human Subjects at VPI&SU. Prior to engaging in the study protocol, each participant

read an informed consent form, asked questions if desired, received answers from an investigator to questions, if applicable, and provided written informed consent.

### Procedures

During a two-hour testing session, anthropometric, body composition, dietary intake, physical activity, and personal data were collected. Each participant also provided fasting blood and second-void urine samples. The study protocol was carried out in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation Laboratory, VPI&SU. All data were collected between September 10, 1999, and April 13, 2000. Sixty participants completed all testing procedures and were included in this analysis.

### Anthropometric data

Body height was measured with a wall-mounted digital stadiometer (Heightronic™, Measurement Concepts, North Bend, WA, U.S.A.). A calibrated electronic scale (Scaletronix, Wheaton, IL, U.S.A.) was used to measure body weight. Each participant was lightly clothed and shoeless during height and weight measurements. Standing heights and weights were recorded to the nearest 0.01cm and 0.01 kg, respectively. Body height and weight measurements were used to calculate body mass index (BMI) as weight in kg/height<sup>2</sup> in m (BMI = kg/m<sup>2</sup>), for each participant.

### Bone mineral density and soft tissue mass measurements

Total body (TB), lumbar spine (LS, L<sub>2</sub>-L<sub>4</sub>), total proximal femur (TPF), including the femoral neck (FN), trochanter (Troch), and Ward's triangle (WT), and total forearm (TF, radius + ulna, including the ultradistal, mid, and proximal one-third forearm) BMD (g/cm<sup>2</sup>) were measured by dual-energy X-ray absorptiometry (DXA, QDR-4500A; Hologic, Inc., Bedford, MA, U.S.A.) using version 8.25a of the Whole Body Fan Beam software and standard spine, hip,

and forearm protocols, respectively. Fat-free soft tissue (FFST) mass, fat mass, and percent body fat (BF) were calculated from the TB scan. All scans were analyzed by a single investigator. Quality control procedures were completed prior to testing on each testing day throughout the duration of the study. A phantom spine was scanned 49 times during the seven months of testing, with a coefficient of variation (CV) of 0.34%.

### Dietary intake

Participants completed a food frequency questionnaire (FFQ; 18) in an interview format. Participants were asked to indicate how frequently and in what quantity individual food items were consumed during the previous 12 months. Three-dimensional food models were used during these interviews to limit variations and to facilitate accuracy in portion size responses. Intake of vitamin and/or mineral supplements during the previous 12 months was also recorded. Average daily nutrient intakes from consumption of foods included in the Block98.2 FFQ and supplements were estimated with the DIETSYS + Plus software (Block Dietary Data Systems, Berkeley, CA, U.S.A., version 5.9).

### Biochemical markers of bone remodeling

After an overnight fast, blood and second-void urine samples were collected from participants between 0800 and 1100 hours to reduce diurnal variation. Within 30 minutes of venous blood draws, samples were centrifuged at 1070 x g for 12 minutes, after which, serum was pipetted into cryovials and frozen at -80° C until later analysis. Serum osteocalcin was measured in batches by radioimmunoassay (RIA; Human Osteocalcin RIA I<sup>125</sup> Kit, Biomedical Technologies, Staughton, MA, U.S.A.). All samples were analyzed in duplicate. The intra- and inter-assay CV for osteocalcin were 7.91% and 7.86%, respectively.

Within one hour of collection, urine samples were pipetted into cryovials and frozen at -80° C until later analysis. Cross-linked N-telopeptide of type I collagen (NTx) was measured by enzyme-linked immunosorbent assay (ELISA; Osteomark, Seattle, WA, U.S.A.). Urinary creatinine was measured by quantitative spectrophotometry (#555A, Sigma Diagnostics, St. Louis, MO). Urinary NTx measurements were reported as bone collagen equivalents (BCE) per mM creatinine. All samples were analyzed in duplicate. The intra- and inter-assay CV were 4.10% and 5.82% respectively.

#### Hormonal assessment

To further support eumenorrheic status of this group, as well as investigate associations between sex-steroid hormones and markers of bone turnover, each participant was asked to recall the first day of her last menstrual period. Serum estradiol (E<sub>2</sub>) and serum progesterone (P<sub>4</sub>) were measured by RIA (Coat-A-Count® Estradiol and Coat-A-Count® Progesterone, respectively, Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). All samples were analyzed in duplicate. The intra- and inter-assay CV for E<sub>2</sub> were 7.0% and 7.9%, respectively. The intra- and inter-assay CV for P<sub>4</sub> were 5.6% and 6.4%, respectively.

#### Physical activity

Participants completed the seven-day physical activity recall (19) in an interview format. This questionnaire was used only to confirm that each participant had not engaged in more than five hours of moderate, hard, and very hard physical activity during the previous week. All 60 participants remained eligible for this study despite this secondary check on recent physical activities.

### Personal data

Each participant self-reported date of birth, date of onset of most recent menstrual cycle, and range of menstrual cycles per year. These variables were used only to confirm a chronological age within the limited age range and normal menstrual cycling (i.e., menses every 26 to 30 days).

### Statistical analyses

Means and standard deviations (SD) were computed for each variable of interest. Pearson correlation coefficients were computed to examine the bivariate relationship between individual anthropometric, soft tissue mass, and nutrient intake variables and BMD measures and biochemical markers of bone formation and resorption. Concentrations of E<sub>2</sub> and P<sub>4</sub> were charted according to day since onset of menstruation. Statistical analyses were completed using the Statistical Analysis System (SAS, SAS Institute, Cary, NC, U.S.A., version 8.0).

### Results

The mean ( $\pm$  SD) age of participants was  $20.3 \pm 1.9$  years. All participants classified themselves as Caucasian with the exception of two individuals with diverse ancestry. One of these participants was of Caucasian maternal descent while the second was of Caucasian paternal descent; thus, these two participants were included in the final analyses, as their exclusion did not change the significance of results.

### Anthropometrics, bone mineral density, and soft tissue mass

Selected characteristics of participants appear in Table 1. According to BMI, four participants were underweight (BMI  $\leq$  19.0), 42 were of normal weight (BMI = 19.1 to 25.0), 12 were overweight (BMI = 25.1 to 29.9), and two were obese (BMI  $\geq$ 30.0; 20).

TABLE 1. Selected characteristics of participants\*.

Variable	Mean $\pm$ SD	Range
Age (years)	20.3 $\pm$ 1.9	18 – 25
Height (m)	1.66 $\pm$ 0.07	1.50 – 1.80
Weight (kg)	63.4 $\pm$ 12.3	46.8 – 125.2
Body mass index (kg/m <sup>2</sup> )	22.9 $\pm$ 4.1	17.8 – 41.0
Total body BMD <sup>†</sup> (g/cm <sup>2</sup> )	1.101 $\pm$ 0.061	0.981 – 1.225
Lumbar Spine BMD (g/cm <sup>2</sup> )	1.116 $\pm$ 0.104	0.922 – 1.376
Total proximal femur BMD (g/cm <sup>2</sup> )	0.967 $\pm$ 0.091	0.758 – 1.228
Femoral neck BMD (g/cm <sup>2</sup> )	0.866 $\pm$ 0.084	0.664 – 1.059
Trochanter BMD (g/cm <sup>2</sup> )	0.733 $\pm$ 0.089	0.554 – 1.017
Ward's triangle BMD (g/cm <sup>2</sup> )	0.819 $\pm$ 0.113	0.611 – 1.118
Total forearm BMD (g/cm <sup>2</sup> )	0.548 $\pm$ 0.038	0.430 – 0.619
Fat-free soft tissue mass (kg)	42.3 $\pm$ 5.4	31.7 – 66.7
Fat mass (kg)	19.4 $\pm$ 7.8	10.4 – 56.8
Body fat (%)	29.6 $\pm$ 5.6	20.5 – 45.0
Osteocalcin (ng/ml)	41.3 $\pm$ 9.0	23.6 – 64.4
NTx <sup>¶</sup> (nM BCE <sup>‡</sup> /mM creatinine) <sup>§</sup>	77.8 $\pm$ 35.9	5.1 – 188.0

\* N = 60.

<sup>†</sup> BMD, bone mineral density.

<sup>¶</sup> NTx, Urinary N-telopeptide cross-link.

<sup>‡</sup> BCE, bone collagen equivalents.

<sup>§</sup> n = 59.



Mean BMD and soft tissue mass measurements of participants are also presented in Table 1. Twenty-three participants (38.3%) had greater than 30% body fat - the upper limit of desirable for women (20); however no participants had a body fat percentage below 20% or below the desired minimum.

Table 2 presents the average daily dietary intakes of selected nutrients. Participants consumed approximately 55%, 14%, and 31% of total energy from carbohydrates, proteins, and lipids, respectively. Seventy-six percent (n = 46) of participants exceeded the dietary protein recommendation of 46 g/d for women of this age group. Sixty percent (n = 36) of participants exceeded 30% of total energy from dietary lipids. Nearly 22% (n = 13) of participants consumed less than 66% of the Adequate Intake (AI) for dietary calcium from food sources alone, while approximately 88% (n = 53) of participants consumed more phosphorus than recommended. Average vitamin D consumption was adequate only when supplement sources were considered. Forty-five percent (n = 27) of participants did not meet 66% of the recommended amount of dietary zinc intake when estimating intake from foods; however, when supplemental zinc was included, only approximately 28% (n = 17) remained below 66% of the dietary recommendation. Mean intake of magnesium met the recommendation when food sources and supplements were estimated. On average, participants consumed adequate dietary iron from food sources, and with the addition of supplemental iron, 20% (n = 12) of participants exceeded the dietary recommendation by two-fold. The mean dietary sodium intake slightly exceeded the recommendation of  $\leq 2400$  mg per day.

TABLE 2. Mean daily dietary intake of selected macronutrients and micronutrients.

Variable	Mean $\pm$ SD	Range
Energy (kJ)	7,913 $\pm$ 2,557	3,791 – 13,987
Carbohydrate (g)	261 $\pm$ 93	122 – 506
Protein (g)	65 $\pm$ 24	21 – 120
Lipid (g)	67 $\pm$ 25	24 – 116
D* Calcium (mg)	992 $\pm$ 468	268 – 2,124
D + S <sup>†</sup> Calcium (mg)	1,203 $\pm$ 569	398 – 2,620
D Phosphorus (mg)	1,223 $\pm$ 436	523 – 2,228
D Vitamin D (IU)	132 $\pm$ 103	12 – 552
D + S Vitamin D (IU)	269 $\pm$ 199	23 – 742
D Zinc (mg)	9.5 $\pm$ 3.9	3.0 – 18.0
D + S Zinc (mg)	14.9 $\pm$ 7.6	3.0 – 33.0
D Magnesium (mg)	275 $\pm$ 106	86 – 551
D+ Z Magnesium (mg)	309 $\pm$ 115	86 – 623
D Iron (mg)	14.8 $\pm$ 6.1	4.0 – 33.0
D + S Iron (mg)	23.4 $\pm$ 16.6	4.0 – 93.0
Sodium(mg)	2,773 $\pm$ 1,084	1,065 – 6,652
Fiber (g)	18 $\pm$ 9	6 – 52

\* D, from dietary sources alone.

<sup>†</sup> D + S, from dietary plus supplemental sources.

### Biochemical markers of bone turnover and hormone levels

Mean values for biochemical markers of bone remodeling are included in Table 1. One outlying value for NTx was measured; thus, this participant's NTx level was excluded from statistical analyses (NTx, n = 59).

As a group, hormone status was indicative of normal menstrual function as indicated by concentration pattern of E<sub>2</sub> (Figure 1) and P<sub>4</sub> (Figure 2).

### Correlation analyses

Pearson correlation coefficients of anthropometric and soft tissue mass measurements with selected BMD measurements are shown in Table 3. Table 4 includes associations between selected BMD measures and average dietary intakes of nutrients of interest. With the exception of magnesium, which had a negative and significant association with TB BMD, negative, although nonsignificant, associations were found between these selected nutrients and TB BMD. Magnesium intake also had a significant negative association with LS BMD as did dietary protein and iron (from food sources) intakes. Although all associations were not significant for nutrient intakes and TPF BMD, only dietary calcium (from food and supplements) and dietary zinc (from food and supplements) intakes had positive relationships with TPF BMD. Dietary intakes of nutrients were not significantly related to TF BMD. Vitamin D intake from food sources and supplements had a positive association with BMD of the ultradistal forearm (see Figure 3).

Significant associations were not found between serum osteocalcin and urinary NTx levels and anthropometric variables; however, NTx had a negative association with age (see Figure 4). Serum osteocalcin did not have significant relationships with any BMD measurements. Urinary NTx had a significant negative association with LS BMD ( $r = -0.36$ ;

Figure 1. Serum estradiol concentration versus days since onset of most recent menstruation.

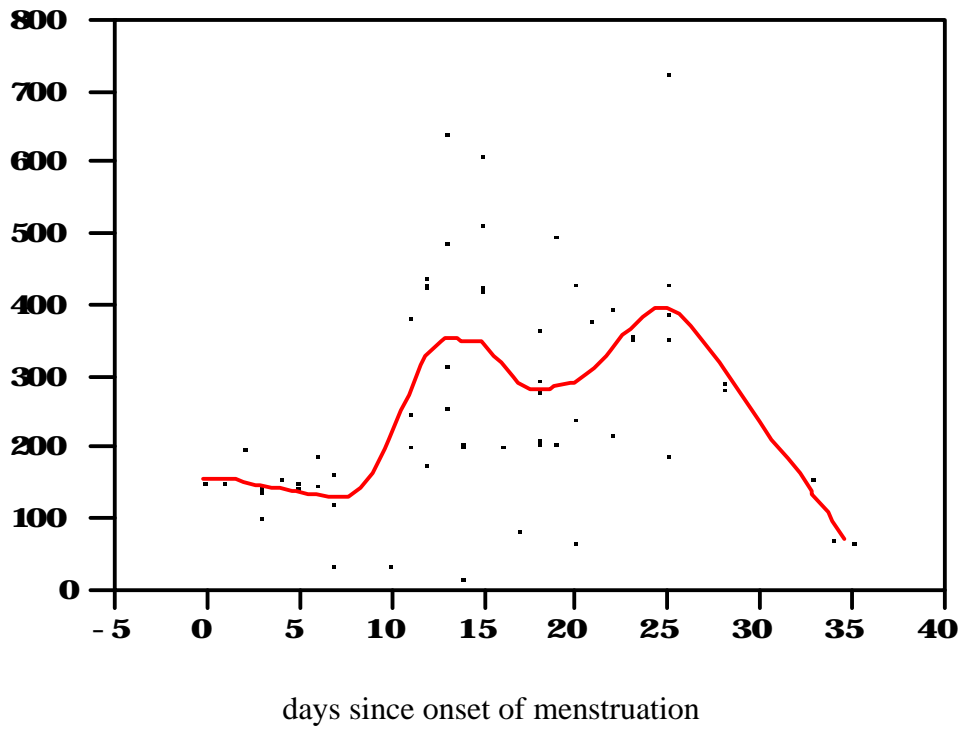


Figure 2. Serum progesterone concentration versus days since onset of most recent menstruation.

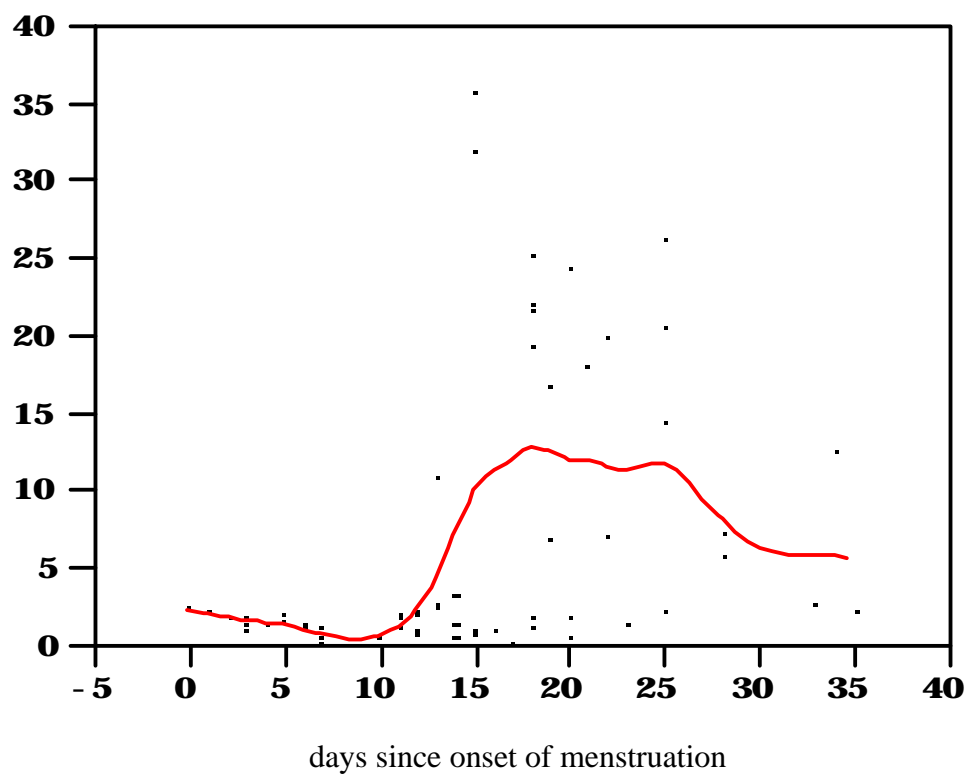


TABLE 3. Pearson correlation coefficients for relationships between anthropometric and soft tissue mass measurements and selected bone mineral density (BMD) measurements.

Anthropometric and soft tissue mass measure						
BMD measure	Height	Weight	BMI <sup>†</sup>	FFST <sup>‡</sup> mass	Fat mass	Body fat %
Total body	0.23	0.28*	0.21	0.42***	0.13	-0.07
Lumbar spine	0.18	0.36**	0.32*	0.36**	0.30*	0.19
Total proximal femur	0.06	0.48****	0.48****	0.52****	0.38**	0.20
Total forearm	-0.17	0.24	0.33**	0.20	0.23	0.21

\* p < 0.05.

\*\* p < 0.01.

\*\*\* p < 0.001.

\*\*\*\* p < 0.0001.

<sup>†</sup>BMI, body mass index.

<sup>‡</sup>FFST, fat-free soft tissue.



TABLE 4. Pearson correlation coefficients for associations between bone mineral density (BMD) measurements and mean intakes of selected nutrients.

Nutrient	BMD measure			
	TB <sup>a</sup> BMD	LS <sup>b</sup> BMD	TPF <sup>c</sup> BMD	TF <sup>d</sup> BMD
Energy	-0.22	-0.13	-0.06	-0.04
Carbohydrate	-0.22	-0.17	-0.04	-0.10
Protein	-0.24	-0.28*	-0.13	-0.06
Lipid	-0.19	-0.05	-0.07	0.06
D <sup>e</sup> Calcium	-0.16	-0.24	-0.12	-0.02
D + S <sup>f</sup> Calcium	-0.12	-0.12	0.01	-0.07
Phosphorus	-0.20	-0.24	-0.09	0.04
Vit. D	-0.08	-0.17	-0.07	0.04
D + S Vit. D	-0.08	-0.14	-0.07	0.10
Zinc	-0.20	-0.24	-0.08	0.04
D + S Zinc	-0.06	-0.15	0.11	0.05
Magnesium	-0.31*	-0.31*	-0.22	0.11
D + S Magnesium	-0.27*	-0.31*	-0.14	-0.06
Iron	-0.25	-0.29*	-0.16	0.04
D + S Iron	-0.09	-0.24	-0.10	-0.08
Sodium	-0.23	-0.21	-0.07	0.04
Fiber	-0.24	-0.25	-0.18	-0.10

<sup>a</sup>TB = total body, <sup>b</sup>LS = lumbar spine, <sup>c</sup>TPF = total proximal femur, <sup>d</sup>TF = total forearm,

<sup>e</sup>D = dietary sources, <sup>f</sup>D + S = dietary + supplemental sources.

\*p < 0.05.

Figure 3. Relationship between total dietary intake of vitamin D (IU) and ultradistal forearm BMD (g/cm<sup>2</sup>).

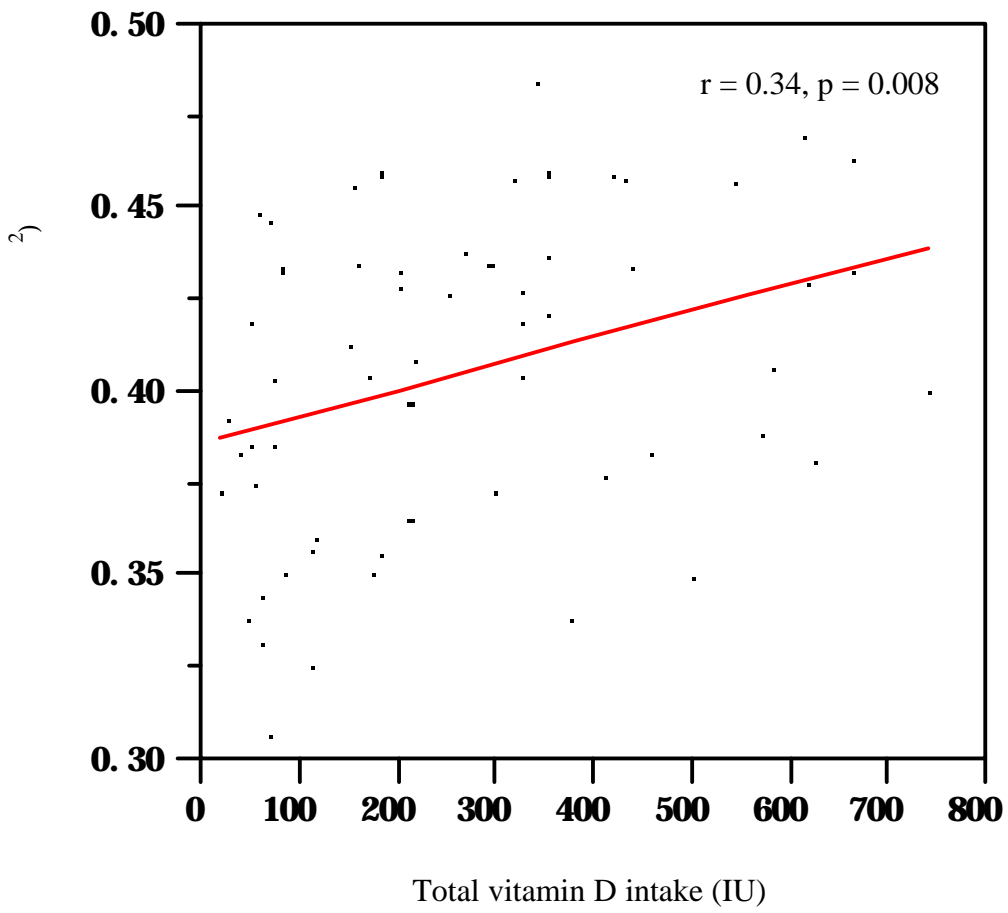
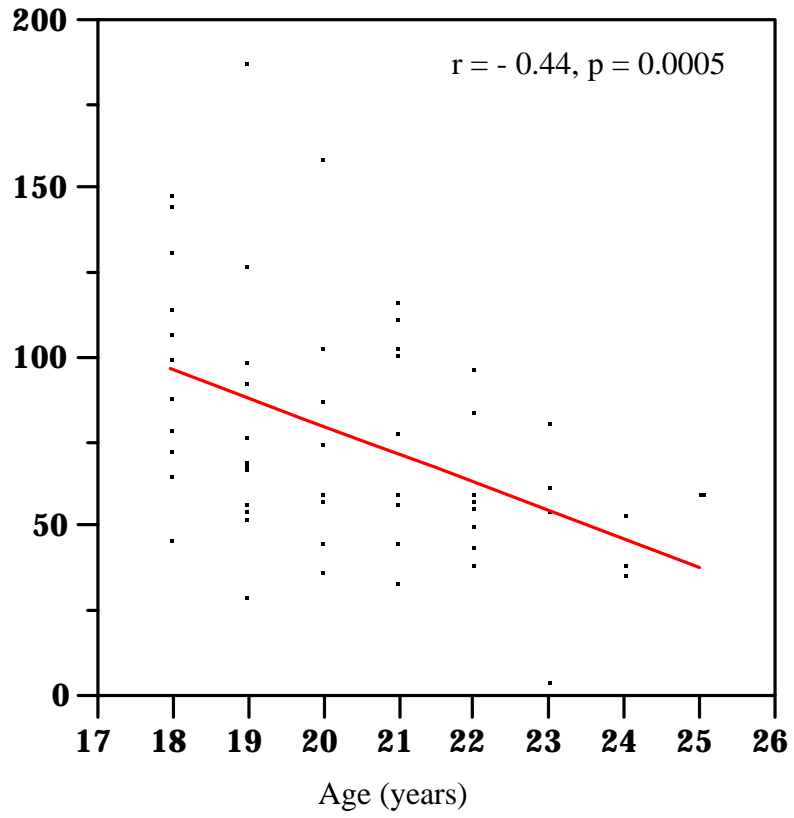


Figure 4. Urinary N-telopeptide crosslinks (NTx; BCE<sup>\*</sup>/mM creatinine) versus age (years).

\*BCE = Bone collagen equivalents.



p = 0.005). Serum osteocalcin and urinary NTx did not have significant associations with any selected nutrients.

## Discussion

Few studies have investigated associations between anthropometric, soft tissue mass, and dietary intakes and comprehensive BMD measurements in healthy young-adult females. Thus, this study provides insight into BMD and related variables in a group of females approaching peak bone mass. Furthermore, this study is unique in that dietary variables are not limited to those nutrients most commonly reported in studies of nutrition and BMD (i.e., calcium, phosphorus, and vitamin D), but rather, includes other nutrients that may have direct or indirect effects on BMD.

Among eumenorrheic young-adult females, aged 18 to 25 years, who engage in fewer than five hours of moderate, hard, and very hard physical activities per week, TB BMD is associated with body weight and FFST mass. Moreover, LS BMD and TPF BMD are also associated with body weight, BMI, FFST mass, and fat mass, while TF FMD is only associated with BMI. Findings from the current study support other studies in which body weight has demonstrated positive associations with BMD measurements (8,9,16). Associations between body weight and BMD are likely related to the mechanical load placed on bone from support and locomotion of body weight that stimulates gains in BMD. The relationships between BMI and LS, TPF, and TF BMD are expected given the dependence of BMI measures on weight. Positive associations between fat mass and LS and TPF BMD are supported by other investigations as well (12-14).

The statistical significance of TB, LS, and TPF BMD and FFST mass associations are expected and supported elsewhere (16). Among young-adult females, FFST mass may provide

greater mechanical forces on bone compared to fat mass weight alone, and thereby, enhance BMD. Body fat percent did not have a statistically significant relationship with BMD measures perhaps suggesting that high body weight and high BMI, specifically in the form of FFST mass, may be important for high BMD in the weight bearing regions of the skeleton such as the hip and spine in young-adult females. The hypothesis that FFST mass is critical to the attainment of peak BMD has been supported by other studies (16).

Several investigations provide evidence of a direct relationship between energy restriction and bone resorption (21-25). These studies used a variety of methods including very-low-calorie diets (21), short-term fasting (22,24), long-term moderate energy restriction (23), and high-fiber, low-fat diets (26) to induce energy restriction and, subsequently, weight loss. Alterations in bone turnover follow fasting (24) as well as reductions in BMD with energy-restriction and weight loss (27,28) have been observed. Collectively, these studies suggest that adequate energy is required to support and preserve BMD. The present study did not find a statistically significant positive association between energy intake and BMD, however. Interestingly, the average energy intake for participants in the present study was 1,731 kJ below estimated energy needs. Because the present study was not a longitudinal study of energy restriction and BMD, conclusions cannot be drawn about the impact of the level of energy intake on BMD at various body sites. Nonetheless, the lack of a significant association between energy intake and BMD may indicate that habitually low energy intake may alter bone metabolism responses and BMD such that a new, lower BMD level is established or that maximum BMD cannot be achieved. For example, the average BMD values for this group of young-adult females were slightly below BMD values reported elsewhere for normal, healthy Caucasian women of similar age (4,29). Further investigations of chronic dieting habits among young-adult

females are warranted to better characterize the relationships between sheer energy shortage and BMD.

Both positive (16,30) and negative (15) associations have been reported between protein intake and BMD. Adequate dietary protein intake is needed for proper synthesis, maintenance, and repair of bone, yet at elevated intakes, dietary protein increases urinary calcium excretion and may alter calcium balance (31). The mean protein intake of participants in the present study was above the recommended level, and protein had a negative correlation with LS BMD, suggesting that the high dietary protein intake may have resulted in high renal calcium excretion and low BMD at the LS. Because dietary protein intake is linked to calcium homeostasis, adequate dietary calcium intake is also important.

Although a large number of investigations show a positive relationship between dietary calcium intake and BMD (15,16,32–34), not all studies do (7,10,13). In the present study, dietary calcium intake, regardless of inclusion of supplemental calcium was not related to any BMD measure. Dietary calcium intake has been shown to be less important than physical activity (11,35) or estrogen status (36,37) to BMD in young-adult females. Given that physical activity and estrogen status were homogenous in the group of participants in the present study, dietary calcium may have been less related to BMD than these other factors. Additionally, dietary calcium is only one component of the diet important for BMD.

Phosphorus is also a fundamental nutrient required by bone to support hydroxyapatite formation. Phosphorus is associated with a reduction in protein-induced calcium excretion (38), yet at high intakes, phosphorus can increase fecal calcium losses as well as lower the serum ionized calcium concentration which results in PTH-induced resorption of bone (39,40). Dietary phosphorus intake was not related to BMD at any body site. Although the mean phosphorus



consumption was high in this group of young-adult females, relationships between the calcium:phosphorus ratio and BMD measures were not found.

Independent and interdependent relationships exist among protein, calcium, and phosphorus that impact BMD. For example, Teegarden and colleagues (16) identified independent, positive associations between dietary calcium, protein, and phosphorus intakes and BMD of the spine and forearm, but when using the calcium:protein ratio or the calcium:phosphorus ratio as independent variables, relationships to BMD were not found. These investigators concluded that there was not a single, overall ratio for these nutrients that was optimal for bone health. Regression equations used to predict TB and LS BMD supported their conclusion by showing that an increase in dietary calcium, while maintaining protein and phosphorus, predicted an increase in BMD and that an increase in protein and phosphorus in proportion to calcium, predicted a decrease in BMD. Moreover, increasing phosphorus without increasing calcium did not predict a reduction in BMD (16).

In contrast to Teegarden's et al. (16) study, Recker and colleagues (4) found that the calcium:protein ratio was the strongest predictor of changes in LS BMD over time. Metz and coinvestigators (15) identified similar relationships between the calcium:protein ratio and regional forearm BMD. In the present study, the ratios of calcium:phosphorus and calcium:protein did not show significant relationships to BMD. However, after elimination of one outlier with a total calcium:protein ratio of nearly twice that of the upper quartile average, a significant relationship was found between the calcium:protein ratio and TPF BMD ( $n = 59$ ;  $r = 0.26$ ;  $p < 0.05$ ) and WT BMD ( $n = 59$ ;  $r = 0.26$ ;  $p < 0.05$ ).

It is clear that vitamin D is essential to BMD (41,42) through its modulation of dietary calcium absorption (43) and influence on bone cells (44). Vitamin D deficiency among older

individuals is associated with low spinal BMD (41), most of which is trabecular bone. The ultradistal region of the forearm is also primarily composed of trabecular bone and may also be highly influenced by dietary vitamin D intake. Thus, although the TF BMD was not associated with dietary vitamin D intake, the ultradistal portion of the forearm was. This suggests that adequate dietary vitamin D intake is important to BMD not only in postmenopausal women but in young-adult females as well.

*In vivo* studies show that zinc stimulates bone synthesis by augmenting osteoblast activity, but at high intakes, zinc disrupts bone structure and reduces overall bone strength (45). Among humans, high supplemental doses of zinc (140 mg/d) reduce intestinal calcium absorption when dietary calcium intake is low ( $\leq 30\%$  RDA; 46). In animal models, addition of zinc to a low-calcium diet, has an additive effect of reducing bone strength (47). In the present study, dietary zinc intake did not have a significant association with any measure of BMD. Mean dietary zinc intake (diet + supplements) was only marginally above the RDA. Perhaps at “subsistence” levels, dietary zinc intake has neither a positive nor a negative association with BMD in light of other nutrients which compete with zinc for absorption, metabolism, and incorporation into bone.

Magnesium is essential for normal functioning of the calcium-regulating hormones that maintain calcium homeostasis (48,49). New and colleagues (50) provide evidence for an indirect relationship between magnesium and markers of bone resorption. A significant negative relationship was found between energy-adjusted magnesium intake as quantified from a FFQ and excretion of pyridinoline cross-links (50). But it is also apparent that at high intakes of calcium, absorption of magnesium may be inhibited (51). Because both magnesium and calcium are essential to bone, yet may interact with one another, it may be concluded that appropriate intakes

of both nutrients are essential for optimal bone health and that an imbalance in either direction can impair bone metabolism. In the present study, dietary magnesium intake had a negative association with both TB and LS BMD. The level of dietary magnesium intake may have been modulated by dietary calcium such that magnesium had an unexpected association with TB and LS BMD.

The role of iron in bone is not well established. Iron has been shown to interact with other minerals found in bone, such as zinc, however (52). Because dietary zinc and iron compete for absorption, high intake of one may inhibit absorption of the other. In the present study, dietary iron had a negative association with LS BMD; however, this relationship was lost when supplemental iron was added to dietary intake. The relevance of dietary iron intake to BMD requires further investigation, but it is important given that many young-adult females consume grossly inadequate levels of dietary iron (53).

High dietary sodium intake increases urinary calcium excretion (31) and is, therefore, thought to be detrimental to bone at excessive intakes. Among the young-adult females in this study, the average sodium intake was moderate and did not have a significant association with any measure of BMD.

Diets high in fiber may adversely affect BMD by inhibiting absorption of minerals essential to bone, particularly calcium (54-56). Fiber intake among this group of females was below recommendations and, thus, might explain why a significant relationship between fiber and BMD was not found.

Assessing the relationships between dietary intakes of single nutrients, ratios of nutrients, and BMD is difficult due to the complexity of food-nutrient and nutrient-nutrient interactions in self-selected diets. Moreover, these interactions are complicated by the addition of vitamin

and/or mineral supplements. More extensive cross-sectional studies that investigate self-selected diets and BMD are needed. Presently, data from longitudinal investigations of changes in dietary intake relative to BMD are limited due to the “controlling” of dietary intake and are, therefore, not often insightful as to the role of self-selected diets on BMD among populations (21,23,26,28). Studies of energy restriction are either of short duration with extreme energy deficit (22,24) or of long duration accompanied by significant weight reductions (23,26). Critically lacking are investigations of associations between long-term energy deficits (or “chronic dieting”) and BMD among young-adult females. Chronic dieting is a continuous or repeated reduction in energy intake that may not be associated with weight loss (57-59). Such diets are highly prevalent among women in western societies (57,60). Nutrients included in the present study paralleled energy intake, and because some unexpected relationships were observed between intakes and BMD, it is important that further research be conducted on self-selected, low energy diets and bone health.

Significant relationships between bone biomarkers and dietary variables were not found. Associations between dietary intake and bone biomarkers may have been modulated through reproductive hormones as nutrient status is related to menstrual function (61,62). Biomarkers of bone turnover fluctuate throughout the menstrual cycle (61,63), and although serum estradiol and progesterone were analyzed in the present study and function appeared normal as a group, significant associations were not observed between estradiol, progesterone, biomarkers of bone turnover, and dietary intake. The Block98.2 FFQ is a tool used to estimate average daily dietary intake of nutrients from recollection of intake over the previous 12 months rather than more recent intake; in contrast, bone biomarkers are relatively current indicators of bone remodeling.

Urinary NTx was directly associated with age. This finding supports Rauch and colleagues' (64) study of excretion of hydroxypyridinium crosslinks in individuals aged 4 to 25 years of age. Rauch et al. (64) found that the concentration of crosslinks was highest in individuals near puberty. The oldest individuals (aged 20 to 25 years) had the lowest concentrations.

The present study has several limitations. Although anthropometric measurements of participants were similar to measures reported elsewhere for women of this age range (4), BMD variables were lower than other reported values of age-matched individuals (15,16). This may be due to the study's selection criteria that limited physical activity. Secondly, bone mass is accumulated during the developmental years. Because this study included a retrospective account of just one year in that developmental process, physical activity, dietary intake, lifestyle habits, and overall health during the previous year may not adequately reflect current BMD status. As with any study, results may be influenced by the investigative techniques. Although FFQs are widely used and have been validated (65,66), several limitations to this method of dietary recall exist including memory, estimation accuracy, and nutrient database precision.

Among these 60 eumenorrheic young-adult females engaging in limited hours of moderate, hard, and very hard physical activities, body weight, BMI, FFST mass, and fat mass all have positive associations with BMD. In particular, TPF BMD is most significantly related to these anthropometric and soft tissue variables suggesting that overall body mass, specifically FFST mass, places added mechanical force on this body site. Dietary protein, magnesium (dietary and dietary + supplement), and iron (dietary only) intakes all have significant negative associations with LS BMD. Additionally, dietary magnesium intake (dietary and dietary + supplement) has a negative relationship with TB BMD. Although the association with dietary

protein intake and LS BMD was expected, the lack of association between other BMD measures and other nutrients and the significant negative relationships with magnesium and iron and BMD were unexpected. It is necessary to further study self-selected diets and their relationships to BMD. Because intakes of individual dietary components paralleled energy consumption, future research that investigates bone health of individuals consuming diets that are adequate and inadequate in energy are necessary. Finally, because inconsistent findings regarding dietary intake and BMD in young-adult females exist, further research regarding BMD in females approaching peak bone mass is warranted.

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DIETARY INTAKE AND BONE MINERAL DENSITY IN YOUNG-ADULT FEMALE  
CHRONIC DIETERS AND NONDIETERS<sup>1</sup>

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<sup>1</sup> Beiseigel JM, Nickols-Richardson SM. 2000. To be submitted to the Journal of the American Dietetic Association.

## Abstract

**Objective** To compare variables that impact bone mineral density (BMD) in young-adult female chronic dieters versus nondieters.

**Design** A cross-sectional study design was selected to identify differences in mean dietary intakes, body composition measures including BMD, biochemical markers of bone turnover, and anthropometric measures between chronic dieters and nondieters. Selected nutrient intakes were estimated from food frequency questionnaires analyzed with the DIETSYS+Plus Analysis Software (version 5.9, 1999, Block Dietary Data Systems, Berkeley, CA). Whole body (WB) dual energy X-ray absorptiometry (DXA; versions 8.25a, 2000, Whole Body Analysis software, QDR 4500A, Hologic Inc., Bedford, MA) scans were completed to measure fat-free soft tissue (FFST) mass, fat mass, and percent body fat as well as WB BMD. Additionally, BMD of the lumbar spine (LS, L<sub>2</sub>-L<sub>4</sub>), nondominant total proximal femur (TPF), and nondominant total forearm (TF) were measured by DXA using standard site-specific spine, hip, and forearm protocols and software (Hologic, Inc., Bedford, MA). Serum osteocalcin was measured by radioimmunoassay (Human Osteocalcin RIA I<sup>125</sup>, Biomedical Technologies, Staughton, MA), while urinary N-telopeptide (NTx) was measured by enzyme-linked immunosorbent assay (Osteomark, Seattle, WA). Body weight and height were measured on an electronic scale and next to a wall-mounted digital stadiometer, respectively, and body mass index was calculated from body weight and height. Each participant's energy need was estimated based on gender, age, body height, body weight, and activity level and calculated with The Food Processor® Nutrition Analysis Software profile system (version 7.4, 1999, esha Research Inc., Salem, OR). A chronic dieter was defined as an individual who consumed < 67% of her estimated energy

need, while a nondieter was defined as an individual who consumed  $\geq 67\%$  of her estimated energy need.

**Subjects/setting** Young-adult female chronic dieters (n = 18) and nondieters (n = 38) were included in this study. Exclusion criteria included younger than age 18 or older than age 25, weekly participation in more than five total hours of moderate, hard, or very hard physical activity, irregular menstrual cycles, use of medications or substances with known effects on bone metabolism, or bone fracture(s) during the previous 12 months. Females with metabolic diseases were also excluded. All participants were Caucasian ethnicity. Testing procedures were completed in the Bone metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory and adjunct facilities, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

**Statistical analyses performed** Means and standard errors of the means were calculated for study variables according to group. Student's t-tests were completed to compare group differences for variables of interest. Cohen's d-values were calculated for all BMD measures to examine the size of the effect between chronic dieters and nondieters.

**Results** Except for vitamin D (food + supplement sources) and iron (food + supplement sources), a significant difference was found in the mean daily dietary intake of energy, protein, carbohydrate, fat, calcium, phosphorus, vitamin D (food sources), zinc, magnesium, iron (food sources), sodium, and fiber ( $p < 0.04$  to  $p < 0.0001$ ) between chronic dieters and nondieters. Although chronic dieters consumed significantly less of the previously identified nutrients, WB BMD was significantly higher in chronic dieters compared to nondieters ( $p < 0.05$ ,  $1.125 \pm 0.011$  g/cm<sup>2</sup> versus  $1.090 \pm 0.010$  g/cm<sup>2</sup>, respectively). Significant differences in FFST mass, fat mass, percent body fat, serum osteocalcin, urinary NTx, body weight, body height, and body mass index were not found between groups ( $p > 0.05$  for all measures).

***Applications/conclusions*** Nutrient intake is linked to energy intake. As total energy intake increases so may the intake of nutrients that have negative associations with BMD such as dietary protein and sodium. Use of nutrient supplements may not be ideal when energy intake is adequate as excess nutrients may interact with one another and be less available to support BMD. Dietitians should encourage young-adults females, approaching the age of peak bone mass, to consume a variety of foods in balance and moderation.

**KEYWORDS:** Bone mineral density, Chronic dieting, Dietary intake, Young-adult females

### Introduction

Young-adult females between the late second and early third decades of life are at a stage of the life span during which peak bone mass is achieved (1). Although largely influenced by genetics, several modifiable factors, such as physical activity and dietary calcium intake, are associated with attainment of peak bone mass and, ideally, maximal bone mass (2,3). By maximizing bone mineral density (BMD) by the end of the young-adult years, an individual is, theoretically, better able to withstand losses of BMD and bone strength and structure and, ultimately, prevent bone fractures associated with osteoporosis in older age.

Attention has been directed toward the role of calcium in bone mineral accrual during childhood and adolescence (1,4-6) and calcium and attenuation of BMD in the postmenopausal years (7-9), yet calcium and other dietary factors have not been well investigated among females in the young-adult years. Because many women in the young-adult age group are conscientious of body weight, shape, and image, patterns of energy restriction are common (10). Severely malnourished individuals and females suffering from anorexia nervosa have significantly less bone mass than age-matched women who are well-nourished or do not suffer from anorexia nervosa (11). Metabolism of bone has also been shown to respond quickly to drastic reductions

in energy intake (12,13), and direct cause and effect relationships between energy restriction and reductions in BMD have been observed in longitudinal studies using both animal models (14,15) and human models (16-19). In all of these previous studies, however, reductions in bone mass were induced by severe or moderate, acute or chronic energy-restricted diets in which significant amounts of body weight were lost.

A more typical pattern of “dieting” or energy restriction among young-adult females is associated with little change in body weight; thus, the implications for bone health from these previous studies (16-19) may not be relevant to the majority of young-adult females who engage in chronic or long-term energy restriction without subsequent weight changes. Because micronutrient intake of vitamins and minerals are linked to macronutrients or overall energy intake, young-adult females who chronically diet may place their bones at risk for osteoporosis by consuming inadequate amounts of nutrients needed to achieve peak BMD. Presently, indicators of bone health have not been investigated in healthy, young-adult women reporting chronic dieting habits. Therefore, the purpose of the present cross-sectional investigation was to compare dietary intake, BMD measures, and biochemical markers of bone turnover in young-adult eumenorrheic females with limited physical activity who did or did not chronically restrict dietary energy intake.

## Materials and Methods

### Study participants

Students and employees from the Virginia Polytechnic Institute and State University (VPI&SU) campus and young-adult females residing in the surrounding cities were invited, via e-mail notices, posted flyers, and personal contacts to participate in this study, advertised as an investigation of bone density and diet in young-adult females. Initial screening of interested



participants was conducted by use of an investigator-designed questionnaire. Exclusion criteria included younger than age 18 or older than age 25, weekly participation in more than five total hours of moderate, hard, or very hard physical activity, amenorrhea, oligomenorrhea, or disruption of regular menstrual cycles, use of medications affecting bone metabolism, including oral contraceptives, cigarette smoking, or bone fractures during the last 12 months. Females with metabolic conditions, such as diabetes and thyroid disorders, were also excluded. All participants were of Caucasian ethnicity.

#### Data collection

Written informed consent was given by each participant after the project protocol and procedures were explained by an investigator. Written informed consent was obtained from each participant prior to her engagement in any procedure. The study protocol, all procedures, and informed consent requirements were approved by the Institutional Review Board for Research Involving Human Subjects at VPI& SU.

Testing of participants was completed between September, 1999, and April, 2000. General demographic and health, dietary intake, body composition (including BMD), blood and urine samples, anthropometric, and physical activity data were collected from each participant during individual two-hour testing periods. Data collection procedures were conducted in the Bone metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory and adjunct facilities, VPI&SU.

#### General demographic and health data

In an interview format, each participant self-reported her average number of menstrual cycles during the previous 12 months, date of the first day of the most recent menstrual flow, and birth date. Eumenorrhea (26 to 30 day cycles) and chronological age within the age restriction

were confirmed by an investigator with these data, but these data were not further used in statistical analyses.

### Dietary intake

In interview format, a food frequency questionnaire (FFQ; 20) was completed by each participant. An investigator (JMB) trained by a Registered Dietitian (SNR) conducted FFQ interviews. Participants indicated the frequency and quantity with which various food and beverage items were consumed over the last 12 months. During FFQ interviews, food models (three-dimensional) were used to assist participants in identifying accurate portion sizes of food and beverage items. Vitamin and/or mineral supplement consumption during the last 12 months was also reported by each participant. The DIETSYS+Plus Analysis Software (version 5.9, 1999, Block Dietary Data Systems, Berkeley, CA) was used to estimate average daily dietary intakes of selected nutrients from foods and beverages alone as well as from foods, beverages, and supplements combined.

### Body composition

Whole body (WB) dual energy X-ray absorptiometry (DXA) scans were conducted to measure the fat-free soft tissue (FFST) mass, fat mass, and percent body fat of each participant (version 8.25a, 2000, Whole Body Analysis software, QDR 4500A, Hologic Inc., Bedford, MA). The standard spine, hip, and forearm protocols and version 8.25a of the Whole Body Analysis software were used to measure BMD ( $\text{g}/\text{cm}^2$ ) of the lumbar spine (LS, L<sub>2</sub>-L<sub>4</sub>), nondominant total proximal femur (TPF), including the femoral neck (FN), trochanter (Troch), and Ward's triangle (WT) regions, nondominant total forearm (TF), including the mid-forearm, proximal 1/3, and ultradistal forearm, and WB, respectively.

Quality control procedures were conducted in the morning, and prior to testing of participants, on each testing date. Forty-nine phantom LS scans were completed resulting in a coefficient of variation (CV) of 0.34%. One investigator analyzed all DXA scans to ensure consistency.

#### Biochemical markers of bone turnover

Between 8:00 to 11:00 a.m., participants provided fasting blood and second-void urine samples. All samples were collected within a three-hour time block and in the morning to reduce diurnal variation among samples. Whole venous blood was drawn from each participant by a Certified Phlebotomist; within 30 minutes of the blood draw, the blood sample was centrifuged at 1200 RPM for 12 minutes. Serum was then pipetted into three, 1 mL cryovials and frozen at  $-80^{\circ}$  C until later analysis. In batches, serum osteocalcin, a biochemical marker of bone formation, was measured by radioimmunoassay (RIA, Human Osteocalcin RIA  $I^{125}$ , Biomedical Technologies, Staughton, MA). The inter- and intra-assay CV for osteocalcin were 7.86% and 7.91%, respectively.

Second-void urine samples were provided by each participant. Within one hour of collection, urine samples were transferred into cryovials and frozen at  $-80^{\circ}$  C. Crosslinked N-telopeptide of type I collagen (NTx), a biochemical marker of bone resorption, was measured in batches by enzyme-linked immunosorbent assay (ELISA, osteomark, Seattle, WA) from these urine samples. Quantitative spectrophotometry was completed to measure urinary creatinine (#555, Sigma Diagnostic, St. Louis, MO). Urinary NTx measurements were reported as bone collagen equivalents (BCE) per mM creatinine. One chronic dieter had a NTx value beyond the acceptable range; thus, this participant's NTx level was excluded from statistical analysis ( $n = 17$

for NTx in chronic dieters). The inter- and intra-assay CV for NTx were 4.10% and 5.82%, respectively. All serum osteocalcin and urinary NTx samples were analyzed in duplicate.

#### Anthropometric data

Each participant was shoeless and lightly clothed during measurement of body weight and standing height. Body weights and standing heights were measured on a calibrated electronic scale (ScaleTronix, Wheaton, IL) and next to a calibrated wall-mounted, digital, stadiometer (Heightronic™, Measurement Concepts, North Bend, WA) to the nearest 0.01 kg and 0.01 cm, respectively. Body mass index (BMI) was calculated by an investigator for each participant from the participant's weight and height measurements ( $BMI = \text{kg}/\text{m}^2$ ).

#### Physical activity

To confirm a limitation in moderate, hard, and very hard physical activities in which participants engaged, the seven-day physical activity recall (21) was completed. Each participant indicated to an investigator the number of hours that she engaged in various types of activities during the previous week. As all 60 participants continued to meet the limited physical activity requirement, these data were not further used in statistical analyses.

#### Chronic dieting status

Participants were instructed to self report estimated amount of time during the previous year which was spent dieting. Responses included greater than 50% of the previous year, less than 50% of the previous year, or never during the previous year (22). From further analysis of FFQs, it was found that those who reported dieting greater than 50% of the previous year were not found to have estimated energy restriction. Estimated energy intake did not differ according to response, thus, an alternate classification method was used.

Participants were divided into two groups – chronic dieters and nondieters. To categorize each participant into one of these two groups, three steps were completed by an investigator. First, each participant's estimated daily energy need was calculated based on her age, height, weight, and activity level. The Food Processor<sup>®</sup> Nutrition Analysis Software profile system (version 7.4, 1999, *esha* Research Inc., Salem, OR) was used to establish each participant's estimated energy need. Secondly, the average daily energy intake was extracted from the FFQ analysis. Thirdly, average daily energy intake from the Block98.2 FFQ was compared to the estimated daily energy need and recorded as a percentage of estimated need (estimated energy intake/estimated energy need). If average daily estimated energy intake was less than or equal to 66.9% of the estimated energy need for a participant, she was placed into the chronic dieters group. Nondieters were those participants whose average daily estimated energy intakes fell between 67% to 125% of estimated energy needs.

Eighteen participants fell into the chronic dieters group while 38 participants were classified as nondieters. Four women consumed greater than 125% of estimated energy needs (outliers) and were, therefore, eliminated from further analysis. Thus, 56 participants were included in subsequent data analyses.

### Statistical analyses

Means  $\pm$  standard error of the mean (SEM) were calculated for study variables according to group. Student's *t*-tests were completed for group comparisons (chronic dieters versus nondieters) for dietary intake, body composition (including BMD), biochemical markers of bone turnover, and anthropometric data. The Statistical Analysis System (SAS) was used to analyze all data (version 8.0, 1999, SAS Software Inc., Cary, NC). Significant differences of  $p < 0.05$  were identified.

The magnitude of differences or effect size for mean BMD values between chronic dieters and nondieters are also reported in addition to p-values for these measurements. Cohen's  $d$  (23) or effect size was calculated as  $[(\text{mean } 1 - \text{mean } 2)/SD_{\text{pooled}}]$  so that the size of the effect, rather than simply p-value, could be evaluated. Small, moderate, and large effects are represented as 0.20, 0.50, and 0.80 SD, respectively (24).

## Results

The average age of these 56 participants was  $20.3 \pm 0.2$  years (range 18 to 25 years). A statistically significant difference in chronological age was not found between chronic dieters ( $n = 18$ ,  $20.4 \pm 0.5$  years) and nondieters ( $n = 38$ ,  $20.3 \pm 0.3$  years). Again, all participants self-reported eumenorrhea and participation in less than 5 hours of moderate, hard, and very hard physical activity per week during the previous 12 months.

### Dietary intake

Table 1 includes average dietary intakes of absolute and relative daily estimated energy intakes, macronutrients, and selected nutrients of importance to BMD. The average daily estimated energy need for participants ranged from 1,892 to 3,636 kilocalories (kcal), while the average estimated absolute energy intake (as calculated from the Block98.2 FFQ data) ranged from 906 to 3,343 kcal or 37% to 124% of energy needs. Because mean absolute energy intake was significantly higher in nondieters compared to chronic dieters ( $p = 0.0001$ ) yet both groups required equal levels of energy, nondieters also had a significantly higher mean relative energy intake compared to chronic dieters ( $p = 0.0001$ ). Participants consumed approximately 14%, 55%, and 32% of total energy from dietary protein, carbohydrate, and fat, respectively. Significant differences in the percent of total energy consumed from the macronutrients were not found between chronic dieters and nondieters. On average, chronic dieters, compared to

TABLE 1. Average daily dietary intakes of selected nutrients in chronic dieters and nondieters <sup>a</sup>.

Nutrients	Chronic dieters	Nondieters	p-value
	(n = 18)	(n = 38)	
Absolute energy intake (kcal)	1,231 ± 51	2,095 ± 92	0.0001
Energy requirement (kcal)	2,342 ± 50	2,300 ± 52	NS <sup>b</sup>
Relative energy intake (% of required)	53 ± 2	92 ± 3	0.0001
Protein (g)	42.8 ± 2.7	72.6 ± 3.3	0.0001
Carbohydrate (g)	168.7 ± 6.8	288.2 ± 12.5	0.0001
Fat (g)	43.7 ± 2.5	73.8 ± 3.5	0.0001
D <sup>c</sup> Calcium (mg)	653 ± 71	1,131 ± 77	0.0001
D + S <sup>d</sup> Calcium (mg)	783 ± 78	1,327 ± 88	0.0003
Phosphorus (mg)	816 ± 56	1,359 ± 61	0.0001
D Vitamin D (IU)	90 ± 15	157 ± 19	0.02
D + S Vitamin D (IU)	223 ± 44	300 ± 33	NS
D Zinc (mg)	5.8 ± 0.4	10.7 ± 0.5	0.0001
D + S Zinc (mg)	11.6 ± 1.7	16.0 ± 1.2	0.04
D Magnesium (mg)	176 ± 12	308 ± 15	0.0001
D + Z Magnesium (mg)	209 ± 14	344 ± 17	0.0001
D Iron (mg)	10.1 ± 0.7	16.1 ± 0.9	0.0001
D + S Iron (mg)	19.7 ± 4.6	24.7 ± 2.5	NS
Sodium (mg)	1,770 ± 96	3,028 ± 128	0.0001
Fiber (g)	10.3 ± 0.8	19.9 ± 1.4	0.0001

<sup>a</sup> Values are mean + SEM; <sup>b</sup> NS, not statistically significant at p < 0.05; <sup>c</sup> D, from dietary sources; <sup>d</sup> D+S, from dietary plus supplement.

nondieters, consumed significantly less dietary protein ( $p = 0.0001$ ), carbohydrate ( $p = 0.0001$ ), fat ( $p = 0.0001$ ), calcium ( $p = 0.0001$ ), phosphorus ( $p = 0.0001$ ), vitamin D ( $p = 0.02$ ), zinc ( $p = 0.0001$ ), magnesium ( $p = 0.0001$ ), iron ( $p = 0.0001$ ), sodium ( $p = 0.0001$ ), and fiber ( $p = 0.0001$ ) from food and beverage sources. After accounting for nutrient intakes from dietary supplements, chronic dieters continued to consume, on average, less dietary calcium ( $p = 0.0003$ ), zinc ( $p = 0.04$ ), and magnesium ( $p = 0.0001$ ) but not vitamin D or iron compared to nondieters.

### Body composition

Significant differences for mean FFST mass ( $43.5 \pm 0.9$  kg vs.  $42.0 \pm 1.0$  kg), fat mass ( $19.8 \pm 1.3$  kg vs.  $19.5 \pm 1.5$  kg), and percent body fat ( $29.8 \pm 1.0\%$  vs.  $29.6 \pm 1.0\%$ ) were not found between chronic dieters compared to nondieters, respectively. Average BMD measurements at various body sites are provided in Table 2. Chronic dieters had significantly higher WB BMD compared to nondieters ( $p = 0.05$ ). Additionally, the magnitude of the effect for WB BMD was moderate ( $d = 0.61$ ). All remaining BMD measures did not achieve statistical significance (based on p-values); however, moderate effects were observed for the differences in LS ( $d = 0.56$ ), Troch ( $d = 0.45$ ), and proximal 1/3 forearm ( $d = 0.74$ ) between chronic dieters and nondieters with chronic dieters having higher BMD values at these sites. The magnitude of the effect for differences between chronic dieters and nondieters in TPF ( $d = 0.37$ ), FN ( $d = 0.29$ ), and WT ( $d = 0.41$ ) BMD were small to moderate.

### Biochemical markers of bone turnover

Statistically significant differences (based on p-values) were not observed for serum osteocalcin ( $n = 18$ ,  $39.9 \pm 1.9$  ng/mL vs.  $n = 38$ ,  $41.9 \pm 1.5$  ng/mL) or urine NTx ( $n = 17$ ,  $68.7 \pm 9.4$  BCE/mM creatinine vs.  $n = 38$ ,  $82.3 \pm 5.8$  BCE/mM creatinine) between chronic dieters and nondieters, respectively. However, chronic dieters had lower levels of serum osteocalcin ( $d =$



TABLE 2. Bone mineral density measurements<sup>a</sup> for chronic dieters and nondieters.

Bone site (g/cm <sup>2</sup> )	Chronic dieters (n = 18)	Nondieters (n = 38)	p-value	d-value <sup>b</sup>
Whole body	1.125 ± 0.011	1.090 ± 0.010	0.05	0.61
Lumbar spine (L <sub>2</sub> -L <sub>4</sub> )	1.156 ± 0.026	1.101 ± 0.015	0.07	0.56
Total proximal femur	0.990 ± 0.019	0.957 ± 0.015	0.21	0.37
Femoral neck	0.883 ± 0.021	0.859 ± 0.013	0.32	0.29
Trochanter	0.759 ± 0.019	0.721 ± 0.014	0.13	0.45
Ward's triangle	0.848 ± 0.030	0.804 ± 0.016	0.19	0.41
Total forearm	0.549 ± 0.008	0.550 ± 0.006	0.94	- 0.03
Mid-forearm	0.568 ± 0.009	0.571 ± 0.006	0.71	- 0.08
Proximal 1/3 forearm	0.663 ± 0.012	0.632 ± 0.006	0.39	0.74
Ultradistal forearm	0.408 ± 0.008	0.409 ± 0.007	0.94	- 0.02

<sup>a</sup> Bone mineral density values are reported as means ± SEM;

<sup>b</sup> d-value = Cohen's d (effect size); ± 0.20 = small, ± 0.50 = moderate, ± 0.80 = large effects.

- 0.22) and urinary NTx ( $d = - 0.37$ ) compared to nondieters when evaluated based on the magnitude of the effect.

#### Anthropometric data

Mean body weight, height, and BMI were marginally higher for chronic dieters compared to nondieters, but averages for these variables were not statistically significantly different between groups. Body weight, height, and BMI averages for chronic dieters were  $65.1 \pm 2.1$  kg,  $168.3 \pm 1.9$  cm, and  $23.0 \pm 0.7$  (BMI), respectively, while body weight, height, and BMI means for nondieters were  $63.2 \pm 2.3$  kg,  $165.7 \pm 1.0$  cm, and  $22.8 \pm 0.8$  (BMI), respectively.

#### Discussion

Young-adult females who are chronic dieters have significantly higher WB BMD compared to nondieters of the same age, weight, height, and BMI. Additionally, effect size data indicate that chronic dieters have moderately higher BMD at the LS, Troch, and proximal 1/3 forearm as well as slightly higher BMD at the TPF, FN, and WT compared to nondieters. These differences in BMD measures exist despite a significantly lower dietary intake of estimated absolute and relative energy, calcium, and other nutrients important to BMD in chronic dieters versus nondieters. Moreover, due to the lack of significant differences in FFST mass, fat mass, body weight, and height, between these two groups, and the inclusion of only eumenorrheic and relatively sedentary females, dietary intake differences may be important to the differences observed in BMD measures between these groups.

Several previous investigations of dietary intake and BMD have focused on nutrients that may be lacking in the diet (25-27); however, results from the present study suggest that over-availability of some nutrients may also pose a concern for BMD. For example, mean daily dietary protein consumption among nondieters was estimated at 158% of the Recommended

Dietary Allowance (RDA) for females in this age range (28) compared to chronic dieters who consumed approximately 93% of the RDA for daily dietary protein. Such a high consumption of dietary protein is not atypical; national surveys consistently show protein consumption in typical American diets as well above recommended intakes (29). High dietary protein intake is associated with increased urinary calcium excretion (30), possibly altering calcium homeostasis with subsequent increases in bone calcium resorption or decreases in bone formation and mineralization to maintain a normal plasma calcium concentration. A perpetually high dietary protein intake may impair accrual of bone mineral and/or enhance breakdown of the bone matrix over time (30,31). Thus although dietary calcium intake was sufficient (32) according to the Adequate Intake (AI) in nondieters, the high dietary protein intake may have overshadowed benefits to BMD of adequate dietary calcium intake.

Although a high dietary protein intake is a feasible explanation for the differences in BMD measures observed between these two groups, other dietary components are also linked to BMD. Mean daily dietary phosphorus intake of nondieters was nearly twice the Dietary Reference Intake (DRI) while chronic dieters exceeded the DRI for phosphorus (32) by only 17%. Phosphorus is associated with low urinary calcium losses (33); however, phosphorus may lower the serum calcium concentration as well as decrease intestinal absorption of calcium, both of which actions trigger secretion of parathyroid hormone (PTH) resulting in bone calcium resorption (34,35).

Vitamin D serves dual roles in bone health. Vitamin D assists in calcium absorption but also allows PTH to trigger bone resorption (36,37). Both groups of participants met the AI for vitamin D (32) when all sources were considered; however, nondieters consumed over 75 more IU of vitamin D, on average, than did chronic dieters. At dietary intakes beyond the AI for

vitamin D, PTH effects on bone, induced by vitamin D, may override the role of vitamin D at the intestine. Such a contention requires further investigations, however.

Zinc, magnesium, and iron are essential for normal bone formation (38), maintenance of calcium-regulating hormones (39,40), and bone strength (25), respectively. These three nutrients may interact with one another (41) as well as compete with calcium for absorption (42). Thus, balanced levels of these nutrients in the diet are important for bone health. In the present study, mean daily dietary intake of zinc in nondieters was 25% higher than the RDA (28), and the average daily dietary iron intake was 66% higher than the RDA (28). The mean daily dietary intake of magnesium from all sources was only 10% above the DRI (32) for nondieters; yet with over-availability of dietary zinc and iron, adverse outcomes for BMD may be possible due to nutrient-nutrient interactions and competition for absorption and utilization.

Similar to protein, sodium is capable of increasing urinary calcium excretion. Thus, dietary intake of high sodium levels may prevent maximal mineralization of bone (30). Although the average daily dietary intake of sodium was not excessive in nondieters (43), nondieters consumed 58% more sodium in the diet compared to chronic dieters.

Dietary fiber is associated with fecal excretion of minerals important for bone strength including calcium, zinc, and iron (44,45). Dietary fiber intakes in both groups were within an acceptable range; however, nondieters consumed approximately twice as much dietary fiber, on average, than chronic dieters.

Nondieters met greater than 67% of the recommendations for all nutrients from foods alone. Without accounting for supplement use, nondieters consumed, on average, greater than 100% of the recommendations for dietary protein, calcium, phosphorus, iron, and sodium. When including supplements, intakes of applicable nutrients by nondieters exceeded recommended

levels. Nondieters had nutrient intakes in excess of 133% of recommendations for protein, phosphorus, vitamin D, and iron. Approximately 56% of nondieters consumed nutrient supplements.

Foods alone did not provide greater than 66% of recommendations for calcium, vitamin D, zinc, and magnesium for chronic dieters, but when accounting for supplement use, only calcium and magnesium remained below recommendations (78% and 67%, respectively). Average daily dietary intakes among chronic dieters did not exceed 133% of the recommendation for any nutrient. Approximately 56% of chronic dieters consumed nutrient supplements.

Among nondieters, bone turnover or remodeling biomarkers was slightly higher compared to chronic dieters. This suggests that both bone formation and resorption occurred at a greater rate in nondieters versus chronic dieters. One explanation for this observation is that with a lack of adequate energy intake compared to energy needs (as seen in chronic dieters), bone turnover may be down-regulated. This contention is supported by human studies that have shown reductions in markers of bone formation (12,13) and markers of bone resorption (13) following acute periods of fasting.

The implication of the small differences in serum osteocalcin and urinary NTx between chronic dieters and nondieters is unclear given the unexpected differences in BMD measures between these two groups. However, it may be possible that the BMD values among chronic dieters represent peak BMD at these sites in light of the lower levels of bone biomarkers, while nondieters may still be accruing BMD at the various body sites measured. Additional studies of longitudinal design are warranted and essential to the further investigation of the role of energy

restriction and nutrient intakes on BMD and biomarkers of bone turnover in young-adult women reaching peak BMD.

The present study has several limitations. Bone mineral accrual occurs throughout the developmental years. Thus, it is not clear if differences observed between groups in this cross-sectional study are due to dietary intakes during the previous year or are a result of the cumulative effects of dietary intakes and other factors throughout earlier formative years. The FFQ is a widely used tool for estimating dietary intake data over a period of time (46,47); however, the FFQ is limited by the memory and estimation capability of participants and nutrient evaluation database. Moreover, underreporting is a widespread phenomenon associated with dietary recall data collection (48). Inaccuracies due to underreporting may flaw the current study and, therefore, a future recommendation would be to incorporate a more direct method of energy intake, such as doubly labeled water, in order to more appropriately classify individuals who are chronically restricting energy. Interesting is that a relationship between energy restriction and reported frequency of dieting during the previous year was not found. Among those classified as chronic dieters, 12 (67%) reported never dieting during the previous year, whereas among nondieters, 17 (45%) reported dieting during the previous year. Therefore, it was felt that FFQ responses were not skewed according to self-reported frequency of dieting because individuals who reported dieting during the previous year did not consistently report energy restriction.

A lack of a standard definition or measurement tool for chronic dieting required that a proxy measure be defined for this study. As stated above, there are potential flaws with the classification method, but for the purposes of this study, it was felt that this method was most appropriate because, unlike previous studies (22,49), it did not rely on personal interpretation of the term “dieting” by the participant. This method normalized dieting to overall energy

restriction. In order to more appropriately identify energy restricters, though, a more direct method of identification is recommended. Lastly, findings from this study are limited to Caucasian, young-adult females with normal menstrual function and limited activity and cannot be generalized to a wider population. Nonetheless, this study is unique because it is the first to investigate chronic dieting in relation to bone health in otherwise healthy, young-adult women. It is important to investigate BMD in this age group of women because dieting and weight control practices are common among many females who are approaching attainment of peak BMD. Although it appears that chronic dieters have higher BMD values at several body sites despite lower intakes of energy, macro-, and micronutrients compared to nondieters, great caution is needed when extrapolating results suggestion that chronic dieting among young-adult eumenorrheic women with limited physical activity may not be detrimental to bone health.

#### Applications

High energy intake may be associated with high intake of nutrients that adversely impact bone such as protein and sodium. Furthermore, because absorption and utilization of many nutrients are interrelated, high intakes of nutrients related to BMD, such as calcium, phosphorus, zinc, magnesium, and iron may interfere or compete with one another. Nutrient interactions may be further complicated by consumption of vitamin and/or mineral supplements. With additional intake of individual nutrients through supplements, the appropriate profile of all important nutrients to BMD may become unbalanced. To promote attainment of peak BMD in young-adult females, dietitians should refer to the fundamentals of nutrition – variety, balance, and moderation. Counseling females to consume adequate but moderate intakes of energy with nutrient dense foods from a variety of food sources is crucial to osteoporosis prevention. Nutrient supplements may not be ideal when energy intake is adequate as excess nutrients may

interact with one another and be less available to support BMD in eumenorrheic, young-adult females with limited physical activity.

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## CHAPTER V

### SUMMARY AND FUTURE DIRECTIONS

Anthropometric, body composition, bone mineral density (BMD), and dietary intake variables, as well as biomarkers of bone turnover were investigated in a group of young-adult females (N = 60; Chapter 3). Fat-free soft tissue mass and body weight had positive associations with whole body (WB), lumbar spine, and total proximal femur (TPF) BMD. Fat mass and body mass index (BMI) were positively related to lumbar spine and TPF BMD. Body mass index and forearm BMD also had a positive association. Anthropometric and body composition data did not relate to biomarkers of bone turnover, but significant inverse relationships were observed between urinary N-telopeptide crosslinks (NTx) and age and NTx and lumbar spine BMD. Lumbar spine BMD has a negative association with dietary protein, iron, and magnesium intake. Dietary magnesium intake was also shown to have a negative relationship with WB BMD.

Comparisons in bone-related variables of interest between young-adult female chronic dieters and nondieters were also examined (Chapter 4). A chronic dieter was defined as an individual consuming on average  $\leq 66.9\%$  (n = 18) of her estimated recommended energy requirements. Nondieters (n = 38) consumed between 67% and 125% of their estimated energy requirements. Statistically significant differences were not observed between chronic dieters and nondieters for age, anthropometric or body composition variables. Chronic dieters had significantly lower consumption of energy, macronutrients, and but significantly higher WB BMD (p = 0.05) compared to nondieters

Several overall conclusions from these studies may be drawn. Although both fat mass and fat-free soft tissue mass have positive associations with BMD among young-adult females, it appears that fat-free soft tissue mass may be more beneficial to BMD than is fat mass. This first

study (Chapter 3) supports previous research by finding a negative association between protein intake and BMD. Research investigating the relationship between iron and BMD is scarce, but the negative relationship observed in this study may be explained by the ability of iron to inhibit absorption of other nutrients important to bone. Although this study is cross-sectional and not experimental, the relationships observed here contradict previous studies that have found positive associations between magnesium and bone health. This is indicative of a need for further investigation of the role of magnesium, as well as other nutrients, in bone health, particularly among young-adult women.

Because of the established importance of certain nutrients (i.e., calcium and vitamin D) for bone health, and studies which have demonstrated negative effects of energy restriction and weight loss on BMD, the comparative investigation of BMD and bone turnover in chronic dieters and nondieters was warranted. Chronic dieting is common among many young-adult females and, therefore, an understanding of the relationships between chronic dieting and bone health was a fundamental addition to this overall investigation of bone health in young-adult females.

Findings from the second investigation (Chapter 4) were unexpected in that chronic dieters had higher WB BMD than controls. It was observed that nutrient consumption of chronic dieters more closely resembled dietary recommendations for this age group than did nondieters. This may indicate that perhaps among young-adult females, overall energy intake may be less important for bone mass than are the individual nutrients that energy provides. An important finding from this study is that many nutrients exceeded recommendations, particularly among nondieters. Thus, in conjunction with higher energy intakes, were higher protein, phosphorus, and sodium intakes which may have a negative impact on BMD. Furthermore, higher nutrient

intakes may enhance the susceptibility for nutrient-nutrient interactions which may also be detrimental to bone health.

It is clear from these studies that further research must be conducted to more thoroughly investigate bone health in young-adult females. Animal models have shown age-related differences in bone metabolism, but these animal studies, and results of human studies including females of other age groups, do not necessarily apply to females in the second and third decades of life. These present studies indicate a need for further investigation of relationships between dietary factors and BMD among young-adult females. Finally, the high prevalence of weight control tactics among females, particularly college-aged females, must be further investigated in relation to bone health. Diets that result in elevated or reduced intakes of individual nutrients must be studied in order to determine their ultimate effect on bone.

## APPENDIX

General health questionnaire and data collection sheet

Subject ID # \_\_\_\_\_

Investigator initials \_\_\_\_\_

Date of testing \_\_\_\_\_

Participant's Date of Birth \_\_\_\_\_

Age \_\_\_\_\_

Weight: \_\_\_\_\_ (kg)

Height: \_\_\_\_\_ (m)

BMI: \_\_\_\_\_

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1. Have you engaged in > 5 hours of physical activity per week during the previous year?

YES      NO

2. Have you ever had a bone fracture or broken bone?

YES      NO

3. Have you ever been diagnosed as having bone disease such as osteoarthritis, etc.?

YES      NO

4. Have you even been diagnosed with a metabolic disorder such as diabetes, PKE, etc.?

YES      NO

5. Have you ever been diagnosed with the following chronic illnesses?

(Circle any that are present)

Chron's disease

Cancer

Ulcerative colitis

Lactase deficiency

Malabsorption

Asthma

Renal disease

Thyroid disease

Other \_\_\_\_\_

6. Have you started your menstrual cycles?

YES      NO

If yes, do you menstruate:

12-14 times per year

9-11 times per year

6-8 times per year

3-5 times per year

0-2 times per year

7. Are you taking an oral contraceptive?

YES      NO



8. Are you taking any other medications (prescription and non-prescription)?

YES NO

If yes, what medication? \_\_\_\_\_

9. Are you pregnant?

YES NO

10. Are you lactating?

YES NO

11. During the previous year, how often have you gone on a diet to lose weight?

Never

< 50% of the time

> 50% of the time

Time of blood draw: \_\_\_\_\_

Time of urine collection: \_\_\_\_\_

## VITA

Jeannemarie M. Beiseigel, daughter of William and Annamae Beiseigel, was born September 23, 1976 in Philadelphia, PA. Jeannemarie received a Bachelor of Science degree in Nutrition and Foods Systems Management from Hood College, Frederick, MD in May, 1998. During her time at Virginia Polytechnic Institute and State University, she has been funded through a departmental Graduate Teaching Assistantship. Jeannemarie has also been the recipient of the Mildred B. Davis Fellowship and the Jewell L. Taylor Fellowship, both funded by the American Association of Family and Consumer Sciences as well as the Meszaros Travel Scholarship, funded by Provost Peggy Meszaros. Jeannemarie will remain at Virginia Tech in pursuit of her Doctoral Degree in Human Nutrition. From there, she plans to continue research in the nutritional sciences while instructing at a university.