BONE MINERAL DENSITY AND BIOMARKERS OF BONE TURNOVER IN YOUNG-ADULT FEMALES WITH AND WITHOUT COGNITIVE EATING RESTRAINT

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BONE MINERAL DENSITY AND BIOMARKERS OF BONE TURNOVER IN YOUNG-ADULT FEMALES WITH AND WITHOUT COGNITIVE EATING RESTRAINT
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The early adult years are critical as they provide the final window of opportunity to maximize peak bone mass and help prevent osteoporosis later in life. Dietary habits of young women are often molded by social pressures to be thin. Negative implications for cognitive eating restraint (CER) on bone health have been shown, but direct evidence to support such contentions is limited. Therefore, this study was conducted to further investigate the relationships between CER and bone health in young women. Women aged 18 to 25 years with normal body mass index and limited physical activity participated in this study. Body composition and anthropometric variables, dietary intake, endocrine factors, biomarkers of bone turnover, and measurements of bone mineral content (BMC) and bone mineral density (BMD) were examined cross-sectionally in women with high (n = 31) and low (n = 34) CER scores. High CER participants possessed more fat mass (FM) ($p < 0.05$) and percent body fat (BF%) ($p = 0.01$) and consumed a greater number of servings of fruits and vegetables ($p < 0.05$) per day than the CER participants. No differences in biochemical measurements, BMC or BMD were found between groups. Using similar methods, a study was conducted to compare high (n = 27) and low (n = 26) CER groups at baseline and after 6-months. At baseline, the high CER group possessed significantly higher FM ($p < 0.05$) and BF% ($p = 0.01$) and lower biochemical markers of bone formation ($p < 0.05$) than the low CER group; no other group differences were apparent at baseline. Using repeated measures ANOVA, a significant Group x Time interaction was identified for salivary cortisol concentrations ($p < 0.05$). Mean salivary cortisol concentrations were significantly lower at 6-months versus baseline in the high CER group ($p < 0.05$) but did not differ between time points in the low CER group. No other significant Group x Time interactions were found. Overall, despite finding a lower serum osteocalcin concentration in the high CER group at baseline, evidence of compromised BMC or BMD between women with high versus low CER scores over 6 months was not found.

DEDICATION

This dissertation is dedicated to my Mom, Dad, sisters, Barbara and Nancy, and brother, Matthew. A lot of work went into this project but none would have been possible without their support and encouragement. They are a constant reminder of who I am and who I want to be. Forever and always, I love you. Thanks!

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TABLE OF CONTENTS

LIST OF TABLES

APPENDIX

CHAPTER 1

INTRODUCTION

Osteoporosis is a metabolic bone disease in which the microarchitecture and mass of the skeleton are dramatically impaired and risk of fracture is increased (Khosla and Kleerepoper 1996). It is the most prevalent bone disease in developed countries (Wasnich 1996). Osteoporosis is typically classified as a "woman's disease" because it afflicts nearly four times more women than men ("Position of the American…1999, Turner et al. 1998). Osteoporosis is not only painful but often life-threatening. Nearly 20% of patients die within one year after sustaining a hip fracture (Drinkwater 1994).

While osteoporosis is one of the most prevalent diseases afflicting women, the etiology is poorly understood. The operational definition of osteoporosis according to the World Health Organization (WHO) is a bone mineral density (BMD) measurement > 2.5 standard deviations below the mean peak bone mass for young healthy adults (Lindsay 1996). Due to limited availability of effective treatment options and costs associated with options that are available, prevention remains the most efficient means of dealing with this disease as a nation. Effective prevention occurs from a combination of maximizing BMD prior to the age of peak bone mass and minimizing bone loss throughout the remaining adult years.

The age at which peak BMD is achieved varies between individuals as well as between bone sites, but it is generally understood to occur by the late twenties (Matkovic 1991, Recker et al. 1992). While genetics account for the majority of peak bone mass potential, modifiable lifestyle factors such as dietary intake and physical activity can influence the achievement of peak bone mass.

Adequate dietary calcium intake has long been recognized for its important role in achievement of peak bone mass. Other dietary factors such as phosphorus, vitamin D and protein intake have also been investigated among young-adult females in regard to bone health (Metz et al. 1993, Recker et al. 1992, Teegarden et al. 1998). Beyond these select nutrients, though, is a lack of research, particularly among young-adults.

In addition to intake of individual nutrients, adequate energy intake appears important to bone health. Maintaining a normal or above normal body mass is positively associated with BMD (Bakker et al. 2003, Rollins et al. 2003), whereas severely undernourished individuals,

such as individuals with anorexia nervosa, have dramatically reduced bone mass (Mazess, Barden, and Ohlrich 1990). Furthermore, weight loss is associated with a loss of BMD (Avenell et al. 1994, Compston et al. 1992, Ramsdale and Bassey 1994), even when exercise is included in the weight-loss strategy (Anderson et al. 1997, Svendsen et al. 1993). Even periods of acute fasting have been shown to induce a quick response from bone cells identified by rapid changes in biochemical markers of bone turnover (Grinspoon et al. 1995, Talbott and Shapses 1998).

It is well recognized that chronic dieting behaviors are common among young-adult females due to societal pressures to be thin (Biener and Heaton 1995, Grodner 1992, Manore 1996). It remains unclear if chronic dieting without changes in body weight affects bone health. Therefore, it is necessary to investigate relationships between chronic dieting behaviors and bone health in young-adult females and yet there is currently no consistent method of identifying chronic dieters. However, cognitive eating restraint (CER) has been implicated in relation to bone health. Females with high CER have been shown to have subclinical menstrual cycle disturbances (Barr, Janelle and Prior 1994, Barr, Prior and Vigna 1994), elevated cortisol concentrations (Anderson et al. 2002, McLean, Barr and Prior 2001a), and reduced whole body bone mineral content (BMC) (McLean, Barr and Prior 2001b, Van Loan and Keim 2000). Differences in BMD have not been shown and longitudinal investigations of CER and bone health have not yet been published. In an attempt to fill some of these voids, the current study was designed to: (1) ascertain if chronic dieters can be successfully identified from the CER subscale of the Eating Inventory questionnaire (Stunkard and Messick 1988), (2) conduct cross-sectional and longitudinal investigations to examine differences in dietary intake, physiological measurements, and BMD in young-adult females with and without CER, and (3) examine relationships between dietary intake, physiological measurements and BMD in young-adult females. The first study found that females with high CER possess higher fat mass and percent body fat (BF%), but do not chronically restrict energy intake and as a result, do not have reduced resting energy expenditure or differences in physiological measurements compared to low CER counterparts. Therefore, we conclude that having high CER does not necessarily equate with chronic dieting and this questionnaire is not a useful tool for identifying chronic dieters among this population (Chapter 3). We also found that, with the exception of fat mass and BF %, no differences in body composition, including BMC and BMD, or biochemical parameters exist between high and low CER groups (Chapter 4). Over 6-months, although significant changes in

2

dietary intake, BMC, BMD and biochemical measurements were noted among all participants, CER groups did differ in any measure over time with the exception of salivary cortisol in which high CER participants had a significant reduction in mean salivary cortisol concentration while the low CER group remained constant (Chapter 5). Among all participants, significant relationships were found between select lifestyle, anthropometric, dietary and biochemical variables and bone measurements. The importance of dietary adequacy is addressed (Chapter 5) and directions for future research are discussed (Chapter 6).

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CHAPTER II

REVIEW OF LITERATURE

Osteoporosis – Prevalence and Prevention

Osteoporosis is a prevalent and costly disease in the United States (U.S.) and worldwide. It is typically classified as a "woman's disease" due to the majority of osteoporotic fractures occurring in women. Despite this classification, osteoporosis affects both genders and is on the rise in both males and females (Huuskonen et al. 2000). Historically, osteoporosis has been classified as a disease primarily affecting older individuals, yet possibly due to a combination of lifestyle changes and improved methods of detecting low bone mineral density (BMD), osteoporosis is becoming more common among younger generations. Further research is needed to improve the understanding of lifestyle and bone interactions in order to effectively prevent and treat this disease regardless of age and gender.

Osteoporosis is primarily a result of an imbalance in bone resorption and bone formation. When bone resorption is favored over bone formation as a result of a hypogonadal state (particularly menopause), lack of weight-bearing exercise, inadequate dietary intake, smoking, or use of certain medications, the ultrastructure of bone is compromised and the risk of bone fractures is amplified.

Techniques recommended for osteoporosis prevention are designed to achieve peak bone mass early in life, maintain bone density throughout adulthood and detect bone loss or low bone mass prior to fracture. In order to successfully prevent osteoporosis, all of these methods must be employed; thus, osteoporosis prevention strategies should be incorporated into all decades of life, but particularly in early adulthood, prior to the age of peak bone mass.

Peak Bone Mass

Attainment of peak bone mass entails a combination of non-modifiable internal factors such as genetics and modifiable external factors such as physical activity, dietary intake and lifestyle (Dombrowski 2000). Data show that bone calcium deposition is favored over bone resorption until the second and third decades of life (Bronner and Abrams 1998, Matkovic 1991) at which time, an equilibrium is met and sustained until around the age of menopause when bone

resorption follows the withdrawal of endogenous estrogen. Therefore, research aimed at attainment of peak bone mass must be targeted towards adolescents and young adults.

Determinants of Bone Mass

Throughout the lifespan, bone is in a constant state of remodeling. Osteoclasts are working to resorb bone while osteoblasts are rebuilding bone. If a balance in these processes are met, bone mass is relatively stable. The control of bone resorption and formation are mediated by both internal, non-modifiable factors and external, modifiable factors.

Genetics and, to some extent, endocrine factors are non-modifiable factors that affect bone metabolism (Bonjour et al. 1991). A genetic inheritance of low bone mass has been shown in daughters of osteoporotic mothers (Seeman et al. 1998). The strong genetic component of bone mass was further demonstrated by Pocock and colleagues (1987) who showed high correlation coefficients in BMD between sets of twins, but found significantly stronger correlations between the BMD of monozygotic twins compared with dizygotic twins. Although exact genetic controls of BMD have yet to be identified, genes are believed to directly influence skeletal size and mass. Genes also help determine non-skeletal characteristics that influence bone mass such as body size and shape, muscle strength, nutrient absorption, and endocrine function (Rizzoli, Bonjour, and Ferrari 2001). Still, genetics is not the only factor important for attainment and maintenance of BMD. Prevention and treatment options must be aimed at both modifiable and non-modifiable factors. Table 1 includes both modifiable and non-modifiable risk factors for osteoporosis (Wasnich 1996). While several factors have been deemed important for bone, research is still needed to more fully understand and to better make recommendations for osteoporosis prevention.

Before identifying research directions and efforts to further understand effects of lifestyle on osteoporosis prevention, it is necessary to understand the basics of bone structure and bone metabolism, including internal and external mediating factors.

Bone Structure

Bone is a living tissue essential for human survival. It serves four main purposes: (1) support and locomotion, (2) protection of vital organs, (3) site of hematopoiesis, and (4) reservoir for metabolically active ions (Baron 1996).

Table 1. Factors Associated with Increased Risk of Osteoporosis

Ethnicity	Caucasian, Asian
Age	Women: postmenopausal Males: $\geq$ 65 years
Gender	Female
Genetic	Familial prevalence
Physical Characteristics	Small body frame Low body mass
Endogenous Hormones	Estrogen deficiency Androgen deficiency Hyperthyroidism Hypercortisolism
Lifestyle	Nutritional inadequacies Lack of sun exposure Low physical activity Cigarette smoking Alcohol use
Medications	Corticosteroids Thyroid medications Laxatives Diuretics Antacids

Bone tissue is comprised of two main types – compact and cancellous. All vital cellular and extracellular components required for normal functioning of cortical and trabecular bone are the same, yet they differ in structure and function. Approximately 90% of compact bone (a.k.a., cortical bone) is calcified, whereas up to 25% of cancellous (a.k.a., trabecular bone), is calcified. Cortical bone plays mainly a structural role, providing support and protection. Trabecular bone houses bone marrow and is highly vascular with abundant amounts of connective tissue; therefore, cancellous bone is considered the "metabolically active bone" (Baron 1996).

Bone contains a protein matrix, 90% of which is comprised of type I collagen fibers (Knott and Bailey 1998). To provide strength, collagen fibers are densely cross-linked in a lamellar structure. Crystallized mineral structures, known as hydroxyapatite $[3Ca_3(PO4)_2 \cdot (OH)_2]$ are located on and around collagen fibers to further strengthen the ultrastructure of bone (Baron 1996).

Three types of bone cells, osteocytes, osteoblasts, and osteoclasts carry out skeletal activity. Osteocytes begin as bone forming cells (osteoblasts), but become entrapped in newly formed bone matrix and undergo calcification (Baron 1996). Osteocytes remain embedded deep within the bone, in the protein matrix, until bone resorption occurs by osteoclastic activity. Osteocytes play a vital role in normal bone activity as these osteocytes appear to initiate bone resorption by communicating through gap junctions to other osteocytes as well as to bone surface receptors (Baron 1996).

Bone Resorption

Osteoclasts are large multinucleated cells primarily responsible for bone resorption. Single osteoclasts are located on the surface of bone independently or are found in small groups. Osteoclasts possess ruffled borders which secrete protons required to reduce pH at the resorptive site which, in turn, dissolves hydroxyapatite and exposes the protein matrix of the bone. Osteoclasts also secrete both lysosomal and non-lysozomal enzymes which resorb the protein matrix (Mundy 1996).

A reversal phase follows osteoclastic resorption. During this phase, osteoblasts migrate to the area of newly exposed bone on the surface where osteoblasts then act to produce more bone (Baron 1996).

<u>Bone Formation</u>

Osteoblasts are mononucleated cells responsible for bone formation. They produce both collagenous and non-collagenous products for the matrix of bone (Puzas 1996). Approximately 20% of the protein produced by osteoblasts is type I collagen (Puzas 1996). Osteocalcin, which constitutes approximately 1% of bone matrix protein, is the most abundantly produced non-collagenous protein secreted by osteoblasts (Eriksen et al. 1995). Once bone matrix components are produced from osteoblasts, mineralization occurs.

Receptors for a variety of calcium and bone regulating hormones, prostaglandins, and cytokines have been found on osteoblasts. Receptors for calcitriol, parathyroid hormone (PTH), and interleukin-6 (IL-6), for example, lie on the surface of these bone forming cells. These agents have been shown to induce bone resorption, so the presence of such receptors on osteoblasts suggests a role for osteoblasts in regulation of bone resorption (Martin and Ng 1994).

Endocrine Function and Bone

<u>Calcium Homeostasis</u>

All but 1% of the body's calcium is located in bone (Volpe 1999). Calcium is a major component of bone's crystallized mineral matrix, hydroxyapatite, which gives strength and structure. The process of mineralization occurs when the blood calcium concentration rises. The blood calcium concentration is tightly regulated and normally maintained within a narrow range of 9 to 11 mg/dl or 2.5 mmol/L (Bronner and Pansu 1999, Volpe 1999). A rise in the serum calcium concentration above normal results in the secretion of calcitonin from the thyroid gland, specifically from C-cells. Release of calcitonin suppresses osteoclastic bone resorption allowing calcium precipitate to form on the bone matrix. The mineralization process returns the serum calcium concentration to normal (Delftos 1996).

On the other hand, when the serum calcium concentration falls below normal, bone provides a readily available source of calcium for restoration of the blood concentration (Volpe 1999). In response to even a slight serum calcium decrement, the parathyroid gland responds by rapidly secreting PTH. The three main mechanisms by which PTH restores the serum calcium concentration are: (1) resorption of skeletal calcium, (2) reabsorption of renal calcium, and (3) synthesis of active vitamin D $(1,25(OH)_2D_3)$ in the kidneys to facilitate intestinal calcium absorption (Holick 1996, Kronenberg 1996).

Surprisingly, receptors for PTH are not located on osteoclasts, but rather on osteoblasts. Parathyroid hormone directly inhibits osteoblastic bone formation (McSheehy and Chambers 1986). When triggered by PTH, osteoblasts produce and release factors that induce osteoclastic bone resorption so that the serum calcium concentration is restored (McSheehy and Chambers 1986).

In response to a sub-normal serum calcium concentration, PTH increases renal reabsorption of calcium, funneling calcium back into circulation and reducing urinary calcium output. Conversion of inactive to active vitamin D, dihydroxycholecalciferol or calcitriol, occurs within the renal tubules with PTH stimulation. Calcitriol increases calcium absorption in the small intestine and enhances osteoclastic bone resorption to help restore the serum calcium concentration to the normal range (Holick 1994).

While dihydroxycholecalciferol is important for restoring the blood calcium concentration when low, through a transient activation of bone resorption, calcitriol also plays an important role in the maintenance of bone integrity (Holick 1996). Provitamin D_3, found in the skin, is converted to previtamin D when exposed to solar ultraviolet-B (UVB) radiation. Previtamin D then converts to inactive vitamin D. Both dietary sources of vitamin D and solar radiation-converted vitamin D undergo an initial hydroxylation in the liver and a second hydroxylation step in the kidneys, resulting in 1,25-dihydroxyvitamin D_3, the active form (Holick 1996).

While vitamin D does not appear to directly drive bone mineralization, vitamin D plays an indirect role by maintaining the normal blood calcium concentration and prevents skeletal disorders (Holick 1996). Vitamin D is essential for adequate intestinal calcium absorption (Pansu et al. 1983) as it is required for expression of the intracellular calcium binding protein, calbindin, and the vitamin D-dependent calcium pump located at the plasma membrane (Johnson and Kumar 1994). Low dietary intake and/or inadequate subcutaneous conversion of vitamin D will lead to reduced calcium absorption and impaired mineral balance. If calcitriol production is reduced and calcium absorption is impaired, PTH secretion will be stimulated and osteoclastic bone resorption will occur to normalize the serum calcium concentration (McSheehy and Chambers 1986). These homeostatic controls adjust as needed in response to fluctuations in the serum calcium concentration and nutrient intakes so that calcium balance is maintained in healthy individuals.

Estrogen and Bone

It is well documented that estrogen is positively associated with BMD ("Position of the American..." 1999, Dombrowski 2000). Puberty, a time of significant increases in circulating estrogen, is marked by the largest gains in BMD and bone mineral content (BMC) (Bonjour et al. 1991, Dombrowski 2000). Throughout the gynecological lifespan of a woman, estrogen protects bone (Dombrowski 2000). Second only to natural aging, lack of estrogen is the most acknowledged cause of bone loss among women (Dombrowski 2000). While estrogen deficiency is clearly associated with menopausal bone loss, lack of estrogen during any stage of a woman's life can have devastating consequences for the skeleton (Wasnich 1996).   When endogenous estrogen production wanes and menstruation is interrupted, such as with menopause, lactational amenorrhea, or anorexia nervosa, an upsurge in bone resorption and a reduction of bone formation occurs (Gallagher et al. 1980). The end result is reduced BMD and BMC with increased risk of osteoporotic fractures. Amenorrhea, an extended cessation of menstrual cycling, mimics menopause in that both are associated with reduced estrogen synthesis and increased bone resorption, leading to BMD losses (Rigotti et al. 1991). Amenorrhea is usually found in association with radical weight loss or severe deficits of energy intake and stores (Cassidy 1999) but may also result from mild energy restriction and weight loss even when a healthy weight is maintained (Rencken et al. 1996).

The positive effects of estrogen replacement therapy (ERT) for preventing, and possibly reversing, bone loss in peri- and post-menopausal women are well documented ("Position of the American..." 1999, Prestwood et al. 1999). Bone mass of amenorrheic women shows positive gains when estrogen-containing oral contraceptives are applied, but questions remain regarding any additional positive benefits of supplemental estrogen on BMD among eumenorrheic premenopausal females (MacDougall et al. 1999).

Estrogen has been shown to directly (Erikson et al. 1988) and indirectly (Gallagher et al. 1980) impact bone metabolism. Serum calcium (Muneyyirci-Delale et al. 1998) and bone formation marker (Gorai et al. 1998) concentrations oscillate in response to estrogen fluctuations throughout the menstrual cycle. The identification of estrogen reseptors on both osteoblasts (Erikson et al. 1988, Komm et al. 1988) and osteoclasts (Pensler et al. 1990) provides evidence of direct effects of estrogen on bone cells. Yet, receptor-mediated events are probably

responsible for fluctuations in the serum osteocalcin concentration that has been associated with the serum estradiol concentration (Nielsen et al. 1990).

Estrogen indirectly affects bone by increasing calcitriol production, thereby increasing active calcium absorption (Gallagher et al. 1980). A fluctuating estrogen concentration is also partly responsible for changing the serum magnesium concentration throughout the menstrual cycle (Muneyyirci-Delale et al. 1998). Estrogen increases cellular uptake and bone deposition of magnesium (Seelig 1993). Magnesium deficiencies are common in postmenopausal osteoporosis due to a reduced estrogen concentration (Seelig 1990) which impairs magnesium absorption (Cohen et al. 1983). Because calcium and magnesium metabolism is interrelated (Seelig 1993, Alcock and MacIntyre 1962) and magnesium is required for normal function of PTH and vitamin D (Seelig 1993), magnesium deficiency results in bone calcium losses and reduced intestinal calcium absorption (Seelig 1993, Zofkova and Kancheva 1995). Therefore, adequate magnesium consumption is essential for women receiving concurrent ERT and calcium therapy (Seelig 1993, Sojka 1995).

Cortisol and Bone

Cortisol is the predominant glucocorticoid produced endogenously (Findling, Aron and Tyrrell 1998). Elevated urinary cortisol excretion has been shown in elderly individuals who have sustained bone fractures (Greendale et al. 1999). The exact mechanistic action of glucocorticoids on bone is not well understood, but the catabolic actions of elevated glucocorticoids (i.e., cortisol) have been documented (Greendale et al. 1999). Glucocorticoids shift bone metabolism in favor of bone resorption (Lukert and Raisz 1990). While glucocorticoids directly affect bone cells, they also affect skeletal structure by decreasing intestinal calcium absorption (Morris et al. 1990). In addition, glucocorticoids regulate PTH. Some studies have shown that PTH is elevated among individuals with elevated glucocorticoid concentrations (Lukert and Raisz 1990, Manelli and Giustina 2000). However, other investigators have found concentrations of PTH to be within the normal range (Wichers 1999). Still, parathyroidectomy has been found to prevent glucocorticoid-induced bone resorption (Canalis 1996, Hattersley et al. 1994) indicating that glucocorticoids augment bone resorption via increased secretion or increased activity of PTH.

Glucocorticoids inhibit bone formation as demonstrated by a reduced serum concentration of a bone formation biomarker (i.e., osteocalcin) found in association with

elevated glucocorticoids (Peretz et al. 1989). This likely occurs due to direct effects on osteoblasts (Canalis 1996) and to indirect effects on glucocorticoid-induced reductions in sex-steroid synthesis (Lukert and Raisz 1990). There is also speculation that glucocorticoids interfere with the growth hormone-insulin-like growth factor-1 (IGF-1) axis (Canalis 1996). Glucocorticoids have complex actions on cells of the osteoblast lineage. Glucocorticoids have been found to reduce cell replication of this cell type, thereby reducing the number of cells capable of forming bone matrix (Canalis 1996, Delaney, Gabbatis and Canalis 1995). Furthermore, glucocorticoids increase collagen degradation by upregulating the synthesis of matrix metalloproteinases responsible for bone resorption (Delaney et al. 1995, Rifas et al 1994). These two factors, reduced collagen synthesis and increased bone turnover, are key pathways by which glucocorticoids contribute to low BMD and, ultimately, osteoporosis.

Growth Hormone, Insulin-like Growth Factor-1 and Bone

During early puberty, linear growth of the skeleton is stimulated by circulating sex steroids (Van Coeverden et al. 2002) which stimulate production of growth hormone (GH) (Krabbe et al. 1982). Circulating GH and its primary mediator, IGF-1, enhance bone growth directly (Van Coeverden et al. 2002) and indirectly by stimulating further production of sex steroids (Holmes and Shalet 1996, Krabbe et al. 1982). Sex steroids, GH, and IGF-1 are all positively associated with BMC and accumulations in BMC during puberty (Van Coeverden et al. 2002).

Wright and colleagues (1995) studied GH and bone parameters for four weeks in two groups of 3 week-old GH knock-out dwarf rats: an untreated control group and a group treated with recombinant GH. Serum alkaline phosphatase (a marker of bone formation), IGF-1, body weight, total body BMD, and bone volume were all significantly lower in control rats compared with GH-treated rats.

Insulin-like growth factor-1 is required for adequate growth and development (Yakar et al. 2002) and most bone related activities of GH are believed to be carried out through IGF-1. Hematopoiesis, sex steroid production, and growth of bone, cartilage and muscle tissue are all stimulated by the presence of IGF-1 (Boonen et al. 2002, Styne 1997a). Produced in most tissue, IGF-1 can act in autocrine and paracrine fashions, whereas the majority of endocrine IGF-1 is produced in the liver and has a systemic effect (Styne 1997a). In circulation, IGF-1 is complexed with binding proteins, primarily insulin-like growth factor binding protein-3 (IGFBP-

3) and an acid labile subunit (Yakar et al. 2002, Styne 1997a). This acid labile subunit is not found in extravascular fluid; thus, it is believed that the role of IGFBP-3 is to regulate IGF-1 passage from circulation to the site of biological activity (Yakar et al. 2002).

Reduced IGF-1 (Jehle et al. 2003; Sugimoto et al. 1997) and IGFBP-3 (Jehle et al. 2003; Johansson et al. 1997) concentrations have been associated with reduced BMD in osteoporotic patients. Mice with knockouts of both liver IGF-1 and the acid-labile subunit have a significantly increased GH production and an 85% reduction in circulating IGF-1. Despite elevated GH, a severe attenuation of linear growth, a 10% reduction in BMD, and a 35% reduction in periosteal circumference and cortical thickness occurred (Yakar et al. 2002). With 4 weeks of IGF-1 treatment, total expected height of the proximal growth plate was achieved among these knockout mice (Yakar et al. 2002). These results demonstrate the importance of IGF-1 in regulating growth in mice.

Serum IGF-1 may also be responsible for modulating some bone-related activities of estrogen. For example, while PTH is typically known for its catabolic effects on bone, in conjunction with estrogen, PTH can have anabolic effects through stimulation of osteoblast function. These PTH-modulating effects of estrogen seem to involve IGF-1 (Nasu et al. 2000). *In vitro*, the presence of an anti-IGF-1 antibody inhibits the effects of estrogen and IGF-1 on bone. But, when estrogen activity is blocked by an anti-estrogen agent (i.e., tomoxifen), pretreatment with IGF-1 allows for estrogen-like activity (Nasu et al. 2000).

The importance of adequate dietary intake on GH and IGF-1 are apparent from research. Both mild (Norrelund, Riis and Moller 2002) and severe (Jacoangeli et al. 2002) undernutrition are associated with alterations in GH and IGF-1 concentrations. During reduced-energy weight-loss diets, GH is elevated and appears to help preserve body protein stores (Norrelund, Riis and Moller 2002). In healthy middle-aged and elderly men, dietary intakes of protein and the minerals potassium, zinc, magnesium, calcium, and phosphorus as well as total energy have been positively associated with serum IGF-1 concentrations (Giovannucci et al. 2003). Because nutritional intake is associated with these bone-mediating endocrine factors, it is necessary to include these variables in investigations of dietary intake as it relates to bone health.

Dietary Intake and Bone

Bone serves as a reservoir for several minerals available from the diet (Broadus 1996). In addition, dietary constituents are essential for production and activity of enzymes (Czajka-Narins

1992), hormones (Seelig 1993, Zofkova and Kancheva 1995), and bone cells (Czajka-Narins 1992) required for bone metabolism. The integrity of bone is affected by inadequacies and imbalances of nutritional components, including, but not limited to, calcium and vitamin D. Therefore, the relationships between dietary intake and bone health must be better understood.

<u>Calcium and Bone</u>

The skeleton serves as a reservoir for ~99% of the body's calcium (Volpe 1999). Calcium accounts for 40% of bone mineral (Bronner and Pansu 1999); therefore, it is understandable why dietary calcium is promoted for improvement and maintenance of bone integrity.

Both active and passive transport systems are involved in calcium absorption (Bronner and Pansu 1999). Along the apical membrane of the duodenum, calcium ions are actively transported through transcellular calcium channels. Once within the intestinal cell, calcium is rapidly bound to calbindin in order to maintain tight regulation of the intracellular calcium ion concentration. Calbindin then transports calcium to the ATP-dependent calcium pump located on the basolateral surface of the intestinal cell. Against electrochemical and concentration gradients, calcium is pumped out of intestinal cells into serum (Bronner and Pansu 1999).

Dietary intake of calcium determines transport system usage. When dietary calcium intake or bioavailability is low, active transcellular calcium transporters predominate (Bronner and Pansu 1999), but when dietary calcium intake is above 800 mg/d, the active transport processes are downregulated (Buckley and Bronner 1980) and passive transport is favored (Pansu et al. 1993).   Passive, paracellular transport occurs primarily in the jejunum and ileum down an electrochemical gradient (Bronner and Pansu 1999).

Levels of calcium intake alone do not fully determine absorption. Calcium absorption is enhanced by vitamin D intake, lactose and glucose availability, an intact and optimally functioning digestive tract, and in times of increased need (i.e., pregnancy) (Volpe 1999). Calcium bioavailability is hindered by high dietary fiber, phytate, and oxalate intakes (Volpe 1999). Urinary calcium excretion is increased with high dietary protein and sodium intake (Volpe 1999), and diets high in phosphorus have been shown to reduce the serum ionized calcium concentration, thereby inducing PTH secretion and consequently resulting in bone demineralization (Calvo and Park 1996).

16

A number of studies demonstrate a direct relationship between dietary calcium intake and BMD (Black et al. 2002, Rubin et al. 1999, Salamone et al. 1996, Turner et al. 1998). In light of robust causal data linking dietary calcium intake and BMD, Adequate Intake (AI) recommendations for calcium were recently increased (Food and Nutrition Board 1999). A study by Black and colleagues (2002) exemplifies the importance of adequate dietary calcium intake for children, in particular. While milk is not the only dietary source of calcium, it is a major source in the United States. A group of children who avoided milk were compared to age- and sex-matched children who regularly consumed milk (Black et al. 2002). Among the milk avoiders, the mean calcium intake was low (443 $\pm$ 230 mg Ca/d) indicating that other high-calcium foods were not consumed to replace calcium missing from milk avoidance. Compared to milk consumers, milk avoiders were significantly shorter and had significantly smaller skeletons. Milk avoiders also had significantly lower BMC and BMD compared to milk consumers. Furthermore, of these fifty children who avoided milk, twelve had previously sustained fractures on one or more occasions. Annual distal forearm fractures among children are expected in 1% of this population (Jones et al. 2000); yet, among these children, the annual incidence was 3.5% (Black et al. 2002).

While the relationship between calcium and bone health has been the focus of much research, many other dietary factors are important to bone metabolism and bone integrity. Other dietary components play a role in bone health by directly affecting bone or playing roles in preserving calcium economy (New et al. 2000, Rubin et al. 1999). Several dietary components with known roles for bone health are listed in Table 2 (Dhonukshe-Rutten 2003, Kleerekoper and Avioli 1996, New et al. 2000, "Position of the American…" 1999, Rubin et al. 1999). Nutrients and dietary compounds most commonly associated with BMD are discussed below.

Phosphorus and Bone

Calcium and phosphorus are the two major components of hydroxyapatite, the main crystalline structure of bone. Hydroxyapatite crystals hold approximately 85% of total bone phosphorus (Broadus 1996). Therefore, it is clear that phosphorus is essential to bone structure.

Passive diffusion is the route of phosphorus absorption (Calvo and Park 1996). In the United States, where phosphorus consumption is rarely inadequate, deficiency is not of general concern (Czajka-Narins 1992). Due to the abundance of phosphorus contained in carbonated beverages and processed foods, over-consumption of phosphorus is of concern with regard to

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	Total energy
Fluoride	Vitamin D	Oxalates	
Iron	Vitamin K	Phytates	
Magnesium	Vitamin B-12	Phytoestrogens	
Phosphorus		Protein	
Potassium			
Sodium			
Zinc			

bone health (Calvo and Park 1996). Furthermore, while phosphorus is essential to bone and can reduce urinary calcium output, a balanced calcium to phosphorus ratio is critical. Unfortunately, foods typically high in phosphorus, such as soft drinks, are often low in calcium (Calvo and Park 1996). A ratio of dietary phosphorus to calcium intake greater than 1:1 can be detrimental to bone. High dietary phosphorus consumption can reduce the serum ionized calcium concentration, thereby inducing PTH secretion and resulting in bone loss (Calvo and Park 1996). While these detrimental effects of high dietary phosphorus intakes on bone are debated, concern regarding the potential for high phosphorus intakes to alter calcium-regulating hormones is justified.

Magnesium and Bone

The third major mineral component of bone is magnesium. Approximately 67% of total body magnesium is found in the skeleton (Broadus 1996). Calcium and magnesium share a common absorption pathway in the small intestine, and several metabolic functions of calcium and magnesium are intertwined (Alcock and MacIntyre 1962, Seelig 1993). Magnesium is critical for regulation of PTH, vitamin D, and calcitonin (Seelig 1993, Zofkova and Kancheva 1995) and, thus, plays an essential role in normal bone metabolism. Due to the reliance on magnesium for maintaining these calcium-regulating hormones, hypocalcemia and subsequent bone loss can result when dietary intake is inadequate, absorption is impaired, or retention is altered (Rude et al. 1978). New et al (2000) found an inverse relationship between dietary magnesium intake and urinary pyridinoline excretion, a marker of osteoclast activity, suggesting that osteoporosis may be caused, in part, by inadequate magnesium intake.

Because a common absorption pathway is shared, a high calcium intake can impair magnesium absorption (Alcock and MacIntyre 1962, Cohen et al. 1983). Moreover, high dietary calcium intakes may not optimally benefit bone if magnesium absorption is impaired. An optimal calcium to magnesium ratio for dietary intake has not been determined, yet due to the essential nature of these minerals for skeletal health and their interactions, consensus regarding the optimal ratio is needed.

Zinc and Bone

The amount of zinc in the human body is between 1.5 and 2.5 grams. Nearly 30% of the total body zinc is contained in bone (Volpe 1999). In the small intestine, zinc absorption occurs by carrier-mediated processes and diffusion (Czajka-Narins 1992). Several dietary components

affect zinc absorption. When zinc is consumed with dietary protein, amino acids form zinc chelates and enhance zinc absorption (Czajka-Narins 1992, Lonnerdal 2000). Zinc absorption is reduced when consumed in a meal containing fiber and phytates. Diets high in copper or iron also inhibit zinc absorption due to the competition of these minerals with zinc for absorption. (Abdel-Mageed and Oehme 1991, Czajka-Narins 1992).

Zinc and calcium metabolism are inter-related. Osteoblast differentiation is stimulated by zinc, and synthesis of bone proteins is promoted by zinc (Chen et al. 1998, Hashizume and Yamaguchi 1993, Yamaguchi et al. 1988). *In vitro*, zinc has been shown to inhibit osteoclastic resorption (Moonga and Dempster 1995). *In vivo* studies demonstrate a zinc-induced stimulation of bone synthesis, but with excess intakes of zinc, this action does not occur. With excessive zinc intake, bone microarchitecture is undermined and overall bone integrity is decreased (Kawamura et al. 2000). Furthermore, excessive supplemental doses of zinc (140 mg/d) impair calcium absorption at the small intestine when dietary calcium intake is inadequate (Spencer et al. 1997).   It has been shown from animal research that zinc added to a low-calcium diet reduces BMC and bone strength beyond that of a low-calcium diet without added zinc (Kenney and McCoy 1997). Thus, while zinc is an essential component of bone that aids in formation of bone, its benefits are generally realized only with adequate dietary calcium intake. When dietary calcium intake is inadequate, high zinc intake may not be advantageous to bone structure and strength.

Fat, Protein, Sodium and Bone

The aforementioned information demonstrates that several nutrients are important for bone, not only because of skeletal storage and for structural support, but because they have important functions in calcium homeostasis and bone metabolism (New et al. 2000, Rubin et al. 1999). Other nutrients also impact BMD via effects on calcium balance (New et al. 2000, Rubin et al. 1999). Dietary fat, protein, and sodium are consumed in amounts typically well above recommended levels in American diets (Kennedy et al. 1999). While the effects of such high dietary intakes of these nutrients on BMD are not well described, concern is justified. Multifaceted nutrient-nutrient interactions exist between dietary fat, protein, sodium, and calcium, respectively (Heaney 1993). Dietary fat may prevent calcium absorption by binding to calcium and forming calcium soaps which are passed along the gastrointestinal tract for removal (Bronner and Pansu 1999). An inverse relationship between the dietary calcium to protein ratio

and BMD in young women has been shown even when calcium intake is adequate but protein is high (Teegarden et al. 1998). Renal calcium excretion is stimulated by high dietary intakes of both protein and sodium (Packard and Heaney 1997). Research regarding appropriate dietary fat, protein, and sodium intakes is on-going, notably in relation to calcium requirements and for optimal bone health. At present and with current dietary intakes in Americans, the most recent recommendation for calcium intake is supported (Food and Nutrition Board 1999).

Fiber and Bone

The typical American diet contains far less dietary fiber than is recommended (Hendricks and Herbold 1998). Improved health and protection against disease are reasons to support increased dietary fiber intake (Davidsson et al. 1996). However, bone health does not appear to benefit from a high dietary fiber intake as dietary fiber may interfere with mineral absorption (Davidsson et al. 1996) which may explain the inverse association between dietary fiber consumption and BMD (Lloyd et al. 1987). Hoffman et al (1999) examined the effects of a fat/fiber diet verses sugar/starch (control) diet on BMC in growing foals. At completion of this year-long study, foals provided the fat/fiber diet had significantly lower BMC than foals provided the sugar/starch diet. They (1999) concluded that calcium-binding to fat and fiber in the experimental diet impaired the bioavailability of calcium. In humans, however, it appears that when combined with an overall healthy diet, fiber consumption is not detrimental to bone. New and colleagues (2000) found that among premenopausal women, those who consumed higher quantities of fruits and vegetables earlier in life had higher BMD at 40 to 60 years if age compared to women with lower consumption of fruits and vegetables during childhood. While fruits and vegetables contain fiber, they also contain an abundance of other nutrients, particularly zinc, magnesium, potassium, and vitamin C which may positively influence BMD and override the negative effects of fiber. This assertion is backed by previous research that shows diets high in zinc, magnesium, potassium, and vitamin C were associated with high BMD in premenopausal women aged 44 to 49 years (New et al. 1997).

In addition to reduced intestinal calcium absorption with a high fiber diet (Davidsson et al. 1996), fiber may further affect bone health by reducing serum estrogen levels. Both mild and extremely restrictive dieting behaviors are commonly associated with an increased intake of dietary fiber, often from fruits, vegetables or fiber supplements while eliminating other foods. Elevated fecal estrogen excretion (Dorgan 1996, Goldin et al. 1981, 1982) and reduced serum

estrogen concentrations (Bagga 1995) have been reported in women consuming high fiber diets. Because of the critical role of estrogen on bone health (Rubin et al. 1999, Salamone et al. 1996), further investigations of fiber intake and estrogen status are warranted.

Understanding the role of individual nutrients in bone health is difficult due to the fact that absorption, metabolism, and function of many nutrients are affected by each other. Disagreement between epidemiological and experimental data regarding the influence of dietary components on bone health suggest that positive and/or negative effects of single nutrients may be modulated by total dietary intake and components (Spencer et al. 1997). Thus, it is necessary to consider the total diet as well as individual components when assessing the impact of nutrients on bone health.

Dieting and Bone

Body weight is positively associated with BMD (Orozco and Nolla 1997) and overweight and obese individuals have a reduced risk of osteoporosis compared to underweight individuals (Wasnick 1996). This positive association between body mass and BMD is likely a result of increased strain on bone from carrying weight (Rubin et al. 1999; Salamone et al. 1996). In postmenopausal years when estrogen tends to be low, overweight women are further protected against osteoporosis due to the ability of adipose tissue to convert androgens to estrogens and provide a natural source of estrogen otherwise lacking in normal weight and underweight postmenopausal women (Davidsson et al. 1996). While high body mass is protective of BMD, excess weight carries with it many negative health risks and, therefore, weight loss is often required.

Dieting, in order to reduce body weight, can have significant effects on bone. Weight loss, anthropometric changes, nutritional adjustments, energy requirement modifications, and hormonal alterations can occur with dieting. Both animal and human models have been studied in order to examine the effects of acute and chronic dieting, with and without weight loss, on BMD.

Evidence from Animal Studies

Lane and colleagues (1995) demonstrated that long-term (6 years) moderate food restriction in juvenile male rhesus monkeys resulted in shortened stature and reduced BMC, but not BMD. This suggests the importance of adequate energy intake to support bone growth throughout the years of skeletal modeling.

Ndiaye et al. (1995) fed groups of rats energy restricted diets at 0, 20, 40, and 60% below energy needs and observed the effects on bone formation. Following four weeks of experimental diets, serum osteocalcin, calcitriol, PTH, calcium, and phosphorus were analyzed from pooled serum collections. All three experimental groups (20, 40, 60% energy restricted groups) had significantly lower serum osteocalcin, calcium, and phosphorus concentrations than controls (0% group). No significant differences in serum PTH or calcitriol concentrations were noted between groups. These results indicated that a reduction in bone formation, independent of parathyroid or vitamin D status, was instigated by energy restriction in rats (Nyiade et al. 1995).

Talbott and colleagues (1998) conducted a nine-week investigation of the effects of energy restriction, calcium restriction, and energy plus calcium restriction on bone remodeling in a group of three-month- and ten-month-old female rats. A single group of rats from each age group was fed diets adequate in energy and calcium to serve as controls. Energy restriction was induced by reducing the carbohydrate content of the diet, while levels of dietary fat, protein, vitamins, minerals, and fiber remained equal between groups. Calcium restricted rats consumed 15 mg of calcium daily while control rats were fed 78 mg of calcium daily. Phosphorus was decreased in relation to calcium to maintain equal calcium to phosphorus ratios of all diets (Talbott et al. 1998).

Urinary tritiated tetracycline concentrations, a marker of bone resorption, were measured weekly throughout the study. 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-weeks (Talbott et al. 1998). In energy restricted rats of both ages, body weight was compromised after the 9-week study period. Despite age, urinary tritiated tetracycline excretion was significantly greater in all energy, calcium and energy plus calcium restricted groups versus controls, but after the 6th week, bone resorption was decreased to levels similar to control levels in all energy and calcium restricted groups. No additive effect was observed in those animals receiving the restricted energy plus calcium diets. Despite age, rats in the energy restricted groups had higher serum concentrations of osteocalcin versus rats in the calcium restricted and control groups (Talbott et al. 1998).

An increase in BMD was observed in all 3-month-old rats during the 9-week study. Compared to control rats, however, 3-month-old calcium restricted rats had significantly smaller increases in BMD. Compared to control rats, 3-month-old energy restricted rats possessed final

BMD values that were comparable to controls; however, body weight in energy restricted rats remained lower. These results suggest that in growing rats, energy restriction may prevent body mass increases, but may not negatively impact BMD if calcium intake meets needs. Conversely, with restricted dietary calcium in young rats, bone mineral acquisition is attenuated (Talbott et al. 1998).

In the 10-week-old rats, BMD increased significantly over the 9-week study in control rats only, but not in any of the energy or calcium restricted rats. Urinary tritiated tetracycline excretion was significantly higher in the 10-week-old energy-restricted animals. Findings from this study indicate that bone remodeling in response to restricted calcium and/or energy is elevated regardless of age, but that the effect of energy restriction on BMD may be dependent on age (Talbott et al. 1998).

Evidence from Human Studies

Severe, chronic undernutrition, such as that associated with anorexia nervosa has been shown to impart drastic effects on bone mass (Mazess et al. 1990). For example, it has been shown that young-adults with anorexia nervosa have 25% lower total BMC than healthy, age-matched women (Mazess et al. 1990). These effects are likely due to a combination of factors negatively influencing bone metabolism such as low body weight, inadequate nutrient intake, and disturbed hormonal balance. While these factors are extreme in women with anorexia nervosa, where the body is starved of food, similar, yet less extreme, effects can occur among healthy populations following moderate weight loss dieting.

Ramsdale and Bassey (1994) studied the effects of a long-term, moderate energy-restricted diet with resulting weight loss on BMD. Participants (n = 45) followed a low-fat, low-sugar diet for 6 months in order to attain a healthy BMI of 20 to 25. Weighted food records were kept for three days prior to dietary adjustments and again at 4 months. At 0, 3, and 6 months, DXA measurements were taken to assess total body, lumbar spine, and proximal femur BMD. Total energy consumption decreased approximately 27%. Reductions in micronutrient intakes, including calcium, phosphorus, and vitamin D, occurred in conjunction to energy reductions. Body mass was significantly reduced (-3.4 $\pm$ 3.1 kg; mean $\pm$ SD) over 6 months. Significant loss of total body and lumbar spine BMD also occurred over the 6 months, but femoral BMD measurements did not significantly change. Over 50% of individuals (n = 23) lost greater than

5% of initial body weight. Loss of total body and lumbar spine BMD was approximately twice as great among this group compared with those who lost less than 5% of initial body weight.

Compston and coworkers (1992) demonstrated that rapid weight loss was associated with significant reductions in whole body BMD. In this study, a group of 13 obese women aged 37 to 60 years were placed on a very-low-calorie (405 kcals/d) diet for 10 weeks followed by a 10-month period during which subjects returned to original pre-diet weights. Whole body BMD measurements were completed at weeks 11, 23, and 57. During the 10-week diet period, significant weight loss occurred. Accompanying the weight reduction were significant reductions in whole body BMD measurements, but as weight returned to pre-diet levels, return of BMD to baseline values were also observed. This study demonstrates that bone mass is lost in response to reduced body mass, but with subsequent weight gain, whole body BMD increases as well (Compston et al. 1992).

Results from this study are interesting yet limited. The use of whole body BMD analysis is insightful but does not provide information regarding sites of common osteoporotic fractures such as the hip or spine. Thus, although it appears that weight cycling would not detrimentally impact BMD due to the return of BMD with return of body weight, it is unclear if bone mass is lost and re-gained from the same skeletal site. Thus, from this study (Compston et al. 1992), the overall effects of weight cycling on risk of bone fracture remain unclear.

Avenell and colleagues (1994) helped delineate questions with regard to loss and gain of body weight and changes in site-specific BMD. In order to lose 20% of excess body weight, 16 overweight postmenopausal women adhered to a low-calorie (1200 kcal/d), low-fat (21% kcals from fat), high fiber (19 g cereal fiber/d) diet for six months. A subsequent six months were allowed for participants to return to baseline weights. Forty-six age- and weight-matched non-dieting women were included as controls. Lumbar spine and femoral neck BMD measurements were performed by DXA at months 0, 3, 6, and 12 for dieters and at months 0 and 12 for controls. A loss of lumbar spine and femoral neck BMD occurred for both dieters and controls. While differences between the groups in femoral neck BMD changes were not observed, the dieting group had a loss of spinal BMD at 6 months twice that of the interpolated 6-month reductions among controls. With continued loss of spinal BMD among dieters, despite weight regain, 12 month reductions in spinal BMD among dieters was more than two-fold greater than controls. This study supports other reports of reductions in BMD when body weight is reduced.

This study further implies that when weight is returned following weight loss, bone mineral will not necessarily be restored to its original site and, therefore, BMD may remain compromised (Avenell et al. 1994). Since weight cycling is a common phenomenon in Western societies, these results have widespread implications.

Exercise is often recommended along with dietary restrictions in order to achieve weight loss. Weight-bearing activities benefit bone mass and are associated with higher BMD among younger groups (Teegarden et al. 1996) and maintenance of bone mass in adults (Packard and Heaney 1997). It is logical to question if exercise added to dietary restriction would negate the negative effects of weight loss on BMD. Therefore, Svendsen and colleagues (1993) utilized a group of overweight postmenopausal women in order to examine the effects of weight loss induced by diet alone or diet plus exercise on BMD. Participants in this 3-month intervention were randomly assigned to one of three groups: controls (n = 21) who maintained their usual diet and activity level; a 4,200 kJ/d diet (n = 51); or a 4,200 kJ/d diet with combined aerobic plus anaerobic exercise (n = 49). At baseline and after 12 weeks, DXA scans were performed for analysis of body composition and whole body, lumbar spine, and forearm BMD. Compared to controls, both diet groups experienced significant weight loss. Although total weight loss was not significantly different between the two experimental groups, for those women in the diet-plus-exercise group fat mass accounted for a significantly greater proportion of weight lost compared to non-dieters (fat mass lost = 9.6 kg versus 7.8 kg, respectively). Over the 12 weeks, both diet groups had a trend for reductions in whole body BMD. The diet-plus-exercise group experienced significant reductions in spinal BMD which did not occur in either the diet-alone group or controls (Svendsen et al. 1993). These results challenge other reports which consistently tout exercise as a means of preserving and/or enhancing BMD by showing that exercise may actually increase the amount of bone lost in conjunction to weight reduction.

Andersen, Wadden, and Herzog (1997) performed a similar investigation of the effects of diet or diet plus resistance exercise on bone mass. Participants included 21 obese postmenopausal women who were placed on a 24-week diet consisting of 925 to 1,500 kcals. The diet alone group (n = 9) was asked to limit overall physical activity and specifically refrain from any resistance training. The remaining women (n = 12) engaged in regular resistance exercise sessions throughout the study. Significant losses of body weight, fat mass and lean body mass occurred within both groups with no significant differences between groups. At

completion of the study, both groups had significant reductions in femoral neck BMC and BMD and trochanter BMC. In addition, the exercise group also had significant reductions in trochanter BMD. When loss of BMD was calculated as an absolute value, no significant differences were noted between groups but when loss was calculated as a percentage of initial BMD, the exercise group experienced a two-fold greater reduction in BMD at the femoral neck and trochanter compared to the non-exercise group. These results support those of Svendsen and colleagues (1993) indicating that resistance training does not reduce or prevent bone loss accompanying diet-induced weight loss and may, in fact, enhance bone loss (Andersen, Wadden, and Herzog 1997).

Most research examining the effects of weight loss diets on BMD have utilized overweight (Avenell et al. 1994, Svendsen et al. 1993) or obese individuals (Andersen, Wadden, and Herzong.1997). It is unclear if similar effects would be found in normal weight dieters. Salamone and colleagues (1999) attempted to fill this void in research by investigating effects of weight loss on BMD and bone metabolism in a group of 236 normal weight (BMI $\leq$ 24.44), overweight (BMI = 24.45 to 26.44) and obese (BMI $\geq$ 26.45) females. Participants included healthy, premenopausal women aged 44 to 50 years who were randomly assigned to a dietary intervention (n = 115) or control (n = 121) group. Individual moderate weight loss goals based on initial BMI were established for those in the dietary intervention group. In order to achieve weight loss, women were directed to follow a diet containing less than 25% of total energy from fat and to increase moderate physical activities such as walking. Calcium supplements (1,200 mg/d) were recommended. When weight loss goals were attained, participants were advised to maintain weight by slowly increasing energy intake. At baseline, and after 18 months, serum osteocalcin and urinary N-telopeptide concentrations were measured and BMD of the whole body, lumbar spine, and total proximal femur (TPF) was analyzed by DXA.

Dieters were successful at reducing dietary fat intake and increasing physical activity while controls maintained baseline dietary intake and physical activity throughout the 18 months. Dieters lost significant amounts of body weight (-3.2 $\pm$ 4.7 kg). Both groups experienced significant BMD loss of the lumbar spine and TPF. Loss of TPF BMD among dieters was approximately twice that of controls (-0.81 $\pm$ 1.3% and $-$ 0.42 $\pm$ 1.1%, respectively). Changes in spinal BMD showed a similar, but non-significant difference between groups (-0.70 $\pm$ 1.4% and -0.37 $\pm$ 1.5% in the weight-loss and control groups, respectively). Interestingly, among those

with the greatest levels of physical activity, there appeared an attenuation of spinal BMD losses, yet this effect was not seen for TPF BMD. Biochemical markers of bone turnover were not significantly different between groups but among those with the greatest loss of initial body weight (> 5.3%, n = 17), nearly significant (p = 0.052) annual percent changes in N-telopeptides (+13.6%/year) were found when compared to remaining dieters (n = 51; +2.0%/year) and controls (-2.4%/year). Similar differences in osteocalcin were not observed. These results add to data showing a direct relationship between weight loss and BMD loss. Furthermore, these weight loss-induced changes in BMD and bone metabolism occurred in women regardless of initial BMI. Therefore, these results have implications for obese, overweight, and normal weight dieting women (Salamone et al. 1999).

These aforementioned studies have reported a loss of bone mass when body mass is reduced, likely resulting, in part, from the reduced load placed on the bone. However, reducing dietary intake results in biochemical changes that may affect bone and result in bone mass changes. Talbott and Shapses (1998) assessed fasting-induce biochemical changes in bone metabolism. A group of college-aged male rowers (n = 14) was placed on a 24-hour water-only fast. A group of 13 male rowers served as controls and kept 24-hour food logs. Blood and urine were collected before and after the 24-hour fast to assess bone formation by serum osteocalcin and bone resorption by total urinary pyridinium cross-links. Following this 24-hour fast, markers of bone formation and resorption were significantly reduced indicating a rapid metabolic response to energy restriction by osteoblasts and osteoclasts. Energy consumption among controls varied widely, placing some individuals in negative energy balance. Regression analyses showed a significant inverse correlation between energy intake and biomarkers of bone resorption, and a significant positive correlation between energy intake and markers of bone formation. This provides additional support for the association between energy intake and bone turnover (Talbott and Shapses 1998).

Grinspoon and colleagues (1995) conducted an investigation of the effects of a 4-day fast and related acidosis on bone metabolism. Fourteen women aged 18 to 26 years followed a complete, water-only, 4-day fast. A calcium deplete multivitamin was provided to all subjects. Participants were equally divided into two groups. Half consumed an oral potassium bicarbonate ($KHCO_3$) solution to counteract acidosis. The remaining control group did not receive this neutralizing solution. At baseline and completion of the study, serum was analyzed for

bicarbonate, calcium, ionized calcium, and PTH concentrations as well as venous pH. Bone formation was assessed through measurements of serum osteocalcin and type-I procollagen carboxy-terminal propeptide. Bone resorption was assessed through measurements of urinary pyridinoline and deoxypyridinoline. Those not receiving $KHCO_3$ had significant reductions in serum bicarbonate levels, indicative of metabolic acidosis which was further evident from a reduction in venous pH. Serum and total ionized calcium concentrations and urinary calcium excretion increased significantly in those not receiving a neutralizing agent. Acidosis among this group was also related to a significant reduction in PTH concentration. Despite changes in calcium balance, markers of bone resorption did not change significantly for either group over the 4-day fast. Interestingly, markers of bone formation declined significantly in both groups indicating that acid-base balance was not to blame for this change in bone metabolism. These results indicate that metabolic acidosis may increase bone mineral dissolution, thereby altering calcium balance independent of osteoclast activity or PTH. Furthermore, reduced osteoblast activity induced by fasting occurs independent of acid-base balance (Grinspoon et al. 1995).

Dieting is a common practice for many young-adult females (Story et al. 1991, Grunewald 1985). Evidence shows that energy restricted diets accompanied by weight loss negatively affect BMD (Avenell et al. 1997, Ramsdale and Bassey 1994, Salamone et al. 1999) and that acute fasting disrupts bone turnover (Grinspoon et al. 1995, Talbott and Shapses 1998). Weight reduction and maintenance diets are practiced by many women, yet it remains in question whether self-prescribed chronic energy restriction or food avoidance, with or without weight loss, impact bone health. Nickols-Richardson and Beiseigel (2003) investigated the relationships between chronic dieting and bone in young-adult females. They originally sought to identify chronic dieters. A group of 56 young-adult eumenorrheic females were separated into two groups based on self-reported dietary intake data collected from food frequency data. Participants were classified as chronic energy restrictors (n = 18) if average daily energy intake was less than 67% of estimated energy needs. Nonrestrictors (n = 38) were women whose daily energy consumption was between 67% and 125% of estimated energy needs. Interestingly, it was found that energy restrictors had significantly higher total body BMD than nonrestrictors (Nickols-Richardson and Beiseigel 2003). While the methods used in this report are questionable due to the potential for underreporting of food intake and the fact that the study was cross-sectional, the question remains as to whether structured weight-loss diets affect bone in the same

29

way as chronic energy restriction that may or may not be associated with weight reduction. Because young-adult females are nearing the age of peak bone mass, and attainment of peak bone mass is a necessary factor for osteoporosis prevention, the need for further research into the relationship between chronic dieting and bone health among young-adult females is needed.

Dietary Intakes of College Women

Dietary practices among young-adult females affect current and future health, specifically bone health. Osteoporosis prevention should be aimed at younger females, and yet a 1994 report by Kasper and colleagues found that while 90% of college females had heard of osteoporosis, less than half had been given information from educators or physicians. Most females in this study were able to identify some risk factors for osteoporosis, but inadequate calcium intake and a sedentary lifestyle were correctly identified by only 6.7%. A majority of these females did not perceive themselves to be at risk for osteoporosis and felt that osteoporosis was trivial compared to other diseases (Kasper et al. 1994).

Women are commonly affected by negative, and often distorted, body images. Bailey and Goldberg (1989) found that in a group of 59 young-adult, normal weight women, 85% viewed themselves as overweight. Schulken and colleagues (1997) asked a group of college women to choose from a group of body silhouettes the silhouette found most desirable. An underweight image was chosen by 80% of participants.

Among young-adult females, dieting and weight loss practices are driven more by a desire for thinness than health. This, in addition to a lack of knowledge concerning risk factors for common diseases that affect women, may further increase risk factors for disease, particularly osteoporosis, among young-adult females which will follow them into later life.

Few studies have reported the overall dietary intakes of American women in college. Inconsistent eating patterns due to daily schedule changes, social pressures, and school-related stresses make dietary assessment difficult. With regard to bone health, insight into the dietary practices of college-aged women is valuable as they are nearing the age of peak bone mass and diet may influence the attainment of peak BMD.

Twenty-four hour dietary recall data of females aged 20 to 29 years was collected in the National Health and Nutrition Examination Survey of 1988 to 1991 (NHANES III) from which daily intake averages were calculated (Hendricks and Herbold 1998). For women in this age group, fat and saturated fat intakes were higher than recommendations while dietary fiber,

calcium, zinc, iron, and folate were lower than recommended (Hendricks and Herbold 1998). These findings may be attributed to the report that food consumption outside the home came primarily from fast food restaurants, vending machines and convenience stores (Sneed and Holdt 1991) where foods are commonly high in fat and saturated fat and lacking in other nutrients.

Two-day dietary records were used to evaluate dietary intakes of 335 college women (Vickery et al. 1985). Dietary analysis was performed for protein, carbohydrate, fat, thiamin, riboflavin, niacin, calcium, iron, vitamin A, and vitamin C content. Over 75% of these women were found to have consumed greater than 67% of the 1980 Recommended Dietary Allowances (RDA) for all nutrients except niacin, iron, and vitamin A (Vickery et al. 1985). While these findings demonstrate fairly good dietary intakes among college-aged women, these findings are limited in that a two-day dietary record does not truly evaluate the overall dietary intake, particularly in a group prone to consuming foods outside the home at irregular schedules.

Since this research was published in 1985, the Adequate Intake (AI) of calcium for females in this age range has increased from 800 mg/d to 1,000 mg/d (Food and Nutrition Board 1999). Using the current recommendations for calcium, it appears that, in fact, a much greater proportion of these participants were consuming diets low in calcium.

Food choices in the United States are not driven solely by hunger and taste preference, but often by psychosocial influences, particularly among women. Koszewski and Kuo (1996) surveyed 141 college-aged females and found that more than half were unhappy with their current body weight. For these women, dieting and patterns of food restriction began at about the age of 15. Of these females, 40% had lost and re-gained 10 pounds over the previous 24 months and unhealthy weight-loss practices, including use of laxatives (9%) and purging (10%) were not uncommon (Koszewski and Kuo 1996).

Due to America's obsession with being thin, dieting for weight loss is a widespread practice (Beals and Manore 1999). Two reports published in 1993 found that approximately 40% of American women were currently dieting to lose weight (Horm and Anderson 1993; Serdula et al. 1993). College women are, perhaps, more susceptible to unhealthy practices in their motivation to be thin due to increased social pressures. A survey conducted of 166 college women, 18 to 24 years of age, found that > 18% reported dieting > 50% of the time over the previous eight months and were, therefore, classified as chronic dieters. Periodic dieting, dieting < 50% of the time over the previous eight months, was reported by 45.2% of these women. Only

one-third reported not dieting during the previous eight months (Grunewald 1985). Furthermore, this study asked participants to self-report weight loss strategies used over the previous eight months. An astounding 58 (34.9%) had fasted/starved for a period of time, 56 (33.7%) utilized diet pills or diet supplements, 9 (5.4%) purged, and 9 (5.4%) used laxatives (Grunewald 1985). When seeking information on dieting practices, lay-magazines and newspapers (44.0%) and family or relatives (26.5%) were the top two sources used (Grunewald 1985). These resources often dispense misleading and unreliable information with regard to healthy weight-loss practices.

The 1995 National College Health Risk Behavior Survey provides information specifically about US undergraduate students (Lowry et al. 2000). Questionnaire data were obtained from 4,609 students from 136 two- and four-year colleges. According to BMI, 29.9% of females were overweight or obese, yet 48.9% saw themselves as being overweight or obese. Of the 2,823 females included in this study, 1,688 (59.8%) wanted to lose weight. In order to lose or maintain current weight, 62.6% were exercising, 42.1% were dieting, 7.0% used diet pills, and 4.2% used laxatives and/or induced vomiting. Dietary intake assessment found approximately 85% of females consumed ≤ 2 servings of high-fat foods per day, yet only 25% consumed ≥ 5 servings of fruits and vegetables daily (Lowry et al. 2000).

Liebman and colleagues (2001) found a similarly high percentage of college females dieting. When asked "Have you dieted in the past 12 months with the express purpose of losing weight?" (p. 52, Liebman et al. 2001), 84 (38%) of the 210 females responded "yes". Similar to findings from Lowry and coworkers (2000), dietary fat avoidance was high among females, particularly those who reported dieting (Liebman et al. 2001). Horvath and coworkers (2000) showed that dietary calcium intake was reduced when female college runners followed a low-fat diet, but these results differed from other findings (Arsenault and Cline 2000, Dixon et al. 1997; Gorbach et al. 1990), which have shown a switch to reduced fat food options (i.e. from whole to skim milk) such that mineral consumption was not compromised.

Little information is available regarding current dietary trends among young-adult females, particularly with regard to bone health. Recent data supporting a high prevalence of dieting or weight control practices among college students (Liebman et al. 2001, Lowry et al. 2000) are cause for concern if dietary quality is compromised. The effects of reducing or altering dietary intake among young-adults may have implications for bone health resulting from

altered nutrient intake, weight changes, or hormonal alterations. Research investigating chronic dieting and bone health among young-adult females is warranted.

Dietary Restriction and Endocrine Function

Severe malnutrition disrupts menstrual function (Styne 1997b) and has serious consequences for BMD (Mazess 1990) due to a loss of estrogen. Linear growth (Aron, Findling, and Tyrrell 1997) and bone mineralization (Bachrach et al. 1998) are dependent on GH. The actions of GH on bone appear carried out through insulin-like growth factors and their binding proteins, primarily IGF-1 and IGFBP-3 (Aron, Findling, and Tyrrell 1997). Actions of estrogen also seem to be modulated, in part, through IGF-1 (Nasu et al. 2000). Malnutrition can interfere with the GH-IGF-1 axis evident by increased GH concentrations (Smith et al. 1974, Soliman et al. 1986, Thissen, Ketelslegers and Underwood 1994) and reduced IGF-1 concentrations (Hintz et al. 1978, Mohan and Jaya Rao 1979) in malnourished individuals. Bergendahl and coworkers (1999) found that acute fasting increased cortisol secretion and reduced IGF-1 concentrations despite increased secretion of GH. With increased cortisol and reduced IGF-1 the negative implications for bone health are great. Grinspoon and colleagues (1995) did not examine cortisol or GH concentrations but did show significant reductions in serum IGF-1 concentrations following a 4-day fast in young women. These women also had increased bone mineral dissolution and reduced osteoblastic activity following the fast (Grinspoon et al.1995). Among normal weight women following a short-term very-low calorie diet, serum concentrations of GH and IGFBP-3 were elevated, results not found in aged-matched obese women (Rasmussen et al. 1995). Elevated IGFBP-3 has been shown in adolescent females with anorexia nervosa (Audi et al. 2002) and osteoporotic adults (Jehle et al. 2003).

It appears that severe energy restriction can alter endocrine function which thereby affects bone health. Still, the hormonal status of chronic dieters has not been fully investigated. It is unclear if chronic dieting has implications for bone health which may be carried out through altered endocrine function.

Cognitive Eating Restraint

While a fair amount of research has been conducted, examining effects of structured, successful weight loss diets on BMD as well as effects of complete fasting on bone metabolism, it remains unclear how chronic dieting, a more prevalent form of dieting in this society, affects bone health. Chronic dieting involves the incessant limitation of energy and/or food intake in

order to maintain or achieve weight loss. Researchers are aware that chronic dieting habits are practiced by many, yet methods of identifying chronic dieters remain inconsistent and unreliable. Story and colleagues (1991) asked the question "How many times have you gone on a diet over the past year?" (p 995). Chronic dieters were identified as individuals who reported dieting greater than 10 times over the past year or were "always" dieting. By questioning the number of episodes of dieting over a time frame, the total amount of time spent dieting is not identified and, therefore, excludes those individuals who go on a limited number of diets but remain on them for long periods. The method used by Grunewald (1985) to identify chronic dieters asks the question: "Over the past year, how often have you been on a diet?"   Response selections include: (a) "never", (b) "less than 50% of the time", and (c) "greater than 50% of the time" (p.1446, Grunewald 1985). Those selecting choice "c" would be identified as chronic dieters. While this method may be useful because it eliminates the previous problem, both methods use the term "diet" which may limit their usefulness. The term diet is associated with distinct weight loss efforts with defined start and stop points. While many individuals, particularly chronic dieters, limit energy intake or refrain from certain foods in order to lose or maintain weight, they do not identify their behaviors with dieting since it has become a constant effort and therefore, a way of life.   Both methods above would exclude these individuals who to not identify with the term "diet" or have an academic understanding of the definition of dieting.

Both of the above methods were used in a study involving young-adult females by Nickols-Richardson and Beiseigel (2000, unpublished data). Dietary intake from four-day food records or 12-month food frequencies did not correlate with either method. Still, food record data from 56 participants showed that 18 (32%) were consuming less than 67% of estimated energy requirements (Nickols-Richardson and Beiseigel 2003). While dietary intake assessment is often met with under-reporting, the amount of energy restriction found among these young women was likely a result of actual limitations in food intake rather than under-reporting alone. Nevertheless, these findings support the need for a better method of identifying chronic dieters, particularly among young-adult female who, despite continual limitations in food intake in order to maintain or lose weight, view these behaviors as a lifestyle. Because chronic dieters make continual efforts toward restricting food intake, cognitive processes with regard to food behaviors may likely differ from those of non-dieters. Therefore, the use of a cognitive eating restraint (CER) assessment may better identify chronic dieters because it eliminates the problems

associated with the term "diet" and examines mental processes and habits associated with food intake (Lowe 1993). Still, CER questionnaires remain to be evaluated as a tool for identifying chronic dieters.

The Eating Inventory (Stunkard and Messick 1988) is a two-part questionnaire with Part I containing 36 true/false questions and Part II containing 15 rating-scale questions. The questionnaire is easy to administer and takes approximately 10 to 15 minutes to complete. It was produced in order to evaluate three constructs of eating patterns: (a) cognitive restraint with regard to eating, (b) disinhibition, and (c) hunger. These three dimensions of eating behavior are included as sub-scales within the test and are thereby easily assessed.

Cognitive eating restraint is the limitation of food intake with the purpose of preventing weight gain or enhancing weight loss (Van Loan and Keim 2000). People who exhibit characteristics of restrained eating are consciously aware of their dietary intakes. They are more likely to limit or avoid fat intake, use "diet foods" or reduced-calorie foods, experience weight fluctuations which might be attributed to variability in energy intake, and they may have impaired internal regulation of food intake (Tepper, Trail and Shaffer 1996, Tuschl et al. 1990).

Cognitive Eating Restraint and Bone

Few studies have investigated the relationships between CER and bone health (Barr, Prior, and Vigna 1994; Van Loan and Keim 2000). Barr, Prior and Vigna (1994) studied relationships between CER score, menstrual cycle length, and BMD in a cohort of 27 normally menstruating women. Women were divided into three groups based upon CER score. Women in the upper (n = 9) and lower (n = 9) score tertiles were compared. It was found that both groups were similar in menstrual cycle length but women in the upper tertile had significantly shorter luteal phase length compared to the lower tertile (8.6 ± 0.9 vs. 10.8 ± 0.5 days, respectively). When BMD measurements were compared between groups, no differences were found. Yet, implications for effects on bone health exist considering earlier research such as that of Prior and colleagues (1990) who found that women who experienced either anovulatory and/or shortened luteal phase cycles during a year-long study lost BMD, while women with normal menstrual cycles maintained BMD (Prior et al. 1990). A shortened luteal phase will reduce overall estrogen exposure throughout the menstrual cycle (Goldfien and Monroe 1997) and indicate the onset of menstrual cycle disturbances. While it is not exactly clear why women with high CER scores experience a shortened luteal phase, Barr, Prior and Vigna (1994)

speculate that females with high CER experience increased stress related to food and body image. Increased stress results in an increase in corticotropin releasing hormone (CRH) (Findling, Aron and Tyrrell 1997) which can disrupt luteinizing hormone (LH) secretion thereby disrupting the menstrual cycle and the hormonal profile necessary for maintenance of bone mass. Furthermore, elevated CRH may increase cortisol production which, in itself, has negative implications for bone health.

The contention that high CER is associated with elevated cortisol is supported by two recent studies examining such relationships (Anderson et al. 2002; McLean et al. 2001a). McLean, Barr and Prior (2001a) found significantly higher 24-hour urinary cortisol excretion per mmol of creatinine among women with high CER scores than women with low CER scores. Anderson and coworkers (2002) had similar findings when measuring salivary cortisol levels. College-age women with high CER scores were found to have significantly higher salivary cortisol concentrations than women with low CER scores.

Positive correlations between CER scores and salivary (Anderson et al. 2002) and urinary cortisol (McLean, Barr and Prior 2001a) concentrations can have negative implications for bone. Excess cortisol production can pose serious risk for bone (Findling, Aron and Tyrrell 1997), but due to the complex relationship between cortisol and other hormones involved in bone metabolism, evaluation of cortisol alone provides limited insight. Current published data regarding relationships between CER, bone health, and/or hormones have failed to investigate other hormones important for bone metabolism. Elevated cortisol concentrations found in individuals with high CER have been attributed to persistent psychological stress associated with food and weight control (Anderson et al. 2002; McLean, Barr and Prior 2001a). Growth hormone and IGF-1 concentrations have not been studied in association to CER-related stress. In cases of extreme stress, GH levels can be high (Chatterton et al. 1997; Richter et al. 1996), but Malarkey and colleagues (1991) found GH was not altered in relation to everyday stress as represented by "academic stress". Therefore, without directly researching the effects of CER on GH and IGF-1 concentrations, it remains unclear, based on previous research, how these factors relate.

In addition to Barr, Prior, and Vigna's work (1994), other cross-sectional investigations have examined relationships between CER and BMD. Van Loan and Keim (2000) found that among a group of women with body weights between 90% to 150% of their ideal body weight,

those with highest CER scores had significantly lower whole body BMC than those with the lowest CER scores when body weight was included as a covariate. Among those women who weighed ≤ 71.0 kg, women with high CER scores (≥9) had significantly lower whole body BMC than women with low CER scores (< 9). Despite these differences, no significant differences in BMD were found between groups.

McLean, Barr, and Prior (2001b) had similar findings when investigating a group of normally menstruating women aged 20 to 35 years. It was found that women with high eating restraint exercised more than women with low eating restraint. When exercise was included as a covariate, whole body BMC was significantly greater in women with low eating restraint than women with high eating restraint. Multiple regression analyses showed that both exercise and eating restraint scores explained 11.3% of the variance in whole body BMC and BMD.

Despite the absence of significant differences in BMD between women with high and low CER scores from these studies (Barr, Prior, and Vigna 1994, McLean, Barr, and Prior 2001b, Van Loan and Keim 2000), the association between eating restraint and BMD measures are still plausible. The above studies are cross-sectional rather than longitudinal. Barr, Prior, and Vigna (1994) included only 18 women of later gynecological age (40.6 ± 0.9 years) in their comparisons. While Van Loan and Keim (2000) had a large sample size, they also included women who ranged in age from 19 to 50 years. Also, body weight, which clearly influences bone mass (Orozco and Nolla 1997) was not controlled for in the initial subject recruitments efforts. Finally, Van Loan and Keim (2000) only measured whole body BMC and BMD which does not allow for evaluation of significant differences in BMD of regional body sites. Despite these flaws, these studies support the need for further investigation of relationships between CER and bone health.

Methods of Evaluating Bone Mineral and Bone Metabolism

General Methods

Methods used to evaluate BMD and bone metabolism are advantageous in studying cross-sectional populations and monitoring individuals over time. Measurements of BMD are useful in establishing baseline values for individuals and diagnosing bone disorders such as osteopenia and osteoporosis. Treatment effectiveness can be monitored through follow-up BMD measurements as well as measurements of bone metabolism. Cross-sectional and experimental research of bone health are also made possible by techniques used to evaluate BMD and bone

metabolism. Some of the more commonly used methods for evaluating bone mineral and bone metabolism are described here.

Bone Mineral Content and Density

Dual energy X-ray absorptiometry is the gold standard for measurement of BMC and BMD. The DXA machine emits photons at two different wavelengths and absorbed by body tissues. Attenuation of these photons distinguishes between tissue types and allows for calculation of whole-body and site-specific BMC and BMD, lean body mass, and fat mass. A DXA scan yields results with greater precision and accuracy in a relatively short period of time compared to earlier measurement tools (i.e., single photon absorptiomentry, dual photon absorptiometry) (Shore and Poznanski 1996). Bone scans performed by DXA are also better at monitoring changes in BMC and BMD over time as well as identifying those at risk for bone fractures (Cumings and Black 1995, Rizzoli et al. 1995).

Measurements of BMC report total body or site-specific bone mass, while BMD partially accounts for the surface area of the measured site. A measurement of BMD by DXA is not a true three-dimensional (g/cm^3) volumetric measurement but rather a two dimensional areal BMD (g/cm^2) (Katzman et al. 1991). While volumetric BMD measurements can be achieved from combined anterior-posterior and lateral measurements, they are seldom found in published research making areal BMD measurements the standard in clinical and research settings (Cassidy 1999).

Biomarkers of Bone Turnover

The cycle of bone resorption and bone formation is referred to as bone turnover. Rates of bone turnover can be assessed by analyzing enzymatic activity of osteoblasts and osteoclasts or by measuring components byproducts of bone resorption or formation in body fluids (Eyre 1996). Techniques uses to measure bone turnover have limited sensitivity. This in combination with the wide range of accepted normal values limits the usefulness of single time point measurements for an individual. Biochemical analyses of markers of bone turnover are valid and more insightful for monitoring bone metabolism, including skeletal disease progression and treatment effectiveness, over time as well as for cross-sectional investigations of bone health (Beck-Jensen 1997).

Byproducts of osteoblast activity found in urine or serum are measured as biomarkers of bone formation. Osteoblasts are bone cells responsible for production of the protein matrix of

bone (Puzas 1996). Evidence suggests that osteoblasts are regulated, in part, via receptor-medicated interactions of PTH (McSheehy and Chambers 1986), vitamin D (Puzas 1996) and estrogen (Erikson et al. 1998). During bone formation, osteoblasts secrete bone matrix proteins. The most abundant non-collagenous bone matrix protein is osteocalcin which constitutes 1% to 2% of total bone protein (Gundberg 1983). During bone formation, a small quantity of newly synthesized osteocalcin enters into circulation (Erikson et al. 1995) and can be measured by radioimmunoassay (RIA; Delmas 1993). Osteocalcin is likely the most validated biomarker of bone formation due, in part, to the belief that serum osteocalcin is not of product of bone breakdown but rather of *de novo* synthesis (Beck-Jensen 1997, Marcus 1996, Eriksen et al. 1995).

Byproducts of osteoclast activity found in serum or urine are measured as biomarkers of bone resorption. Osteoclasts are cells which dissolve the crystalline mineral structure and disassemble the protein matrix of bone (Baron 1996). Calcitonin (Baron 1996) and estrogen (Erikson et al. 1988) receptors have been identified on the surface of osteoclasts. When these ligands are bound to their respective receptors, osteoclastic activity is inhibited. Alternating alignment of collagen fibers forms the lamellar structure of bone (Baron 1996). Condensation reactions of lysyl and hydroxylysyl residues form covalent cross-links of amino acid side-chains which add strength to the lamellar structure. Two principal crosslinks are present in type I collagen. N-telopeptide (NTx) occurs from the linkage of two aminopeptides located near residue 930; C-telopeptide (CTx) occurs from the linkage of two carboxytelopeptides near residue 87 (Calvo et al. 1996). During osteoclastic bone resorption, degredation byproducts are metabolized by the liver and kidney. These crosslinks are small enough to bypass further degradation and are excreted into the urine intact where they can be measured as markers of bone resorption.

Commercial enzyme-linked immunosorbent assay (ELISA) kits make it easy to detect NTx in urine, a method useful in monitoring bone turnover. Difficulty can be encountered when comparing results of bone resorption analyzed by different markers. Urinary NTx differs from other biochemical markers of bone resorption such as free or total pyridinolines in that NTx concentrations increase more in response to estrogen withdrawl and decrease more in response to estrogen therapy and other antiresorptive agents (Calvo et al. 1996). Commercial ELISA kits are also more practical in that they require less time than other techniques for analyses because no

pretreatment or hydrolyses of samples are required. Because urinary NTx concentrations are dependant on hydration status and urinary output, values are typically normalized to creatinine measurements (Calvo et al. 1996).

Summary

Osteoporosis prevention must be aimed at young-adults prior to the age of peak bone mass. Several dietary components are important to bone health. Body weight is positively correlated with BMD and weight loss is associated with loss of BMD. Dieting for weight loss may also alter dietary intake and disrupt hormonal balance necessary for maintaining bone integrity. Dieting is a common practice among young-adults, yet identifying individuals who chronically restrict energy intake in order to maintain or lose body weight is difficult. The use of a simple CER questionnaire may serve as an appropriate proxy for identifying individuals who chronically diet. By identifying these individuals, it would then be possible to assess longitudinal changes in dietary intake, hormone levels and bone measurements in order to comprehensively evaluate the effect of chronic dieting on bone health in young adults. Therefore, the purpose of this research was to: (1) examine if the CER subscale of the Eating Inventory enables identification of chronic dieters and non-dieters based on select physiological and dietary variables, (2) examine cross-sectional differences in dietary intake, hormonal variables, biomakers of bone turnover, and BMC and BMD in young-adult females with and without CER, and (3) examine changes in BMC and BMD in young-adult females with and without CER over 6-months.

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RESTING ENERGY EXPENDITURE DOES NOT DIFFER ACCORDING TO COGNITIVE EATING RESTRAINT SCORES OR A SIMPLE DIETING QUESTION RESPONSES IN YOUNG-ADULT FEMALES[1]

Abstract

Background: Chronic dieting habits are common among women in developed countries, yet due to inconsistent methods of identifying chronic dieters, little is known about the health implications of such behaviors. Because chronic dieters would be expected to suppress food intake over time, it is expected that resting energy expenditure (REE) would be reduced among chronic dieters compared to non-dieters. Specifically, the ability of cognitive eating restraint (CER) scores and a simple dieting question to separate individuals based on indicators of suppressed intake, including REE, has not been adequately examined.

Objective: We investigated the ability of the CER subscale of the Eating Inventory and a simple dieting question to distinguish differences in REE, body composition, salivary and urinary cortisol, dietary intake, and physical activity in a group of young women.

Design: Women (mean age = 20.4 ± 2.3 y; n = 65) were divided into high (score >9; n = 31) and low (score ≤9; n = 34) CER groups and some (n = 27) and never (n = 38) dieting groups based on questionnaire responses. Indirect calorimetry was used to measure REE and dual-energy X-ray absorptiometry to measure body composition. Salivary and 24-h urinary cortisol were measured by enzyme-linked immunosorbent assay and radioimmunoassay, respectively. Food Frequency Questionnaires, physical activity recalls, and anthropometric measures were completed.

Results: Women in the high CER group possessed more fat mass (FM) ($p < 0.05$) and higher body fat percent (BF%) ($p = 0.01$) than women in the low CER group, despite a lack of differences in body mass index (BMI). No differences were found between high and low CER groups in REE, fat-free soft tissue (FFST) mass, salivary or urinary cortisol concentrations, energy and macronutrient intake, and h of physical activity. Women in the group with some dieting possessed more FM ($p < 0.01$) and higher BF% ($p < 0.05$) and had higher weight ($p < 0.01$) and body mass index ($p < 0.01$) than women in the never dieting group. Dietary protein intake (%) was higher ($p < 0.05$) in women reporting some dieting vs never dieting.

Conclusions: The CER subscale and a simple dieting question were useful in separating women based on body fat and weight-related indicators of restrained eating at one point in time, but not based on other physiological and behavioral indicators.

KEY WORDS: Chronic dieting, cognitive eating restraint, cortisol, dietary intake, dieting question, resting energy expenditure, women

55

Introduction

At any point in time, nearly 50% of all women in North America are "dieting" (1). Clinical eating disorders such as anorexia nervosa and bulimia nervosa have defined criteria for diagnoses with objective signs and symptoms that allow for adverse health effects to be clearly evaluated. Yet, clinically diagnosed eating disorders affect only a small proportion of women (<10%) in the United States (U.S.) (2,3). The majority of women in Westernized societies who alter dietary intake to manipulate body weight or composition do not develop eating disorders from their chronic dieting efforts.

Chronic dieters continuously monitor and limit food intake to achieve or maintain an average or below-average body weight (4,5). Chronic energy restriction may reduce intake of vitamins and minerals and adversely impact health by increasing risks for acute illnesses and chronic diseases. Unlike eating disorders, however, identification of individuals who chronically diet is difficult due to a lack of objective measures with which to apply to physiological and behavioral signs and symptoms. Simple questions designed to identify chronic dieters are tenuous as responses vary due to phrasing of questions (6). Other, more involved, questionnaires must rely on individuals' responses regarding eating attitudes (7).

Because chronic dieting is a reality for many women, an understanding of potential health outcomes and complications associated with chronic dieting is important to achieve. Therefore, a valid method of identifying chronic dieters must be established. Chronic dieters have been defined as individuals with abnormal eating patterns and behaviors (8) or high dieting frequency (9,10), yet the usefulness of these approaches remains in question.

Long-term energy restriction can reduce resting energy expenditure (REE) (11,12); thus, chronic dieters would be expected to have a lower REE compared to non-dieting counterparts. Body composition would be expected to differ between chronic dieters and non-dieters (13-15). Cognitive eating restraint (CER) has been associated with elevated concentrations of the stress hormone, cortisol; thus, measures of this hormone should vary in chronic dieters and non-dieters (16-18). Finally, dietary intake has been shown to be limited in women with high CER (18-20), but not consistently so (14,21-23). The objective of this study was, therefore, to investigate the ability of the CER subscale of the Eating Inventory questionnaire (24) and a simple dieting question (9) to consistently identify "chronic dieters" based on differences in REE, body composition, salivary and urinary cortisol, dietary intake, and physical activity in young women.

These variables represent a range of physiological and behavioral factors associated with CER and dieting.

Subjects and Methods

<u>Subjects</u>

Young women, aged 18 to 25 y, were recruited to participate in this study that was approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University (VPI&SU). Subjects were recruited from the VPI&SU campus and surrounding locations by use of paper flyers, electronic mail notices, and personal contacts. Each woman, before participating in any procedure, provided written informed consent.

Each subject completed and returned an investigator-designed general health screening and demographic questionnaire prior to final enrollment into the study. Exclusion criteria included self-reported: age <18 and >25 y; weekly participation in >7 h of hard and very hard physical activity; amenorrhea, oligomenorrhea, or any disruption of menstrual cycles within the previous years; use of oral contraceptives for <18 mo (if used); use of medications to correct metabolic disorders; history of eating disorders, metabolic disorders, or chronic diseases; parity ≥ 1; and cigarette smoking. Self-reported body height and weight estimations were included in this screening questionnaire from which body mass index (BMI) was calculated (BMI = kg/m^2) for each subject. Individuals with BMI of <18 or >25 were excluded because higher REE has been found in overweight and obese individuals compared to normal weight persons (25). Women were also excluded if weight fluctuations of >2.27 kg, ≥ 3 times in the past 2 y were reported, due to effects on REE (26).

<u>Procedures</u>

A cohort of 65 women participated in the study. A power analysis conducted prior to the study indicated that 29 subjects per CER group was required to detect significant differences with a power of 0.80. Due to rolling admission, data were collected between February 2001 and October 2002. During individual, 2-h testing periods, fasted subjects (10 to 12 h) completed questionnaires and the testing protocol. Each woman underwent testing on one day that fell between the 4th and 10th d of her menstrual cycle (follicular phase) due to documented differences in energy expenditure during menstrual cycle phases (27). Fifty-three subjects returned 6 mo later to repeat the Eating Inventory questionnaire and a simple dieting question to

compare scores and answers over time. All procedures were carried out in the Bone metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory at VPI&SU between 0700 – 1100 h and within 1.5 h of subject's awaking.

Cognitive eating restraint

Subjects completed the Eating Inventory, after which an investigator scored the CER subscale according to established guidelines (24) (Stunkard & Messick 1988). The Eating Inventory includes the 21-item CER subscale designed to assess effort to control food intake for the purpose of manipulating body weight (i.e., to lose weight or to avoid weight gain). Based on the median CER score of our subjects and consistent with median CER scores reported by other investigators (28,29), women were categorized into high or low CER groups if their scores were >9 (n = 31) or ≤9 (n = 34), respectively.

Simple dieting question

Subjects completed a simple dieting question (9), indicating the time spent dieting for weight loss (>50% of time, or <50% of time, or never) during the previous 12 mo. Only 2 subjects indicated dieting >50% of time; thus, they were collapsed into the <50% of the time group for division based on some dieting (n = 27) and never dieting (n = 38).

Resting energy expenditure

Following the overnight fast, REE was measured by indirect calorimetry using a ventilated hood system (VMAX 29N, Sensor Medics, Yorba Linda, CA). This open-circuit instrument was calibrated prior to each measurement. After a 30-min period of rest, gas exchange (oxygen consumption in mL/min and carbon dioxide production in mL/min) from normal breathing was measured with the subject in a supine position and neutral temperature room while awake and refraining from voluntary movement. Gas exchange readings were maintained for ≥15 to 20 min before calculating the mean REE (kJ/d) from the abbreviated Weir equation (30) for the 5 min during which the most consistent steady state was achieved.

Body composition

Dual-energy X-ray absorptiometry (DXA) scans were completed to measure soft tissue mass of the total body (version 8.25a, 2000, Whole Body Analysis Software, QDR4500A, Hologic Inc., Bedford, MA). Fat-free soft tissue (FFST) mass (kg), fat mass (kg), and percent body fat (BF) (%) were measured. Quality control procedures for soft tissue mass were performed by weekly scans of an external tissue bar (31). One investigator completed and

analyzed all total body DXA scans to eliminate inter-tester variation. Test-retest reliability with 24 young-adult men and women resulted in coefficients of variation (CV%) of 1.07%, 1.75%, and 1.79% for FFST mass, fat mass, and BF%, respectively (31).

Salivary and urinary cortisol

Prior to the completion of any questionnaire or procedure, each subject was instructed to gently chew on a 3 cm^2 piece of dental gauze for ~30 s. Saturated gauze was then placed in a sterile tube and centrifuged at 1,540 x g for 5 min, allowing for saliva to collect at the bottom of the tube while dry gauze and extra-salivary particles remained at the top. Saliva was aliquotted into cryovials and frozen at –80°C until later analysis for salivary cortisol by enzyme-linked immunosorbent assay (Salimetrics LLC, State College, PA). The normal reference range for morning salivary cortisol in women of this age range is 0.27 – 1.35 μg/dL (32). Inter- and intra-assay CV for salivary cortisol in our laboratory was 8.2% and 8.5%, respectively.

Urine collection containers with a 4-L capacity were provided to each subject at the end of the 2-h testing session. Because cortisol is not influenced by menstrual cycle phases (33,34), and to increase convenience and facilitate more complete urine collections, subjects were allowed to select the 24-h period following the 2-h testing session for urine collection. Subjects were instructed to record the time of the first morning void at the start of urine collection but to discard this urine (17). For the subsequent 24-h period, all urine was collected in the provided container and stored in a cool place throughout the 24-h period. The first urine of the following morning upon awakening was collected to complete the 24-h sample collection (17). Subjects returned filled containers to the laboratory after which total urine volume was measured, and 1 mL samples were aliquotted and frozen at –80°C until analysis for urinary cortisol by radioimmunoassay (Diasorin, Stillwater, MN). The normal reference range for 24-h urinary cortisol in women of this age range is 80.0 – 600.0 nmol/24-h (17). Intra-assay CV for urinary cortisol in our laboratory was 6.1%. Creatinine was also measured (Sigma Diagnostics #555, St. Louis, MO), and urinary cortisol was recorded as a ratio of cortisol (nmol) to creatinine (mmol). The normal reference range for creatinine in women of this age range is 7.0 – 16.0 mmol/24-h (35). Inter- and intra-assay CV for creatinine in our laboratory was 4.4% and 1.9%, respectively.

Dietary intake

In interview format, each subject completed the Food Frequency Questionnaire (FFQ) (36). Information regarding frequency and quantity of food and beverage items consumed

during the previous 12 mo was gathered. Two- and three-dimensional food models were used to assist subjects with portion size estimations and to limit variability in portion size judgments among subjects. An investigator trained by a Registered Dietitian conducted FFQ interviews. Average daily dietary intakes of energy (kJ/d) and macronutrients (g/d and % of total kJ/d) from foods and beverages were estimated using the DIETSYS+Plus Analysis Software (version 5.9, 1999, Block Dietary Data Systems, Berkeley, CA).

Subjects were also instructed to complete 4-d (3 weekdays + 1 weekend d) dietary records in the days following their testing sessions. Dietary records included dates of record keeping, time of day of food and beverage ingestion, ingredients for any items prepared at home, names of restaurant items or brand names of pre-prepared items. In addition, consumption of condiments and alcoholic and non-alcoholic beverages were recorded. Portion sizes were estimated with the assistance of two-dimensional portion guides. Individual food records were analyzed for average daily energy and nutrient intake using the Food Processor® Nutrition Analysis Software profile system (version 7.4, 1999, *esha* Research Inc., Salem, OR).

Physical activity

Current physical activity was evaluated based on initial screening questionnaires that required each subject to describe weekly exercise habits, including intensity and type of activity, frequency, and duration per occasion. Only individuals reporting ≤ 7 h of activity per wk were included in the study (17). Each subject completed the 7-d physical activity recall (37) as part of the 2-h study protocol. Hard and very hard physical activity (h/wk) were estimated from these self-reported questionnaires.

Anthropometric data

Using a stadiometer (Detecto, Webb City, MO), body height, without shoes, was measured to the nearest 0.1 cm. Body weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Scaletronix, Wheaton, IL) with the subject wearing lightweight clothing. Body height and weight were measured by an investigator after which BMI was calculated (BMI $= kg/m^2$); this calculated BMI (not calculated BMI from self-reported body height and weight measures) was used in data analysis.

Statistical analyses

Group statistics for study variables were calculated as means $\pm$ standard deviations (SD). Comparisons between high vs low CER groups and some vs never dieting groups were

conducted using student's *t*-tests for selected demographic, REE, body composition, salivary and urinary cortisol, dietary intake, physical activity, and anthropometric variables. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (version 10.0, SPSS Inc., Chicago, IL), and statistical significance was set at $p < 0.05$.

Results

Characteristics of all study subjects are shown in **Table 1**. When categorized into high and low CER groups, significant differences between groups were observed only in total body fat mass and BF% (**Table 2**). When categorized by a simple dieting question, significant differences between groups were identified in total body fat mass, BF%, protein intake as a % of total energy intake, body weight, and BMI (**Table 3**).

A subset of women in the high CER (n = 24) and low CER (n = 24) groups completed detailed 4-d food records. Significant differences in average daily energy or macronutrient intakes were not detected; however, women in the high CER group reported significantly greater ($p < 0.05$) fruit and vegetable intake (4.9 ± 1.8 servings/d) compared to women in the low CER group (3.8 ± 1.8 servings/d).

Because CER scores lie on a continuum, it may be questioned whether individuals with mid-range scores (i.e., 7 – 11) have distinct characteristics of either high or low eating restraint. While we and other investigators have separated groups based on median scores, some researchers have excluded subjects within a center range to eliminate potential crossover between groups (14,17). Therefore, CER groups were further divided into very high (upper 70th percentile; score ≥12; n = 21) and very low (lower 30th percentile; score ≤6; n = 20) CER groups (14). Based on these CER comparisons, significant differences between groups in REE or REE per kg FFST mass were not observed, and significant differences in body composition variables were not apparent. Salivary and urinary cortisol did not significantly differ, and energy and macronutrient intake did not significantly differ between the very high and very low CER groups. However, women in the very high CER group consumed significantly less ($p < 0.05$) dietary saturated fat compared to women in the very low CER group (19.8 ± 7.2 vs 24.8 ± 8.0 g/d, respectively). The very high and very low CER groups differed in duration of physical activity reported during the screening process (3.9 ± 1.7 vs. 2.6 ± 1.9 h/wk, $p < 0.05$, for very

TABLE 1

Subject characteristics (n=65)

Characteristic	Mean ± SD
Age (y)	20.4 ± 2.3
Height (cm)	165.0 ± 5.7
Weight (kg)	58.4 ± 6.6
Body mass index (kg/m^2)	21.4 ± 2.0
Ethnicity (n)	
White	59
Black	2
Other	4
Alcohol intake (drinks/wk)	2.2 ± 2.5
Oral contraceptive use (n)	
Yes	26
No	39
Caffeinated beverage intake (drinks/wk)	6.6 ± 6.7

TABLE 2

Comparison of physiological and behavioral variables associated with suppressed intake between high and low cognitive eating restraint (CER) groups[1]

Variable	High CER (n=31)	Low CER (n=34)	*p*-value
REE (kJ/d)	5,740 ± 841	5,751 ± 834	0.958
REE (kJ/kg FFST mass)	140 ± 21	141 ± 18	0.935
FFST mass (kg)	41.1 ± 4.1	41.0 ± 4.0	0.890
Fat mass (kg)	17.0 ± 4.2	14.5 ± 3.7	0.014
Body fat (%)	28.0 ± 5.0	25.0 ± 4.1	0.010
Salivary cortisol (µg/dL)	0.61 ± 0.30	0.57 ± 0.32[2]	0.566
Urinary cortisol (nmol/mmol creat)[3]	49.2 ± 17.5	52.4 ± 19.8	0.576
Urinary cortisol (nmol/24-h)[3]	397.8 ± 155.0	399.9 ± 147.1	0.964
Urine volume (mL/24-h)[3]	1,305 ± 809	1,047 ± 582	0.246
Creatinine (mmol/24-h)[3]	8.2 ± 2.3	8.3 ± 3.6	0.903
Energy intake (kJ/d)[4]	8,811 ± 3,428	8,799 ± 4,234[2]	0.986
Carbohydrate intake (g/d)	297 ± 111	284 ± 72[2]	0.559
Carbohydrate intake (%)	57.1 ± 6.4	54.2 ± 6.0[2]	0.057
Protein intake (g/d)	73 ± 30	71 ± 19[2]	0.757
Protein intake (%)	14.0 ± 2.0	13.6 ± 2.0[2]	0.564
Fat intake (g/d)	72 ± 33	76 ± 25[2]	0.621
Fat intake (%)	30.3 ± 5.3	32.2 ± 5.2[2]	0.151
Physical activity (h/wk)[5]	3.7 ± 2.3	2.8 ± 2.0	0.145
Age (y)	20.7 ± 2.3	20.2 ± 2.4	0.363

Height (cm)	165.1 ± 5.2	164.9 ± 6.2	0.635
Weight (kg)	59.8 ± 6.4	57.1 ± 6.6	0.103
Body mass index (kg/m^2)	21.9 ± 2.0	21.0 ± 2.0	0.068

[1]Mean ± SD; *p*-value for student's *t*-test between groups. REE, resting energy expenditure; FFST, fat-free soft tissue.
[2]n=33 in low CER group.
[3]n=25 in high CER group and n=19 in low CER group.
[4]Average daily dietary intake data from Food Frequency Questionnaire (36).
[5]Average h/wk of hard and very hard physical activity from 7-d physical activity recall (37).

TABLE 3

Comparison of physiological and behavioral variables associated with suppressed intake between women reporting never and some dieting[1,2]

Variable	Some dieting (n=27)	Never dieting (n=38)	p-value
REE (kJ/d)	5,809 ± 739	5,701 ± 897	0.609
REE (kJ/kg FFST mass)	139 ± 16	142 ± 21	0.647
FFST mass (kg)	41.8 ± 4.3	40.4 ± 3.8	0.172
Fat mass (kg)	17.3 ± 4.2	14.6 ± 3.7	0.006
Body fat (%)	28.1 ± 5.1	25.2 ± 4.1	0.015
Salivary cortisol (µg/dL)[3]	0.54 ± 0.20	0.63 ± 0.37	0.251
Urinary cortisol (nmol/mmol creat)[4]	51.5 ± 22.4	49.8 ± 14.6	0.771
Urinary cortisol (nmol/24-h)[4]	410.2 ± 176.8	389.0 ± 126.4	0.646
Urine volume (mL/24-h)[4]	1,349 ± 780	1,064 ± 662	0.197
Creatinine (mmol/24-h)[4]	8.3 ± 2.7	8.3 ± 3.2	0.966
Energy intake (kJ/d)[5]	8,419 ± 2,835	9,086 ± 2,868[3]	0.359
Carbohydrate intake (g/d)	281 ± 96	298 ± 91[3]	0.453
Carbohydrate intake (%)	56.5 ± 6.4	56.2 ± 6.3[3]	0.825
Protein intake (g/d)	73 ± 24	73 ± 29[3]	0.978
Protein intake (%)	14.9 ± 2.7	13.6 ± 2.0[3]	0.041
Fat intake (g/d)	70 ± 29	78 ± 29[3]	0.265
Fat intake (%)	31.2 ± 5.4	32.8 ± 5.4[3]	0.247
Physical activity (h/wk)[6]	3.4 ± 2.1	3.2 ± 2.3	0.710
Age (y)	20.8 ± 2.3	20.1 ± 2.3	0.185

Height (cm)	165.4 ± 5.4	164.7 ± 6.0	0.853
Weight (kg)	60.9 ± 6.1	56.6 ± 6.4	0.009
Body mass index (kg/m^2)	22.2 ± 2.0	20.9 ± 1.9	0.005

[1]Mean $\pm$ SD; *p*-value for student's *t*-test between groups. REE, resting energy expenditure; FFST, fat-free soft tissue.

[2]Self-reported dieting "<50% of the time" or "never" dieting during the previous 12 mo (9).

[3]n=37 in never dieting group.

[4]n=20 in some dieting group and n=24 in never dieting group.

[5]Average daily dietary intake data from Food Frequency Questionnaire (36).

[6]Average h/wk of hard and very hard physical activity from 7-d physical activity recall (37).

high and very low CER groups, respectively). Significant differences in age, height, weight, and BMI between women in these groups did not exist.

Chronic dieting implies a longitudinal effort toward weight control (5); thus, we also examined CER scores 6 mo after the subject's initial testing date. Of the 65 women, 53 agreed to complete a second Eating Inventory questionnaire. Of these 53 women, 83% (n = 44) had changes in CER scores, with only 19% (n = 10) having a change of ± 4 points. When women were classified into high (>9; n = 28) and low (≤9; n = 25) CER groups based on follow-up scores, significant differences in REE, REE per kg FFST mass, body composition, salivary or urinary cortisol, and anthropometrics were not found. However, women in the high CER group originally reported significantly greater ($p < 0.05$) relative dietary protein intake (14.8 ± 2.6%; n = 28) compared to women in the low CER group (13.3 ± 1.9%; n = 25). Women in the high CER group also reported significantly more ($p < 0.01$) h of hard and very hard physical activity (4.2 ± 1.9 h/wk; n = 28) compared to women in the low CER group (2.7 ± 2.0 h/wk; n = 25). The 12 women who did not repeat the Eating Inventory had a mean CER score of 7.5 and median CER score of 9. Women who did not repeat (n = 12) the Eating Inventory had significantly less ($p < 0.05$) FFST mass compared to women who did repeat (n = 53) the questionnaire (38.6 ± 3.8 vs 41.6 ± 3.9 kg, respectively), but significant differences in all other variables were not observed.

Of the 53 women who repeated a simple dieting question after 6 mo, 7 (13%) switched from the some dieting group to the never dieting group, and 2 (~3%) moved from the never to some dieting group. When placed into some dieting (n = 20) and never dieting (n = 33) groups based on follow-up responses, significant differences in any original physiological or behavioral measures were not found.

Discussion

In our study, differences in REE or REE per kg FFST mass were not observed between women in high and low CER groups or between women who reported some and never dieting during the past 12 mo at baseline or when questionnaires were repeated 6 mo later. With prolonged and marked energy restriction in men and women, REE is reduced (11,12). Yet, conflicting evidence of the effects of "chronic dieting" on REE exists. Weight cycling among normal-weight young-adult women has been associated with reduced REE during periods of

energy restriction (26). When weight returned to normal and was maintained, a sustained reduction in REE below the baseline measurement was still observed, suggesting that episodic dieting and weight cycling have negative long-term effects on REE (26). We excluded individuals who experienced weight fluctuations during the previous 2 y; thus, our findings were limited to women who maintained body weight during the recent past. McCargar and McBurney (1) reported that in a group of 172 chronic dieters only 30 (17%) had REE measurements of ≤85% of predicted and only 31 (18%) had REE values >100% of expected. Although the technique for identifying chronic dieters differed from our methods, both studies collectively suggest that these three classification methods, at least, do not successfully identify individuals who engage in energy restriction either to the length or severity needed to reduce REE.

Tuschl et al (20) reported that normal-weight restrained eaters had lower daily energy intake and lower daily total energy expenditure (TEE) compared to non-restrained eaters. Our findings were not consistent with this previous study for several reasons, including the fact that we measured REE while Tuschl et al (20) measured TEE. Although REE is the major component of TEE, without REE measurement, it cannot be assumed that differences in REE were the cause for differences in TEE observed by Tuschl et al (20). In addition, the lack of significant differences in energy intake and FFST mass between comparison groups in our study supports the lack of significant differences in REE between comparison groups.

Individuals with high CER have been shown to limit or avoid high-fat foods, consume more "diet foods," and have more weight fluctuations compared to individuals without restrained eating (20,23). Limitations in food intake to manipulate body weight have been associated with CER (29). Moreover, individuals with high CER have been shown to have impaired internal regulation of food intake (20,23). In our study, women with high CER or reporting some dieting did not significantly differ from women with low CER or never dieting, respectively, in energy or absolute macronutrient intake. Thus, the method of dietary intake data collection could be suspect. When food intake is averaged over a long period (i.e., by 12 mo FFQ), it is possible that short-term fluctuations in food intake (i.e., restricting and binging) may be inadvertently missed. In order to address this concern, a subset of women in the high CER (n=24) and low CER (n=24) groups completed detailed 4-d food records. Based on short-term dietary intake, we found that women with high CER scores were significantly more likely to consume fruits and vegetables

compared to women with low CER scores; however, no other differences in energy or nutrient intakes were observed from these short-term diet records.

Another explanation for the lack of significant differences in energy and macronutrient intake between groups is that women with high CER scores or reporting some dieting may have correctly perceived that they possessed greater amounts of body fat compared to low CER or never dieting counterparts, respectively. High CER scores or some dieting may be related to a conscious desire to manipulate body fat rather than body weight *per se* and by healthy means such as increased fruit and vegetable consumption vs overall energy restriction. When participants with mid-range (i.e., 7 – 11) CER scores were removed from groups, no differences in body composition were observed between the very high and very low CER groups. This finding suggests that a staging along the CER continuum may exist such that women with mid-range scores may contemplate changes, whereas women with very high CER scores practice behaviors, such as more physical activity, and maintain body compositions equivalent to women with very low CER scores.

Our results support the findings of McCargar et al (38) who reported that Canadian women in mid-life who chronically dieted during the previous 12 mo did not have significantly different REE values compared to non-dieters. Total energy intake between groups also did not differ, and consistent with our study, chronic dieters had more healthy dietary habits, including higher consumption of fruits and vegetables (38).

Other physiological indicators of CER or dieting, with or without energy restriction, may be apparent. For example, higher salivary (16) and urinary (17) cortisol concentrations have been reported in women with high CER. A higher urinary cortisol excretion has been linked to a proposed, increased physiological stress associated with food choices and dieting behaviors (17). Anderson et al (16) reported a significantly elevated salivary cortisol concentration among a high vs low CER group, but the implication for stress-induced elevation was not supported considering that the mean salivary cortisol concentrations for both groups were well below the normal, non-stressed reference range for females. In a separate study, self-selection of food was allowed and monitored by women with high or low CER scores, during a concurrent 24-h urine collection period. Because women with high CER scores are conscious of food intake, monitoring of self-selected food consumption by investigators may have induced stress in women with high CER scores and may have resulted in the higher (but not elevated beyond

normal) urinary cortisol found in women with high CER vs low CER scores (17). In this previous study, women were grouped by very high or very low CER score standards vs median scores which may also explain differences noted in urinary cortisol (17). Subjects who completed 24-h urine collections and who were categorized into our very high (n = 16) and very low (n = 15) CER groups were approximately half the number of this previous study (17); yet, the lack of even a trend (i.e., $p < 0.10$) for statistical significance in urinary cortisol concentrations between groups and lack of differences in salivary cortisol concentrations between our groups suggest that free-living, young-adult females, with high CER scores and specified characteristics, have neither higher nor elevated urinary cortisol excretion compared to low CER counterparts.

In our study, we found that 83% of the 53 subjects who repeated the Eating Inventory questionnaire had a change in CER score. While only 19% had a change of ± 4 points, resulting in CER group classification changes, this suggests that CER scores can fluctuate within a relatively short period of time. Thus, the CER subscale of the Eating Inventory is more reasonably a measure of current behavior rather than chronic behavior. This contention is also supported by Kaufman et al (8) who used two questionnaires designed to evaluate abnormal dietary patterns and found that both questionnaires correlated with current eating behaviors but did not relate to long-term behaviors.

In conclusion, we observed no differences in REE (kJ/d or kJ/kg FFST mass), FFST mass, salivary or urinary cortisol, energy or absolute macronutrient intake, physical activity, age, or height between women in high vs low CER groups or between women who reported some vs never dieting. Results from this study differ from previous studies (20,39) perhaps due to the specific inclusion and exclusion criteria which were applied in this study. Yet, the possibility that the CER subscale of the Eating Inventory has transformed from a tool used to identify individuals with chronically restrained eating to an instrument that identifies individuals who are making healthy lifestyle choices at any point in time is plausible. Because healthy choices often require a conscious effort, women who practice healthy behaviors, such as higher fruit and vegetable intake and lower saturated fat intake, may be inappropriately identified as "chronic dieters," based on CER scores. In fact, it appears from these data that women with the highest CER scores are most successful at their efforts. It is also possible that while the Eating Inventory was practical in identifying chronic dieting behaviors in the 1980s, the usefulness of the

questionnaire for this purpose has become outdated. Food and nutrition messages for optimal health have changed from the time that the questionnaire originated; thus, women with high CER scores may simply reflect a secular change in restrained eating due to disordered eating vs restrained eating for health promotion.

While there may be a subgroup of women with high CER scores that do chronically diet via continually restricting dietary intake and practicing unhealthy dieting behaviors, the CER subscale alone is not sufficient for identifying chronic dieters among other health-conscious women. Chronic dieting, defined as individuals restricting intake in order to lose weight over a relatively long period of time, cannot be successfully or consistently identified from the use of CER scores or a simple dieting question.

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BONE MINERAL MEASURES AND MARKERS OF BONE TURNOVER DO NOT DIFFER BETWEEN WOMEN WITH LOW AND HIGH COGNITIVE EATING RESTRAINT SCORES[1]

Abstract

A high cognitive eating restraint (CER) score has been implicated in low bone mineral content (BMC) of the total body (TB). No studies have shown compromised bone mineral density (BMD) in women with high CER. The purpose of this cross-sectional study was, therefore, to examine differences in anthropometric measures, dietary intake, BMC and BMD, biomarkers of bone turnover, and hormone concentrations in women (age = 20.4 ± 2.3 y) with low (n = 34) and high (n = 31) CER scores, established by the Eating Inventory. Average daily dietary intakes of select nutrients were estimated from food frequency questionnaires and 4-d food records. Dual energy X-ray absorptiometry was used to measure BMC (g) and BMD (g/cm^2) of the TB and skeletal sites as well as fat-free soft tissue mass, fat mass (FM), and body fat percent (BF%). Serum osteocalcin and urinary N-telopeptide concentrations were measured by radioimmunoassay and enzyme-linked immunosorbent assay, respectively. Salivary and urinary cortisol, and serum estradiol, progesterone, growth hormone, insulin-like growth factor (IGF)-1, and IGF binding protein-3 concentrations were measured by bioassays. Women in the low versus high CER group possessed significantly less FM ($p < 0.05$) and BF% ($p = 0.01$) and consumed significantly fewer ($p < 0.05$) servings of fruits and vegetables (combined) per d. Significant differences in BMC, BMD, biomarkers of bone turnover, and hormone concentrations were not found between groups. Bone mass, measures of bone turnover, and hormonal mediators of bone metabolism do not differ in young women with low versus high CER.

Key Words: Bone turnover — Bone density — Cognitive eating restraint — Cortisol — Dietary intake

Introduction

Cognitive efforts to lose or maintain body weight are a routine part of daily life for many women in Westernized societies. Such efforts can include restriction of total energy intake and dietary adjustments such as increased consumption of fruits and vegetables or reduced intake of high-fat foods in favor of reduced fat alternatives (1-3). The cognitive eating restraint (CER) subscale of the Eating Inventory (4) was designed to easily identify individuals who regulate food intake. A high prevalence of CER in women in the United States has been reported (5); therefore, it is necessary to understand the physiological effects of CER, particularly with regard to women's health.

Osteoporosis is a devastating and prevalent disease that affects women, and previous investigations have implicated both direct and indirect effects of CER on bone health. Cross-sectional investigations have identified subclinical menstrual cycle disturbances (6,7) high cortisol excretion (8,9), and low total body bone mineral content (BMC) (10,11) in women with high CER scores. Yet, the implications for bone are not well understood and even contradictory since other researchers have found no differences in cortisol secretion (12) or bone mineral density (BMD) (11) between groups of women with low and high CER scores. Moreover, within these studies that have investigated such relationships, incomplete pictures have been drawn because of a lack of dietary information (8), limited bone mineral measurements (11), or a lack of evaluation of other endocrine factors known to mediate bone metabolism. The purpose of this study, therefore, was to examine the cross-sectional relationship between CER, anthropometric measures, dietary intake, hormonal factors, and bone in a group of young women.

Experimental Subjects

Prior to initiation of this research, approval was granted by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute & State University (VPI&SU), Blacksburg, VA, U.S.A. Young women, aged 18 – 25 y, from the VPI&SU campus and surrounding areas were recruited by personal contacts, paper bulletins, and electronic mailings to participate in this study. Potential subjects had questions and concerns addressed by researchers, and prior to participation, all potential subjects read and signed informed consent forms. Subjects were not financially compensated for participation in this study but were provided comprehensive, individualized results upon completion of the study.

An investigator-designed general health questionnaire was completed by all potential subjects prior to acceptance into this study. Exclusion from this study was based on the following: (1) participation in > 7 h of hard and very physical activity per wk; (2) self-reported menstrual cycle length of < 21 d or > 35 d; (3) cigarette smoking; (4) use of medications or agents known to affect bone metabolism; (5) a history of metabolic or eating disorders. Women using oral contraceptives were permitted into this study only if they had been continuously taking these medications for ≥ 18 mo. Self-reported height and weight were used to calculate body mass index (BMI) [BMI = weight (kg)/height (m^2)]. Women found to have a BMI < 18 or > 25 were excluded from participation. Because weight fluctuations have been shown to impact BMD (13), subjects were excluded if they had gained or lost > 2.3 kg ≥ 3 times during the previous two y.

Materials and Methods

Procedures

A total of 65 women (59 = Caucasian, 2 = Black, 4 = Other) met the enrollment criteria and were included in this study. Data were collected between 0700 – 1100 h, from February 2, 2002 to October 30, 2002.

Individual, 2-h appointments were scheduled with subjects during which time, data regarding CER, anthropometrics, dietary intake, physical activity patterns, and bone mineral status, as well as urine and blood samples were collected. Due to the influence of the menstrual cycle on hormone concentrations and markers of bone turnover (14), all data were collected during the follicular phase (4 – 10 d after onset of menses) of the subjects' menstrual cycles. All procedures were carried out in the Bone Metabolism, Osteoporosis and Nutrition Evaluation Laboratory (BONE Lab) at VPI&SU. A power analysis conducted prior to the study indicated that 30 subjects per CER group was required to detect significant differences in lumbar spine (LS) BMD with a power of 0.80.

Cognitive eating restraint

Subjects were instructed to arrive at the BONE Lab after an overnight (≥ 10 h) fast. Subjects were then instructed to complete the Eating Inventory questionnaire that was scored according to established guidelines (4). Using the CER subscale of the Eating Inventory questionnaire, to assess desire to regulate food intake in order to control or reduce body weight, subjects were divided into two groups based on the median score of all subjects. This method

was consistent with procedures and scores reported by other researchers (11,15). Women with scores ≤ 9 were placed in the low CER group (n = 34) while women with scores > 9 were placed in the high CER group (n = 31). Subjects were unaware of their individual CER scores.

Anthropometrics

Wearing lightweight clothing, without shoes, weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Scaletronix, Wheaton, IL, U.S.A.) and height was measured to the nearest 0.1 cm using a stadiometer (Detecto, Webb City, MO, U.S.A.). These heights and weights were then used to calculate each subject's BMI and were used in data analyses rather than BMI calculated from self-reported heights and weights obtained during the screening process.

Dietary intake

Dietary intake data were gathered using the Food Frequency Questionnaire (FFQ) (16). In an interview format, subjects answered questions regarding frequency and quantity of multiple food items and beverages consumed during the previous 12 mo. Two and three-dimensional food images and models were used to assist in portion size estimations and to minimize variation in estimates of portion sizes among subjects. The DIETSYS+Plus software (version 5.9, 1999, Block Dietary Data Systems, Berkeley, CA, U.S.A.) was used to estimate average daily dietary intake of energy (kJ/d), macronutrients (g/d and % of total kJ/d), and selected micronutrients.

Subjects were instructed on completion of 4-d (3 weekdays and 1 weekend day) food records. Record details included time of d, estimated portion sizes (with two-dimensional portion size images for reference), and any other pertinent information such as ingredients used in foods prepared at home, names of restaurants and food preparation establishments when applicable, brand names, and added condiments. All alcoholic and non-alcoholic beverages consumed were recorded as well. Food records were analyzed using the Food Processor[®] Nutrition Analysis Software profile system (version 7.4, 1999, *esha* Research Inc., Salem, OR, U.S.A.) and examined for estimated average daily dietary intake of energy (kJ/d), macronutrients (g/d and % of total kJ/d), selected micronutrient intake, and food intake patterns.

Physical activity

Current physical activity patterns were assessed from initial screening questionnaires that asked subjects to describe physical activities, durations and frequencies per wk. All subjects accepted into the study self-reported ≤ 7 h/wk. The 7-d physical activity recall (17) was

79

administered during the subject's testing session and confirmed ≤ 7 h of hard and very hard physical activity per wk by subjects.

Bone mineral and soft tissue mass measurements

Using dual-energy X-ray absorptiometry (DXA) (QDR-4500A, Hologic, Inc., Bedford, MA, U.S.A.), BMC (g) and BMD (g/cm^2) of the total body (TB), Lumbar Spine (LS) (L_1-L_4), total proximal femur [(TPF), including the femoral neck (FN), trochanter (Troc), and Ward's triangle (WT)], and total forearm [(TF) = radius + ulna, including the ultradistal (UD), Mid, and proximal one-third (Prox) forearm] were measured. Measurements were conducted and analyzed using version 8.25a of the TB software and standard protocols for LS, TPF and TF measurements, respectively. From TB DXA scans, fat-free soft tissue (FFST) mass (kg), fat mass (FM) (kg), and body fat percent (BF%) were calculated. All DXA scans were conducted and analyzed by one investigator to eliminate inter-tester error. Quality control procedures using a phantom LS were performed on the morning of each testing d, producing a coefficient of variation (CV) of 0.52%. Test-retest reliability for TB BMD, LS BMD, TPF BMD, TF BMD, FFST mass, FM, and BF% produced CVs of 0.73, 0.92, 0.69, 1.09, 1.07, 1.75, and 1.79%, respectively, in 24 young-adult men and women (18).

Biochemical assessments

Biomarkers of bone turnover

Venous blood samples (~34 mL) were drawn from subjects by a medical technologist between 0800 – 1100 h to limit diurnal variations and within 1.5 h of each subject's awaking. Samples were centrifuged at 1,070 *x g* for 12 min. Serum was separated into cryovials and frozen at -80° C until later analysis. Serum osteocalcin (ng/mL) was measured by radioimmunoassay (RIA) (Human Osteocalcin RIA I^{125} Kit, Biomedical Technologies, Staughton, MA, U.S.A.). All samples were analyzed in duplicate. The intra- and inter-assay CVs for osteocalcin were 6.0% and 2.7%, respectively.

Second void urine samples were also collected between 0800 - 1100 h. Samples were refrigerated until separated into cryovials and frozen at -80° C until later analysis. Urinary cross-linked N-telopeptides of type I collagen (NTx) were quantified by enzyme-linked immunosorbent assay (ELISA) (Osteomark, Seattle, WA, U.S.A.). Urinary creatinine excretion was measured by quantitative spectrophotometry (#555A, Sigma Diagnostics, St. Louis, MO, U.S.A.). Urinary NTx measurements were reported as bone collagen equivalents (BCE) per

mmol creatinine. All samples were assayed in duplicate. The intra- and inter-assay CVs were 6.5% and 7.9%, respectively, for urinary NTx and 4.4% and 1.9%, respectively, for creatinine.

Salivary and urinary cortisol

Prior to completion of all other procedures, subjects were instructed to gently chew on a 3 cm^2 piece of cotton dental gauze for ~30 sec. Each subject placed her saturated gauze into a sterile centrifuge tube. All tubes were spun at 1,540 $x\ g$ for 5 min, extracting saliva from gauze. Aliquotted saliva was stored at -80°C until analyzed for salivary cortisol (μg/dL) by ELISA (Salimetrics LLC, State College, PA, U.S.A.). Intra- and inter-assay CVs for salivary cortisol were 8.5% and 8.2%, respectively.

Near the completion of the 2-h testing session, subjects were provided with 24-h urine collection containers. Subjects were instructed to record the time of their first void upon waking for the 24-h period but to discard this sample (9). All urine excreted during the following 24-h was pooled into their individual containers and refrigerated. Samples were complete after collection of the first morning void of the subsequent morning (9). Because cortisol concentrations have been shown to be unaffected by the menstrual cycle (19,20), subjects were permitted to select the 24-h period most convenient for urine collection. Upon return of samples to the BONE Lab, total urine volume (mL) was measured, and aliquots were pipetted into 1mL cryovials that were frozen at −80°C until analyzed for urinary cortisol by RIA (Diasorin, Stillwater, MN, U.S.A.). Intra-assay CV for urinary cortisol in our laboratory was 6.1%. Creatinine was also measured from the 24-h urine samples, as previously described, and urinary cortisol concentrations were recorded as a ratio of cortisol (nmol) to creatinine (mmol).

Serum hormone concentrations

Serum samples were analyzed for estradiol (pg/mL), progesterone (ng/mL), and GH (ng/mL) by RIA (Coat-A-Count® Estradiol, Coat-A-Count® Progesterone, and Double Antibody hGH, respectively, Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). Samples containing undetectable levels of estradiol (n = 25) and GH (n = 22) were assigned the minimum values detectable by these assays (20 pg/mL and 1 ng/mL, respectively). Serum insulin-like growth factor-1 (IGF-1) (μg/L) was measured by RIA and according to the methods of Weber et al (21). Serum IGF binding protein-3 (IGFBP-3) (mg/L) was measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, U.S.A.). All samples were analyzed in duplicate. Intra- and inter-assay CVs were: 7.2% and 5.0%, respectively, for serum estradiol;

6.5% and 7.3%, respectively, for serum progesterone; 6.3% and 2.1%, respectively, for serum GH; 11.0% and 10.8%, respectively, for serum IGF-1, and 4.4% and 9.0%, respectively, for serum IGFBP-3.

Statistical analyses

The Statistical Package for Social Sciences (SPSS) (version 10.0, 1999, SPSS Inc., Chicago, IL, U.S.A.) was used for all statistical procedures. Descriptive statistics were completed for variables of interest. Group comparisons (low versus high CER) were made using unpaired t-tests. Pearson correlation coefficients were determined to examine the bivariate relationships between variables of interest. A two-tailed level of significance was set at $p < 0.05$. Stepwise linear regression analyses were conducted to determine significant prediction models for BMD measures and biomarkers of bone turnover. Stepwise regression entered variables at $\alpha = 0.10$ and removed variables at $\alpha = 0.15$.

Results

Table 1 provides characteristics of all study subjects and characteristics of women in low and high CER groups. Groups were significantly different in FM and BF% but not in other descriptive characteristics. A trend for higher BMI ($p < 0.07$) in women in the high compared to low CER group was observed.

Estimated average daily dietary intakes of selected nutrients based on FFQ (n = 64) are presented in **Table 2**. Group comparisons show that women in low and high CER groups did not significantly differ in dietary intake. Trends for a greater % of energy consumed from carbohydrate ($p < 0.06$) and higher daily dietary fiber intake ($p < 0.08$) were observed in the high versus low CER group. Average daily dietary intake of nutrients estimated from 4-d food records (n = 49) showed no significant differences between CER groups in intake of any nutrients (**Table 3**). A trend for a higher vitamin C intake ($p < 0.06$) was found in the high versus low CER group, likely due to a significantly greater ($p < 0.05$) consumption of fruits and vegetables combined (4.9 ± 1.8 versus 3.8 ± 1.8 servings/d, respectively).

Measurements of TB and site-specific BMC and BMD are presented in **Table 4**. Significant differences in bone measures between groups were not identified. Biochemical markers of bone turnover are also presented in Table 4. A trend ($p < 0.07$) for a lower serum osteocalcin concentration in the high versus low CER group was observed.

Table 1. Descriptive characteristics of all study subjects and by cognitive eating restraint (CER) group

Variable		All Subjects (n = 65)	Low CER (n = 34)	High CER (n = 31)	P-value
Age (y)		20.4 ± 2.3^a	20.2 ± 2.4	20.7 ± 2.3	0.363^b
Weight (kg)		58.4 ± 6.6	57.1 ± 6.6	59.8 ± 6.4	0.103
Height (cm)		165.0 ± 5.7	164.9 ± 6.2	165.1 ± 5.2	0.858
Body mass index (kg/m^2)		21.4 ± 2.0	21.0 ± 2.0	21.9 ± 2.0	0.068
FFST^c mass (kg)		41.0 ± 4.0	41.0 ± 4.0	41.1 ± 4.1	0.890
Fat mass (kg)		15.7 ± 4.1	14.5 ± 3.7	17.0 ± 4.2	0.014
Body fat (%)		26.4 ± 4.7	25.0 ± 4.1	28.0 ± 5.0	0.010
Exercise (h/wk)^d		3.3 ± 2.2	2.8 ± 2.0	3.7 ± 2.3	0.145
Alcohol (drinks/wk)^e		2.2 ± 2.5	2.5 ± 2.7	1.9 ± 2.4	0.278
Oral Contraceptive Use	*Yes*	26	12	14	
	No	39	22	17	
Multivitamin Use^f	*Yes*	20	10	10	
	No	36	21	15	
	Other^f	9	3	6	

[a]Mean $\pm$ SD.
[b]*p*-value for student's *t*-test between CER groups.
[c]FFST = fat-free soft tissue.
[d]Average h/wk of hard and very hard physical activity from 7-d physical activity recalls (Blair et al. 1985).
[e]Average weekly number of alcoholic drinks/wk consumed based on initial screening questionnaire.
[f]n = 64; Multivitamin use on a regular basis; "other" indicates use of single vitamin or mineral.

Table 2. Estimated average daily dietary intake from Food Frequency Questionnaires of all study subjects and by cognitive eating restraint (CER) group

Nutrient	All Subjects (n = 64)	Low CER (n = 33)	High CER (n = 31)	P-value
Energy (kJ/d)	8,805 ± 2,847[a]	8,799 ± 2,232	8,811 ± 3,428	0.986[b]
Carbohydrate				
Total (g/d)	290 ± 93	284 ± 72	297 ± 111	0.559
% kJ	55.5 ± 6.3	54.2 ± 6.0	57.1 ± 6.4	0.057
Fat				
Total (g/d)	74 ± 29	76 ± 25	72 ± 33	0.621
% kJ	31.3 ± 5.3	32.2 ± 5.2	30.3 ± 5.3	0.151
Saturated fat (g/d)	23 ± 10	23 ± 8	22 ± 11	0.561
Protein				
Total (g/d)	72 ± 25	71 ± 19	73 ± 30	0.757
% kJ	13.8 ± 2.3	13.6 ± 2.0	14.0 ± 2.0	0.564
Fiber (g/d)	21 ± 8	19 ± 8	23 ± 7	0.074
Minerals (mg/d)				
Calcium	1,089 ± 437	1,028 ± 331	1,153 ± 525	0.257
Iron	16.4 ± 5.4	15.9 ± 5.0	16.9 ± 5.8	0.484
Magnesium	328 ± 108	314 ± 101	342 ± 114	0.306
Phosphorus	1,359 ± 441	1,336 ± 331	1,384 ± 510	0.672
Potassium	3,463 ± 1118	3,305 ± 1,011	3,632 ± 1,216	0.245
Sodium	3,027 ± 1,119	3,069 ± 977	2,982 ± 1,267	0.760
Zinc	10.7 ± 4.0	10.8 ± 3.4	10.5 ± 4.5	0.784
Vitamins				
Vitamin A (IU/d)[c]	11,887 ± 7,280	10,756 ± 5,670	13,090 ± 8,608	0.202
Vitamin C (mg/d)	176 ± 102	157 ± 82	196 ± 118	0.122
Vitamin D (IU/d)	168 ± 111	179 ± 120	155 ± 100	0.391

[a]Mean ± SD.
[b]*p*-value for student's *t*-test between CER groups.
[c]IU = International Units.

Table 3. Estimated average daily dietary intake from 4-d food records of all study subjects and by cognitive eating restraint (CER) group

Nutrient or Food Group	All Subjects (n = 49)	Low CER (n = 24)	High CER (n = 25)	P-value
Energy (kJ/d)	7,579 ± 1,601[a]	7,619 ± 1,694	7,554 ± 1,537	0.867[b]
Carbohydrate				
Total (g/d)	256 ± 56	253 ± 56	259 ± 56	0.731
% kJ	56.7 ± 7.5	55.8 ± 5.4	57.9 ± 9.1	0.347
Fat				
Total (g/d)	60 ± 21	60 ± 18	59 ± 23	0.811
% kJ	29.4 ± 6.6	29.8 ± 5.2	29.0 ± 7.8	0.679
Protein				
Total (g/d)	66 ± 17	67 ± 19	66 ± 15	0.747
% kJ	14.7 ± 2.4	14.8 ± 2.5	14.7 ± 2.4	0.787
Fiber (g/d)	18 ± 20	18 ± 8	18 ± 7	0.993
Minerals (mg/d)				
Calcium	834 ± 297	844 ± 354	824 ± 238	0.818
Iron	15.9 ± 1.6	14.4 ± 6.6	17.3 ± 15.1	0.400
Magnesium	177 ± 71	164 ± 69	189 ± 72	0.210
Phosphorus	853 ± 267	839 ± 318	865 ± 212	0.737
Potassium	1,937 ± 668	1,794 ± 650	2,075 ± 670	0.144
Sodium	3,180 ± 858	3,260 ± 988	3,104 ± 724	0.534
Zinc	7.0 ± 3.1	7.1 ± 3.7	6.9 ± 2.5	0.830
Vitamins				
Vitamin A (IU/d)[c]	7,680 ± 4,988	7,055 ± 4,414	8,281 ± 5,506	0.395
Vitamin C (mg/d)	97 ± 63	79 ± 53	113 ± 68	0.056
Vitamin D (IU/d)	118 ± 78	128 ± 87	108 ± 70	0.384
Food Groups (servings/d)				
Fruits	1.6 ± 1.1	1.4 ± 1.0	1.9 ± 1.2	0.127
Grains	6.6 ± 1.6	6.6 ± 1.3	6.7 ± 1.9	0.954
Meat	1.4 ± 0.9	1.5 ± 1.1	1.3 ± 0.7	0.621
Milk	1.8 ± 0.9	1.9 ± 1.1	1.7 ± 0.7	0.651
Vegetables	2.7 ± 1.1	2.5 ± 1.1	3.0 ± 1.1	0.082

[a]Mean ± SD.
[b]p-value for student's t-test between CER groups.
[c]IU = International Units.

Table 4. Measures of bone mineral content (BMC), bone mineral density (BMD), and biomarkers of bone turnover of all study subjects and by cognitive eating restraint (CER) group

Variable	All Subjects (n = 65)	Low CER (n = 34)	High CER (n = 31)	P-value
BMC (g)				
Total body	2,145 ± 243[a]	2,141 ± 248	2,149 ± 242	0.899[b]
Lumbar spine (L$_1$-L$_4$)	56.6 ± 8.7	56.3 ± 9.3	57.0 ± 8.2	0.729
Total proximal femur	31.9 ± 5.4	32.6 ± 5.8	31.1 ± 4.8	0.270
Femoral neck	4.3 ± 0.6	4.3 ± 0.6	4.3 ± 0.6	0.905
Trochanter	7.2 ± 1.4	7.1 ± 1.5	7.2 ± 1.2	0.751
Ward's triangle	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.876
Total forearm	11.9 ± 1.4	11.9 ± 1.6	11.9 ± 1.1	0.894
Ultradistal	2.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.2	0.629
Mid	6.5 ± 0.9	6.5 ± 1.0	6.4 ± 0.8	0.821
Proximal	3.2 ± 0.3	3.2 ± 0.4	3.2 ± 0.2	0.784
BMD (g/cm^2)				
Total body	1.12 ± 0.08	1.11 ± 0.08	1.11 ± 0.07	0.735
Lumbar spine (L$_1$-L$_4$)	1.01 ± 0.09	1.02 ± 0.08	1.00 ± 0.09	0.370
Total proximal femur	0.98 ± 0.10	0.99 ± 0.10	0.97 ± 0.10	0.445
Femoral neck	0.85 ± 0.10	0.86 ± 0.09	0.85 ± 0.10	0.948
Trochanter	0.75 ± 0.09	0.75 ± 0.10	0.74 ± 0.10	0.449
Ward's triangle	0.84 ± 0.13	0.85 ± 0.13	0.84 ± 0.13	0.753
Total forearm	0.56 ± 0.04	0.56 ± 0.04	0.57 ± 0.04	0.634
Ultradistal	0.42 ± 0.05	0.42 ± 0.06	0.42 ± 0.03	0.967
Mid	0.59 ± 0.04	0.58 ± 0.04	0.59 ± 0.04	0.381
Proximal	0.67 ± 0.06	0.67 ± 0.07	0.68 ± 0.04	0.390
Biomarkers of bone turnover				
Osteocalcin (ng/mL)[c]	9.5 ± 2.3	10.0 ± 2.4	9.0 ± 2.0	0.065
NTx (BCE/mmol creat)[c]	75.1 ± 26.6	78.2 ± 28.7	71.9 ± 24.2	0.352

[a]Mean ± SD.
[b]*p*-value for student's *t*-test between CER groups.
[c]n = 64.
NTx = urinary N-telopeptide; BCE = bone collagen equivalents; creat = urinary creatinine.

All subjects self-reported being in the follicular phase of their menstrual cycles, yet after evaluation of serum estradiol and progesterone concentrations, one subject possessed a progesterone concentration (16 ng/mL) consistent with the luteal phase of the menstrual cycle. Therefore, with the exception of salivary and urinary cortisol, hormonal data from this subject were excluded from statistical analyses. Serum IGFBP-3 concentrations for all subjects were higher than the normal range (2.67 – 5.58 mg/L versus 3.65 – 7.99 mg/L, manufacturer's range versus BONE Lab range). However, considering our narrow inter- and intra-assay CVs for IGFBP-3 as well as the positive association between IGFBP-3 and IGF-1 ($p \leq 0.001$), IGFBP-3 values were deemed valid and usable. Significant differences in hormone concentrations between low and high CER groups were not observed (**Table 5**).

Table 6 displays correlation coefficients for relationships between BMC, BMD, and bone biomarkers with anthropometric and soft tissue mass measurements for all 65 subjects, except where noted. Height and FFST mass were positively associated with all measures of BMC ($p \leq 0.01 – p \leq 0.001$), except WT BMC. Weight was positively associated with all BMC measures ($p \leq 0.01 – p \leq 0.001$). Body mass index had a positive relationship with UD forearm BMC ($p \leq 0.05$). Fat mass was positively associated with WT BMC ($p \leq 0.05$) and UD forearm BMC ($p \leq 0.05$). Height was positively associated with LS BMD ($p \leq 0.01$) as were weight ($p \leq 0.01$) and FFST mass ($p \leq 0.05$). Height ($p \leq 0.01$), weight ($p \leq 0.05$), and FFST mass ($p \leq 0.05$) each had a positive relationship with FN BMD. Height ($p \leq 0.05$) and FFST mass ($p \leq 0.05$) both had a positive association with WT BMD. Weight, BMI, and FM were all positively related to TF BMD ($p \leq 0.05 – p \leq 0.01$), UD forearm BMD ($p \leq 0.05 – p \leq 0.01$), and Mid forearm BMD ($p \leq 0.05 – p \leq 0.01$). Fat-free soft tissue mass was also positively related to TF BMD ($p \leq 0.05$) and Mid forearm BMD ($p \leq 0.05$). Body fat % was negatively associated with serum osteocalcin ($p \leq 0.05$).

Table 7 displays correlation coefficients between bone variables and select FFQ variables. While several nutrients, including vitamin D, calcium, phosphorus, protein, zinc, magnesium, and potassium ($p \leq 0.05 – p \leq 0.01$), had significant and positive associations with certain BMC measures, only estimated average daily dietary vitamin D and calcium intakes were positively related to BMD ($p \leq 0.05 – p \leq 0.01$) and only to sites of the forearm. Estimated average daily dietary fiber, iron, magnesium, phosphorus, and potassium intakes were all

Table 5. Mean hormone concentrations for all subjects and by cognitive eating restraint (CER) group

Hormone	All Subjects (n = 64)	Low CER (n = 33)	High CER (n = 31)	P-value
Salivary cortisol (μg/dL)	0.59 ± 0.31[a]	0.57 ± 0.32	0.61 ± 0.30	0.530[b]
Urinary cortisol (nmol/mmol creatinine)[c]	50.6 ± 18.4	52.4 ± 19.8	49.2 ± 17.5	0.614
Estradiol (pg/mL)	33.1 ± 19.4	37.2 ± 21.4	31.7 ± 16.3	0.254
Progesterone (ng/mL)	0.66 ± 0.22	0.70 ± 0.24	0.62 ± 0.19	0.170
Growth Hormone (ng/mL)	3.9 ± 3.7	4.1 ± 4.0	3.6 ± 3.4	0.614
IGF-1[d] **(μg/L)**	57.1 ± 30.6	59.5 ± 30.2	54.5 ± 31.3	0.517
IGFBP-3[e] **(mg/L)**	5.4 ± 0.9	5.2 ± 0.9	5.6 ± 0.9	0.094

[a]Mean $\pm$ SD.
[b]*p*-value for student's *t*-test between CER groups.
[c]n = 19 in Low CER group and n = 25 in High CER group.
[d]IGF-1 = insulin-like growth factor-1.
[e]IGFBP-3 = insulin-like growth factor binding protein-3.

Table 6. Pearson correlation coefficients for total body and site-specific bone mineral measures and biomarkers of bone turnover with anthropometric and soft tissue mass measures[a]

	TB BMC (g)	TB BMD (g/cm^2)	LS BMC (g)	LS BMD (g/cm^2)	TPF BMC (g)	TPF BMD (g/cm^2)	FN BMC (g)	FN BMD (g/cm^2)	Troc BMC (g)	Troc BMD (g/cm^2)	WT BMC (g)	WT BMD (g/cm^2)
Height (cm)	0.63***	0.18	0.42***	0.34**	0.57***	0.20	0.59***	0.38**	0.57***	0.14	0.24	0.31*
Weight (kg)	0.52***	0.11	0.40**	0.35**	0.43***	0.16	0.48***	0.29*	0.36**	0.11	0.33**	0.23
BMI (kg/m^2)	0.15	-0.01	0.17	0.17	0.10	0.05	0.15	0.07	0.01	0.03	0.21	0.06
Fat mass (kg)	0.24	-0.01	0.22	0.21	0.17	0.01	0.24	0.11	0.16	0.08	0.27*	0.05
FFST mass (kg)	0.51***	0.10	0.37**	0.29*	0.48***	0.20	0.48***	0.32*	0.35**	0.06	0.21	0.27*
BF%	0.04	-0.06	0.08	0.10	-0.02	-0.09	0.05	-0.03	0.02	0.05	0.18	-0.07

	TF BMC (g)	TF BMD (g/cm^2)	UD BMC (g)	UD BMD (g/cm^2)	Mid BMC (g)	Mid BMD (g/cm^2)	Prox BMC (g)	Prox BMD (g/cm^2)	Osteocalcin[b] (ng/mL)	NTx[b] (BCE/mmol creatinine)
Height (cm)	0.58***	0.13	0.36**	0.12	0.65***	0.17	0.36**	0.15	0.11	0.11
Weight (kg)	0.49***	0.35**	0.48***	0.35**	0.49***	0.37**	0.37**	0.24	-0.04	-0.08
BMI (kg/m^2)	0.15	0.33**	0.30*	0.33**	0.09	0.32**	0.17	0.18	-0.11	-0.17
Fat mass (kg)	0.22	0.26*	0.29*	0.30*	0.21	0.27*	0.12	0.17	-0.22	-0.03
FFST mass (kg)	0.49**	0.25*	0.40**	0.23	0.49***	0.26*	0.40**	0.17	0.18	-0.05
BF%	0.06	0.17	0.16	0.21	0.05	0.19	-0.01	0.12	-0.30*	-0.07

[a]n = 65. [b]n = 64. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; TB = total body; BMC = bone mineral content; BMD = bone mineral density; LS = lumbar spine (L$_1$-L$_4$); TPF = total proximal femur; FN = femoral neck; Troc = trochanter; WT = Ward's triangle; TF = total forearm; UD = ultradistal forearm; Mid = mid forearm; Prox = proximal forearm; NTx = urinary N-telopeptide; BCE = bone collagen equivalents; BMI = body mass index; FFST = fat-free soft tissue; BF% = body fat percent.

Table 7. Pearson correlation coefficients for total body and site-specific bone mineral measures and biomarkers of bone turnover with estimated average daily dietary intake of selected nutrients from Food Frequency Questionnaires[a]

	TB BMC (g)	TB BMD (g/cm²)	LS BMC (g)	LS BMD (g/cm²)	TPF BMC (g)	TPF BMD (g/cm²)	FN BMC (g)	FN BMD (g/cm²)	Troc BMC (g)	Troc BMD (g/cm²)	WT BMC (g)	WT BMD (g/cm²)
Kilojoules	0.07	0.05	0.19	0.12	0.01	-0.05	0.07	0.04	-0.01	-0.10	-0.02	0.07
Carbohydrate (g)	0.06	0.05	0.18	0.12	0.01	0.03	0.11	0.08	0.04	-0.06	0.01	0.09
Fat (g)	0.02	0.05	0.16	0.10	-0.06	-0.10	-0.01	-0.03	-0.06	-0.10	-0.06	0.03
Protein (g)	0.20	0.08	0.24	0.13	0.14	-0.06	0.15	0.10	0.04	-0.09	0.03	0.06
Fiber (g)	-0.04	0.08	0.11	0.09	-0.12	0.04	0.02	0.06	-0.11	-0.01	0.03	0.06
Calcium (mg)	0.24	0.12	0.28*	0.20	0.22	0.09	0.24	0.16	0.20	0.08	0.08	0.14
Iron (mg)	0.06	0.06	0.09	0.04	0.02	0.03	0.02	0.06	-0.01	-0.09	0.01	0.10
Magnesium (mg)	0.07	0.08	0.18	0.11	-0.02	-0.01	0.05	0.01	-0.06	-0.08	0.06	0.07
Phosphorus (mg)	0.23	0.12	0.26*	0.17	0.17	-0.01	0.19	0.11	0.11	-0.04	0.08	0.13
Potassium (mg)	0.08	0.04	0.24	0.17	0.03	0.05	0.20	0.06	-0.03	-0.03	0.09	0.11
Sodium (mg)	0.07	0.07	0.15	0.10	0.04	-0.04	0.01	0.05	-0.05	0.10	0.04	0.09
Zinc (mg)	0.12	0.07	0.17	0.09	0.04	-0.01	0.08	0.09	-0.01	-0.09	0.06	0.11
Vitamin A (IU)	-0.03	-0.02	0.12	0.04	0.10	0.15	0.07	0.13	-0.01	0.05	0.02	0.02
Vitamin C (mg)	0.01	0.03	0.08	0.07	0.01	0.08	0.12	0.08	0.01	0.03	0.04	0.03
Vitamin D (IU)	0.33*	0.23	0.09	0.08	0.39**	0.16	0.18	0.13	0.22	0.10	0.14	0.22

Table 7. Continued

	TF BMC (g)	TF BMD (g/cm^2)	UD BMC (g)	UD BMD (g/cm^2)	Mid BMC (g)	Mid BMD (g/cm^2)	Prox BMC (g)	Prox BMD (g/cm^2)	Ostoecalcin[b] (ng/mL)	NTx[b] (BCE/mmol creatinine
Kilojoules	0.19	0.16	0.24	-0.04	0.17	0.16	0.16	0.21	-0.21	0.28*
Carbohydrate (g)	0.16	0.14	0.21	0.01	0.15	0.15	0.10	0.18	-0.20	0.33**
Fat (g)	0.16	0.17	0.22	-0.06	0.12	0.17	0.17	0.24	-0.18	0.22
Protein (g)	0.29*	0.19	0.32*	0.01	0.25	0.21	0.23	0.21	-0.24	0.13
Fiber (g)	0.05	0.07	0.12	0.04	0.05	0.08	0.01	0.09	-0.43***	0.02
Calcium (mg)	0.31*	0.30*	0.37**	0.14	0.29*	0.35**	0.16	0.25*	-0.19	0.06
Iron (mg)	0.12	0.09	0.14	0.19	0.14	0.10	0.01	-0.08	-0.28*	0.20
Magnesium (mg)	0.23	0.16	0.25*	-0.01	0.22	0.17	0.15	0.20	-0.35**	0.10
Phosphorus (mg)	0.35**	0.17	0.37**	0.01	0.34**	0.20	0.24	0.24	-0.25*	0.15
Potassium (mg)	0.19	0.11	0.28*	0.01	0.17	0.12	0.13	0.14	-0.32**	0.15
Sodium (mg)	0.16	0.07	0.22	-0.02	0.14	0.08	0.11	0.10	-0.23	0.17
Zinc (mg)	0.26*	0.15	0.27*	-0.06	0.26*	0.16	0.19	0.24	-0.21	0.17
Vitamin A (IU)	-0.03	0.05	0.18	0.22	-0.06	0.06	-0.06	-0.06	-0.21	-0.22
Vitamin C (mg)	0.02	0.07	0.12	0.01	-0.01	0.10	0.01	0.11	-0.18	0.27*
Vitamin D (IU)	0.38**	0.27*	0.44**	0.34**	0.39**	0.31*	0.19	0.05	-0.09	-0.02

[a]n = 64. [b]n = 63. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; TB = total body; BMC = bone mineral content; BMD = bone mineral density; LS = lumbar spine (L$_1$-L$_4$); TPF = total proximal femur; FN = femoral neck; Troc = trochanter; WT = Ward's triangle; TF = total forearm; UD = ultradistal forearm; Mid = mid forearm; Prox = proximal forearm; NTx = urinary N-telopeptide; BCE = bone collagen equivalents; IU = International Units.

negatively associated with serum osteocalcin ($p \leq 0.05 - p \leq 0.001$), while energy, carbohydrate, and vitamin C had significant and positive associations with urinary NTx ($p \leq 0.05 - p \leq 0.01$). Correlation coefficients for associations between bone measures and select nutrients from 4-d food records (n = 49) are presented in **Table 8**. Significant and positive associations between estimated average daily dietary phosphorus, vitamin C, vitamin D, servings of fruit, and servings of milk ($p \leq 0.05 - p \leq 0.01$) and certain BMC measures were found. Estimated average daily dietary phosphorus, vitamin D, vitamin C, and potassium intakes as well as servings of fruit were positively related to certain BMD measures ($p \leq 0.05 - p \leq 0.01$). Servings of meat per d was negatively associated with Prox forearm BMC ($p \leq 0.05$) and TF BMD ($p \leq 0.05$). Significant relationships between bone biomarkers and estimated nutrient intakes from 4-d food records were not observed.

Pearson correlation coefficients for associations between bone measures and hormones are displayed in **Table 9**. Salivary cortisol was not related to any bone measure; however, urinary cortisol was positively related to LS BMC ($p \leq 0.05$) and LS BMD ($p \leq 0.05$) and UD forearm BMD ($p \leq 0.05$). Serum estradiol was positively associated with FN BMD ($p \leq 0.05$), and serum progesterone was not associated with any BMC or BMD measure. Growth hormone had significant inverse relationships with FN ($p \leq 0.05$), Troc ($p \leq 0.05$), and WT ($p \leq 0.05$) BMC and FN BMD ($p \leq 0.05$). Significant and negative associations between IGF-1 ($p \leq 0.05$) and IGFBP-3 ($p \leq 0.05$) and WT BMC were found. Serum osteocalcin was not associated with any BMC or BMD measure, but urinary NTx was positively associated with WT BMD ($p \leq 0.05$).

Table 10 presents correlation coefficients for age, CER score, anthropometric and soft tissue mass measurements with bone biomarkers and hormones. Significant inverse associations between age and IGF-1 ($p \leq 0.01$), progesterone ($p \leq 0.01$), urinary NTx ($p \leq 0.001$), and urinary cortisol ($p \leq 0.05$) concentrations were found. A positive association between CER and IGFBP-3 ($p \leq 0.05$) and negative relationships between CER and osteocalcin ($p \leq 0.05$) and urinary NTx ($p \leq 0.05$) were observed. Estradiol ($p \leq 0.05$) and progesterone ($p \leq 0.05$) were positively associated with FFST mass, and progesterone was negatively related ($p \leq 0.05$) to BF%. Estradiol was positively related to progesterone ($p \leq 0.001$) and serum osteocalcin ($p \leq 0.05$), while progesterone was positively associated with urinary NTx ($p \leq 0.05$). Growth hormone was

Table 8. Pearson correlation coefficients for total body and site-specific bone mineral measures and biomarkers of bone turnover with estimated average daily dietary intake of selected nutrients and food groups from 4-d food records[a]

	TB BMC (g)	TB BMD (g/cm²)	LS BMC (g)	LS BMD (g/cm²)	TPF BMC (g)	TPF BMD (g/cm²)	FN BMC (g)	FN BMD (g/cm²)	Troc BMC (g)	Troc BMD (g/cm²)	WT BMC (g)	WT BMD (g/cm²)
Kilojoules	0.01	0.05	0.11	0.11	0.04	-0.06	0.05	0.01	-0.04	-0.15	-0.03	0.02
Carbohydrate (g)	0.13	0.18	0.14	0.13	0.13	0.06	0.12	0.06	0.05	-0.03	0.03	0.15
Fat (g)	-0.07	-0.06	0.11	0.09	-0.05	-0.17	-0.03	-0.07	-0.06	-0.20	-0.05	-0.10
Protein (g)	-0.01	0.05	0.08	0.15	-0.02	0.18	0.16	0.28	0.02	0.06	0.01	0.10
Fiber (g)	0.01	0.18	-0.11	0.03	0.01	0.22	0.01	0.11	-0.12	0.11	0.11	0.18
Calcium (mg)	0.15	0.24	0.08	0.20	0.06	0.24	0.16	0.17	-0.01	0.20	0.25	0.19
Iron (mg)	0.02	-0.07	-0.03	-0.07	-0.04	-0.12	-0.05	-0.10	-0.06	-0.13	0.01	-0.05
Magnesium (mg)	0.20	0.25	0.09	0.09	0.06	0.28	0.14	0.20	0.07	0.18	0.19	0.23
Phosphorus (mg)	0.31*	0.34*	0.15	0.23	0.17	0.36*	0.27	0.33*	0.12	0.25	0.38**	0.30*
Potassium (mg)	0.22	0.26	0.15	0.20	0.10	0.38**	0.25	0.27	0.19	0.30*	0.22	0.36*
Sodium (mg)	-0.08	-0.07	0.04	0.05	-0.08	-0.11	-0.08	0.03	-0.14	-0.21	-0.21	-0.10
Zinc (mg)	0.15	0.17	0.04	0.05	-0.05	0.07	0.05	0.11	-0.03	0.04	0.11	0.01
Vitamin A (IU)	-0.05	0.16	0.06	0.12	-0.04	0.22	-0.07	0.03	-0.15	0.12	0.13	0.13
Vitamin C (mg)	0.14	0.14	0.30*	0.34**	0.11	0.35*	0.31*	0.33*	0.22	0.32*	0.20	0.24
Vitamin D (IU)	0.26	0.29*	-0.04	0.09	0.35*	0.32*	0.24	0.29*	0.11	0.22	0.36*	0.31*
Fruit[b]	0.28	0.34*	0.28*	0.39**	0.14	0.41**	0.31*	0.32*	0.21	0.38*	0.28*	0.45**
Grains[b]	0.07	-0.04	0.20	0.01	0.11	-0.06	0.07	0.01	0.09	-0.15	-0.25	0.03
Meat[b]	-0.10	-0.06	-0.01	0.10	-0.01	0.11	0.07	0.22	0.01	0.06	-0.08	-0.02
Milk[b]	0.19	0.27	0.03	0.12	0.19	0.22	0.18	0.19	0.11	0.24	0.35*	0.24
Vegetables[b]	-0.06	0.03	0.14	0.18	-0.05	0.26	0.16	0.27	0.05	0.21	0.09	0.18

Table 8. Continued

	TF BMC (g)	TF BMD (g/cm^2)	UD BMC(g)	UD BMD (g/cm^2)	Mid BMC (g)	Mid BMD (g/cm^2)	Prox BMC (g)	Prox BMD (g/cm^2)	Osteocalcin[c] (ng/mL)	NTx[c] (BCE/mmol creatinine)
Kilojoules	-0.02	-0.03	0.10	-0.18	-0.04	0.01	-0.05	0.18	0.14	0.13
Carbohydrate (g)	0.05	0.03	0.09	-0.09	0.06	0.06	-0.02	0.18	0.07	0.14
Fat (g)	-0.03	-0.04	0.10	-0.21	-0.07	-0.04	0.02	0.17	0.23	0.12
Protein (g)	-0.15	-0.14	0.01	-0.12	-0.16	-0.10	-0.21	-0.01	0.10	0.05
Fiber (g)	-0.04	0.09	-0.01	0.22	-0.04	0.10	-0.06	-0.05	-0.09	-0.06
Calcium (mg)	0.12	0.11	0.05	-0.01	0.14	0.14	0.05	0.16	0.06	0.01
Iron (mg)	0.08	-0.01	0.01	0.10	0.11	0.01	0.05	-0.15	-0.01	0.04
Magnesium (mg)	0.17	0.20	0.15	0.20	0.17	0.22	0.12	0.06	-0.12	-0.12
Phosphorus (mg)	0.23	0.25	0.25	0.13	0.23	0.28	0.14	0.20	-0.07	-0.03
Potassium (mg)	0.14	0.05	0.11	0.03	0.17	0.07	0.01	0.08	-0.09	0.05
Sodium (mg)	-0.13	-0.07	0.02	-0.09	-0.16	-0.05	-0.09	0.01	0.07	0.06
Zinc (mg)	0.03	0.09	-0.10	0.07	-0.07	0.01	-0.01	0.01	-0.15	-0.02
Vitamin A (IU)	-0.07	0.05	-0.03	0.06	-0.07	0.02	-0.09	0.01	-0.21	-0.20
Vitamin C (mg)	-0.06	-0.14	-0.05	-0.08	-0.04	-0.03	-0.13	0.13	-0.23	-0.18
Vitamin D (IU)	0.18	0.15	0.10	0.03	0.22	0.15	0.02	0.05	0.01	0.01
Fruit[b]	0.09	0.06	0.07	0.06	0.14	0.08	-0.06	0.14	-0.13	-0.04
Grains[b]	0.11	-0.01	0.16	0.16	0.10	0.09	0.06	-0.02	0.07	0.01
Meat[b]	-0.26	-0.30*	-0.07	-0.16	-0.27	-0.27	-0.28*	-0.09	0.04	-0.03
Milk[b]	0.22	0.22	0.12	-0.04	0.24	0.22	0.13	0.23	0.07	0.10
Vegetables[b]	-0.20	-0.10	-0.01	0.17	-0.21	-0.04	-0.27	-0.18	-0.23	-0.14

[a]n = 49. [b]servings per day. [c]n = 48. *p ≤ 0.05; **p ≤ 0.01; TB = total body; BMC = bone mineral content; BMD = bone mineral density; LS = lumbar spine (L$_1$-L$_4$); TPF = total proximal femur; FN = femoral neck; Troc = trochanter; WT = Ward's triangle; TF = total forearm; UD = ultradistal forearm; Mid = mid forearm; Prox = proximal forearm; NTx = urinary N-telopeptide; BCE = bone collagen equivalents; IU = International Units.

Table 9. Pearson correlation coefficients for total body and site-specific bone mineral measures and hormones[a]

	TB BMC (g)	TB BMD (g/cm²)	LS BMC (g)	LS BMD (g/cm²)	TPF BMC (g)	TPF BMD (g/cm²)	FN BMC (g)	FN BMD (g/cm²)	Troc BMC (g)	Troc BMD (g/cm²)	WT BMC (g)	WT BMD (g/cm²)
Salivary cortisol (µg/dL)	-0.08	0.04	-0.01	0.05	0.03	0.04	-0.14	-0.09	0.02	0.08	-0.05	-0.02
Urinary cortisol (nmol/mmol creatinine)[b]	0.28	0.19	0.37*	0.36*	0.12	0.23	0.26	0.18	0.27	0.19	0.01	0.09
Estradiol (pg/mL) [c]	0.10	0.01	0.15	0.13	0.18	0.20	0.20	0.27*	0.23	0.01	0.01	0.10
Progesterone (ng/mL) [c]	0.22	0.08	0.12	0.12	0.24	0.19	0.22	0.24	0.20	0.05	0.02	0.07
Growth Hormone (ng/mL)[c]	-0.18	-0.01	-0.19	-0.15	-0.25	-0.12	-0.25*	-0.30*	-0.28*	-0.07	-0.27*	-0.07
IGF-1[c,d] (µg/L)	-0.04	-0.02	-0.13	0.01	0.01	-0.14	-0.02	-0.05	-0.01	-0.22	-0.31*	-0.16
IGFBP-3[c,e] (mg/L)	-0.02	-0.06	-0.02	0.04	-0.14	-0.14	-0.04	-0.09	-0.10	-0.24	-0.27*	-0.17
Osteocalcin (ng/mL)[c]	-0.01	-0.11	0.03	-0.07	0.08	-0.03	0.07	0.03	0.13	-0.11	0.10	-0.04
NTx (BCE/mmol creatinine)[c]	0.03	-0.05	0.04	-0.01	0.06	0.06	0.17	0.13	0.17	-0.02	0.05	0.26*

	TF BMC (g)	TF BMD (g/cm²)	UD BMC (g)	UD BMD (g/cm²)	Mid BMC (g)	Mid BMD (g/cm²)	Prox BMC (g)	Prox BMD (g/cm²)
Salivary Cortisol (µg/dL)	-0.11	-0.02	-0.13	-0.04	-0.14	-0.04	0.01	0.03
Urinary cortisol (nmol/mmol creatinine)[b]	0.24	0.01	0.23	0.37*	0.24	-0.04	0.14	-0.18
Estradiol (pg/mL) [c]	0.10	0.10	0.01	-0.07	0.10	0.07	0.13	0.13
Progesterone (ng/mL) [c]	0.14	-0.03	0.13	-0.05	0.15	-0.01	0.08	0.01
Growth Hormone (ng/mL)[c]	-0.14	-0.09	-0.16	-0.17	-0.11	-0.09	-0.19	-0.03
IGF-1[c,d] (µg/L)	-0.01	-0.10	-0.07	-0.24	0.03	-0.04	-0.08	0.10
IGFBP-3[c,e] (mg/L)	-0.01	-0.17	-0.02	-0.21	0.03	-0.15	-0.08	-0.09
Osteocalcin (ng/mL)[c]	-0.02	-0.02	-0.07	-0.03	-0.03	-0.05	0.05	-0.05
NTx (BCE/mmol creatinine)[c]	0.05	0.09	0.03	0.04	0.04	0.04	0.07	0.10

[a]n = 64. [b]n = 44. [c]n = 63. [d]IGF-1 = insulin-like growth factor-1. [e]IGFBP-3 = insulin-like growth factor binding protein-3.
*p≤0.05; TB = total body; BMC = bone mineral content; BMD = bone mineral density; LS = lumbar spine (L$_1$-L$_4$); TPF = total proximal femur; FN = femoral neck; Troc = trochanter; WT = Ward's triangle; TF = total forearm; UD = ultradistal forearm; Mid = mid forearm; Prox = proximal forearm; NTx = urinary N-telopeptide; BCE = bone collagen equivalents.

Table 10. Pearson correlation coefficients for age, cognitive eating restraint (CER) score, anthropometric measures, soft tissue mass measures, and hormone concentrations[a]

	Salivary cortisol (µg/dL)	Growth hormone[b] (ng/mL)	IGF-1[b] (µg/L)	IGFBP-3[b] (mg/L)	Estradiol[b] (pg/mL)	Progesterone[b] (ng/mL)	Osteocalcin (ng/mL)	NTx (BCE/mmol creatinine)	Urinary cortisol[c] (nmol/mmol creatinine)
Age (y)	-0.22	0.01	-0.31**	-0.24	-0.06	-0.43**	-0.15	-0.45***	-0.30*
CER score	0.03	-0.13	-0.17	0.25*	-0.08	-0.14	-0.32*	-0.25*	-0.03
Height (cm)	-0.23	-0.28*	0.04	0.15	0.16	0.21			0.17
Weight (kg)	-0.23	-0.20	-0.10	-0.01	0.13	0.12			0.15
Body mass index (kg/m^2)	-0.10	-0.03	-0.10	-0.14	0.04	-0.03			0.03
Fat mass (kg)	-0.14	-0.18	-0.17	-0.13	-0.05	-0.16			0.05
FFST mass (kg)	-0.23	-0.13	-0.01	0.08	0.27*	0.32*			0.16
Body fat (%)	-0.07	-0.13	-0.18	-0.16	-0.14	-0.29*			-0.01
Salivary cortisol	1.00	0.23							
Growth hormone	0.23	1.00							
IGF-1	-0.01	0.04	1.00						
IGFBP-3	-0.04	0.02	0.46***	1.00					
Estradiol	0.11	-0.20	0.26*	-0.07	1.00				
Progesterone	0.19	-0.05	0.27*	0.21	0.44***	1.00			
Osteocalcin	0.09	-0.08	0.23	-0.08	0.30*	0.08	1.00		
NTx	0.15	-0.07	0.13	-0.01	0.23	0.26*	0.34**	1.00	
Urinary cortisol	0.29	-0.06	-0.12	0.12	0.29	0.28	0.01	0.01	1.00

[a]n = 64. [b]n = 63. [c]n = 44.

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; IGF-1 = insulin-like growth factor-1; IGFBP-3 = insulin-like growth factor binding protein-3; NTx = urinary N-telopeptide; BCE = bone collagen equivalents.

negatively associated with height ($p \leq 0.05$). Insulin-like growth factor-1 was positively associated with IGFBP-3 ($p \leq 0.001$), estradiol ($p \leq 0.05$), and progesterone ($p \leq 0.05$). Serum osteocalcin and urinary NTx were positively correlated ($p \leq 0.01$).

Results of stepwise linear regression analyses with individual BMD measures and bone biomarkers as dependent variables are displayed in **Table 11**. Independent variables included CER score, age, all anthropometric and soft tissue mass measures, selected nutrients from FFQ data, h/wk of hard and very hard physical activity, alcohol intake (drinks/wk) based on screening questionnaires, and hormone concentrations (except urinary cortisol due to the limited number of samples). Data from 63 participants were included in regression analyses due to the combination of missing hormonal and dietary data for two participants.

Discussion

Cognitive eating restraint

Adverse effects of CER on bone health have been suggested, but only a few studies have been conducted that directly examined BMC and BMD in women with and without eating restraint (10,11). Our study compared bone mineral measures between women with low and high CER as well as examined lifestyle and physiological variables that impact bone. In this group of young-adult women with healthy BMI and moderate physical activity, significant differences between low and high CER groups were not observed in any measure of BMC or BMD. Groups did differ, however, in FM and BF%. Although FFST mass appears more important for maintaining and achieving peak bone mass in early adulthood (22), FM is also positively associated with BMD in premenopausal women (23,24). Therefore, analysis of covariance was conducted using BMC and BMD as dependent variables with height, weight, BMI, FM and FFST mass as covariates and group as the fixed factor (data not shown), and again, differences in bone measures between CER groups were not detected.

We identified a trend for a lower mean serum osteocalcin concentration in women with high compared to low CER. Despite a documented association between serum osteocalcin and estradiol (14,25), mean estradiol concentrations did not differ between CER groups in the current study. While we did not examine gonadotropin concentrations or monitor body temperature throughout the menstrual cycle to evaluate subclinical menstrual cycle disturbances, other investigators have identified such disturbances in young-adult females with high CER (6,7).

Table 11. Stepwise linear regression models for bone mineral density (BMD[a]) and biomarkers of bone turnover

Dependent Variable	Predictor	R^2	Model Adjusted R^2	Unstandardized β (Standard Error)	Standardized β	P-value	Model P-value
Total body BMD			**0.03**				**0.078**
	Constant			1.088 (0.018)		<0.001	
	Vitamin D[b] (IU/d)	0.05		0.0001589 (0.000)	0.224	0.078	
Lumbar spine (L$_1$-L$_4$) BMD			**0.10**				**0.005**
	Constant			0.733 (0.095)		<0.001	
	Weight (kg)	0.12		0.004727 (0.002)	0.351	0.005	
Total proximal femur BMD			**0.15**				**0.007**
	Constant			0.807 (0.61)		<0.001	
	Calcium:Protein ratio[b]	0.09		0.007779 (0.003)	0.320	0.009	
	Progesterone (ng/mL)	0.05		0.133 (0.056)	0.294	0.020	
	IGF-1 (µg/L)	0.04		-0.000702 (0.000)	-0.218	0.080	
Femoral neck BMD			**0.23**				**<0.001**
	Constant			-0.359 (0.314)		0.257	
	Height (cm)	0.17		0.00726 (0.002)	0.438	<0.001	
	Alcohol[c] (drinks/wk)	0.06		-0.00898 (0.004)	-0.242	0.040	
	Estradiol (pg/mL)	0.04		0.0009756 (0.001)	-0.198	0.085	
Trochanter BMD			**0.19**				**0.003**
	Constant			0.138 (0.312)		0.442	
	Calcium:Protein ratio	0.10		0.005061 (0.003)	0.223	0.068	
	IGF-1 (µg/L)	0.05		-0.000741 (0.000)	-0.246	0.037	
	Alcohol (drinks/wk)	0.04		-0.00997 (0.004)	-0.276	0.028	

Variable		Model R²	B (SE)	β	p	Model p
Height (cm)	0.05		0.00360 (0.002)	0.224	0.068	
Ward's triangle BMD		**0.18**				**0.002**
Constant			-0.365 (0.431)		0.401	
Height (cm)	0.11		0.0071 (0.003)	0.314	0.009	
IGF-1 (μg/L)	0.05		-0.00106 (0.000)	-0.249	0.035	
NTx (BCE/mmol creatinine)	0.06		0.001207 (0.001)	0.249	0.037	
Total forearm BMD		**0.17**				**0.003**
Constant			0.438 (0.049)		<0.001	
Vitamin D (IU/d)	0.07		0.0001117 (0.000)	0.322	0.009	
BMI (kg/m²)	0.07		0.005722 (0.002)	0.298	0.014	
Exercise[d] (h/wk)	0.07		-0.00468 (0.002)	-0.267	0.030	
Ultradistal forearm BMD		**0.29**				**<0.001**
Constant			0.326 (0.060)		<0.001	
Vitamin D (IU/d)	0.11		0.0001594 (0.000)	0.372	0.001	
BMI (kg/m²)	0.09		0.005014 (0.003)	0.211	0.060	
Alcohol (drinks/wk)	0.06		-0.00522 (0.002)	-0.280	0.013	
IGF-1 (μg/L)	0.07		-0.000420 (0.000)	-0.270	0.017	
Mid forearm BMD		**0.19**				**0.002**
Constant			0.456 (0.050)		<0.001	
Vitamin D (IU/d)	0.10		0.000132 (0.000)	0.369	0.003	
BMI (kg/m²)	0.07		0.005760 (0.002)	0.289	0.015	
Exercise (h/wk)	0.06		-0.00475 (0.002)	-0.261	0.031	
Proximal forearm BMD		**0.15**				**0.003**
Constant			0.669 (0.022)		<0.001	
Iron[b] (mg/d)	0.06		-0.00523 (0.002)	-0.498	0.004	
Zinc[b] (mg/d)	0.08		0.008477 (0.002)	0.597	0.001	

Osteocalcin (ng/mL)		**0.39**				**<0.001**
	Constant			6.685 (2.538)		0.011	
	Fiber[b] (g/d)	0.19		-0.139 (0.30)	-0.464	<0.001	
	NTx (BCE/mmol creatinine)	0.12		0.02870 (0.008)	0.339	0.001	
	Fat mass (kg)	0.05		-0.163 (0.001)	-0.289	0.007	
	Fat-free soft tissue mass (kg)	0.07		0.1505 (0.001)	0.265	0.012	
NTx (BCE/mmol creatinine)		**0.44**				**<0.001**
	Constant			93.126 (31.421)		0.004	
	Age (y)	0.21		-2.968 (1.150)	-0.263	0.012	
	Osteocalcin (ng/mL)	0.07		4.276 (1.191)	0.362	0.001	
	Vitamin A[b] (IU/d)	0.04		-0.00131 (0.000)	-0.357	0.003	
	Vitamin C[b] (mg/d)	0.04		0.102 (0.033)	0.391	0.003	
	Iron (mg/d)	0.04		1.376 (0.598)	0.277	0.025	
	Calcium:Phosphorus ratio	0.03		-28.639 (16.753)	-0.193	0.093	

[a]BMD in g/cm^2.
[b]Estimated average daily dietary intake based on food frequency questionnaires.
[c]Estimated average weekly number of alcoholic drinks/wk based on initial screening questionnaires.
[d]Average h/wk of hard and very hard physical activity from 7-d physical activity recalls (Blair et al. 1985).
IU = International Units; IGF-1 = insulin-like growth factor-1; NTx = urinary N-telopeptide; BCE = bone collagen equivalents

100

Menstrual cycle disturbances with reduced estradiol concentrations may reduce markers of bone formation that, over time, may compromise BMD (26,27).

The trend for a lower mean serum osteocalcin concentration in women with high CER may be linked to the trend for a greater estimated average daily dietary fiber intake among the high versus low CER subjects (based on FFQ). Dietary fiber has been shown to increase urinary (28) and fecal estradiol excretion (29,30) and to reduce serum estradiol concentrations (31). Yet, a difference in the mean estradiol concentrations between groups was not found, and a correlation between estradiol and estimated average daily dietary fiber intake was not observed.

Dietary fiber may also affect bone formation through its ability to impair intestinal calcium absorption as well as absorption of other nutrients important for bone metabolism (32-34). The significant and negative correlation between estimated mean daily dietary fiber intake and serum osteocalcin (based on FFQ) indicates an inverse relationship between these two variables. Furthermore, stepwise regression analysis indicates that estimated average daily dietary fiber intake accounts for nearly 20% of the variation in serum osteocalcin. When comparing groups with high ($\geq$ 20 g/d) and low (< 20 g/d) dietary fiber intake, the mean serum osteocalcin concentration was significantly lower in the high fiber group compared to the low fiber group (8.5 $\pm$ 1.9 versus 10.5 $\pm$ 2.2 ng/ml, respectively, $p < 0.001$). Thus, if high CER is associated with high fiber intake, bone formation may be reduced, and over time, may compromise BMD.

Women in the low and high CER groups did not differ significantly in any other physiological variable measured. This is in contrast to previous findings that individuals with high CER have higher cortisol measures compared to individuals with low CER (8,9). In our study, salivary cortisol was measured in 64 subjects, and samples were collected prior to any other procedure, such as blood draw or body weight measurement, to avoid imposing a stress-response. Anderson and colleagues (8) reported a significantly higher salivary cortisol concentration in restrained eaters compared to non-restrained eaters. While the average concentration was indeed higher in the restrained versus non-restrained group, this concentration was not "elevated" as noted by the investigators (8). Moreover, body weights were measured prior to collection of saliva samples, perhaps imposing stress in individuals with eating restraint, especially in light of their higher BMI compared to non-restrained eaters (8). The impact of a cortisol concentration at the high end of the normal range on bone is unknown.

Pirke et al (12) found no difference in mean cortisol concentrations between high and low CER groups, but measurements were taken overnight, when food-related stress was low. McLean, Barr and Prior (9) collected 24-h urine samples to examine urinary cortisol excretion. While McLean, Barr and Prior (9) did find significantly higher cortisol excretion in high versus low CER subjects, the study protocol may have impacted results. Subjects were allowed to choose foods consumed during the urine collection period from a wide selection, but food intake was monitored by researchers to assess nutrient intake during this time (9). While monitoring of food intake would likely not impose stress for a non-eating restrained woman, such monitoring by an unfamiliar person in a controlled setting may likely impose stress for an eating restrained woman. We also examined 24-h urinary cortisol excretion in 44 subjects, and no differences were found in mean urinary cortisol concentrations between CER groups. Our results would likely not have differed with a larger number of urine samples considering that our mean salivary cortisol concentrations did not differ between CER groups. We question whether daily food-related situations pose greater stress versus other daily stress (e.g., academic and social stress) in high CER women compared to women with low CER with comparable ages and lifestyles. Free-living young women with high CER scores who do not restrict energy intake may not possess higher cortisol concentrations compared to similar women with low CER.

Implications of the impact of CER on bone health are cloudy considering results showing a positive correlation between CER score and IGFBP-3 and negative correlations between CER score and biomarkers of bone turnover. The role of IGFBP-3 is to protect IGF-1 in circulation, thereby extending the half-life of IGF-1 and assisting in the delivery of IGF-1 to target tissue, including bone where it exerts positive effects on bone formation (35,36). In osteoporotic patients receiving rhIGF-1 treatment, IGFBP-3 given in equimolar proportions helped to augment the physiological effects of IGF-1 without negative side effects (37). Therefore, the positive correlation between IGFBP-3 and CER score has positive implications for bone. Yet, considering the inverse relationships found between CER score and biochemical markers of bone turnover, negative implications for bone exist.

Correlation analyses showed an inverse relationship between CER score and mean urinary NTx concentration. While it may appear beneficial that bone resorption is inversely associated with CER score, the average serum osteocalcin concentration was also inversely related to CER score, indicating a reduced rate of bone turnover with a high CER score. Further

attempts to delineate differences between low and high CER groups by elimination of the middle 40% of scores was completed. Data between women scoring in the lower 30% (CER score = $\leq$ 6, n = 21, very low CER) and women scoring in the upper 70% (CER score = $\geq$ 12, n = 20, very high CER) on the CER scale were evaluated. Compared to the very low CER group, women in the very high CER group had a significantly higher mean IGFBP-3 concentration (5.20 $\pm$ 0.88 versus 5.81 $\pm$ 0.93 mg/L, respectively, $p = 0.038$) and a significantly lower mean urinary NTx concentration (65.9 $\pm$ 22.2 versus 81.1 $\pm$ 25.7 BCE/mmol creatinine, respectively, $p = 0.05$). Together, these results are favorable for bone health in very high CER subjects over time, supporting the need for a longitudinal investigation of CER and bone health. When comparing groups based on these separation criteria, the h per wk of physical activity (based on screening) was significantly higher in the very high versus very low CER group (3.7 $\pm$ 1.8 versus 2.5 $\pm$ 1.9 h/wk, respectively, $p = 0.048$). However, very high and very low CER groups did not differ significantly in any other variable, and even soft tissue mass differences that were previously detected based on a median score separation of CER were no longer present.

Low CER subjects were not found to have significantly different energy or nutrient intakes compared to high CER subjects. A trend for a difference ($p < 0.06$) in relative energy intake from carbohydrate (based on FFQ) was found in the high versus low CER group, despite a lack of difference in estimated average daily dietary total carbohydrate intake (g/d).   According to 4-d food records, the only trend ($p < 0.06$) in nutrient intake was for a higher average estimated daily dietary vitamin C intake among high versus low CER subjects. This was probably a result of a significantly higher ($p < 0.05$) combined average daily dietary intake of fruits and vegetables in the high versus low CER group. Our findings were consistent with results of Tepper et al (2) who reported a higher intake of fruits and vegetables in subjects with restrained eating versus without restrained eating. Significant differences in energy intake between these groups were also not observed (2), further corroborating our results.

Correlation analyses

Because significant differences in bone measures or hormone concentrations that impact bone were not found between CER groups, bivariate correlation analyses were conducted to examine relationships between select variables for the total sample of women.

Anthropometric and soft tissue mass measures

Our data support findings of positive relationships between body height (38,39) and weight (22,40,41) and BMD. It also appears that in weight-bearing regions, FFST mass is more important for BMD than FM, also supported by previous research (22,24,38).

Fat mass was positively associated with some forearm BMD measures, but BF% was not associated with any measure of BMD. Positive associations between body FM and BMD have been previously reported (23,24,42), but body fat may be detrimental to bone due to the significant inverse association found between BF% and serum osteocalcin. Hla et al (43) reported an inverse association between FM and biochemical markers of bone turnover (i.e., serum osteocalcin and urinary NTx). While leptin concentration was not measured in the current study or by Hla et al (43), it can be speculated that these results may be related to an increase in leptin circulation associated with a high proportion of FM, both of which have been inversely associated with BMD (44) and reported to inhibit bone formation (45,46).

Total FFST mass was positively associated with BMC at nearly all sites and positively associated with BMD of the LS, FN, WT, TF, and Mid forearm region. These positive associations between FFST and BMC and BMD are likely attributable to the mechanical stimuli associated with FFST mass on bone and support the findings of other investigators (22,24,38).

Dietary intake

Dietary variables from FFQ that had positive correlations with certain BMC measures included estimated average daily dietary protein, calcium, phosphorus, potassium, vitamin D, zinc, and magnesium intakes; yet, only estimated average daily dietary vitamin D and calcium intakes had positive associations with certain BMD variables and only at the forearm. Previous investigations of dietary intake and bone health in young adults have supported the importance of adequate dietary calcium (22), while other studies have not reported benefits of calcium in this stage of the life span (47,48). Maintaining a normal serum vitamin D concentration through endogenous or exogenous sources is important for maintaining calcium balance and BMD (49,50). In our study, both vitamin D and calcium were positively associated with BMD but only at the forearm. Because the forearm is not the recipient of frequent weight-bearing activity, effects of dietary factors, such as calcium and vitamin D, may be more apparent at this region in young women.

Significant and negative correlations were not found between dietary variables and bone mineral measures. Positive correlations between FFQ variables and serum osteocalcin were not

present, but negative associations were found between serum osteocalcin and estimated average daily dietary intakes of phosphorus, iron, magnesium, potassium and fiber. Phosphorus is important for bone as it is the second major mineral component of the skeleton. Yet, with inadequate calcium intake, a high phosphorus intake may reduce the serum calcium concentration, thereby limiting the availability of calcium for bone formation (51). Iron and magnesium are important for bone strength (52) and calcium regulating hormones (53,54), respectively, but iron (55) and magnesium (56) may compete with calcium for absorption and metabolism. Most evidence regarding potassium intake and bone health demonstrates a positive association between potassium intake and BMD (57-59). It has been suggested that potassium and food sources of potassium, such as fruits and vegetables, can help neutralize the serum pH and prevent acid-induced bone loss (60). Oral potassium supplementation has also been shown to reduce urinary calcium excretion associated with a high sodium diet (61). The relationship reported here may indicate an overall reduced rate of bone turnover with high potassium intake.

Dietary fiber has the ability to reduce intestinal calcium absorption (62) and has been associated with reduced BMD (34,63), explaining its inverse relationship with serum osteocalcin. Still, without an increase in urinary NTx excretion associated with these dietary variables, the true implications cannot be explained from this cross-sectional investigation. Conversely, negative associations were not present for dietary variables and urinary NTx, but urinary NTx was positively related to estimated average daily dietary energy, carbohydrate, and vitamin C intakes. The relationship between energy and carbohydrate intake and bone resorption is difficult to assess as energy and carbohydrate may represent a number of different dietary variables. Short-term fasting alters bone turnover (64) and reduced energy weight loss diets reduce BMD (13,65,66), yet the estimated average daily energy intake of our subjects was not indicative of fasting, nor was the method for collection of dietary intake data adequate for identification of dramatic energy intake extremes. In addition, subjects self-reported body weight stability for the two y prior to the study, indicating energy balance. The positive association between energy intake and urinary NTx seen in this study may support the contention that adequate energy intake is important for bone turnover and that with even moderate dietary restriction, the rate of bone turnover in young-adults may be reduced. Dietary carbohydrate is a vague dietary intake variable as it may represent a high consumption of simple sugars and low-nutrient foods, which have been inversely associated with BMD (67). Carbohyhdrate may also

represent a high consumption of fruits and vegetables which have been shown to positively correlate with BMD (58,59), or carbohydrate might negatively relate to bone health as it may contain a high amount of fiber and phytate from cereal grains, both of which are associated with reduced calcium absorption (62). As energy and carbohydrate intake were both positively associated with intakes of other nutrients, it is difficult to delineate these associations and further investigation is warranted.

Vitamin C is essential for bone health as it is required for hydroxylation of lysyl and prolysyl residues of collagen (68). The vitamin C deficiency disease, scurvy, is associated with weakening of bone structure (68). Vitamin C is also required for osteoblastogenesis (69) and osteoclastogenesis (70,71) and may be necessary for maintaining balance between bone formation and resorption (71).

Dietary variables from 4-d food records that were positively related to BMD were vitamin C, vitamin D, phosphorus and potassium. While a high phosphorus intake with low calcium intake can be detrimental, our result of a positive relationship between certain BMD measures and phosphorus intake is in agreement with previous findings (22). It is necessary to note, however, that the phosphorus intake according to 4-d food record analyses was relatively low compared to the typical American diet (72). Upon further investigation of food records it was found that the foods contributing most to the phosphorus content of these diets included milk and other dairy products. While meat is most frequently the highest contributor to phosphorus intake, estimated average daily dietary phosphorus intake did not correlate with daily servings of meat, yet did correlate significantly with daily servings of milk products (r = 0.63, p < 0.0001), followed by fruits and vegetables (combined) (r = 0.45; p < 0.001). This may be interpreted that adequate intakes of phosphorus, within a normal range, are beneficial for bone mass. Yet, considering the near-significant (p = 0.052) inverse relationship between Mid forearm BMD and meat consumption, it is recommended that phosphorus intake be derived from food sources other than meat. Support for this positive association between dairy foods and bone are confirmed by the positive association between certain BMD measures and vitamin D intake, which coincides with results related to FFQ data. Nonetheless, these positive associations with phosphorus are contradictory to our findings of a negative relationship between phosphorus (from FFQ) and serum osteocalcin. While phosphorus is the second most abundant mineral contained within bone and is, therefore, essential for bone, its ability to bind calcium and reduce

the serum calcium concentration (51) provides negative implication for high phosphorus consumption.

Finally, an interesting association with certain BMD measures was observed with estimated average number of servings of fruit per day that likely accounted for the positive association between certain BMD measures and vitamin C and potassium. These results are supported by previous research (58,59) in which high intakes of fruits and vegetables or of nutrients contained within these foods were associated with high BMD. Still these data contradict our aforementioned finding of an inverse relationship between magnesium, potassium and dietary fiber and serum osteocalcin as fruits and vegetables are good sources of these nutrients. Furthermore, there are implications for bone health in women with high CER in light of their significantly higher intake of fruits and vegetables (based on 4-d food records) and trends for a higher fiber intake according to FFQ and a lower serum osteocalcin concentration compared to women with low CER.

Hormones

Surprisingly, bivariate correlations showed an inverse relationship between GH and BMC of the FN, Troc and WT and FN BMD. These results are contradictory to expected, considering the documented positive influence of GH on bone formation (73-75). A positive relationship between urinary cortisol excretion and LS BMC and LS BMD and UD forearm BMD was also surprising. Elevated cortisol can have detrimental effects on bone, but previous research does not address any negative effects, if any, of a cortisol concentration at the high end of the normal range on bone. Considering the influence of cortisol on bone cells (76,77), when within a normal physiological range, high cortisol may be positive for bone health, but when elevated above the normal range, such as with glucocorticoid use or in diseased states, bone may be compromised.

Estradiol was positively associated with FN BMD. Estrogen is protective of bone mass throughout the gynecological life of a woman (26), and with reduced estrogen exposure, bone resorption is enhanced (78). Estrogen replacement in post-menopausal years is beneficial for preserving bone mass because of its anti-resorptive effects on bone (79). Estrogen has also been shown to enhance bone formation (80), and biomarkers of bone formation fluctuate in response to changes in the serum estradiol concentration throughout the menstrual cycle (14). We found a significant and positive correlation between serum estradiol and serum osteocalcin

107

concentrations that is supported by previous research (14). Some of the osteogenic properties of estradiol on bone are believed to be mediated, in part, by serum IGF-1 (81). A trend for a positive correlation between serum IGF-1 and osteocalcin concentrations ($p < 0.08$) which, in light of the positive association between serum estradiol and IGF-1 concentrations, leads to the conclusion that these endocrine factors are working together to promote bone formation. In osteoporotic patients, IGF-1 has been shown to significantly correlate with BMD (82,83), and treatment with IGF-1 and IGFBP-3 has been shown to increase bone mass in elderly patients following a hip fracture (37,84). While we found significant associations between estradiol and FN BMD and estradiol and IGF-1, we did not find a significant and positive association between IGF-1 and BMD, but rather, a negative association between IGF-1 and WT BMC. Few studies have investigated IGF-1 in relation to BMD in young-adult females. When comparing BMD and endocrine factors in young-adult females with anorexia nervosa to age-matched healthy females, a significantly lower BMD and lower IGF-1 concentration in young-adult female anorexic patients existed; however, the mean IGF-1 concentration was not associated with mean BMD (85). In our study, serum IGFBP-3 was positively associated with IGF-1 but negatively associated with WT BMC. This inverse relationship with BMC and lack of a significant bivariate association with BMD does not enable us to draw conclusions regarding the role of IGF-1 and IGFBP-3 in bone health of young women.

Growth hormone is required for achievement of maximal linear height (86). After cessation of linear growth, it would not be expected that GH would correlate with height except in individuals who possess high skeletal muscle mass as a result of height (87). In our study, we found an inverse relationship between GH and height but no significant relationship between GH and FFST mass. To our knowledge this GH-height relationship cannot be explained from previous research. Age was inversely associated with IGF-1, progesterone, NTx, and urinary cortisol concentrations. The serum IGF-1 concentration peaks around the age of puberty and slowly declines thereafter (35) which may explain the relationships seen here. Similarly, puberty is a time of high bone turnover, and biochemical markers of bone turnover peak during puberty and decline thereafter (88, 89).

Regression analyses

Stepwise linear regression analyses were conducted to identify significant predictors of BMD and biomarkers of bone turnover. Estimated average daily dietary vitamin D intake

emerged as the only predictor of TB BMD ($p < 0.08$) but explained only 5% of the variance in TB BMD. Estimated average daily dietary vitamin D intake ($p < 0.01$) was also a positive predictor of TF BMD as well as UD ($p = 0.001$) and Mid ($p < 0.01$) forearm BMD. Previously, we found that vitamin D was the only nutrient that was significantly and positively associated with BMD in a group of premenopausal women of the same age range as women in the current study (24). Estimated average daily dietary vitamin D and calcium intakes were the only two nutrients associated with BMD in our bivariate correlation analysis, indicating the importance of foods rich in calcium and vitamin D, such as fortified milk products, for bone. From this research, it cannot be conclusively stated if current intake of vitamin D impacts current BMD status of if the intake represented here reflects a lifetime consumption of vitamin D-rich foods such as dairy products. Other research examining the bone health of adults living in Northern latitudes such as in Finland (90) and Canada (91) have found associations between low blood vitamin D concentration and low BMD values. While we did not measure serum vitamin D concentrations, our findings further support the need for adequate vitamin D status and consumption of vitamin D-rich foods to augment BMD.

Body weight was the only significant predictor of LS BMD ($p < 0.01$), accounting for ~12% of the variation and demonstrating the importance of maintaining adequate body weight to support bone health (40,41). It was recently shown that among small body-framed women, individuals with a normal body weight had significantly higher FN and LS BMD versus lower weight women (92).

The mean daily dietary ratio of calcium:protein intake was a positive predictor of TPF ($p < 0.01$) and Troc BMD ($p < 0.07$). Bivariate correlations showed that the ratio of calcium:protein was positively related to Troc BMD (r = 0.25, $p < 0.05$) and Mid forearm BMD (r = 0.28, $p < 0.03$) and that the ratio of calcium:phosphorus was also positively associated with Mid forearm BMD (r = 0.25, $p < 0.05$). Our findings agree with those of Teegarden et al. (22) who also identified dietary ratios of calcium:protein and calcium:phosphorus as positive predictors of BMD. It has been suggested that a high calcium intake is needed with high protein consumption in order to offset urinary calcium losses and to maintain calcium homeostasis (22,93). Serum progesterone ($p < 0.05$) was also a positive predictor of TPF BMD, and while estrogen has received most of the attention for its positive influence on bone, receptors for

109

progesterone have been identified in the nucleus of bone cells, indicating a role for progesterone in regulating bone metabolism (94).

Although IGF-1 was not significantly related to any BMD site in bivariate correlation analyses, IGF-1 was negatively associated with TPF ($p = 0.08$), Troc ($p < 0.05$), WT ($p < 0.05$), and UD forearm ($p < 0.02$) BMD in regression analyses, where IGF-1 contributed $4 - 7\%$ of the variation in BMD measures. This finding was not expected, given the positive role of IGF-1 on bone, particularly as a mediator of the osteogenic properties of estrogen and GH (81,95). Moreover, GH had a significant inverse relationship with FN BMD. A possible explanation for these findings may relate to the inverse relationship between age and IGF-1 and age and urinary NTx. It is plausible that women included in our study were nearing or at the age of peak bone mass, and thus, bone metabolism approached or achieved an adult steady state.

Estimated average alcohol intake was a significant and negative predictor of FN ($p < 0.05$), Troch ($p < 0.05$), and UD forearm ($p < 0.05$) BMD. Previous research has suggested negative associations between alcohol intake and bone health (96), but other data suggest positive associations (97). Subjects in this study had low to moderate alcohol consumption by study design; therefore, negative implications for bone health from moderate alcohol consumption in young-adult women may exist.

Body height predicted 17%, 5%, and 11% of the variance in FN ($p < 0.001$), Troc ($p < 0.07$), and WT ($p < 0.01$) BMD, respectively. Body height has been shown to be positively associated with BMD at weight-bearing skeletal sites (38,39), which is in accordance with findings presented here.

Estrogen has both anti-resorptive (79) and osteogenic properties (80). Loss of estrogen is associated with increased bone resorption (78). Our results demonstrate that even among normally menstruating young-adult women, estrogen exposure is beneficial for BMD as serum estradiol emerged as a predictor, albeit a weak one, of FN BMD.

Puberty is a period of high bone turnover which slowly declines as peak bone mass is achieved (98). Bone resorption is necessary for adequate bone mineral deposition. Urinary NTx accounted for 6% of the variance in WT BMD ($p < 0.05$), which indicates that bone resorption during the young-adult years benefits bone mass as it is likely followed by a period of bone formation which restores and enhances BMD at the site of resorption (99).

Athletes possess higher BMD compared to non-athlete controls (100), and the training effects on BMD can be appreciated from lateral differences in BMD between dominant and non-dominant upper limbs of racquetball players (101). The benefits of moderate activity in non-athletic women have been shown in premenopausal (102,103) and postmenopausal (104) women. In contrast to most studies (102,103), hours of hard and very hard exercise per week was an inverse predictor of TF ($p < 0.05$) and Mid ($p < 0.05$) forearm BMD. The benefits of exercise are believed to be the result of an adaptive response from bone to the mechanical load placed upon it (105). These negative associations were found in a region of the body that was less likely to receive the mechanical load from most forms of exercise performed by women included in this study (e.g., jogging, aerobic dance). Research has shown that muscle strength at a particular bone site relates to the BMD of that region, but not of other regions (106). Previous research has also shown that exercise is associated with high bone mass in weight-bearing regions and lower bone mass in non-weight-bearing regions of the skeleton (107) and may explain results observed here.

Body mass index was a positive predictor of TF ($p < 0.02$), UD forearm ($p = 0.06$), and Mid ($p < 0.02$) forearm BMD. While the forearm is not typically considered a weight-bearing region of the body, body mass does appear beneficial for BMD at this region. Other investigators have shown positive relationships between BMI and BMD at the LS and TPF (92), and our data suggest that even within a healthy range, a higher BMI may be beneficial for forearm BMD.

Proximal forearm BMD was predicted by both estimated average daily dietary iron ($p < 0.01$) and zinc ($p = 0.001$) intakes. Zinc has been proven important to bone metabolism via stimulation of osteoblast differentiation and synthesis of bone matrix proteins (108,109). At the same time, zinc inhibits osteoclastic bone resorption (110), but these effects are only seen when zinc intake is adequate, but not high. At high intakes, bone microarchitecture is compromised (111). Iron and zinc may have contrasting effects on Prox forearm BMD, but may more likely reflect the competition between dietary iron and zinc for absorption. At high dietary iron intake, zinc absorption can be impaired and vice versa (112,113).

Surprisingly, no other dietary variables, including calcium intake, emerged as significant predictors in models of BMD. Fat mass and FFST mass were not found in any BMD model, despite documented positive associations between muscle mass and BMD (22). Overall,

independent variables account for no more than 30% of the variation in BMD. This is not surprising considering the strong genetic component of BMD (40,41) and the fact that all parameters examined were reflective of the previous 12 mo or less. While women in this study were at or near the age of peak BMD, the most rapid age for accumulation of BMD is in the post-pubertal years (114,115). Chronological age was included in regression models, and age was not a significant predictor of BMD at any site. This is supported by other research that shows that menarcheal, rather than chronological, age is strongly correlated to BMD (116). It appears that dietary factors positively related to bone include vitamin D intake and the dietary calcium:protein ratio, while alcohol intake is negatively related to bone. The complex relationship between iron and zinc warrants further investigation considering food sources for these nutrients are often the same. Despite the negative associations found between exercise and forearm BMD variables, it is not recommended that exercise, particularly at the level included in this study, be restricted considering the overall health benefits of moderate physical activity, and the well-documented positive effects of exercise on weight-bearing regions of the skeleton (41,117).

Insight into the cellular actions of bone can be examined from serum osteocalcin and urinary NTx. The model predicting serum osteocalcin concentration included dietary fiber ($p < 0.001$, negative) and FM ($p < 0.01$, negative) and urinary NTx ($p = 0.001$, positive) and FFST mass ($p < 0.02$, positive). The negative association with fiber can be explained by the ability of fiber to bind nutrients important for bone and to limit their absorption (33,34) as well as its ability to reduce circulating estrogen concentrations (28,31). The negative association with FM may be a result of less mechanical strain on bone from FM, whereas FFST mass, which was a positive predictor, may cause a mechanical stimuli that encourages bone formation. Still, the inverse association between FM and serum osteocalcin is contrary to research that suggests positive associations between serum leptin, a hormone produced from adipose tissue, and BMD (118-120). Our findings support other research, however, by showing that at a given body weight, possession of greater amounts of FM (and higher serum leptin) are inversely related to BMD among premenopausal women (121). Considering the higher FM and BF% possessed by the high CER subjects, implications for bone health are present for women with high CER.

It is no surprise that urinary NTx predicted serum osteocalcin and vice versa considering that bone turnover is a coupled process (98). Higher rates of bone turnover favor overall bone

formation during adolescence and early-adulthood (98) and result in bone loss at menopause (122); thus, the coupled process is essential to maintain high BMD and sound bone microarchitecture. Despite the narrow age range of subjects, the NTx was negatively associated with age, perhaps indicating a slowing down of bone turnover as subjects reach the age of peak bone mass. In addition, vitamin A ($p < 0.01$) and the calcium:phosphorus ratio ($p < 0.10$) were negative predictors of NTx. Interpreting relationships with NTx can be complex due to the positive associations between NTx and osteocalcin, yet high rates of bone resorption may impair bone status. The inverse association between vitamin A intake ($p < 0.01$) and NTx was not expected considering that vitamin A has been shown to increase bone resorption and suppress bone formation (123), although other researchers have shown no association between vitamin A and BMD (124). Iron ($p < 0.05$) was positively associated with urinary NTx indicating an overall negative effect of a high iron intake on bone. Little research is available to suggest positive or negative associations of iron with bone health, but in light of the current research as well as previous research citing an inverse relationship between dietary iron and LS BMD in young women (24), it appears that more research into the associations between iron and bone are needed, particularly when evaluating menstruating women.

In conclusion, in this group of young women, it does not appear that CER directly compromises bone health. Data collected adds confusion to definitive statements regarding CER and bone health. High CER subjects did not possess lower BMC or BMD at any body site including the TB, and salivary cortisol was not elevated in women in this group compared to women in the low CER group. Moreover, we found positive correlations between urinary cortisol excretion and BMD in a subset of subjects. Indirect relationships between CER score and urinary NTx may benefit bone over time, but if serum osteocalcin is reduced as well, BMD may be compromised. In addition, the high CER group had significantly higher FM and BF%, both of which had negative implications for bone from their observed inverse relationships with serum osteocalcin. Still, we observed a positive association between FM and BMD that confounds these results. A higher average intake of fruits and vegetables found in the high CER group may benefit bone health as these foods are good sources of nutrients associated with BMD; yet, with higher fiber intake, these positive effects on bone may be lost. Further studies, including longitudinal investigations are clearly warranted considering the divergence of these findings.

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A PROSPECTIVE EVALUATION OF DIETARY INTAKE, BONE MINERAL, AND
MARKERS OF BONE METABOLISM IN WOMEN WITH HIGH AND LOW COGNITIVE
EATING RESTRAINT[1]

MicroAbstract

Cognitive eating restraint (CER) has been linked to low bone mineral content, but not low density. Bone mineral measures were examined at baseline and after 6 months in women (18–25 y) with high (n=27) and low (n=26) CER. Group x Time interactions were not found. A high CER score did not impact bone.

Abstract

Introduction: Cross-sectional studies suggest negative effects of cognitive eating restraint (CER) on bone mineral content (BMC) in women; however, longitudinal investigations have not been conducted to support sustained negative relationships between CER and bone. The purpose of this study was to prospectively examine bone mineral measures and factors related to bone in women with high and low CER scores.

Materials and Methods: Young women (20.7 ± 2.4 years) of healthy body mass index and with limited physical activity participated in this 6-month investigation. Subjects were placed into high (n = 27) and low (n = 26) CER groups based on responses to the CER subscale of the Eating Inventory. Body height and weight were measured, and dietary intakes of select nutrients were estimated from food frequency questionnaires. Total body and site-specific BMC (g) and bone mineral density (BMD) (g/cm^2) were measured by absorptiometry as were soft tissue mass measures. Salivary and urinary cortisol and serum estradiol, progesterone, growth hormone, insulin-like growth factor (IGF)-1, and IGF binding protein-3 concentrations were measured by bioassays. Data were collected at baseline and after six months. Unpaired *t*-tests and repeated measures analysis of variance were used to evaluate differences between groups and over time.

Results: Of all variables measured, a significant Group x Time interaction was observed only in salivary cortisol concentration ($p < 0.05$). Women with high CER had a significantly greater

125

decrease in salivary cortisol from baseline to follow-up (0.61 ± 0.31 to 0.46 ± 0.24 µg/dL, $p < 0.05$) compared to women with low CER (0.58 ± 0.28 to 0.60 ± 0.29 µg/dL, $p > 0.05$).

Conclusion: Dietary intake, BMC and BMD, biomarkers of bone turnover, and hormonal mediators of bone metabolism, except salivary cortisol, do not differ in young women with high versus low CER over a short period of time.

Key Words: Bone mineral, Cognitive eating restraint, Cortisol, Dietary intake, Premenopausal women

Introduction

Maximizing bone mineral density (BMD) early in life may protect against osteoporotic fractures later in life. While genetics play a large role in determining peak bone mass potential, achievement of peak bone mass occurs through a combination of modifiable and non-modifiable factors.

The age at which peak bone mass is achieved is understood to vary according to bone site, but it is generally agreed that the majority of bone is attained by the third decade of life (1,2). Many women, in the young-adult years, are concerned with body image and composition and often make conscious efforts to modify dietary intake to reduce or maintain body weight. Recent evidence suggests that such cognitive eating restraint (CER) may have direct and indirect implications as a modifiable risk factor for bone health. For example, in eumenorrheic women with high CER scores, subclinical menstrual cycle disturbances were identified (3,4). Higher salivary and urinary cortisol concentrations have been reported in women with high CER scores compared to women with low CER scores (5,6). Furthermore, two studies have reported lower total body (TB) bone mineral content (BMC) in women with high CER compared to their low CER counterparts (7,8). Negative implications for skeletal health, specifically BMC, in women with high CER exist based on findings from previous studies. Yet, BMD has not been shown to be compromised in women with high CER. Thus, it is necessary to further examine the role of CER in bone health of women. To date, no studies have been published that have comprehensively examined differences in anthropometric measures, dietary intake, bone mass and density, indicators of bone turnover, and selected hormones in women with high and low CER. Moreover, previous studies of CER and bone have been limited to cross-sectional evaluations. The purpose of this study was, therefore, to prospectively examine anthropometrics, dietary intake, TB and site-specific BMC and BMD measures, biomarkers of bone turnover, and hormonal mediators of bone metabolism in young women with high and low CER.

Materials and Methods

Subjects

Young women, aged 18 to 25 years, were recruited to participate in this 6-month investigation of CER and bone health. This study was approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute & State

University (VPI&SU). Subjects were recruited from the campus and surrounding locations by use of personal contacts, paper bulletins, and electronic notices. Prior to participation in any procedure, each subject read and signed an informed consent form.

Potential subjects were screened for inclusion by use of an investigator-designed general health screening questionnaire. Women were excluded from this study based on self-reported: age < 18 and > 25 years; participation in > 7 hours of hard and very hard physical activity per week; amenorrhea, oligomenorrhea, or any disruptions in menstrual cycles during the previous 12 months; a history of metabolic disorders, eating disorders, or chronic diseases known to affect bone; use of medications known to affect bone metabolism; cigarette smoking; or parity ≥ 1. Subjects using oral contraceptives were included if consecutive use of these medications was of ≥ 18 months duration prior to the start of the study.

Each subject reported current height and weight, from which body mass index (BMI) was calculated (BMI = kg/m^2). Women with a BMI < 18 or > 25 were excluded because of the positive association between body mass and BMD (9,10). Weight changes affect BMD (11); thus, subjects were excluded if they self-reported weight changes of > 2.3 kg, ≥ 3 times during the two years prior to the study.

Procedures

Fifty-three women met inclusion criteria and participated in this 6-month investigation. Baseline data were collected between February 2, 2002 and October 30, 2002, and follow-up data were collected between August 1, 2002 and April 30, 2003. At baseline and follow-up, each subject was scheduled for an individual, two-hour testing session between the hours of 0700 – 1100 and during the first 4 – 10 days since the onset of menses (follicular phase) to limit diurnal variations and menstrual cycle effects (12) on biochemical measurements. Each testing session took place following an overnight fast (≥ 10 hours) and within 1.5 hours of the subject's awaking. During each testing session (at baseline and follow-up), data regarding CER, anthropometrics, dietary intake, physical activity, and bone mineral were collected. Saliva, blood, and urine samples were also collected for biochemical assessments. All procedures were carried out in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation Laboratory (BONE Lab) at VPI&SU.

Cognitive eating restraint

Cognitive eating restraint was assessed from responses to the CER subscale of the Eating Inventory (13). Established scoring guidelines were followed, and subjects were categorized into high or low CER groups, based on the median score of subjects and consistent with methods of other investigators (8,14). The high CER group (n = 27) consisted of women who scored > 9 on the CER scale, while the low CER group (n = 26) was comprised of women who scored ≤ 9 on the CER scale.

Anthropometric measures

Body height (at baseline testing session only) and weight of each subject were measured with the subject wearing lightweight clothing and without shoes. Height was measured to the nearest 0.1 cm using a stadiometer (Detecto, Webb City, MO, USA), and weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Scaletronix, Wheaton, IL, USA).

Dietary intake

Using the food frequency questionnaire (FFQ) (15), average daily dietary intake at baseline was estimated from responses to the FFQ, representing dietary intake during the previous 12 months. In an interview format, the frequency and quantity of consumption of numerous food and beverage items was ascertained. Food models (2- and 3-dimensional) were used to facilitate the accurate and consistent estimation of portion sizes. At the 6-month follow-up session, FFQ data were collected using the same procedure as baseline; however, food and beverage consumption represented the previous six months. Average daily dietary intakes of energy (kJ/d), macronutrients (g/d and % of total kJ/d), and select micronutrients were estimated using The DIETSYS+Plus software (version 5.9, 1999, Block Dietary Data Systems, Berkeley, CA, USA).

Physical activity

Initial screening questionnaires required each participant to self-report the type, frequency, and duration of physical activity per week. Women participating in > 7 hours of physical activity per week were excluded, and the 7-day physical activity recall (16) was used to confirm limited physical activity of subjects at baseline and follow-up. Hours engaged in hard and very hard physical activities per week were calculated from 7-d physical activity recalls.

Bone mineral and soft tissue mass measurements

Dual-energy X-ray absorptiometry (DXA) (QDR-4500A, Hologic, Inc., Bedford, MA, USA) scans were performed to measure BMC (g) and BMD (g/cm^2) of the TB, lumbar spine

(LS) ($L_1 - L_4$), total proximal femur [(TPF), including the femoral neck (FN), Trochanter (Troc), and Ward's triangle (WT)], and total forearm [(TF) = radius + ulna, including the ultradistal (UD), Mid, and proximal one-third (Prox) forearm]. Version 8.25a of the TB software and standard protocols for LS, TPF and TF measurements were used to conduct and analyze respective DXA scans. Fat-free soft tissue (FFST) mass (kg), fat mass (FM) (kg) and body fat percent (BF%) were calculated from TB DXA scans. One investigator conducted and analyzed all DXA scans to eliminate inter-tester variation. On the morning of each testing date and prior to any subject's scans, quality control procedures were performed using a LS phantom and producing a coefficient of variation (CV) of 0.48%. Test-retest reliability for TB, LS, TPF, and TF BMD and FFST mass, FM, and BF% produced CVs of 0.73%, 0.92%, 0.69%, 1.09%, 1.07%, 1.75%, and 1.79%, respectively, in 24 men and women (17).

Biochemical assessments

Biomarkers of bone turnover

Fasting venous blood samples (~34 mL) were drawn between 0800 – 1100 hours by a medical technologist. At the follow-up testing session, blood samples were collected no > 60 minutes of the baseline sample collection time to limit variability in measurements due to diurinal variations. Samples were centrifuged at 1070 x g for 12 minutes. Serum samples were aliquotted into 1 mL cryovials and frozen at -80° C until later analysis. Serum osteocalcin (ng/mL), a biochemical marker of bone formation, was measured by radioimmunoassay (RIA) (Human Osteocalcin RIA I^{125} Kit, Biomedical Technologies, Staughton, MA, USA). All samples were analyzed in duplicate. Intra- and inter-assay CVs for osteocalcin in our laboratory are 6.0% and 2.7%, respectively.

Subjects also provided second-void urine samples between the hours of 0800 – 1100. Samples were refrigerated until separated into cryovials and frozen at -80° C until later analysis. Urinary cross-linked N-telopeptides of type I collagen (NTx), a marker of bone resorption, were quantified by enzyme-linked immunosorbent assay (ELISA) (Osteomark, Seattle, WA, USA). Urinary NTx were normalized to urinary creatinine excretion, which was measured by quantitative spectrophotometry (#555A, Sigma Diagnostics, St. Louis, MO, USA) and reported as bone collagen equivalents (BCE) per mM creatinine. All samples were assayed in duplicate. Intra- and inter-assay CVs in are laboratory are 6.5% and 7.9%, respectively for urinary NTx and 4.4% and 1.9%, respectively, for creatinine.

Salivary and urinary cortisol

Prior to any other procedure, each subject gently chewed a 3 cm^2 piece of cotton dental gauze for ~30 seconds. Saturated gauze was then placed into individual sterile centrifuge tubes, which were centrifuged at 1,540 *x g* for 5 minutes to extract saliva from gauze and from extra-salivary particles. Saliva samples were stored in 1 mL cryovials at -80° C until analyzed for salivary cortisol (μg/dL) by ELISA (Salimetrics LLC, State College, PA, USA). Intra- and inter-assay CVs for salivary cortisol in our laboratory are 8.5% and 8.2%, respectively.

Urine collection containers were distributed to subjects at the end of the baseline testing session. Written and verbal instructions for a 24-hour urine collection were provided and were based on standard methods (6). Studies have shown that cortisol production is not influenced by the menstrual cycle (18,19); therefore, each subject was allowed to select the 24-hour period most convenient for her collection. Subjects returned 24-hour urine containers to the BONE Lab after completion. Total volume (mL) was measured, and 1 mL samples were aliquotted and frozen at −80°C until analyzed for urinary cortisol by RIA (Diasorin, Stillwater, MN, USA). Intra-assay CV for urinary cortisol in our laboratory is 6.1%. Creatinine was also measured from 24-hour urine samples as previously described, and urinary cortisol concentrations were recorded as a ratio of cortisol (nM) to creatinine (mM).

Serum hormone concentrations

Serum estradiol (pg/mL), progesterone (ng/mL), and growth hormone (GH) (ng/mL) were measured by RIA (Coat-A-Count® Estradiol, Coat-A-Count® Progesterone, and Double Antibody hGH respectively, Diagnostic Products Corporation, Los Angeles, CA, USA). Samples containing undetectable concentrations of estradiol (n = 19 at baseline and n=14 at 6 months) were assigned the minimum value detectable by this assay (20 pg/ml). Samples containing undetectable concentrations of GH (n = 14 at baseline and n = 5 at 6 months) were assigned minimum values (1 ng/mL). Serum insulin-like growth factor (IGF)-1 concentrations (μg/L) were measured by RIA using methods described by Weber et al (20). Serum IGF binding protein-3 (IGFBP-3) concentrations (mg/L) were measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). All samples were analyzed in duplicate. Intra- and inter-assay CVs in our laboratory are: 7.2% and 5.0%, respectively, for serum estradiol; 6.5% and 7.3%, respectively, for serum progesterone; 6.3% and 2.1%, respectively, for serum GH;

11.0% and 10.8%, respectively, for serum IGF-1, and 4.4% and 9.0 %, respectively, for serum IGFBP-3.

<u>Statistical analyses</u>

Statistical analyses were performed using The Statistical Package for Social Sciences (SPSS) (version 10.0, 1999, SPSS Inc., Chicago, IL, USA). Baseline comparisons of CER groups were made using unpaired student t-tests. A 2 x 2 analysis of variance (Group x Time) with repeated measures on the Time factor was used to examine between group differences from baseline to follow-up. Significant interactions were further examined using paired student t-tests for comparisons within groups over time. Paired student t-tests were also used for comparison of all subjects at the baseline and 6-month time points. Bivariate Pearson correlation coefficients were calculated to examine simple relationships between baseline bone mineral measures as well as changes in bone measurements with variables of interest. Statistical significance was set at the $p \leq 0.05$ level, using two-tailed comparisons.

Relationships between baseline bone mineral measures and anthropometrics, dietary intake, biochemical, and lifestyle variables were further examined using stepwise linear regression. Stepwise linear regression analyses were also conducted using change in BMC and BMD over time as dependent variables with anthropometric, dietary intake and lifestyle variables from the 6-month time point as well as baseline hormone measurements (due to 4 subjects foregoing blood draws at the 6 month time point) as independent variables. Variables were entered and removed from models at $\alpha = 0.10$ and 0.15, respectively.

Results

Scores on the CER subscale in subjects averaged 9.0 ± 5.1 with a median score of 9 (range of possible scores $= 0 - 21$). Baseline and 6-month anthropometric and soft tissue mass data for all subjects and by CER group are presented in Table 1. The mean age of subjects in this study at baseline was 20.7 ± 2.4 years, and CER groups did not significantly differ in age. At baseline, the high CER group possessed significantly greater FM ($p < 0.05$) and BF% ($p = 0.01$), with a trend for significantly higher BMI (p = 0.051). Significant Group x Time interactions were not identified for these variables, and significant changes in anthropometric and soft tissue mass measures did not occur over time for all 53 subjects.

Table 1. Anthropometric and soft tissue mass measures of all subjects and by cognitive eating restraint (CER) group

Variable	All subjects (n = 53)			High CER (n = 27)		Low CER (n = 26)		P-value (Group x Time)[2]
	Baseline	6-month	P-value[1]	Baseline	6-month	Baseline	6-month	
Height (cm)	165.4 ± 5.6[3]			165.4 ± 4.6		165.3 ± 6.5		
Weight (kg)	59.0 ± 6.3	59.0 ± 5.8	0.959	60.5 ± 6.1	60.2 ± 5.9	57.4 ± 6.3	57.7 ± 5.6	0.442
BMI (kg/m^2)	21.6 ± 2.0	21.6 ± 2.0	0.878	22.1 ± 1.9	22.0 ± 1.9	21.0 ± 2.0	21.1 ± 2.0	0.336
Fat mass (kg)	15.8 ± 4.1	15.7 ± 4.4	0.731	17.2 ± 4.3	17.1 ± 4.1	14.3 ± 3.4[4]	14.2 ± 4.3	0.811
FFST mass (kg)	41.6 ± 3.9	41.5 ± 3.6	0.870	41.6 ± 4.0	41.7 ± 3.8	41.5 ± 3.9	41.4 ± 3.5	0.732
Body fat (%)	26.2 ± 4.9	26.4 ± 4.8	0.370	27.9 ± 5.1	27.9 ± 5.0	24.5 ± 4.1[5]	24.9 ± 4.1	0.204

BMI, Body mass index; FFST, fat-free soft tissue.
[1]Baseline vs. 6-month using paired t-tests;
[2]Group x Time interaction using repeated measures analysis of variance;
[3]Mean ± standard deviation (SD);
[4]$p < 0.05$, high vs. low CER at baseline using unpaired t-test;
[5]$p \leq 0.01$, high vs. low CER at baseline using unpaired t-test.

Table 2 displays estimated average daily dietary intake data for select nutrients and physical activity data. Significant differences in nutrient intakes between CER groups at baseline were not found. Significant Group x Time interactions were not observed in estimated average daily dietary intake of any nutrients. In all 53 subjects, significantly lower estimated average daily dietary energy ($p < 0.01$), total fat ($p < 0.01$), and sodium ($p < 0.05$) intakes and significantly higher estimated average number of alcoholic drinks per week ($p < 0.05$) were found at 6-months compared with baseline.

Mean BMC and BMD measures for all subjects are provided in Table 3. In all 53 subjects, significant increases in WT ($p < 0.05$), TF ($p < 0.01$), Mid forearm ($p < 0.05$), and Prox forearm ($p < 0.02$) BMC and a significant decrease in TPF BMD ($p < 0.05$) over time were found. Comparisons of mean BMC and BMD measures by CER group are presented in Table 4. Baseline differences were not found between CER groups. Significant Group x Time interactions for bone mineral measures were not observed, and when using analysis of covariance with anthropometric and soft tissue mass measures as covariates, significant Group x Time interactions were still not detected.

Although all subjects indicated that they were between days 4 and 10 since the onset of menses, the serum progesterone concentration for one subject at baseline suggested that she was in the luteal phase of her menstrual cycle; thus, with the exception of salivary and urinary cortisol concentrations, her biochemical data were excluded. Therefore, biochemical data were available for 52 subjects at baseline. At their 6-month testing sessions, four subjects did not provide blood samples; therefore, for serum analyses, data were available for 48 subjects for both baseline and follow-up, except where noted in the indicated tables. Table 5 provides baseline and 6-month mean concentrations for biochemical measures in all 53 subjects. Mean serum osteocalcin ($p < 0.01$), estradiol ($p < 0.05$), progesterone ($p = 0.05$), and IGF-1 ($p = 0.001$) concentrations were significantly lower at baseline compared to follow-up.

Group comparisons of biochemical measures are presented in Table 6. At baseline, the high CER group had a significantly lower ($p < 0.05$) mean serum osteocalcin concentration compared to the low CER group. Significant Group x Time interactions were not detected in these biochemical measures, except for a significant Group x Time interaction for salivary cortisol. The mean salivary cortisol concentration in women with high CER significantly decreased over time ($p = 0.011$) but did not change over time in women with low CER.

Table 2. Estimated average daily dietary intake and hours of exercise of all subjects and by cognitive eating restraint (CER) group

Variable	All subjects (n = 53)		P-value[1]	High CER (n = 27)		Low CER (n = 26)		P-value (Group x Time)[2]
	Baseline	6-month		Baseline	6-month	Baseline	6-month	
Kilojoules	8,487 ± 2,457[3]	7,662 ± 2,433	0.008	8,308 ± 2,673	7,212 ± 1,947	8,672 ± 2,560	8,130 ± 2,815	0.355
Carbohydrate (g)	281 ± 81	261 ± 87	0.062	281 ± 90	249 ± 68	282 ± 72	273 ± 104	0.289
Carbohydrate (%)	55.9 ± 6.0	57.1 ± 6.9	0.166	57.0 ± 6.0	58.1 ± 7.1	54.7 ± 6.0	55.6 ± 6.7	0.909
Fat (g)	70 ± 25	60 ± 21	0.002	68 ± 27	57 ± 20	73 ± 24	63 ± 22	0.770
Fat (%)	30.9 ± 4.9	29.6 ± 5.8	0.108	30.4 ± 5.1	29.5 ± 6.5	31.5 ± 4.7	30.0 ± 5.0	0.606
Protein (g)	70 ± 22	65 ± 24	0.077	70 ± 24	60 ± 19	71 ± 19	70 ± 28	0.128
Protein (%)	14.0 ± 2.3	14.1 ± 2.3	0.778	14.2 ± 2.7	13.9 ± 2.2	13.9 ± 2.0	14.3 ± 2.4	0.223
Calcium (mg)	1,095 ± 414	1,035 ± 486	0.352	1,116 ± 489	1,013 ± 377	1,073 ± 329	1,096 ± 553	0.293
Iron (mg)	16.2 ± 4.7	16.3 ± 8.0	0.941	16.1 ± 5.6	15.5 ± 7.5	16.4 ± 4.8	17.1 ± 8.5	0.474
Magnesium (mg)	323 ± 97	301 ± 99	0.061	326 ± 99	296 ± 80	320 ± 95	306 ± 118	0.517
Phosphorus (mg)	1,334 ± 394	1,238 ± 425	0.077	1,321 ± 426	1,163 ± 305	1,347 ± 366	1,316 ± 516	0.235
Potassium (mg)	3,352 ± 964	3,173 ± 1,070	0.141	3,419 ± 970	3,092 ± 792	3,283 ± 971	3,256 ± 1,311	0.215
Sodium (mg)	2,944 ± 1,015	2,693 ± 1,076	0.021	2,840 ± 1,004	2,478 ± 978	3,051 ± 1,034	2,916 ± 1,146	0.285
Zinc (mg)	10.3 ± 3.2	10.7 ± 7.2	0.611	9.8 ± 3.3	9.0 ± 2.9	10.8 ± 3.2	12.5 ± 9.6	0.102
Vitamin A (IU)	12,126 ± 7,598	10,840 ± 7,273	0.238	11,358 ± 6,015	10,889 ± 8,903	12,865 ± 8,918	10,806 ± 5,438	0.463
Vitamin C (mg)	171 ± 93	167 ± 90	0.743	181 ± 96	165 ± 77	161 ± 90	169 ± 103	0.280
Vitamin D (IU)	171 ± 113	165 ± 123	0.666	145 ± 98	153 ± 95	198 ± 123	177 ± 148	0.346
Alcohol (drinks/wk)[4]	2.1 ± 2.4	2.8 ± 3.0	0.030	1.5 ± 1.9	2.1 ± 2.5	2.8 ± 2.7	3.6 ± 3.3	0.656
Exercise (h/week)[5]	3.4 ± 2.1	3.4 ± 2.1	0.956	3.7 ± 2.3	3.7 ± 2.3	3.0 ± 1.9	3.0 ± 1.9	0.895

IU, international units.

[1]Baseline vs. 6-month using paired *t*-tests;

[2]Group x Time interaction using repeated measures analysis of variance;

[3]Mean ± SD;

[4]Estimated average number of alcoholic drinks/wk based on initial screening questionnair

[5]Average h/wk of hard and very hard physical activity from 7-d physical activity recalls (16)

Table 3. Bone mineral measures of all subjects at baseline and 6-month time points[1]

	Baseline	6-month	P-value[2]
Total body			
BMC	2,169.9 ± 235.1[3]	2,173.5 ± 32.6	0.451
BMD	1.12 ± 0.08	1.13 ± 0.08	0.462
Lumbar spine (L$_1$-L$_4$)			
BMC	56.8 ± 8.7	57.0 ± 8.8	0.271
BMD	1.00 ± 0.09	1.01 ± 0.08	0.753
Total proximal femur			
BMC	32.9 ± 5.4	32.9 ± 5.3	0.909
BMD	0.99 ± 0.10	0.98 ± 0.10	0.028
Femoral neck			
BMC	4.37 ± 0.58	4.34 ± 0.54	0.245
BMD	0.86 ± 0.10	0.86 ± 0.10	0.638
Trochanter			
BMC	7.30 ± 1.4	7.40 ± 1.4	0.331
BMD	0.75 ± 0.09	0.75 ± 0.09	0.119
Ward's triangle			
BMC	0.99 ± 0.15	1.01 ± 0.17	0.030
BMD	0.84 ± 0.12	0.84 ± 0.12	0.755
Total forearm			
BMC	12.0 ± 1.3	12.1 ± 1.3	0.008
BMD	0.57 ± 0.04	0.57 ± 0.04	0.191
Ultradistal forearm			
BMC	2.19 ± 0.24	2.19 ± 0.25	0.709
BMD	0.43 ± 0.05	0.42 ± 0.04	0.450
Mid forearm			
BMC	6.56 ± 0.90	6.61 ± 0.89	0.012
BMD	0.59 ± 0.04	0.59 ± 0.04	0.209
Proximal forearm			
BMC	3.25 ± 0.29	3.28 ± 0.28	0.015
BMD	0.68 ± 0.04	0.68 ± 0.05	0.838

BMC, bone mineral content (g); BMD, bone mineral density (g/cm^2).
[1]n = 53;
[2]Baseline vs. 6-month using paired *t*-tests;
[3]Mean ± SD.

Table 4. Bone mineral measures of high and low cognitive eating restraint (CER) groups at baseline and 6-month time points[1]

Variable	Baseline	P-value[2] (baseline)	6-month	P-value[3] (Group x Time)
Total body BMC (g)				
High CER	$2,174.1 \pm 222.6$[4]		$2,171.6 \pm 229.4$	
Low CER	$2,165.5 \pm 251.8$	0.895	$2,175.5 \pm 249.9$	0.194
Total body BMD (g/cm^2)				
High CER	1.11 ± 0.07		1.12 ± 0.07	
Low CER	1.13 ± 0.09	0.614	1.13 ± 0.08	0.099
Lumbar spine (L$_1$-L$_4$) BMC (g)				
High CER	57.8 ± 8.3		58.3 ± 8.5	
Low CER	55.7 ± 9.2	0.407	55.7 ± 9.0	0.194
Lumbar spine (L$_1$-L$_4$) BMD (g/cm^2)				
High CER	1.03 ± 0.08		1.02 ± 0.08	
Low CER	1.00 ± 0.09	0.259	1.00 ± 0.09	0.526
Total proximal femur BMC (g)				
High CER	32.5 ± 4.6		32.7 ± 5.0	
Low CER	33.3 ± 6.2	0.261	32.1 ± 8.3	0.318
Total proximal femur BMD (g/cm^2)				
High CER	0.98 ± 0.10		0.98 ± 0.09	
Low CER	0.99 ± 0.11	0.546	0.98 ± 0.10	0.769
Femoral neck BMC (g)				
High CER	4.38 ± 0.54		4.38 ± 0.48	
Low CER	4.36 ± 0.64	0.888	4.31 ± 0.61	0.388
Femoral neck BMD (g/cm^2)				
High CER	0.86 ± 0.10		0.86 ± 0.09	
Low CER	0.86 ± 0.10	0.948	0.86 ± 0.09	0.421
Trochanter BMC (g)				
High CER	7.40 ± 1.20		7.41 ± 1.40	
Low CER	7.22 ± 1.65	0.687	7.39 ± 1.41	0.485
Trochanter BMD (g/cm^2)				
High CER	0.75 ± 0.09		0.75 ± 0.09	
Low CER	0.75 ± 0.09	0.921	0.75 ± 0.09	0.587
Ward's triangle BMC (g)				

High CER	0.99 ± 0.10		1.02 ± 0.14	
Low CER	0.98 ± 0.16	0.848	1.02 ± 0.20	0.875

Ward's triangle BMD (g/cm^2)

High CER	0.83 ± 0.11		0.84 ± 0.10	
Low CER	0.84 ± 0.13	0.871	0.84 ± 0.14	0.160

Total forearm BMC (g)

High CER	11.9 ± 1.0		12.0 ± 1.0	
Low CER	12.1 ± 1.6	0.727	12.2 ± 1.6	0.438

Total forearm BMD (g/cm^2)

High CER	0.57 ± 0.03		0.57 ± 0.04	
Low CER	0.57 ± 0.04	0.918	0.57 ± 0.04	0.785

Ultradistal forearm BMC (g)

High CER	2.19 ± 0.19		2.18 ± 0.17	
Low CER	2.18 ± 0.29	0.873	2.20 ± 0.32	0.090

Ultradistal forearm BMD (g/cm^2)

High CER	0.42 ± 0.03		0.43 ± 0.04	
Low CER	0.42 ± 0.05	0.740	0.42 ± 0.04	0.834

Mid forearm BMC (g)

High CER	6.51 ± 0.70		6.55 ± 0.67	
Low CER	6.60 ± 1.09	0.711	6.67 ± 1.06	0.538

Mid forearm BMD (g/cm^2)

High CER	0.59 ± 0.03		0.59 ± 0.04	
Low CER	0.59 ± 0.05	0.733	0.59 ± 0.04	0.749

Proximal forearm BMC (g)

High CER	3.23 ± 0.23		3.26 ± 0.24	
Low CER	3.27 ± 0.35	0.564	3.30 ± 0.32	0.665

Proximal forearm BMD (g/cm^2)

High CER	0.68 ± 0.04		0.68 ± 0.04	
Low CER	0.68 ± 0.05	0.821	0.68 ± 0.05	0.900

BMC, bone mineral content (g); BMD, bone mineral density (g/cm^2).
[1]n = 53;
[2]High CER group vs. Low CER group at baseline using unpaired student *t*-tests;
[3]Baseline vs. 6-month using paired *t*-tests;
[4]Mean ± SD.

Table 5. Biochemical measures of all subjects[1] at baseline and 6-month time points

	Baseline	6-month	P-value[2]
Osteocalcin (ng/mL)	10.0 ± 2.1^3	11.2 ± 2.6	0.003
Urinary NTx (BCE/mM creat)	72.8 ± 24.3	78.1 ± 39.9	0.302
Salivary cortisol[4] (μg/dL)	0.60 ± 0.29	0.53 ± 0.27	0.112
Urinary cortisol[5] (nM/mM creat)	51.2 ± 18.8	NA	NA
Estradiol (pg/mL)	34.8 ± 18.1	48.6 ± 37.2	0.013
Progesterone (ng/mL)	0.67 ± 0.22	0.75 ± 0.30	0.050
Growth hormone (ng/mL)	3.7 ± 3.7	5.0 ± 5.0	0.111
Insulin-like growth factor-1 (μg/L)	60.5 ± 33.0	75.4 ± 35.0	0.001
Insulin-like growth factor binding protein-3 (mg/L)	5.3 ± 0.9	5.1 ± 0.7	0.155

NTx, urinary N-telopeptide; BCE, bone collagen equivalents; creat, creatinine; NA, not available.
[1]n = 48;
[2]Baseline vs. 6-month using paired *t*-test;
[3]Mean ± SD;
[4]n = 53;
[5]n = 40.

Table 6. Biochemical measures by cognitive eating restraint (CER) group at baseline and 6-month time points[1]

Variable	Baseline	P-value (Baseline)[2]	6-month	P-value (Group x Time)[3]
Osteocalcin (ng/mL)				
High CER	9.3 ± 1.8[4]		10.3 ± 2.5	
Low CER	10.6 ± 2.3	0.011	12.0 ± 2.5	0.613
Urinary NTx (BCE/mM creatinine)				
High CER	68.3 ± 22.1		78.5 ± 53.3	
Low CER	78.1 ± 26.3	0.166	78.3 ± 18.9	0.251
Salivary cortisol[5] (μg/dL)				
High CER	0.61 ± 0.31		0.46 ± 0.24	
Low CER	0.58 ± 0.28	0.689	0.60 ± 0.29	0.037
Urinary cortisol[6] (nM/mM creatinine)				
High CER	49.4 ± 17.8		NA	
Low CER	53.7 ± 20.2	0.483	NA	NA
Estradiol (pg/mL)				
High CER	32.9 ± 15.3		45.6 ± 36.5	
Low CER	36.6 ± 20.6	0.539	51.4 ± 38.4	0.849
Progesterone (ng/mL)				
High CER	0.65 ± 0.19		0.68 ± 0.28	
Low CER	0.69 ± 0.24	0.347	0.82 ± 0.31	0.222
Growth hormone (ng/mL)				
High CER	3.2 ± 3.4		4.4 ± 3.7	
Low CER	4.8 ± 4.1	0.657	5.3 ± 5.9	0.945
Insulin-like growth factor-1 (μg/L)				
High CER	56.9 ± 35.1	0.384	71.0 ± 28.6	
Low CER	62.0 ± 30.8		79.3 ± 40.8	0.712
Insulin-like growth factor binding protein-3 (mg/L)				
High CER	5.5 ± 0.9		5.0 ± 0.8	
Low CER	5.2 ± 0.9	0.149	5.2 ± 0.7	0.113

NTx, N-telopeptide; BCE, bone collagen equivalents; NA, not available.
[1]n = 23 in High CER group and n = 25 in Low CER group;
[2]Baseline High vs. Low CER group using unpaired student *t*-test;
[3]Group x Time interaction from repeated measures analysis of variance;
[4]Mean ± SD; [5]n = 27 High CER and n = 26 Low CER; [6]n = 23 High CER and n = 17 Low CER.

Because significant Group x Time interactions were not found in BMC and BMD measures, and because most research regarding bone health and dietary intake in young adults has been limited to calcium, phosphorus, and vitamin D, bivariate correlation analyses were conducted to examine relationships between bone mineral measures and select variables for the total sample (n = 53). Table 7 displays Pearson correlation coefficients for comparisons between bone mineral measures and bone biomarker concentrations with anthropometric and soft tissue mass measures at the 6-month time point. Body height, weight, and FFST mass were significantly and positively associated with all measures of BMC ($p \leq 0.05 - p \leq 0.001$), except for Troc BMC, where only height was related and for WT BMC, where no measures were associated. Body height was also positively related to LS and FN BMD (both $p \leq 0.05$). Body weight was positively associated with LS and Prox forearm BMD (both $p \leq 0.05$). Age had an inverse relationship with FN BMD ($p \leq 0.05$), and BMI was positively associated with UD forearm BMD ($p \leq 0.05$). Serum osteocalcin was inversely related to CER score ($p \leq 0.01$), and age was inversely associated with urinary NTx ($p \leq 0.05$).

Table 8 displays relationships between estimated average daily dietary intake for select nutrients with BMC, BMD, and bone biomarker measures at baseline. Average calcium intake was positively associated with LS BMC ($p \leq 0.05$) and UD forearm BMC ($p \leq 0.05$). Average phosphorus intake was positively related to LS, TPF, TF, UD forearm, and Mid forearm BMC (all $p \leq 0.05$). Average vitamin D intake was positively related to TB, TPF, Troc, TF, UD forearm, and Mid forearm BMC ($p \leq 0.05 - p \leq 0.01$) and TPF and Mid forearm BMD (both $p \leq 0.05$). Serum osteocalcin was negatively associated with estimated average daily dietary fiber intake ($p \leq 0.01$) and magnesium intake ($p \leq 0.05$), whereas urinary NTx was negatively related to vitamin A intake ($p \leq 0.05$).

Table 9 presents correlation analyses for hormone concentrations and bone mineral measures and bone biomarkers at baseline. A negative association was observed between IGFBP-3 and UD forearm BMD ($p \leq 0.05$). A positive relationship between serum estradiol and FN BMD was observed ($p \leq 0.05$), while positive associations between serum progesterone and TB, TPF, and FN BMC (all $p \leq 0.05$) and FN and WT BMD (both $p \leq 0.05$) were noted. The Urinary NTx was positively associated with Troc BMD ($p \leq 0.05$), and urinary cortisol

Table 7. Pearson correlation coefficients for anthropometric and soft tissue mass variables with bone mineral measures and biomarkers of bone turnover at the 6-month time point[1]

	TB BMC (g)	TB BMD (g/cm^2)	LS BMC (g)	LS BMD (g/cm^2)	TPF BMC (g)	TPF BMD (g/cm^2)	FN BMC (g)	FN BMD (g/cm^2)	Troc BMC (g)	Troc BMD (g/cm^2)	WT BMC (g)	WT BMD (g/cm^2)
Age (y)	-0.03	-0.01	-0.05	-0.15	-0.18	-0.24	-0.18	-0.29*	-0.19	-0.20	-0.08	-0.22
CER score	0.06	0.05	0.14	0.15	0.02	0.06	0.02	0.01	-0.02	0.01	-0.02	0.01
Height (cm)	0.61***	0.14	0.43***	0.30*	0.70***	0.27	0.59**	0.29*	0.54***	0.23	0.18	0.22
Weight (kg)	0.47***	0.07	0.34*	0.31*	0.46***	0.15	0.39**	0.18	0.18	0.06	0.22	0.16
BMI (kg/m^2)	0.05	-0.04	0.04	0.12	-0.02	-0.04	-0.01	-0.03	-0.20	-0.10	0.10	0.01
Fat mass (kg)	-0.01	0.19	0.24	0.20	0.15	-0.01	0.03	0.04	0.01	0.13	0.05	0.25
FFST mass (kg)	0.49***	0.07	0.28*	0.21	0.52***	0.18	0.40**	0.20	0.20	0.03	0.17	0.16
BF%	0.02	-0.05	0.09	0.11	-0.04	-0.09	-0.00	-0.05	-0.04	-0.02	0.05	-0.03

	TF BMC (g)	TF BMD (g/cm^2)	UD BMC (g)	UD BMD (g/cm^2)	Mid BMC (g)	Mid BMD (g/cm^2)	Prox BMC (g)	Prox BMD (g/cm^2)	Osteocalcin[2]	NTx[2] (BCE/mmol creatinine)
Age (y)	0.13	0.18	0.13	0.10	0.09	0.20	0.16	0.18	-0.24	-0.30*
CER score	0.06	0.11	-0.01	0.12	0.07	0.12	-0.02	0.01	-0.42**	-0.09
Height (cm)	0.59***	0.02	0.28*	-0.15	0.66***	0.02	0.40**	0.23	0.02	0.06
Weight (kg)	0.52***	0.25	0.48***	0.18	0.50***	0.25	0.42***	0.31*	-0.11	-0.18
BMI (kg/m^2)	0.13	0.26	0.31*	0.30*	0.05	0.26	0.18	0.16	-0.13	-0.23
Fat mass (kg)	0.25	0.24	0.31*	0.24	0.23	0.24	0.16	0.20	-0.10	-0.22
FFST mass (kg)	0.51***	0.07	0.36**	-0.02	0.51***	0.08	0.44**	0.19	-0.13	-0.06
BF%	0.07	0.21	0.18	0.24	0.05	0.22	0.01	0.13	-0.06	-0.23

TB, total body; BMC, bone mineral content; BMD, bone mineral density; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; NTx, urinary N-telopeptide; BCE, bone collagen equivalents; CER, cognitive eating restraint; BMI, body mass index; FFST, fat-free soft-tissue; BF%, body fat percent.
[1]n = 53.
*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

Table 8. Pearson correlation coefficients for baseline estimated average daily dietary intake and bone mineral content (BMC), bone mineral density (BMD), and biomarkers of bone turnover[1]

	TB BMC (g)	TB BMD (g/cm^2)	LS BMC (g)	LS BMD (g/cm^2)	TPF BMC (g)	TPF BMD (g/cm^2)	FN BMC (g)	FN BMD (g/cm^2)	Troc BMC (g)	Troc BMD (g/cm^2)	WT BMC (g)	WT BMD (g/cm^2)
Kilojoules	0.02	0.02	0.12	0.12	0.06	0.02	0.05	0.04	0.06	0.01	-0.05	-0.01
Carbohydrate (g)	-0.02	-0.01	0.18	0.11	0.04	0.07	0.07	0.08	0.10	0.10	-0.02	0.05
Fat (g)	0.03	0.08	0.20	0.15	0.02	-0.01	0.01	0.01	0.02	-0.05	-0.05	-0.04
Protein (g)	0.15	0.03	0.23	0.10	0.22	0.06	0.11	0.06	0.10	0.02	-0.06	-0.01
Fiber (g)	-0.16	0.01	0.10	0.06	-0.12	0.09	-0.07	0.01	-0.13	0.10	-0.07	0.03
Calcium (mg)	0.16	0.03	0.28*	0.17	0.26	0.18	0.20	0.09	0.23	0.19	-0.04	0.07
Iron (mg)	-0.06	-0.03	0.07	-0.01	-0.01	0.06	-0.09	0.01	0.01	0.04	-0.12	-0.04
Magnesium (mg)	0.02	0.05	0.20	0.10	0.063	0.09	0.01	-0.01	-0.02	0.05	-0.02	0.02
Phosphorus (mg)	0.20	0.07	0.27*	0.15	0.28*	0.12	0.17	0.09	0.19	0.09	0.01	0.06
Potassium (mg)	0.01	-0.02	0.25	0.15	0.10	0.14	0.08	0.05	0.03	0.11	-0.01	0.05
Sodium (mg)	0.08	0.06	0.14	0.10	0.05	0.02	-0.06	0.02	-0.02	-0.05	-0.11	-0.07
Zinc (mg)	0.08	0.08	0.18	0.09	0.14	0.09	0.04	0.03	0.06	0.05	-0.07	0.02
Vitamin A (IU)	-0.14	-0.13	0.08	-0.03	0.08	0.13	-0.01	0.07	-0.06	0.03	-0.10	-0.03
Vitamin C (mg)	-0.09	-0.05	0.09	0.05	0.02	0.12	0.08	0.06	0.02	0.15	-0.01	0.07
Vitamin D (IU)	0.37**	0.24	0.13	0.10	0.44**	0.28*	0.20	0.16	0.27*	0.19	0.15	0.19

143

Table 8 continued

	TF BMC (g)	TF BMD (g/cm^2)	UD BMC (g)	UD BMD (g/cm^2)	Mid BMC (g)	Mid BMD (g/cm^2)	Prox BMC (g)	Prox BMD (g/cm^2)	Ostoecalcin[2] (ng/mL)	NTX[2] (BCE/mmol creatinine)
Kilojoules	0.13	0.07	0.18	0.02	0.11	0.07	0.09	0.15	-0.07	0.08
Carbohydrate (g)	0.04	0.02	0.11	0.02	0.04	0.02	-0.03	0.07	-0.13	0.14
Fat (g)	0.17	0.17	0.24	0.10	0.13	0.17	0.18	0.24	0.03	0.03
Protein (g)	0.22	0.03	0.25	-0.02	0.21	0.05	0.16	0.14	-0.12	-0.09
Fiber (g)	-0.09	-0.03	0.01	0.07	-0.09	-0.05	-0.12	-0.04	-0.46***	-0.21
Calcium (mg)	0.23	0.15	0.31*	0.08	0.23	0.19	0.07	0.22	-0.13	-0.02
Iron (mg)	-0.08	-0.07	-0.01	0.04	-0.05	-0.06	-0.19	-0.13	-0.27	-0.05
Magnesium (mg)	0.17	0.07	0.20	0.04	0.17	0.07	0.08	0.13	-0.30*	-0.15
Phosphorus (mg)	0.30*	0.09	0.32*	0.01	0.30*	0.12	0.17	0.20	-0.13	-0.05
Potassium (mg)	0.11	0.03	0.20	0.04	0.10	0.03	0.02	0.07	-0.25	-0.09
Sodium (mg)	0.08	0.03	0.16	0.03	0.07	0.03	0.03	0.05	-0.13	-0.05
Zinc (mg)	0.19	0.06	0.22	0.03	0.18	0.06	0.14	0.13	-0.18	-0.05
Vitamin A (IU)	-0.12	-0.05	0.11	0.08	-0.14	-0.05	-0.18	-0.07	-0.25	-0.29*
Vitamin C (mg)	-0.10	-0.01	0.01	0.01	-0.12	-0.01	-0.12	-0.01	-0.14	0.13
Vitamin D (IU)	0.38**	0.24	0.45**	0.22	0.38**	0.28*	0.18	0.21	-0.04	-0.12

TB, total body; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; NTx, urinary N-telopeptide; BCE, bone collagen equivalents; IU, International Units.
[1]n = 53;
*p $\leq$ 0.05; **p $\leq$ 0.01; ***p $\leq$ 0.001

Table 9. Pearson correlation coefficients for baseline hormone concentrations and bone mineral measures and bone biomarkers[1]

	TB BMC (g)	TB BMD (g/cm^2)	LS BMC (g)	LS BMD (g/cm^2)	TPF BMC (g)	TPF BMD (g/cm^2)	FN BMC (g)	FN BMD (g/cm^2)	Troc BMC (g)	Troc BMD (g/cm^2)	WT BMC (g)	WT BMD (g/cm^2)
Salivary cortisol[1] (µg/dL)	-0.07	0.09	0.03	0.09	0.01	-0.01	-0.09	-0.05	0.02	0.03	0.01	0.01
GH[2] (ng/mL)	-0.16	0.04	-0.17	-0.16	-0.23	-0.02	-0.21	-0.23	-0.26	0.01	-0.18	-0.12
IGF-1[2] (µg/L)	-0.08	0.04	-0.16	-0.01	-0.03	-0.06	-0.07	-0.01	-0.03	-0.12	-0.24	-0.12
IGFBP-3[2] (mg/L)	0.03	0.04	0.01	0.11	-0.09	-0.10	-0.04	-0.04	-0.09	-0.14	-0.23	-0.14
Estradiol[2] (pg/mL)	0.13	0.11	0.17	0.18	0.23	0.20	0.22	0.31*	0.25	0.12	0.06	0.20
Progesterone[2] (ng/mL)	0.31*	0.17	0.17	0.21	0.34*	0.27	0.31*	0.33*	0.23	0.12	0.19	0.28*
Osteocalcin[2] (ng/mL)	-0.01	-0.08	0.01	-0.09	0.04	0.01	0.07	-0.17	-0.10	-0.17	-0.10	-0.05
NTX[2] (BCE/mM creat)	0.06	0.01	0.07	-0.03	0.13	0.09	0.22	0.19	0.27*	0.13	0.10	0.26
Urinary cortisol[3] (nM/mM creat)	0.29	0.20	0.37*	0.35*	0.28	0.24	0.25	0.19	0.26	0.25	0.03	0.10

Table 9 continued

	TF BMC (g)	TF BMD (g/cm²)	UD BMC (g)	UD BMD (g/cm²)	Mid BMC (g)	Mid BMD (g/cm²)	Prox BMC (g)	Prox BMD (g/cm²)	Osteocalcin (ng/mL) (g)	NTx (BCE/mmol creatinine)
Salivary cortisol[1] (µg/dL)	-0.15	-0.06	-0.14	-0.02	-0.17	-0.08	-0.06	-0.05	-0.01	0.16
GH[2] (ng/mL)	-0.15	-0.11	-0.18	-0.15	-0.12	-0.08	-0.21	-0.07	-0.08	-0.05
IGF-1[2] (µg/L)	-0.15	-0.06	-0.14	-0.02	-0.17	-0.08	-0.06	-0.05	0.22	0.11
IGFBP-3[2] (mg/L)	0.07	-0.19	0.02	-0.28*	0.11	-0.14	-0.02	-0.10	-0.11	0.17
Estradiol[2] (pg/mL)	0.10	0.15	0.03	0.08	0.08	0.14	0.18	0.13	0.23	0.19
Progesterone[2] (ng/mL)	0.18	0.02	0.19	-0.01	0.17	0.03	0.11	0.06	0.06	0.17
Osteocalcin[2] (ng/mL)	-0.05	-0.12	-0.09	-0.18	-0.06	-0.08	0.04	-0.05	1.00	0.48***
NTX[2] (BCE/mM creat)	-0.01	0.02	0.02	0.11	-0.02	-0.02	0.03	-0.05	0.48***	1.00
Urinary cortisol[3] (nM/mM creat)	0.28	0.01	0.23	0.02	0.29	-0.02	0.15	0.19	0.01	0.16

TB, total body; BMC, bone mineral content; BMD, bone mineral density; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; NTx, urinary N-telopeptide; BCE, bone collagen equivalents.

[1]n = 53;
[2]n = 52;
[3]n = 40.
*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

was positively related to LS BMC and BMD (both $p \leq 0.05$). Serum osteocalcin and urinary NTx concentrations were positively associated ($p \leq 0.001$).

Correlation analyses between anthropometric and soft tissue mass measures and change in bone mineral measures are presented in Table 10. An inverse association was observed for CER score and change in LS BMD ($p \leq 0.05$). Body weight, FM, and BF% each had an inverse relationship with change in Troc BMD ($p \leq 0.05 - p \leq 0.01$). A negative association between BF% and change in TF BMC ($p \leq 0.05$) was also noted.

Table 11 displays correlation analyses for dietary intake and change in bone mineral measures. Estimated average daily energy intake was positively associated with change in TB BMC ($p \leq 0.05$). Dietary fat intake was positively related to change in TB BMC ($p \leq 0.05$). Fiber ($p \leq 0.05$) and iron ($p \leq 0.05$) intake each had a positive association with change in WT BMD. Phosphorus ($p \leq 0.05$) and zinc ($p \leq 0.05$) intake both had positive relationships with UD forearm BMC. Vitamin C intake was positively associated with TB BMC ($p \leq 0.05$).

Correlation analyses for hormone concentrations and change in bone mineral measures are presented in Table 12. A positive association between GH and change in FN BMD ($p \leq 0.05$) was observed. Negative relationships between estradiol and change in Troc BMD ($p \leq 0.05$), urinary cortisol and change in FN BMC ($p \leq 0.05$), and salivary cortisol and change in Prox forearm BMD ($p \leq 0.001$) were found.

Stepwise linear regression analyses were conducted for baseline BMC (Table 13) and BMD (Table 14) with select baseline variables. Final regression models for changes in BMC and BMD over time are found in Tables 15 and 16, respectively. Significant models were not generated for baseline TB BMD or change in TB, TF, Mid forearm and Prox forearm BMD with stepwise linear regression analyses.

Discussion

The primary purpose of this research was to longitudinally investigate BMC and BMD in women with high and low CER. At baseline, the high CER group possessed significantly higher body FM and BF% versus the low CER group (Table 1). Estimated average daily dietary intake of nutrients did not differ between groups at baseline (Table 2), and mean hormone concentrations did not differ between groups at baseline (Table 6), although mean serum osteocalcin was significantly lower in the high CER group versus low CER group at baseline

Table 10. Pearson correlation coefficients for anthropometric and soft tissue mass variables with change in bone mineral measures at the 6-month time point[1]

	Δ TB BMC (g)	Δ TB BMD (g/cm²)	Δ LS BMC (g)	Δ LS BMD (g/cm²)	Δ TPF BMC (g)	Δ TPF BMD (g/cm²)	Δ FN BMC (g)	Δ FN BMD (g/cm²)	Δ Troc BMC (g)	Δ Troc BMD (g/cm²)	Δ WT BMC (g)	Δ WT BMD (g/cm²)
Age (y)	-0.09	0.06	-0.20	0.02	0.09	-0.01	0.05	0.04	0.02	0.04	-0.14	0.02
CER score	-0.09	0.17	0.08	-0.30*	0.06	-0.12	0.02	-0.15	-0.17	-0.16	-0.05	0.07
Height (cm)	0.03	0.01	0.05	-0.19	0.15	0.06	-0.03	-0.09	-0.15	-0.19	-0.02	-0.16
Weight (kg)	0.09	0.03	0.04	0.01	0.06	-0.05	-0.07	-0.10	-0.16	-0.32*	-0.16	-0.08
BMI (kg/m²)	0.07	0.02	-0.02	0.12	-0.06	-0.12	-0.05	-0.05	-0.06	-0.21	-0.15	0.03
Fat mass (kg)	-0.01	0.02	0.03	0.06	0.03	-0.12	-0.09	-0.13	-0.05	-0.36**	-0.25	-0.11
FFST mass (kg)	0.17	0.03	0.02	-0.03	0.05	0.03	0.02	-0.02	-0.18	-0.12	0.01	0.02
BF%	-0.06	0.01	0.03	0.04	-0.01	-0.14	-0.09	-0.15	-0.01	-0.37*	-0.22	-0.14

	Δ TF BMC (g)	Δ TF BMD (g/cm²)	Δ UD BMC (g)	Δ UD BMD (g/cm²)	Δ Mid BMC (g)	Δ Mid BMD (g/cm²)	Δ Prox BMC (g)	Δ Prox BMD (g/cm²)
Age (y)	0.01	-0.01	-0.05	-0.12	0.02	-0.11	0.10	0.07
CER score	-0.05	0.09	-0.27	0.06	-0.04	0.05	0.21	0.05
Height (cm)	0.01	0.01	-0.06	-0.04	-0.02	0.01	0.08	0.05
Weight (kg)	-0.03	0.04	0.04	-0.04	-0.05	0.08	-0.01	0.15
BMI (kg/m²)	-0.04	0.02	0.09	-0.02	-0.05	0.07	-0.08	0.11
Fat mass (kg)	-0.22	0.01	-0.06	-0.01	-0.21	0.05	-0.16	-0.01
FFST mass (kg)	0.19	0.06	0.10	0.06	0.15	0.09	0.16	0.19
BF%	-0.30*	-0.01	-0.11	0.03	-0.27	0.02	-0.22	-0.08

Δ, change in bone mineral measurement from baseline to 6-months; TB, total body; BMC, bone mineral content; BMD, bone mineral density; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; CER, cognitive eating restraint; BMI, body mass index; FFST, fat-free soft-tissue; BF%, body fat percent.
[1]n = 53.
*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

Table 11. Pearson correlation coefficients for estimated average daily dietary intake from food frequency questionnaires and change in bone mineral content (BMC) and bone mineral density (BMD) measures[1]

	ΔTB BMC (g)	ΔTB BMD (g/cm²)	ΔLS BMC (g)	ΔLS BMD (g/cm²)	ΔTPF BMC (g)	ΔTPF BMD (g/cm²)	ΔFN BMC (g)	ΔFN BMD (g/cm²)	ΔTroc BMC (g)	ΔTroc BMD (g/cm²)	ΔWT BMC (g)	ΔWT BMD (g/cm²)
Kilojoules	0.28*	-0.02	-0.07	0.18	-0.14	-0.10	0.08	0.09	0.12	0.11	0.03	0.05
Carbohydrate (g)	0.27	-0.03	-0.02	0.13	-0.11	-0.10	0.01	0.12	0.13	0.09	0.04	0.06
Fat (g)	0.30*	0.04	-0.14	0.21	-0.15	-0.09	0.15	0.01	0.10	0.15	0.07	0.07
Protein (g)	0.20	-0.02	-0.07	0.18	-0.14	-0.07	0.14	0.10	0.10	0.11	-0.01	0.06
Fiber (g)	0.12	-0.05	-0.08	0.08	0.01	-0.01	0.01	0.18	0.23	0.13	0.04	0.32*
Calcium (mg)	0.05	0.03	-0.11	0.08	-0.12	-0.12	-0.08	0.05	0.04	-0.01	0.19	0.05
Iron (mg)	0.18	-0.07	0.06	0.08	-0.12	-0.06	0.00	0.05	0.12	0.06	0.15	0.27*
Magnesium (mg)	0.19	-0.05	-0.14	0.03	-0.12	-0.10	0.12	0.19	0.20	0.14	0.05	0.21
Phosphorus (mg)	0.18	-0.03	-0.12	0.12	-0.13	-0.08	0.06	0.12	0.05	0.09	0.07	0.06
Potassium (mg)	0.23	-0.04	-0.08	0.12	-0.20	-0.10	0.02	0.15	0.22	0.14	0.05	0.13
Sodium (mg)	0.23	-0.09	-0.10	0.14	-0.17	-0.19	0.04	-0.01	0.13	0.04	0.07	0.01
Zinc (mg)	0.15	-0.07	-0.04	0.01	-0.14	-0.14	0.01	0.11	0.11	0.05	-0.03	-0.07
Vitamin A (IU)	0.13	0.02	-0.17	0.04	0.16	0.08	-0.07	0.04	0.10	0.10	0.04	0.15
Vitamin C (mg)	0.29*	0.04	-0.01	0.10	-0.06	-0.14	-0.18	0.02	0.19	-0.08	0.17	0.11
Vitamin D (IU)	-0.01	0.02	-0.08	0.03	-0.12	-0.03	0.21	0.04	-0.03	0.23	0.13	0.09

Table 11 continued

	ΔTF BMC (g)	ΔTF BMD (g/cm^2)	ΔUD BMC (g)	ΔUD BMD (g/cm^2)	ΔMid BMC (g)	ΔMid BMD (g/cm^2)	ΔProx BMC (g)	ΔProx BMD (g/cm^2)
Kilojoules	0.04	0.08	0.18	0.12	0.02	0.09	-0.11	0.16
Carbohydrate (g)	-0.01	0.07	0.13	0.10	-0.01	0.10	-0.12	0.13
Fat (g)	0.05	0.05	0.17	0.09	0.03	0.04	-0.09	0.15
Protein (g)	0.09	0.01	0.24	0.07	0.07	0.03	-0.11	0.14
Fiber (g)	0.20	-0.01	0.09	-0.03	0.23	0.01	0.04	0.15
Calcium (mg)	-0.07	-0.03	0.25	-0.02	-0.15	-0.13	-0.16	0.13
Iron (mg)	0.06	0.04	0.14	0.05	0.07	0.04	-0.09	0.18
Magnesium (mg)	0.16	-0.05	0.18	-0.05	0.16	-0.02	-0.02	0.14
Phosphorus (mg)	0.06	-0.07	0.30*	-0.01	0.01	-0.07	-0.13	0.13
Potassium (mg)	0.16	0.05	0.15	0.06	0.18	0.09	-0.02	0.13
Sodium (mg)	0.05	0.10	0.20	0.13	0.04	0.14	-0.10	0.16
Zinc (mg)	0.10	-0.04	0.30*	-0.03	0.06	-0.01	-0.11	0.17
Vitamin A (IU)	0.02	0.03	0.03	0.01	0.06	0.04	-0.08	0.11
Vitamin C (mg)	0.16	0.18	0.06	0.22	0.19	0.23	0.02	0.18
Vitamin D (IU)	-0.26	-0.12	-0.04	-0.08	-0.26	-0.15	-0.23	-0.02

Δ, change in bone mineral measurement from baseline to 6-months; TB, total body; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; IU, International Units.
[1]n = 53.
*p $\leq$ 0.05

Table 12. Pearson correlation coefficients for mean hormone concentrations with change in bone mineral measures[1]

	ΔTB BMC (g)	ΔTB BMD (g/cm^2)	ΔLS BMC (g)	ΔLS BMD (g/cm^2)	ΔTPF BMC (g)	ΔTPF BMD (g/cm^2)	ΔFN BMC (g)	ΔFN BMD (g/cm^2)	ΔTroc BMC (g)	ΔTroc BMD (g/cm^2)	ΔWard BMC (g)	ΔWard BMD (g/cm^2)
Salivary cortisol[1] (µg/dL)	-0.12	-0.02	0.22	0.20	-0.12	-0.04	-0.01	0.01	-0.11	0.13	0.11	0.03
GH[2] (ng/mL)	-0.24	-0.24	-0.09	0.14	0.11	0.10	-0.18	0.32*	0.24	0.25	0.17	0.17
IGF-1[2] (µg/L)	0.19	0.03	0.12	0.12	-0.12	0.07	0.13	-0.01	-0.01	0.17	0.15	0.14
IGFBP-3[2] (mg/L)	0.15	-0.03	0.03	-0.06	0.08	0.07	0.08	0.08	0.01	0.09	0.01	0.13
Estradiol[2] (pg/mL)	0.15	0.04	0.16	0.01	-0.12	-0.19	-0.10	-0.21	-0.26	-0.33*	0.10	-0.11
Progesterone[2] (ng/mL)	0.22	0.13	0.16	0.13	0.09	0.11	-0.04	0.04	-0.19	0.04	0.02	-0.04
Osteocalcin[2] (ng/mL)	-0.01	0.13	0.05	0.16	-0.11	0.01	0.03	0.07	-0.26	-0.13	0.13	-0.07
NTx[2] (BCE/mM creat)	0.16	0.05	0.06	-0.05	-0.16	-0.11	-0.03	-0.07	-0.11	-0.23	0.15	-0.17
Urinary cortisol[3] (nM/mM creat)	-0.16	-0.13	0.15	0.11	0.02	-0.01	-0.31*	-0.03	-0.05	-0.15	-0.13	0.10

Table 12 continued

	ΔTF BMC (g)	ΔTF BMD (g/cm^2)	ΔUD BMC (g)	ΔUD BMD (g/cm^2)	ΔMid BMC (g)	ΔMid BMD (g/cm^2)	ΔProx BMC (g)	ΔProx BMD (g/cm^2)
Salivary cortisol[1] (μg/dL)	0.11	-0.14	0.15	0.11	0.09	-0.05	0.02	-0.49***
GH[2] (ng/mL)	0.24	0.04	-0.01	0.03	0.27	0.01	0.16	0.01
IGF-1[2] (μg/L)	-0.06	-0.09	0.16	0.05	-0.11	-0.18	0.12	0.05
IGFBP-3[2] (mg/L)	0.09	0.17	-0.13	0.25	0.04	0.08	-0.01	0.22
Estradiol[2] (pg/mL)	0.03	-0.07	0.11	-0.04	0.05	-0.06	0.09	0.01
Progesterone[2] (ng/mL)	0.06	0.02	0.10	0.01	-0.01	0.07	0.08	0.01
Osteocalcin[2] (ng/mL)	-0.11	-0.08	0.08	-0.10	-0.11	-0.15	0.17	-0.07
NTx[2] (BCE/mM creat)	-0.02	-0.09	0.23	-0.01	-0.03	-0.04	0.05	-0.16
Urinary cortisol[3] (nM/mM creat)	0.11	0.24	-0.13	-0.04	0.08	0.27	0.30	-0.05

Δ, change in bone mineral measurement from baseline to 6-months; TB, total body; BMC, bone mineral content; BMD, bone mineral density; LS, lumbar spine (L$_1$-L$_4$); TPF, total proximal femur; FN, femoral neck; Troc, trochanter; WT, Ward's triangle; TF, total forearm; UD, ultradistal forearm; Mid, mid forearm; Prox, proximal forearm; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; NTx, urinary N-telopeptide; BCE, bone collagen equivalents; Creat, creatinine.
[1]n = 53;
[2]n = 52;
[3]n = 40.
*p ≤ 0.05; ***p ≤ 0.001

Table 13. Stepwise linear regression models for baseline bone mineral content (BMC)[1] (n = 52)

Dependent Variable	Predictor	R^2	Model Adjusted R^2	Unstandardized β (Standard Error)	Standardized β	P-value	Model P-value
Total body BMC			**0.40**				**<0.001**
	Constant			-1875.376 (777.109)		0.020	
	Height (cm)	0.38		21.059 (5.322)	0.495	<0.001	
	Weight (kg)	0.05		9.472 (4.840)	0.245	0.056	
Lumbar spine (L_1-L_4) BMC			**0.25**				**0.001**
	Constant			-66.730 (32.426)		0.045	
	Height (cm)	0.18		0.7227 (0.192)	0.459	<0.001	
	Dietary fat (g/d)[2]	0.06		0.113 (0.044)	0.329	0.014	
	IGF-1 (µg/L)	0.05		-0.0673 (0.035)	-0.243	0.064	
Total proximal femur BMC			**0.58**				**<0.001**
	Constant			-44.515 (17.594)		0.015	
	Height (cm)	0.40		0.586 (0.100)	0.600	<0.001	
	Vitamin D (IU/d)[2]	0.06		0.01598 (0.005)	0.334	0.003	
	Age (y)	0.04		-0.584 (0.217)	-0.259	0.010	
	IGFBP-3 (mg/L)	0.04		-0.00151 (0.001)	-0.245	0.015	
	Vitamin A (IU/d)[2]	0.03		0.0002572 (0.000)	0.360	0.002	
	Iron (mg/d)[2]	0.04		-0.319 (0.135)	-0.273	0.022	
Femoral neck BMC			**0.41**				**<0.001**
	Constant			-8.776 (2.206)		<0.001	
	Height (cm)	0.33		0.06800 (0.011)	0.645	<0.001	
	Alcohol (drinks/wk)[3]	0.04		-0.107 (0.054)	-0.220	0.053	
	BMI (kg/m^{2})	0.04		0.7587 (0.033)	0.256	0.027	
	NTx (BCE/mM creat)	0.05		0.005503 (0.003)	0.229	0.044	
Trochanter BMC			**0.61**				**<0.001**
	Constant			-23.935 (3.897)		<0.001	
	Height (cm)	0.37		0.1946 (0.203)	0.753	<0.001	
	Alcohol (drinks/wk)	0.17		-0.523 (0.108)	-0.437	<0.001	
	NTx (BCE/mM creat)	0.05		0.01061 (0.005)	0.180	0.050	

IGFBP-3 (mg/L)	0.03		-0.000342 (0.000)	-0.211	0.028	
Dietary fat (g/d)	0.04		0.0141 (0.005)	0.203	0.031	
Ward's triangle BMC		**0.19**				**0.003**
Constant			0.624 (0.220)		0.007	
Weight (kg)	0.17		0.009521 (0.003)	0.394	0.003	
IGFBP-3 (mg/L)	0.05		-0.0000382 (0.000)	-0.228	0.077	
Total forearm BMC		**0.44**				**<0.001**
Constant			-18.923 (4.856)		<0.001	
Height (cm)	0.33		0.166 (0.0264)	0.691	<0.001	
BMI (kg/m^2)	0.06		0.151 (0.073)	0.253	0.043	
Dietary fat (g/d)	0.05		0.03945 (0.015)	0.753	0.011	
Kilojoules^2	0.05		-0.129 (0.001)	-0.570	0.050	
Ultradistal forearm BMC		**0.46**				**<0.001**
Constant			1.116 (0.269)		<0.001	
Weight (kg)	0.25		0.01718 (0.004)	0.435	<0.001	
Vitamin D (IU/d)	0.12		0.0008556 (0.000)	0.402	0.001	
Alcohol (drinks/wk)	0.04		-0.0264 (0.011)	-0.264	0.018	
Dietary fat (g/d)	0.04		0.003432 (0.001)	0.363	0.005	
Iron (mg/d)^2	0.07		-0.0171 (0.007)	-0.329	0.014	
Mid forearm BMC		**0.35**				**<0.001**
Constant			14.505 (3.076)		<0.001	
Height (cm)	0.42		11.479 (1.692)	0.704	<0.001	
Dietary fat (g/d)	0.04		0.008061 (0.004)	0.227	0.032	
Age (y)	0.04		0.07281 (0.039)	0.194	0.067	
Proximal forearm BMC		**0.30**				**<0.001**
Constant			-1.083 (1.149)		0.351	
Height (cm)	0.13		2.316 (0.633)	0.439	0.001	
Dietary fat (g/d)	0.05		0.005250 (0.002)	0.466	0.002	
Iron (mg/d)	0.13		-0.0256 (0.009)	-0.406	0.006	
Age (y)	0.04		0.02603 (0.015)	0.214	0.080	
Serum osteocalcin (ng/mL)		**0.50**				**<0.001**
Constant			13.268 (1.797)		<0.001	
NTx (BCE/mM creat)	0.23		0.03050 (0.010)	0.334	0.003	

	Fiber (g/d)^2	0.14		-0.150 (0.037)	-0.483	<0.001	
	Body fat (%)	0.10		-0.148 (0.049)	-0.315	0.004	
	Dietary fat (g/d)	0.04		0.01877 (0.010)	0.215	0.070	
Urinary NTx (BCE/mM creat)			**0.44**				**<0.001**
	Constant			147.060 (37.918)		<0.001	
	Osteocalcin (ng/mL)	0.23		4.067 (1.209)	0.371	0.002	
	Age (y)	0.14		-4.331 (1.134)	-0.428	<0.001	
	IGFBP-3 (mg/L)	0.04		-6.87 (0.300)	-0.249	0.030	
	Carbohydrate (g/d)^2	0.04		0.123 (0.045)	0.408	0.009	
	Sodium (mg/d)^2	0.05		-0.00738 (0.004)	-0.307	0.046	

IGF-1, insulin-like growth factor-1; IU, International Units; IGFBP-3, insulin-like growth factor binding protein-3; BMI, body mass index; NTx, urinary N-telopeptide; BCE, bone collagen equivalents; Creat, creatinine.
[1]BMC in g;
[2]Estimated average daily dietary intake based on food frequency questionnaires (15);
[3]Estimated average weekly number of alcoholic drinks/wk based on initial screening questionnaires.

Table 14. Stepwise linear regression models for baseline bone mineral density (BMD)[1] (n = 52)

Dependent Variable	Predictor	R^2	Model Adjusted R^2	Unstandardized β (Standard Error)	Standardized β	P-value	Model P-value
Lumbar spine (L$_1$-L$_4$) BMD			**0.10**				**0.014**
	Constant			0.139 (0.342)		0.685	
	Height (cm)	0.12		0.005272 (0.002)	0.340	0.014	
Total proximal femur BMD			**0.20**				**0.003**
	Constant			-0.144 (0.371)		0.700	
	Height (cm)	0.09		0.00633 (0.002)	0.368	0.007	
	Alcohol (drinks/wk)[2]	0.10		-0.0112 (0.005)	-0.280	0.038	
	Calcium:phosphorus ratio[3]	0.05		0.125 (0.068)	0.234	0.073	
Femoral neck BMD			**0.31**				**<0.001**
	Constant			-0.311 (0.401)		0.442	
	Height (cm)	0.12		0.006895 (0.002)	0.402	0.002	
	Alcohol (drinks/wk)	0.10		-0.0125 (0.005)	-0.316	0.012	
	Estradiol (pg/mL)	0.07		0.01281 (0.001)	0.249	0.039	
	Age (y)	0.04		-0.0104 (0.005)	-0.262	0.037	
	BMI (kg/m^2)	0.04		0.01048 (0.006)	0.217	0.084	
Trochanter BMD			**0.28**				**<0.001**
	Constant			-0.306 (0.319)		0.342	
	Calcium:protein ratio[3]	0.15		0.005876 (0.003)	0.273	0.032	
	Height (cm)	0.09		0.03961 (0.002)	0.376	0.004	
	Alcohol (drinks/wk)	0.09		-0.0115 (0.005)	-0.316	0.016	
Ward's triangle BMD			**0.24**				**0.003**
	Constant			-0.511 (0.442)		0.253	
	Height (cm)	0.11		0.008315 (0.003)	0.390	0.003	
	Alcohol (drinks/wk)	0.08		-0.0134 (0.006)	-0.273	0.035	
	NTx (BCE/mM creat)	0.06		0.001737 (0.001)	0.357	0.014	
	Osteocalcin (ng/mL)	0.05		-0.0133 (0.007)	-0.249	0.082	
Total forearm BMD			**0.05**				**0.056**
	Constant			0.455 (0.057)		<0.001	
	BMI (kg/m^2)	0.07		0.005179 (0.003)	0.267	0.056	
Ultradistal forearm BMD			**0.17**				**0.004**
	Constant			0.270 (0.054)		<0.001	
	BMI (kg/m^2)	0.13		0.007452 (0.003)	0.379	0.005	

Alcohol (drinks/wk)	0.07		-0.00431 (0.002	-0.268	0.063	
Mid forearm BMD		**0.15**				**0.012**
Constant			0.468 (0.058)		<0.001	
Vitamin D (IU/d)[3]	0.08		0.000171 (0.000)	0.328	0.017	
BMI (kg/m^2)	0.06		0.005367 (0.003)	0.261	0.049	
Exercise (h/wk)[4]	0.06		-0.00490 (0.003)	-0.252	0.063	
Proximal forearm BMD		**0.16**				**0.011**
Constant			0.554 (0.057)		<0.001	
Weight (kg)	0.07		0.001885 (0.001)	0.262	0.047	
Dietary fat (g/d)[3]	0.05		0.001103 (0.000)	0.641	0.008	
Sodium (mg/d)[3]	0.08		-0.0000214 (0.000)	-0.493	0.037	

NTx, urinary N-telopeptide; BCE, bone collagen equivalents; BMI, body mass index; IU, International Units.

[1]BMD in g/cm^2;

[2]Estimated average weekly number of alcoholic drinks/wk based on initial screening questionnaires;

[3]Estimated average daily dietary intake based on food frequency questionnaires (15);

[4]Average h/wk of hard and very hard physical activity from 7-d physical activity recalls (16).

Table 15. Stepwise linear regression models for change in bone mineral content (BMC)[1] over 6-months (n = 48)

Dependent Variable	Predictor	R^2	Model Adjusted R^2	Unstandardized β (Standard Error)	Standardized β	P-value	Model P-value
Δ Total body BMC			**0.07**				**0.031**
	Constant			-26.167 (14.285)		0.073	
	Dietary fat (g/d)[2]	0.09		0.501 (0.226)	0.300	0.031	
Δ Femoral neck BMC			**0.12**				**0.015**
	Constant			0.142 (0.095)		0.139	
	Calcium:Protein ratio[2]	0.09		-0.148 (0.006)	-0.353	0.012	
	Vitamin D (IU/d)[2]	0.07		4.120 (0.000)	0.269	0.051	
Δ Trochanter BMC			**0.09**				**0.035**
	Constant			0.165 (0.253)		0.517	
	Estradiol (pg/mL)	0.07		-0.0120 (0.005)	-0.308	0.028	
	Vitamin C (mg/d)[2]	0.06		0.001987 (0.001)	0.248	0.074	
Δ Ward's triangle BMC			**0.22**				**0.002**
	Constant			0.135 (0.089)		0.137	
	Alcohol (drinks/wk)[3]	0.09		-0.0155 (0.005)	-0.356	0.006	
	Calcium:Protein ratio[2]	0.09		0.007377 (0.003)	0.317	0.014	
	Body fat (%)	0.09		-0.00710 (0.003)	-0.306	0.017	
Δ Total forearm BMC			**0.37**				**<0.001**
	Constant			1.193 (0.296)		<0.001	
	Body fat (%)	0.08		-0.0524 (0.016)	-1.069	0.002	
	Vitamin D (IU/d)	0.16		-0.00113 (0.000)	-0.620	<0.001	
	Potassium (mg/d)[2]	0.10		0.00006084 (0.000)	0.287	0.034	
	Osteocalcin (ng/mL)	0.05		-0.0252 (0.013)	-0.251	0.054	
	Fat mass (kg)	0.04		0.00003273 (0.000)	0.555	0.089	
Δ Ultradistal forearm BMC			**0.42**				**<0.001**
	Constant			-0.104 (0.032)		0.002	
	Δ Weight (kg)	0.12		0.007815 (0.002)	0.384	0.001	
	Phosphorus (mg/d)[2]	0.07		0.0002011 (0.000)	1.169	<0.001	
	Carbohydrate (g/d)[2]	0.07		-0.000653 (0.000)	-0.789	0.001	
	Vitamin D (IU/d)	0.09		-0.000223 (0.000)	-0.377	0.009	

Salivary cortisol (μg/dL)	0.09		0.08230 (0.028)	0.334	0.006	
Alcohol (drinks/wk)	0.04		0.01237 (0.007)	0.205	0.064	
Δ Mid forearm BMC		**0.42**				**0.001**
Constant			0.328 (0.114)		0.006	
Vitamin D (IU/d)	0.08		-0.000756 (0.000)	-0.657	<0.001	
Body fat (%)	0.14		-0.0123 (0.004)	-0.394	0.001	
Potassium (mg/d)[2]	0.12		0.0001190 (0.000)	0.888	<0.001	
Carbohydrate (g/d)	0.07		-0.00132 (0.000)	-0.818	0.002	
Iron (mg/d)[2]	0.05		0.006804 (0.003)	0.371	0.032	
Alcohol (drinks/wk)	0.03		0.02198 (0.013)	0.187	0.091	
Δ Proximal forearm BMC		**0.33**				**<0.001**
Constant			0.269 (0.145)		0.115	
Δ Weight (kg)	0.08		-0.00777 (0.003)	-0.305	0.011	
IGFBP-3 (mg/L)	0.08		0.00002549 (0.000)	0.248	0.020	
Vitamin D (IU/d)	0.11		-0.000352 (0.000)	-0.475	<0.001	
Body fat (%)	0.08		-0.00834 (0.003)	-0.417	0.007	
Osteocalcin (ng/mL)	0.04		-0.00979 (0.005)	-0.240	0.064	

Δ, change from baseline to 6-month; IU, International Units; IGFBP-3, insulin-like growth factor binding protein-3.

[1]BMC in g;

[2]Estimated average daily dietary intake based on food frequency questionnaires (15);

[3]Estimated average weekly number of alcoholic drinks/wk based on initial screening questionnaires.

Table 16. Stepwise linear regression models for change in bone mineral density (BMD)[1] over 6-months (n = 48)

Dependent Variable	Predictor	R^2	Model Adjusted R^2	Unstandardized β (Standard Error)	Standardized β	P-value	Model P-value
Δ Lumbar spine (L$_1$-L$_4$) BMD			0.16				**0.011**
	Constant			-0.0606 (0.028)		0.035	
	CER score[2]	0.09		-0.0112 (0.000)	-0.315	0.019	
	BMI (kg/m^2)	0.06		0.002792 (0.001)	0.305	0.027	
	Salivary cortisol (µg/dL)	0.06		0.01531 (0.008)	0.251	0.065	
Δ Total proximal femur BMD			0.05				**0.064**
	Constant			-0.0137 (0.004)		0.003	
	Alcohol (drinks/wk)[3]	0.07		0.002574 (0.001)	0.259	0.064	
Δ Femoral neck BMD			0.09				**0.021**
	Constant			-0.0110 (0.004		0.017	
	Growth hormone (ng/mL)	0.10		0.001983 (0.001)	0.322	0.021	
Δ Trochanter BMD			0.25				**0.002**
	Constant			0.0102 90.013)		0.452	
	Estradiol (pg/mL)	0.12		-0.000375 (0.000)	-0.367	0.007	
	Fat mass (kg)	0.09		-0.00114 (0.001)	-0.228	0.080	
	Exercise (h/wk)[4]	0.05		0.02220 (0.001)	0.251	0.051	
	IGF-1 (µg/L)	0.06		0.0001587 (0.000)	0.257	0.062	
Δ Ward's triangle BMD			0.13				**0.015**
	Constant			0.03836 (0.020)		0.057	
	Δ Weight (kg)	0.09		0.004844 (0.002)	0.386	0.008	
	NTx (BCE/mM creat)	0.07		-0.000512 (0.000)	-0.280	0.050	
Δ Ultradistal forearm BMD			0.11				**0.021**
	Constant			-0.00834 (0.004)		0.048	
	Alcohol (drinks/wk)	0.09		0.001706 (0.001)	0.310	0.023	
	Vitamin C (mg/d)[5]	0.06		0.00003624 (0.000)	0.246	0.036	

Δ, change from baseline to 6-month; CER, cognitive eating restraint; BMI, body mass index; IGF-1, insulin-like growth factor-1; NTx, urinary N-telopeptide; BCE, bone collagen equivalents; Creat, creatinine.

[1]BMD in g/cm^2; [2]Cognitive eating restraint score from CER subscales of eating inventory (13);
[3]Estimated average weekly number of alcoholic drinks/wk based on initial screening questionnaires;
[4]Average h/wk of hard and very hard physical activity from 7-d physical activity recalls (16);
[5]Estimated average daily dietary intake based on food frequency questionnaires (15).

(Table 6). Because significant differences between groups were not found in all other measures at baseline, the cause for a difference in osteocalcin concentrations between groups could not be explained. Studies investigating subclinical hypercortisolism have found significantly lower biomarkers of bone formation and increased markers of bone resorption (21,22), but cortisol concentrations in our CER groups did not differ. In addition, TB and site-specific BMC and BMD measurements did not differ significantly between groups at baseline (Table 4).

Although reductions in estimated average daily dietary intake of some nutrients did occur in all subjects, dietary intake changes were not observed between groups over time (Table 2). The mean concentrations of serum IGF-1, estradiol, progesterone, and osteocalcin significantly increased over time in all subjects (Table 5), but group differences were not noted in changes in mean hormone concentrations or biochemical markers of bone turnover over time (Table 6). The significant changes over time in identified hormones for all subjects may be attributed to a small shift in testing of many subjects closer to the tenth day of the menstrual cycle (versus closer to the fourth day), as evidenced from the higher mean estradiol and progesterone concentrations. Values still correspond to the follicular phase; however, this may explain the increase in serum osteocalcin and IGF-1 as both fluctuate in response to circulating estradiol concentrations (23,24).

Interestingly, the only Group x Time interaction was found for salivary cortisol (Table 6) and in the opposite direction of expected based on results from previous studies (5,6). Over time, a significant reduction occurred in the mean salivary cortisol concentration in the high CER group such that at 6-months, the low CER group had a trend for a higher mean salivary cortisol concentration ($p = 0.051$). Previous studies have identified significantly higher urinary (6) and salivary (5) cortisol concentrations in women with high CER and have made implications for negative effects on bone health. We did not show significant differences at baseline and, furthermore, showed a reduction in concentration over time only in women with high CER. The effects of high-end cortisol concentration within the normal range on bone are not known. If effects do exist, and a high-end cortisol concentration is not sustained, the negative implication for bone may be lost.

We found no evidence to support negative implications of CER on BMC and BMD as these variables did not differ between CER groups at baseline or over 6-months. Within this group of young women, with normal BMI and limited physical activity, high CER scores were

161

associated with a greater portion of body FM and BF%, and despite a significantly lower mean serum osteocalcin concentration, BMC and BMD at baseline and accrual over time did not differ from women with low CER.

Because Group x Time comparisons did not implicate high CER scores in adverse effects on mediators of BMC and BMD, we subsequently examined relationships between CER scores, dietary intake, and biochemical measures and bone health in this group of young women.

In our study, CER score was inversely related to change in LS BMD in bivariate analysis (Table 10), and CER scores accounted for 9% of the variation in change in LS BMD (Table 16). McLean et al (7) found significantly lower TB BMC in women with high CER scores versus low CER scores and a non-significant trend for lower TB BMD. When included in multiple regression analysis, exercise and CER score combined accounted for 11.3% and 4.5% of the variance in TB BMC and BMD, respectively (7), in this previous cross-sectional study. Results from these investigations suggest that CER has a negative impact on bone health in young women; however, much remains to be discovered about CER and skeletal health.

Changes in BMC and BMD measures did not differ between CER groups over time (Table 4), but in all subjects, significant increases were detected in WT, TF, Mid forearm, and Prox forearm BMC over the 6-month period (Table 3). These increases were expected considering that these women were at an age where bone mineral accrual exceeds losses (25). Still, it was surprising to not find significant increases in TB BMD and even a significant decrease in TPF BMD over time (Table 3) as most experts agree that the age of peak bone mass is at the end of the third decade of life (1). However, Theintz et al (26) found that in women aged 13 to 20 years, the rate of bone mass accrual occurred most rapidly in the years just following menarche with a dramatic attenuation in the rate of bone mass accrual at the FN after age 17 years, and although not statistically significant, the rate of BMD accrual between 17 to 20 years was negative, indicating loss of BMD among some of these individuals. We found an ~1% loss of BMD over 6-months in our subjects, indicating the importance of maximizing peak bone mass early in life and sustaining healthy lifestyle habits to attenuate losses in BMD, even in young women.

Simple bivariate correlations indicated that height was significantly associated with BMC at all skeletal sites except WT (Table 7). Height was also the primary predictor of TB and most site-specific BMC measurements in regression models (Tables 13 and 14), but not change in

bone mineral (Tables 15 and 16). This was expected considering that BMC is a not an areal or volumetric measurement and would, therefore, be expected to relate to bone length. Height was also positively correlated with LS and FN BMD in bivariate evaluations (Table 7) and predicted LS, TPF, FN, Troc, and WT BMD in regression models (Table 14). Similarly, weight was positively correlated with BMC at most skeletal sites (Table 7) but only a positive predictor of TB, WT, and UD forearm BMC (Table 13) and Prox forearm BMD (Table 14). In regression models, with the exception of TB, weight was not included after height was present in the model. Although body weight changed only slightly over time, body weight change was a positive predictor of change in UD forearm BMC (Table 15) and WT BMD (Table 16) over time, and a negative predictor of Prox forearm BMC (Table 15) over time. Body mass index, which controls weight for height, was significantly associated with UD forearm BMC and BMD in bivariate analyses (Table 7), and BMI was a positive predictor of FN and TF BMC (Table 13) and FN, UD forearm, and Mid forearm BMD (Table 14), and change in LS BMD (Table 16) over time. It is well documented that body weight is protective of bone mass (9,10,27) and that with weight loss, bone mineral is lost (11,28,29). Our study supports previous findings by showing that body weight is positively correlated with BMC and BMD, and that even within a healthy range of BMI, body mass is positively associated with bone mass and change in bone mineral status over time.

A positive association between FFST mass and bone mass, likely because of its mechanical properties for stimulating bone remodeling and enhancing bone strength particularly at weight bearing sites, has also been demonstrated (9,10). We found positive associations between FFST mass and BMC at most skeletal sites in bivariate correlation analyses (Table 7), but surprisingly, FFST mass was not a predictor of any BMD measure in regression analyses. This may be explained by the co-linearity of FFST mass with height. Fat mass was correlated with only UD forearm BMC (Table 7), but considering the inverse relationship between BF% and change in Troc and TF BMC (Table 10) as well as the inverse association between BF% and osteocalcin in the regression model (Table 13), it appears that while body weight is positively correlated with BMC and BMD, FFST mass is likely more beneficial than FM for bone health in young adults. Although FFST mass was not included in regression models, exercise which helps promote increases and maintenance of FFST mass and proportionately less FM, was a positive predictor of change in Troc BMD over time (Table 16).

163

Despite the positive associations of age with Prox forearm and Mid forearm BMC in regression models (Table 13), we were surprised by the inverse correlation between age and FN BMD (Table 7) in bivariate correlation analyses and between age and TPF BMC (Table 13) and FN BMD (Table 14) in regression models. The age range of subjects in this study was narrow, but we expected positive associations between age and BMC and BMD due to the expected continued accrual of bone mineral in women of this mean age. We cannot state with certainty that these women have attained peak BMD, and although this may be the case, previous studies have shown that number of years since menarche is a better predictor of BMD than chronological age (30,31). We did not measure menarcheal age in this study. Given that age was significantly and negatively correlated with IGF-1 (r = -0.32, p = 0.020 and r = -0.40, p = 0.004 at baseline and follow-up, respectively) and urinary NTx (r = -0.46, p = 0.001 and r = -0.31, p = 0.025 at baseline and follow-up, respectively) at both time points, we suspect that younger subjects were further from the age of peak bone mass than older subjects. We also suspect that some other unmeasured factor was different between younger and older subjects that could account for the higher FN BMD in younger subjects.

Bivariate correlations showed positive associations between estradiol and FN BMD (Table 9). This was not surprising given the protective effect of estrogen on bone metabolism throughout the gynecological life of a woman (32). Serum progesterone was also positively correlated with FN BMD and BMD and TB, TPF and FN BMC (Table 9). This is interesting since estrogen is more widely recognized for its effect on bone than is progesterone; yet, nuclear receptors for progesterone have been identified in bone cells (33). However, in regression models, it was estrogen, not progesterone, which was present, but with conflicting associations. Estradiol was a positive predictor of FN BMD and change in Ward BMC over time, yet had an inverse correlation coefficient in the bivariate and regression model for change in Troc BMC.

Growth hormone was not significantly related to any baseline BMC or BMD variables, but in regression models, GH was a positive predictor of change in FN BMD over time (Table 16). Growth hormone production is increased during early puberty and enhances bone growth (34). Many of the actions of GH are presumably carried out through IGF-1 (35). Bivariate correlations showed no associations between IGF-1 and bone mineral measurements or changes. However, regression models showed that IGF-1 was an inverse predictor of change LS BMC (Table 13) but a positive predictor of change in Troc BMD (Table 16) over time. A significant

and inverse association was present, however, between serum IGFBP-3 and UD forearm BMD (Table 9). In addition, IGFBP-3 was included in linear regression models as a negative predictor of TPF and Troc BMC (Table 13). This is surprising considering that IGFBP-3 aids in prolonging the half-life and target-tissue delivery of IGF-1, which promotes bone formation (35,36). Yet, IGFBP-3 was included as an inverse predictor of urinary NTx (Table 13), indicating that it is associated with reducing bone resorption. And, while IGFBP-3 was not significantly associated with any change in BMC or BMD through bivariate regression, there was a trend for a positive association between IGFBP-3 and change in UD forearm BMD (r = 0.25, p = 0.076) (Table 12), indicating that although initially associated with lower BMD, IGFBP-3 may be associated with a greater rate of bone mineral accrual at this site. To support this, IGFBP-3 was also included as a positive predictor of change in Prox forearm BMC over time (Table 15). Taken together, these findings suggest that while IGF-1 and IGFBP-3 are inversely associated with BMC measurements, their presence indicates an increase in bone mineral deposition over time.

Glucocorticoid excess is associated with reduced BMC and BMD and increased fracture incidence (37). Glucocorticoids reduce osteoblastic bone formation (38) and shift bone turnover in favor of bone resorption (39). Furthermore, there is some evidence to suggest that excess cortisol can disrupt the GH-IGF-1 axis (38) as well as reduced sex-steroid production (39). Little information is available, however, on bone mineral status among women with high-end cortisol concentrations, within the normal range. To our surprise, we found positive correlations between urinary cortisol excretion and LS BMC and BMD (Table 9). Twenty-four hour urine samples were returned by only 40 women who participated in the 6-month follow-up, so urinary cortisol concentration was not included in regression equations. However, the baseline mean salivary cortisol concentration was positively associated with change in UD forearm BMD (Table 12). While high cortisol concentrations, either from endogenous production or pharmaceutical use, have been associated with increase bone resorption (39), these data indicate that within a normal range, higher cortisol concentrations appear beneficial for bone mass and may be associated with the effect that cortisol has on increasing osteoblast differentiation at normal physiological levels (38).

Calcium is easily the most recognized and researched nutrient in relation to bone health, followed by phosphorus and vitamin D. Information beyond these nutrients is not overly

abundant and often contradictory, especially for the young adult population. However, the young-adult years are quite possibly the final window of opportunity to optimize peak BMD. Therefore, it is necessary that relationships between dietary variables and bone health be investigated since dietary intake can, in most cases, be easily modified.

Bivariate correlations showed that dietary calcium and phosphorus had significant and positive relationships with certain regional BMC measures and that vitamin D had significant and positive associations with certain regional and TB BMC as well as certain BMD measures (Table 8). These results are not surprising given that the most abundant minerals found in bone are calcium and phosphorus and given that vitamin D is a key regulator of calcium homeostasis. With inadequate vitamin D, serum calcium is reduced and intestinal calcium absorption is compromised which results in bone resorption (40). Previous studies have demonstrated positive associations between vitamin D and BMD in young adults (41,42). Vitamin D was also included as a positive predictor of TPF and UD forearm BMC (Table 13) and Mid forearm BMD (Table 14) as well as change in FN BMC (Table 15) over time. However, vitamin D was inversely associated with changes in total and regional forearm BMC over time (Table 15), indicating variable effects of vitamin D on weight bearing and non-weight bearing skeletal sites.

Calcium alone was not present in any regression model, but the calcium to phosphorus ratio was a positive predictor of TPF BMD (Table 14). Teegarden et al (43) found similar positive relationships between the calcium to phosphorus ratio and BMC and BMD. While calcium and phosphorous are both essential to bone, the ability for phosphorus to bind calcium and reduce its availability suggests that calcium must be provided at a somewhat higher proportion to phosphorus, which is supported here by the positive association with TPF BMD. Still, it does not appear that a low phosphorus intake is beneficial considering that phosphorus intake alone was included as a positive predictor of UD forearm BMC change (Table 15), demonstrating that adequate intake of both phosphorus and calcium are important for bone.

Similarly, the calcium to protein ratio was a positive predictor of Troc BMD (Table 14) but was also a negative predictor of change in FN BMC over time (Table 15). It appears that high protein diets can enhance urinary calcium excretion (44), and it has been recommended that individuals consuming high protein diets ensure adequate calcium intakes. But, these recommendations are not always supported research. Researchers argue that high protein consumption does (45) and does not (46) adversely affect bone. In addition, some studies have

demonstrated that a high calcium to protein ratio is associated with higher bone mass (2,43); others have found no association (47), and still others report inverse associations (42). We found conflicting results as well.

While total protein intake was not associated with any bone mineral measurement or marker of bone turnover, total energy intake was. Total energy intake was inversely associated with TF BMC in regression analyses, but only after dietary fat was included in the model (Table 13). Furthermore, energy intake was positively correlated with change in TB BMC over time (Table 11). This is interesting considering that subjects reportedly consumed significantly fewer kJ at 6-months versus at baseline. Moderate energy restriction without weight loss has not been examined in relation to bone, but weight loss diets have clearly demonstrated that a direct association between weight reduction and bone loss exists (11,28,29). Furthermore, acute fasting has also been shown to rapidly alter biochemical markers of bone turnover (48,49). It seems apparent that in addition to weight maintenance, adequate energy intake is beneficial to BMC and BMD and that our findings support a need for further investigation of the effects of chronic dieting on bone since, despite chronic energy restriction, chronic dieters often maintain body weight.

Further implications for dieters come from the positive association between dietary fat intake and TB BMC change in bivariate correlation analyses (Table 11). Furthermore, in regression models, dietary fat was a positive predictor of LS, Troc, TF, UD forearm, Mid forearm and Prox forearm BMC (Table 13), as well as Prox forearm BMD (Table 14), serum osteocalcin (Table 13), and change in TB BMC over time (Table 15). While dietary fat is essential for absorption of fat-soluble nutrients important for bone, such as vitamin D, it is not clear exactly what role dietary fat has in bone mineral accrual or maintenance. Furthermore, high dietary fat intake can reduce intestinal availability of calcium through formation of calcium soaps (50). Still, this is not the only research to suggest positive associations between dietary fat and bone mass. Trichopoulou et al (51) identified positive associations of energy intake and monounsaturated fat intake with BMD in adult men and women, and Gunnes and Lehmann (52) showed that saturated fat was positively associated with BMD in children. These findings are interesting and suggest a need for further research into these associations due to the frequent use of reduced-fat diets for reducing body weight, both of which might impair bone mineral status. Conversely, while dietary fat appears positively associated with bone mass, in regression models,

167

carbohydrate intake was a significant and negative predictor of BMC change in the UD forearm and Mid forearm regions (Table 15). While we did not adjust for simple versus complex carbohydrate consumption, it has been found that older Americans who consume high amounts of candy and sweets have significantly lower BMD than age- and gender-matched counterparts (53).

In addition, carbohydrate food sources are often a good source of dietary fiber. While dietary fiber did not have any significant associations with BMC or BMD measurements, it did have a significant inverse association with serum osteocalcin concentration (Table 8). Other studies have found inverse associations between dietary fiber and BMD in pre-menopausal women (54). Dietary fiber has the ability to bind nutrients and prevent their absorption (55-57) and may compromise bone health by reducing availability of nutrients essential for bone health. Other investigators have reported a higher BMD at a higher consumption of food containing fiber, such as fruits and vegetables, however (52,58,59), and despite our findings of an inverse relationship between dietary fiber and serum osteocalcin, we found a significant positive correlation between dietary fiber intake and change in WT BMD over time (Table 11).

Magnesium was also negatively associated with serum osteocalcin (Table 8). Yet, magnesium is essential for maintaining calcium balance as it is required for function of parathyroid hormone, calcitonin, and calcitriol (60,61). Still, magnesium and calcium metabolism are inter-related, and at higher intakes, it is plausible that magnesium affects calcium balance so that bone formation is reduced but these assumptions are not supported due to the lack of a relationship between magnesium intake and changes in BMC or BMD over time. It is possible though that women with high magnesium intakes have less bone mineral accrual which was not seen in this study due to the relatively short time frame with regard to bone remodeling. But considering that other researchers have found magnesium intake associated with high BMD (58,59,62), we would not necessarily expect to find negative associations even if this study were carried out for a longer time period. To support this, fruits and vegetables are a good source of magnesium and other nutrients found in these foods, such as vitamin C was positively associated with change in TB BMC (Table 11). Moreover, we identified potassium as a positive predictor of certain BMC changes and vitamin C as a positive predictor of change in Troc BMC and UD forearm BMD in regression models. Previous studies support our finding of positive

relationships between these variables and BMD and likely attribute these benefits to a high intake of fruits and vegetables (59,62).

Vitamin A was inversely associated with urinary NTx excretion (Table 8). Vitamin A has been shown to affect bone turnover, but primarily through its role in up-regulation of osteoclastic bone resorption resulting in increased incidence of bone fracture (63). Our results are opposite of what would be expected considering previous findings, but we did not evaluate retinal intake separately from carotenoid consumption. While retinal has been associated with reduced BMD, carotenoids do not appear to have the same effects (63), and if fruits and vegetables account for a large portion of the vitamin A intake (as β-carotene), then benefits for bone are possible.

There is little evidence to suggest a relationship between iron intake and bone, except that iron metabolism may be intertwined with other minerals important for bone metabolism, namely, zinc (64,65). Iron emerged as a negative predictor of TPF, UD forearm, and Prox forearm BMC (Table 13) yet also emerged as a positive predictor of Mid forearm BMC change over time (Table 15). While little direct evident is available, animal studies have found that rats fed iron-deficient diets have compromised bone health and if calcium is concomitantly reduced, BMD is impaired even further (66). Further research is needed to evaluate the relationship between iron intake and bone metabolism, particularly in young women who may be more prone to iron deficiency anemia or supplemental iron usage.

Sodium emerged as a negative predictor of urinary NTx (Table 13) and Prox forearm BMD (Table 14). High sodium diets are associated with increased urinary calcium excretion (44), which may reduce the availability of calcium for bone remodeling and bone mineral accrual.

Finally, we included alcohol consumption as a variable in regression analyses and found that it was a significant and inverse predictor of BMC and BMD at several skeletal sites and change in WT BMC (Table 15), yet alcohol consumption was also a positive predictor of UD forearm BMC (Table 15) and TPF and UD forearm BMD (Table 16). Little information is available on the associations between alcohol consumption and bone mass in young women, and researchers have found both positive (67) and negative (68) associations between alcohol intake and BMD. We did not account for grams of alcohol or type of alcohol consumed but rather number of drinks on average per week. Furthermore, 18 (34%) of our subjects abstained from

alcohol intake while another 10 (19%) consumed < 1 drink per week. Still, it does appear that either alcohol itself or lifestyle factors common in young women who consume alcohol might impact bone, either beneficially of adversely; therefore, these relationships require more attention.

In conclusion, no significant differences were found in BMC or BMD measures between CER groups, and changes in BMC or BMD over time were not significantly different between groups. We did, however, find that CER score was significantly and inversely related to change in LS BMD over time ant that regression analyses indicated a negative relationship between CER score and bone health in these young women. In addition to these direct implications for bone health, indirect associations between CER and bone health can be made from the finding of higher body FM and BF% in the high CER group since these variables were inversely associated with bone mass. Furthermore, serum osteocalcin was significantly lower in the high CER subjects, which indicates a lower rate of bone accrual in these women and may relate to the relationship between CER score and LS BMD change. However, unlike previous studies (7,8) in which significantly higher cortisol concentrations in high CER groups compared to low CER groups have been shown, we did not find that cortisol was higher in women with high CER scores, and we found that cortisol concentrations correlated positively with bone mass. We conclude that evidence does support a negative relationship between CER and bone health but this association cannot be explained by dietary or physiological parameters measured within this study and are not fully supported over time.

Conclusions drawn from our data indicate that dietary intake variables are significantly associated with BMC and BMD. While most research has focused on calcium intake, and public health messages regarding osteoporosis prevention have focused primarily on calcium consumption, it is clear that other dietary factors and behaviors are at work as well. Maximizing bone health early in life may help protect against osteoporotic fractures later in life, and a need for further understanding of the impact of dietary intake, nutrient interactions, healthy weight loss behaviors and bone health are apparent. Young adults are often influenced by a desire to "look good" than to maximize health and prevent chronic disease. As a result, dietary intake may be grossly adjusted. These data here support the contention that dietary adequacy is important. Implications for dieters are vast. It has been shown that a high body mass is protective of bone and that along with weight loss, bone mineral is lost. It also appears that

dietary intake adjustments or extremes, regardless of weight changes, can impact bone. Dieters who drastically limit dietary fat intake may compromise bone health and if low-fat, high sodium processed foods are chosen as replacements, further implications for bone seem apparent. While mixed results regarding dietary fiber are present, fruits and vegetables, which are good sources of fiber, appear to benefit bone mass in young adults. Overall, the message to the public should change and while continuing to emphasize the importance of dietary calcium consumption, the message should stress dietary adequacy to ensure proper intake of a variety of nutrients and avoid imbalances. This message will likely benefit bone as well as protect against other chronic diseases.

Results from this study are limited to moderately active young women of healthy BMI. Much of these data are limited by the accuracy of the tools used and recollection of subjects. Dietary information is also limited to recent intake habits and does not account for intake in the formative years. In recognizing the limitations of this study, results are interesting and beneficial in demonstrating that dietary intake, including nutrients other than calcium, are related to bone health. Further research is needed to identify optimal intakes level to maximize BMD and prevent osteoporosis. In addition, confounding lifestyle factors that might impact nutritional status must be further investigated.

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CHAPTER VI
SUMMARY AND FUTURE DIRECTIONS

The cognitive eating restraint (CER) subscale of the Eating Inventory is not useful for identifying chronic dieters based on physiological and behavioral characteristics (Chapter 3). Women with high CER scores did not possess lower resting energy expenditure (REE) and dietary intake, cortisol concentrations, and exercise habits were not different between groups. Likewise, a simple question of dieting was not successful at identifying chronic dieters in this group of women. Both questionnaires were, however, successful at identifying individuals who may perceive themselves as having a higher fat mass and percent body fat (BF%). These young women likely engage in restrained eating behaviors but when evaluated from food-frequency questionnaires, which evaluates dietary intake over an extended period, average daily dietary intake is not reduced compared to low CER women or women who never diet. Therefore, we were unsuccessful at supporting our hypothesis that the CER subscale of the Eating inventory can serve as a simple, yet appropriate proxy for identifying women who chronically diet.

In Chapter 4, participants were compared cross-sectionally based on having either a high (n = 31) or low CER score (n = 34). In addition to the lack of significant differences found between groups with regard to dietary intake, we also examined salivary and urinary cortisol, and serum growth hormone, insulin-like growth factor-1, and insulin like-growth factor-binding protein-3 and found that concentrations of these hormones were not significantly different between groups. We also analyzed biochemical markers of bone formation (i.e., serum osteocalcin) and bone resorption (i.e., urinary NTx) and total body and site-specific bone mineral content (BMC) and bone mineral density (BMD) and found no apparent differences in these measurements between groups.

A longitudinal investigation (Chapter 5) was conducted to compare high (n = 27) and low (n=26) CER groups at baseline and 6-months. At baseline, the high CER group possessed a greater fat mass and BF% as well as a lower mean concentration of serum osteocalcin which may indicate a lower rate of bone formation in this group. No other significant differences were noted at baseline in any behavioral, physiological, or body composition measurement, including BMC and BMD. Repeated measures analysis of variance showed a significant Group x Time

179

interaction for salivary cortisol. At 6-months, women in the high CER group had significantly lower salivary cortisol compared to baseline, while women in the low CER group had no change significant change in salivary cortisol. This finding was interesting in that other investigators have suggested negative implications for bone health among females with high CER based on findings of higher salivary or urinary cortisol measurements. We do not support such implications due to the lack of significant differences at baseline and a reduced salivary cortisol concentration at 6-months in our high CER group. Furthermore, we found no direct indications for impaired bone health in high CER participants since groups did not differ in BMC or BMD measurements at baseline and Group x Time interactions were not significant. Therefore, despite the significant difference in serum osteocalcin between high and low CER groups, we found no further evidence to support the contention that CER imparts direct or indirect adverse effects on bone health. These results, however, are limited to young women with normal body mass index, regular menstrual cycles, and limited physical activity and who do not lower energy and nutrient intake.

Because no significant group differences in bone measurements were found between high and low CER groups and information regarding dietary intake and bone health is limited for this population, we conducted correlation and regression analyses in order to investigate relationships between select lifestyle, anthropometric, dietary, and biochemical variables with BMC and BMD measurements (Chapters 4 & 5). Not surprisingly, we found that body mass, and variables contributing to body mass, were positively associated with BMC and BMD. Vitamin D was consistently found to be positively associated with BMC and BMD variables at baseline while alcohol intake was inversely associated with baseline measurements. Bivariate regression showed that fruit consumption was positively correlated with BMC and BMD and nutrients abundant in this food group, such as potassium and vitamin C, were similarly associated. Complex, and often conflicting, relationships between individual nutrients with baseline bone measurements and changes over time were common. Therefore, definitive conclusions cannot be drawn regarding specific nutrients, but it is apparent that dietary intake is an important factor related to BMC and BMD. Because young women are the target population of this investigation and dieting is common in this group, implications for dieters were discussed (Chapters 4 & 5).

The effects of chronic dieting on bone health in young women cannot be answered from this investigation because a simple method of identifying chronic dieters remains unidentified. A

tool for easily separating "dieters" from individuals practicing healthy eating behaviors is needed. It is recommended that the CER questionnaire be re-examined and possibly modified as it appears more useful as a tool for identification of individuals who practice positive dietary behaviors rather than restrained eating behaviors. Investigations of food consumption patterns, dietary trends, and weight loss behaviors among young adults are needed and considering the lack of information regarding dietary intake and bone health among young-adults, further research is recommended. Studies focusing on overall diet as well as single nutrients and nutrient interactions beyond those most commonly associated with bone health, such as calcium and phosphorus, are also recommended.

APPENDIX

Repeated Measures Analysis of Variance Tables

Table 1. Repeated measures analysis of variance for bone mineral content (BMC) measurements

DXA Measurement BMC (g)	Variable	DF	Mean Squared	F	p>F
Total Body	Time	1	374.9	0.63	0.451
	Time*Group	1	1037.6	1.73	0.19
	Error (Time)	51	600.3		
	Group	1	148.5	0.001	0.971
	Error (Group)	51	113,184.6		
Lumbar Spine	Time	1	1.8	1.202	0.271
	Time*Group	1	2.5	1.735	0.194
	Error (Time)	51	1.5		
	Group	1	143.0	0.938	0.337
	Error (Group)	51	152.3		
Total Proximal Femur	Time	1	0.4	0.018	0.909
	Time*Group	1	2.1	1.015	0.318
	Error (Time)	51	2.1		
	Group	1	8.4	0.150	0.700
	Error (Group)	51	56.2		
Femoral Neck	Time	1	0.2	1.414	0.245
	Time*Group	1	0.1	0.758	0.388
	Error (Time)	51	0.2		
	Group	1	0.5	0.086	0.771
	Error (Group)	51	0.6		
Ward's Triangle	Time	1	0.3	4.927	0.030
	Time*Group	1	0.0	0.025	0.875
	Error (Time)	51	0.0		
	Group	1	0.0	0.019	0.892
	Error (Group)	51	0.0		
Trocanter	Time	1	0.0	0.977	0.331
	Time*Group	1	0.0	0.496	0.485
	Error (Time)	51	0.0		
	Group	1	0.2	0.055	0.815
	Error (Group)	51	3.8		
Total Forearm	Time	1	0.0	7.7	0.008
	Time*Group	1	0.0	0.612	0.438
	Error (Time)	51	0.0		
	Group	1	0.6	0.178	0.675
	Error (Group)	51	3.5		
Ultradistal Forearm	Time	1	0.0	0.172	0.709
	Time*Group	1	0.0	2.985	0.090
	Error (Time)	51	0.0		
	Group	1	0.0	0.009	0.926
	Error (Group)	51	0.1		

Mid Forearm	Time	1	0.0	6.823	0.012
	Time*Group	1	0.0	0.384	0.538
	Error (Time)	51	0.0		
	Group	1	0.3	0.182	0.692
	Error (Group)	51	1.6		
Proximal Forearm	Time	1	0.0	6.219	0.015
	Time*Group	1	0.0	0.189	0.665
	Error (Time)	51	0.0		
	Group	1	0.0	0.279	0.600
	Error (Group)	51	0.2		

Table 2. Repeated measures analysis of variance for bone mineral density (BMD) measurements

DXA Measurement BMD (g/cm^2)	Variable	DF	Mean Squared	F	p>F
Total Body	Time	1	0.0	0.522	0.462
	Time*Group	1	0.0	2.825	0.099
	Error (Time)	51	0.0		
	Group	1	0.0	0.113	0.783
	Error (Group)	51	0.0		
Lumbar Spine	Time	1	0.0	0.091	0.753
	Time*Group	1	0.0	0.407	0.526
	Error (Time)	51	0.0		
	Group	1	0.0	1.207	0.277
	Error (Group)	51	0.0		
Total Proximal Femur	Time	1	0.0	5.075	0.028
	Time*Group	1	0.0	0.087	0.769
	Error (Time)	51	0.0		
	Group	1	0.0	0.122	0.728
	Error (Group)	51	0.0		
Femoral Neck	Time	1	0.0	0.216	0.638
	Time*Group	1	0.0	0.078	0.781
	Error (Time)	51	0.0		
	Group	1	0.0	0.001	0.977
	Error (Group)	51	0.0		
Ward's Triangle	Time	1	0.0	0.084	0.755
	Time*Group	1	0.0	2.030	0.160
	Error (Time)	51	0.0		
	Group	1	0.0	0.014	0.906
	Error (Group)	51	0.0		
Trocanter	Time	1	0.0	2.441	0.119
	Time*Group	1	0.0	0.299	0.587
	Error (Time)	51	0.0		
	Group	1	0.0	0.025	0.874
	Error (Group)	51	0.0		
Total Forearm	Time	1	0.0	1.707	0.191
	Time*Group	1	0.0	0.076	0.785
	Error (Time)	51	0.0		
	Group	1	0.0	0.051	0.822
	Error (Group)	51	0.0		
Ultradistal Forearm	Time	1	0.0	0.291	0.450
	Time*Group	1	0.0	0.045	0.834
	Error (Time)	51	0.0		
	Group	1	0.0	0.137	0.712
	Error (Group)	51	0.0		

Mid Forearm	Time	1	0.0	1.572	0.209
	Time*Group	1	0.0	0.104	0.749
	Error (Time)	51	0.0		
	Group	1	0.0	0.158	0.692
	Error (Group)	51	0.0		
Proximal Forearm	Time	1	0.0	0.042	0.838
	Time*Group	1	0.0	0.016	0.900
	Error (Time)	51	0.0		
	Group	1	0.0	0.059	0.808
	Error (Group)	51	0.0		

Table 3. Repeated measures analysis of variance for biochemical measurements.

DXA Measurement BMD (g/cm^2)	Variable	DF	Mean Squared	F	p>F
Serum Osteocalcin (ng/ml)	Time	1	35.5	9.875	0.003
	Time*Group	1	0.0	0.259	0.613
	Error (Time)	46	3.6		
	Group	1	52.5	7.632	0.008
	Error (Group)	46	6.9		
Urinary NTx (BCE/mM creat)	Time	1	1017.7	1.4	0.302
	Time*Group	1	963.4	1.352	0.251
	Error (Time)	46	712.6		
	Group	1	75.5	0.047	0.829
	Error (Group)	46	1,607.4		
Salivary cortisol (ug/dL)	Time	1	0.0	2.661	0.112
	Time*Group	1	0.0	4.778	0.033
	Error (Time)	46	0.0		
	Group	1	0.0	0.755	0.389
	Error (Group)	46	0.0		
Serum estradiol (pg/mL)	Time	1	4538.5	6.484	0.013
	Time*Group	1	25.8	0.037	0.849
	Error (Time)	46	699.9		
	Group	1	532.8	0.512	0.478
	Error (Group)	46	1,040.1		
Serum progesterone (ng/mL)	Time	1	0.0	3.885	0.050
	Time*Group	1	0.0	1.535	0.222
	Error (Time)	46	0.0		
	Group	1	0.2	2.113	0.153
	Error (Group)	46	0.1		
Serum growth hormone (ng/mL)	Time	1	36.2	2.130	0.111
	Time*Group	1	0.0	0.005	0.945
	Error (Time)	46	17.0		
	Group	1	16.8	0.774	0.383
	Error (Group)	46	21.6		
Serum insulin-like growth factor-I (ug/mL)	Time	1	5887.5	14.0	0.001
	Time*Group	1	58.1	0.138	0.712
	Error (Time)	46	421.1		
	Group	1	1,080.7	0.562	0.457
	Error (Group)	46	1,922.0		
Serum insulin-like growth factor binding protein-3 (mg/L)	Time	1	1,113.5	2.310	0.155
	Time*Group	1	1,261.0	2.615	0.113
	Error (Time)	46	482.1		
	Group	1	193.8	0.216	0.644
	Error (Group)	46	896.9		

VITA

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