RELATIONSHIPS BETWEEN SERUM LEPTIN AND BONE MINERAL WITH EATING RESTRAINT OR WEIGHT LOSS

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Abstract

Relationships Between Serum Leptin and Bone Mineral with Eating Restraint or Weight Loss

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High body weight seems protective of bone mass, specifically bone mineral content (BMC) and bone mineral density (BMD), thereby reducing the risk of osteoporosis. Cognitive eating restraint (CER), diet composition, and the satiety hormone, leptin, produced by adipocytes, are associated with body mass and may also influence bone mass. Few studies have examined these relationships. To investigate the relationship between leptin and CER score, 36 premenopausal, healthy weight women, as defined by body mass index (BMI) of 18-25 kg/m^2, aged 18-25 years were studied. Women were categorized by baseline Eating Inventory questionnaire scores into either the high CER group (score > 9, n = 20) or low CER group (score $\leq$ 9, n = 16). Serum leptin concentration was significantly lower in the low CER group versus high CER group at baseline. A positive relationship between serum leptin concentration and body fat mass and body fat % in normal weight women despite differences in CER scores was observed. In a separate study, overweight and obese women, (BMI $\geq$ 25 to $\leq$ 43 kg/m^2), aged 32-45 years, were randomly assigned to either a low-carbohydrate, high-protein (LCHP) or low-fat, high-carbohydrate (LFHC) diet for 12 weeks. Serum leptin concentration was significantly greater in the LCHP versus the LFHC diet group at 12 weeks ($p < 0.05$). Over time, significant decreases in serum leptin concentration, BMI, body weight, total lean mass, total fat mass, and body fat % were observed in both diet groups. Serum leptin concentration was positively associated with body weight, fat mass, and body fat % regardless of diet consumed. Both studies are novel in their respective populations and show no direct link between leptin and bone mass when considered in the context of CER or diet composition.

DEDICATION

This thesis is dedicated to James, who helped make this journey possible with his courage, support and love. Thank you for being my best friend and believing in me.

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"Go confidently in the direction of your dreams! Live the life you've imagined." -THOREAU

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CHAPTER I

INTRODUCTION

The percentage of people who are obese in the United States has nearly doubled from 11.6% in 1990 to 22.1% in 2002 (1). Obesity appears to have one benefit to humans. High body weight seems protective of bone mineral density (BMD), thereby reducing the risk of osteoporosis. Osteoporosis affects 25 million Americans, costing more than $6 billion each year to treat (2, 3). Of the approximately 800,000 hip fractures that occur annually, one in five will result in death from secondary complications of the injury and half of those individuals who survive will be dependent on others for help with even the most basic of activities (2, 4).

The ob gene discovered in 1994 is responsible for the production of a hormone called leptin that seems to be associated to both obesity and osteoporosis. Leptin is synthesized mainly by white adipose tissue in response to the amount of energy, stored as fat in adipose tissue, that is available to the body (5). Leptin's interactions with receptors in the hypothalamus control the production of neuropeptides which control energy balance by influencing food consumption through satiety signals (6-9). Because leptin plays a role in maintaining energy balance and homeostasis in the body, leptin may have an important role in obesity (6, 10). Serum leptin concentration tends to increase as body fat increases (8, 10-12). Interestingly, serum leptin is affected by weight change; when body weight decreases, serum leptin decreases (13, 14).

The protective effect of body weight on bone density has been attributed primarily to the stress placed on the skeleton due to mechanical loading (13, 15-19). Theoretically, bone adapts by becoming denser and stronger as the demand from body weight increases. Some researchers, however, believe that factors other than skeletal loading influence bone density (18). To date, a number of hypotheses have been developed regarding whether this observed relationship between body weight and bone sparing could be a result of leptin. Because leptin is related to obesity and obesity to BMD, leptin is suspected to influence BMD.

All the physiologic and metabolic functions for leptin have not yet been elucidated, but leptin receptors are found throughout the body, suggesting that this hormone impacts the body in more ways than just energy metabolism (5). Recently, bone metabolism has been shown to be regulated by leptin but through different routes than those used to regulate energy homeostasis (20-22). *In vivo* studies have demonstrated an inverse relationship between serum leptin concentration and BMD in premenopausal (23) and peri- and post-menopausal women (24).

To date, only one published study has examined the effect of a high- versus low-carbohydrate diet on serum leptin concentration (25). Koutsari et al. (25) reported that in both fasting and post-prandial states, women who consumed a high-carbohydrate diet for three days had a higher mean serum leptin concentration compared to when they consumed a low-carbohydrate diet for three days. Findings from this study suggest that a high-carbohydrate diet

may be detrimental to bone health due to a rise in serum leptin. However, this previous study did not involve weight loss or include a high-protein component of the diet. Energy deficits have been shown to decrease serum leptin (26) which would suggest a benefit to bone mass; however, considered collectively, energy restriction results in adverse changes in bone cell metabolism toward increased bone resorption (27, 28).

Although an increase in serum leptin with consumption of a high-carbohydrate diet was reported, Koutsari et al. (25) did not examine weight loss or fat mass changes. Moreover, cognitive eating restraint (CER) was not included as a variable. Cognitive eating restraint, the conscious control over what one consumes to regulate body weight, has been shown to predict success with weight loss (29). Bone mineral content appears to be linked to CER and has been associated with low BMD in normal weight (30, 31) and overweight/obese premenopausal women (32). Bacon et al. (32) showed that women with higher CER scores have lower bone content. Van Loan et al. (31) also found that BMC varied significantly between women with low and high CER scores with the lowest BMC observed in women weighing <71 kg with high CER.

A study of 25 women, randomized to an Atkins diet group (n = 13) or a traditional diet group (n = 12), demonstrated that women in the Atkins diet group had a significant decrease in hip BMD (1.5%), whereas women in the traditional diet group did not during this 12-week period. While serum leptin would have been expected to decrease similarly in both diet groups due to

comparable body weight and fat mass losses, there may have been differential changes due to diet composition or other factors, such as eating restraint.

Beiseigel et al. (33) indicated that premenopausal women with high CER differed from women with low CER only in body fat mass and body fat percentage. Preliminary data demonstrated that mean serum leptin concentrations in these women were significantly different. Women with high CER scores (n = 5) had significantly higher mean serum leptin concentration compared to women with low CER scores (n = 5). Cognitive eating restraint has been posited to invoke a central stress response, perhaps in conjunction with serum leptin to control eating behavior and energy metabolism and potentially bone metabolism. Thus, changes and differences in BMD may be explained by serum leptin shifts that occur with specific types of diets and with the presence of CER.

With so many conflicting reports, more research is needed regarding the effects of leptin on bone and in relation to eating restraint and dieting for weight loss and control. Both osteoporosis and obesity are major public health concerns; if leptin is related to both diseases it could be a key factor in treatment as well as prevention. Coupled with the epidemic of overweight and obesity and the call for weight reduction, osteoporosis may present an even greater economic and societal burden in the future. The work proposed here is important as it addresses the prevention of two major chronic diseases. Data generated by this research will clarify questions related to the impact of specific

diets for weight loss, actual body mass loss, and CER on bone mineral status in premenopausal women.

KEY WORDS:

Bone mineral content; Bone mineral density; Cognitive eating restraint; Diet; Serum leptin; Women

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CHAPTER II

REVIEW OF LITERATURE

The prevalence of obesity in the United States (U.S.) is increasing at an alarming rate. The percentage of people who are obese in the U.S. has nearly doubled from 11.6% in 1990 to 22.1% in 2002 (1). At this rate, obesity is likely to replace smoking as the leading cause of preventable death in the U.S. (2). Paradoxically, obesity may protect individuals from another costly disease, osteoporosis (3). Osteoporosis affects 25 million Americans, costing more than $6 billion each year to treat (4, 5). Of the approximately 800,000 hip fractures that occur annually, 1 in 5 will result in death from secondary complications of the injury, and half of those individuals who survive will be dependent on others for help with even the most basic of activities (4, 6). Unfortunately, individuals who suffer one fracture are at greater risk of suffering another fracture in the future (7).

Types of Bone

Bone is necessary for life. It provides support to the body, protects vital organs, produces red blood cells and other cells, enables movement, and serves as a reservoir to calcium and other essential micronutrients. Bone is composed of an organic matrix of collagen and mineral salts. Type I collagen fibers account for approximately 90% of this organic matrix (8), while calcium

and phosphorus account for 80-90% of the mineral in bone (9). Magnesium represents about 0.5-1.0% of the mineral in bone, but this amount is highly variable among individuals (10). Collagen fibers and minerals salts are responsible for the strength as well as structural and functional capacity of bone (8, 11).

Two types of bone make up the human skeleton: cortical and trabecular. Cortical bone (also known as compact bone) makes up the majority of the skeleton, most notably the shafts of the long bones. Although trabecular bone (also known as cancellous or spongy bone) accounts for less than one-quarter of the skeleton, it is present in all other bone structures of the body including the ends of the long bones, the iliac crest and the vertebrae. Both trabecular and cortical bone serve in weight-bearing roles and may demonstrate osteoporosis.

Bone Remodeling

Bone is a dynamic, living tissue that is constantly being modified. This modification called bone remodeling, bone turnover, or bone metabolism, begins *in utero* and continues throughout life. Ossification is the first modification event in which bone forms from a cartilage template (12). Bone remodeling is performed by specialized cells and occurs at many sites of the skeleton simultaneously. Resorption occurs by cells called osteoclasts that dissolve existing bone matrix and are partially under endocrine control (3).

Bone formation occurs by osteoblasts and has only recently begun to be understood (13). While these two phases are always occurring, they are occurring at different speeds (3). The "faster" rate involves large quantities of bone being deposited in an area of the skeleton. This speed is used to repair a fracture. However, if bone mass is adequate and remodeling is occurring only as a means of maintenance, the "slower" speed is used, and no net change in bone mass is observed (3).

For approximately the first two decades of life, bone formation will outpace bone resorption resulting in accumulation of bone mass (9). After peak bone mass is reached, bone resorption and bone formation processes are coupled and occur at approximately the same rate resulting in no net change of bone mass. However, as one ages, these processes uncouple and resorption gradually overtakes formation such that bone mass progressively decreases throughout the remainder of life. This uncoupling of bone formation and bone resorption may lead to osteoporosis depending, in part, on the quantity and quality of bone at peak bone mass (PBM).

Osteoporosis or "porous bone" is the most common bone remodeling disease in the developed world; as life expectancy continues to increase, the prevalence of osteoporosis is expected to increase (9). For an individual to be diagnosed with osteoporosis, an individual's bone mineral density (BMD) is compared with the mean BMD of the young-adult reference (9). Table 1 shows the current World Health Organization (WHO) criteria for diagnosis of osteoporosis. Anyone can develop osteoporosis, regardless of the presence or

absence of risk factors (Table 2). Alternatively, an individual can have many risk factors and still not develop osteoporosis.

Table 1: Criteria for Osteoporosis Diagnosis

Bone Mass Category	Standard Deviation (SD) based on T-Score
Normal	0 to -1 SD
Osteopenia	-1 SD to -2.5 SD
Osteoporosis	≥ -2.5 SD

Osteoporosis may be classified by characteristics of the skeleton (14). Type I or postmenopausal (estrogen-related) osteoporosis results more often in vertebral fractures due to loss of trabecular bone in the vertebrae (14). This type of fracture is more likely to occur spontaneously. Type II or senile (age-related) osteoporosis is characterized by weakened cortical bone, although trabecular bone can also be affected, and often results in a hip fracture due to impact, typically a fall (14).

Once osteoporosis is present, further bone deterioration seems inevitable. Whitson et al. (7) found that while bone formation decreased significantly following hip replacement, bone resorption did not change, resulting in a net loss of bone mass. Similarly, Takahashi et al. (14) observed that while both hip and vertebral fractures resulted in increased bone resorption markers, the increase was larger with hip fractures than vertebral fractures. Interestingly, bone formation markers increased in patients with vertebral fractures but decreased in individuals with hip fractures suggesting that the location of the fracture influences the change in BMD (14).

Table 2: Modifiable and Non-Modifiable Risk Factors for Osteoporosis

OSTEOPOROSIS RISK FACTORS

Modifiable	Non – Modifiable
Low body weight [1]	Fracture after age 50 [1]
Low calcium intake [1,2,3]	Family history of fracture [1,3]
Low Vitamin D intake [1,3]	Female [1,2,3]
Current low bone mass [1]	Small body frame [1,2,3]
Sedentary lifestyle [1,2,3]	Age [1,2,3]
Cigarette smoking [1,3]	Family history of osteoporosis [1]
Excessive alcohol consumption [1,3]	Postmenopausal [2]
Anorexia nervosa [1,3]	Caucasian and Asian [1,2,3]
Amenorrhea [1,3]	
Low estrogen level in female [1,3]	
Low testosterone level in male [1,3]	
Some chronic medical conditions [1]	
Medication use (i.e., corticosteroids and anticonvulsants) [1,3]	

[1]Adapted from National Osteoporosis Foundation. Fast facts on Osteoporosis.(17)
[2]Adapted from Centers for Disease Control and Prevention. Bone Health: Introduction.(18)
[3]Adapted from National Institute of Health Osteoporosis and Related Bone Diseases – National Resource Center. (19)

In addition, studies have shown bone mass losses in areas other than the location of the insult (7, 14, 15). Specifically, decreases in BMD of the proximal femur and lumbar spine have both been observed following total hip replacement surgery (7). Adolphson et al. (15) noted that patients undergoing hip surgery, who did not dramatically increase physical activity from presurgery levels, experienced clinically significant decreases in lumbar vertebrae BMD (-8.4% in L1, -5.3% in L2, -5.9% in L3) 6-months after surgery. Inactivity following injury has been shown to reduce bone mass (7). Although spine BMD decreased more following vertebral fracture than hip fracture, and femoral neck BMD decreased more following a hip fracture than vertebral fracture, spine and femoral neck BMD both decreased following a fracture at either location (14). Bone loss in these areas may lead to instability, increased risk of future fall, and fracture, because rapid bone loss has been positively associated with bone fractures independent of BMD (16).

Methods to Measure Bone Remodeling

Because measuring human bone structure *in vivo* is undesirably invasive and technically difficult, measurements of bone mass and turnover are used as proxy measures. Specialized equipment can be used to assess bone mass and bone turnover. X-ray techniques, including X-ray diffraction (XRD) and X-ray microtomography (μCT) are useful in creating three-dimensional (3-D) images of bone microstructure (12). Although they cannot give 3-D images of the bone, radiography, histology, and sonography are useful for viewing structural changes in BMD of developing bone (12). Also, while quantitative computed tomography (QCT) and dual-energy X-ray absorptiometry (DXA) cannot give 3-D images of bone, they are invaluable for studying BMD (12). Quantitative computed tomography converts volumetric (g/cm^3) data obtained from a CT scan into bone density (g/cm^2) (15).

Dual-energy X-ray absorptiometry is a commonly utilized technique for assessing bone mass in the form of bone mineral content (BMC) (g) and BMD (g/cm^2). This procedure is quick, non-invasive, and involves low subject burden. Because a small amount of radiation (approximately 1 millirad for a total body scan) passes through the body, the procedure is not recommended for pregnant women. In addition, individuals possessing a large amount of adiposity around the central or visceral portion of the body and tall individuals (height > 72 inches) present challenges for accurate measurement of bone

mineral. Measures by a trained technologist are typically accurate with only 1-2% error.

Compounds in the blood or excreted in urine can be used as indirect indicators of bone formation and resorption (16). Osteoblast and osteoclast biomarkers are expressed in response to bone formation and bone resorption, respectively, and are useful in determining the relative amount of bone turnover occurring (16). This knowledge can be useful in predicting future bone fractures or bone loss as well as overall risk of osteoporosis (20). Most resorptive markers are produced during the breakdown of bone collagen and are subject to daily circadian rhythm (16, 20). On the other hand, markers of bone formation are proteins synthesized in proportion to the amount of bone formed and are not influenced as much by circadian rhythms (16, 20). In addition, bone formation markers respond to treatments more quickly than do bone resorptive markers (within 3 months vs. between 6 -12 months, respectively) (20). Table 3 shows some common biomarkers of bone metabolism.

Table 3: Markers of Bone Formation and Bone Resorption

BONE METABOLISM BIOMARKERS

Formation Markers	Resorption Markers
Aminoterminal propeptide of type I collagen (PINP)	Crosslinked C-telopeptide of type I collagen (CTX)
Osteocalcin (OC)	Free and total deoxypyridinoline (Dpd)
Carboxyterminal propeptide of type I collagen (PICP)	Free and total pyridinoline (Pyd)
Bone-specific alkaline phosphatase (bAP)	Crosslinked N-telopeptide of type I collagen (NTx)
Bone alkaline phosphatase (B-ALP)	Crosslinked C-telopeptide of type I collagen (ICTP)

Factors Controlling Bone Remodeling

Diet

Osteoporosis is a multifaceted disease influenced not only by the non-modifiable risk factor of genetics but also by environment, lifestyle factors, physical activity, and diet. Diet is perhaps the most modifiable risk factor for osteoporosis. Nutritional intervention may be the best primary strategy for preventing bone loss and resulting osteoporosis. Teasing out the effects of

individual nutrients *in vivo* on bone health is essentially impossible. For example, calcium and Vitamin D are the primary ingredients in the recipe for nutritional preventive therapy, but both of these nutrients are impacted by other nutrients as well as hormones (21). Key bone-related nutrients will be discussed individually, but they are intricately linked, and this must be kept in mind. As Ilich (9) astutely points out, these complicated interactions may be the reason for disagreement among study results and conclusions.

CALCIUM:

Calcium and phosphorus make up 80-90% of mineral content of bone (9). Regardless of race, age, or gender, 99% of body calcium is found in the skeleton (9). This leaves a mere 1% readily available to the rest of the body to participate in crucial life functions. Parathyroid hormone (PTH), calcitonin, Vitamin D (calcitriol) and phosphorus act together to insure that the serum concentration does not extend either too far above or below optimal range (22). Hypercalcemia can result from bone resorption or gut absorption of dietary calcium, while hypocalcemia can be caused by disturbances in either PTH or Vitamin D metabolism and function (23).

Obtaining enough dietary calcium can help prevent its release from the skeleton and subsequent weakening of the bones and future osteoporosis. Dietary calcium is especially important during stages of growth when the majority of the skeleton is formed. Although calcium may be the most important dietary nutrient for bone health, its absorption depends partly on other nutrients such as phosphorus and Vitamin D (24).

PHOSPHORUS:

Dietary phosphorus influences calcium absorption and storage (24). Phosphorus is the second most abundant mineral in the human body with 85% of this mineral located in the skeleton (9). Unlike calcium, phosphate deficiency in the diet is rare because it is widely found in foods (9). In fact, the abundance of phosphorus in the diet may be a concern, because high serum phosphorus concentration may cause lowered serum calcium concentration through release of PTH, ultimately resulting in bone loss (9, 24, 25).

MAGNESIUM:

Another mineral known to influence calcium concentrations in the body is magnesium. Even though a relatively small amount of magnesium is present in the body (approximately 25 g), it is not considered to be a trace nutrient because it comprises 0.5-1.0% of bone ash (9, 10). Between one-half and two-thirds of magnesium is found in the skeleton; the rest is found in soft tissues of the body (9, 10). Magnesium is a primary regulator of calcium metabolism and seems to influence bone strength, preservation, and remodeling (21, 26). Magnesium influences calcium homeostasis and bone mass both directly as part of the bone mineral composition and indirectly by affecting the hormones of bone metabolism, such as PTH (9, 10).

In animal studies, deficiency of magnesium influenced all phases of bone remodeling, while excessive concentrations also had detrimental effects by impairing bone crystallization and, in mice, by stimulating bone resorption (10). In humans, magnesium supplementation seems to prevent fractures and

increase bone density, while individuals with osteoporosis have been observed to have low bone magnesium content (10, 26). Serum magnesium concentration is influenced by action of the kidneys; therefore, any problem with the kidneys can ultimately affect an individual's risk of osteoporosis (23).

ZINC:

Zinc and calcium are thought to compete for absorption, but debate exists as to what level of zinc in the diet can cause a clinically significant lowering of calcium absorption. The body contains 1-2 grams of zinc (9). Zinc is widespread throughout the body and is involved in bone mineralization and collagen formation (9). Zinc deficiency has been associated with low bone formation and osteoporosis in both animals and humans (9). As a cofactor for numerous reactions in the body, zinc concentrations influence growth hormone (GH), insulin-like growth factor-1 (IGF-1), and bone metabolism (27).

NONMINERAL SUBSTANCES:

Absorption of non-mineral substances in the body can also influence bone metabolism. Caffeine and fiber are two food components suspected of influencing calcium content in the body. Caffeine consumption increases excretion of calcium and magnesium (28). In younger women, calcium absorption via the intestines can increase to compensate for any additional calcium excretion and may ultimately have no effect on BMC and osteoporosis risk (28). However, older women are not as effective in absorption of calcium from the intestines making them more susceptible to bone demineralization due to calcium excretion from caffeine intake (28). Older women need to be

more cognizant of obtaining adequate dietary calcium, especially if they consume large amounts of caffeine.

Dietary fiber is often thought to bind to minerals such as calcium and to promote excretion rather than absorption. However, a form of fiber called inulin may have the exact opposite effect. Inulin may improve calcium and magnesium absorption (29). In rats, inulin has been shown to increase BMC in growing rats and prevent bone mass loss in ovariectomized rats (29). For human consumption, inulin has begun to be supplemented in calcium containing dairy products such as yogurt to enhance calcium absorption. Although diet can influence bone mass and metabolism, it does not have as strong of a relationship as estrogen or calcitonin with bone; thus, diet should not be used as a solitary treatment for osteoporosis but rather as a basis from which to build an effective therapy (9, 16).

Hormones

As with most living organisms, bone is affected not only by individual genetics, the environment, and lifestyle factors such as diet and physical activity but also by hormones. Generally, bone remodeling and thus bone mass is kept constant through paracrine and autocrine mechanisms (3). Sex hormones and parathyroid hormone (PTH) are involved in bone resorption, and no single hormone has been shown to completely regulate bone formation (3). In addition to PTH, calcitonin and calcitriol have been considered major regulators of bone metabolism for over 30 years (24). This review will be limited to PTH, Vitamin D, calcitonin, and estrogen.

PARATHYROID HORMONE (PTH):

Parathyroid hormone's main responsibility is to increase the serum calcium concentration if it drops below the functional range (24). This can be accomplished by bone resorption with release of calcium to replenish low serum calcium. In addition, PTH is a regulator of Vitamin D metabolism. Because Vitamin D is necessary for calcium to be absorbed at the gut, a low serum Vitamin D concentration will prevent calcium from moving into the bloodstream and into the skeleton for storage (23). These two mechanisms help maintain the serum calcium concentration within an appropriate range required for many vital functions.

VITAMIN D:

Vitamin D is a dietary nutrient in addition being to a hormone. The active form of Vitamin D known as calcitriol [1,25-$(OH)_2$ vitamin D_3] plays a role in both calcium and phosphorus absorption as well as bone metabolism (9). Vitamin D is inversely related to PTH resulting in calcium assimilation into the skeleton rather than release from the skeleton.

Vitamin D is not an essential micronutrient because it can be made from precursors in the skin upon exposure to sunlight. In addition, many food products are fortified with Vitamin D because it is found "naturally" in relatively few foods. However, deficiency is still a formidable concern to persons residing in higher latitudes who have reduced sunlight in winter months and also for individuals who are lactose intolerant as milk is a major dietary source of Vitamin D. Deficiency of Vitamin D can result in low bone

mineralization known as rickets in children and osteomalacia in adults (9). Vitamin D is also thought to participate in muscular coordination; therefore, a deficiency in this vitamin may increase one's risk of falling or other injury, a serious threat to someone who may have reduced bone mass or osteoporosis (9).

CALCITONIN:

The polypeptide hormone calcitonin is produced by the thyroid gland and lowers the serum calcium concentration by influencing the skeleton and kidneys (24, 30, 31). Calcitonin functions to inhibit bone resorption through action on calcitonin receptors located on osteoclasts and to stimulate urinary calcium excretion (30-32). Calcitonin also increases absorption of calcium from the intestine, increases Vitamin D concentration, and stimulates the movement of calcium into bone (30). In addition, calcitonin may decrease bone loss already occurring and even increase BMD in those with osteoporosis (30, 32).

Calcitonin may impact bone mass indirectly by influencing nutrient consumption through reduced appetite and preventing weight gain (31). On the other hand, calcitonin's effectiveness seems to be influenced by diet. In a review of published clinical trials, Nieves et al (33) found that calcitonin supplementation (in the form of nasal salmon calcitonin) decreased lumbar spine bone mass by 0.2% per year in one study. However, in six studies which combined nasal salmon calcitonin supplementation with calcium

supplementation, the average lumbar spine bone mass increased by 2.1%, showing that calcitonin alone was not enough to favorably impact BMD (33).

Similar results were reported in a clinical trial conducted by Hizmetli et al. (32) involving 107 women. These researchers found beneficial BMD and fracture rate changes in patients who received calcitonin supplementation in addition to calcium supplementation (with adequate Vitamin D). Specifically, patients who received 50 IU or 100 IU of calcitonin had significant increases in BMD, while the control group not receiving calcitonin supplementation had reduced BMD (32). Although not statistically significant, after 24 months, 7 of 31 (22.6%) control subjects suffered new fractures, while 3 of 35 (8.6%) subjects in the 50 IU supplementation group suffered new fractures, and only 2 of 41 (4.9%) subjects in the 100 IU supplementation group suffered new fractures.

ESTROGEN:

Estrogen is present in both men and women (34). Although it is often difficult to separate the effects of estrogen from those of androgen on bone, studies have shown that estrogen has a major role in regulation of bone acquisition, BMD, and bone remodeling in both sexes (20, 34). Some evidence lies in the fact that bone mass decreases after menopause, when estrogen production drastically declines (9). Eastell (20) notes that bone loss of the spine following menopause ranges from 2% to 35% within the first 5 years and is caused by estrogen deficiency (20). In addition, BMD of women with poor

compliance to estrogen therapy was significantly lower than women with good compliance (34).

Serum estrogen concentration influences bone throughout life, not solely during the postmenopausal period. Low serum estrogen results in a concurrent decrease in calcium absorption at the intestine and increase in calcium excretion at the kidney (20). Because estrogen also works to increase secretion of GH, which increases bone size, deficiency during adolescence or young adulthood can inhibit achievement of PBM (20).

If estrogen deficiency occurs after approximately the third decade of life, it can increase both irreversible and reversible bone loss (20). Irreversible bone loss occurs if bone turnover is very rapid or if bone remodeling becomes unbalanced or uncoupled and the actions of osteoclasts exceed that of osteoblasts. Reversible bone loss occurs when a high number of bone remodeling units and "increased remodeling space" are present (20). Also, there is a delay between degradation and mineralization resulting in a period of bone deficit. Luckily, effects of estrogen deficit can be reversed with exogenous estrogen administration (20).

Numerous hormone therapies have been developed to prevent the onset or progression of osteoporosis. Estrogen replacement therapy, nasal calcitonin, and PTH can prevent fracture in women with estrogen deficiency (20). Both estrogen and calcitonin therapies work to decrease the rate of bone remodeling. In contrast, PTH stimulates bone formation rather than inhibiting bone resorption via remodeling. Of these, PTH therapy shows the greatest

BMD increases and greatest reduction of fracture risk compared to estrogen or calcitonin (20).

The Relationship between Leptin and Indices of Adiposity

The *ob* gene discovered in 1994 is responsible for the production of a hormone called leptin that has been associated with both obesity and osteoporosis. This polypeptide (146 amino acids) hormone is synthesized mainly by white adipose tissue but also by brown adipose tissue, the placenta, stomach, mammary epithelium, pituitary, hair follicles, and bone (35). Adipocytes are directed by the *ob* gene to produce and secrete leptin in response to the amount of energy, stored as fat in adipose tissue, which is available to the body. Leptin's interactions with receptors in the hypothalamus control the production of neuropeptides which control energy balance in both mice and humans (36-38) by influencing food consumption through satiety and hunger signals (37-39).

In mice, a defect in the *ob* gene that reduces circulating leptin results in obesity. Administering recombinant leptin to these mice causes weight loss (38). In humans, the defective *ob* gene is rare. In fact, increased expression of the *ob* gene is often found in obese subjects (38). The human leptin concentration normally ranges from 2 to 30 ng/mL, with individuals with levels >30 ng/mL considered leptin resistant (40). The finding of increased serum leptin in obese subjects suggests decreased sensitivity to leptin. If leptin's actions in humans are similar to that of rodents, adipocytes should signal the

amount of adipose tissue to the brain such that appetite decreases and energy expenditure increases ultimately resulting in weight loss. However, it appears that obesity in humans is more likely due to central regulation of food intake and energy expenditure than to defective signaling by adipocytes.

To determine whether leptin could be detected in human serum and what effects weight loss had on serum leptin levels (SLL), Considine et al. (38) studied 275 subjects who were divided into two groups: obese (n = 139, 99 female, 40 male, age = 37 ± 11 y) and lean (n = 136, 84 female, 52 male, age = 29 ± 7 y). Obesity was defined as approximately 120% of ideal body weight or body mass index (BMI) >27.3 and >27.8 kg/m^2 for males and females, respectively. Subjects were not taking medications, had no known metabolic disorders (other than obesity), and reported 'stable' body weight $\geq$3 months prior to study. Leptin was detected in all obese subjects (lowest concentration detected was 1.7 ng/mL) but undetected in 7% of lean subjects. Mean serum leptin concentrations were 31.3 ± 24.1 ng/mL in obese subjects and 7.5 ± 9.3 ng/mL in lean subjects. When obesity was defined by BMI, SLL were significantly higher in lean and obese women than lean and obese men. However, this significance disappeared when the gender groups of equivalent body fat percentage [evaluated by bioelectrical impedance analysis (BIA)] were compared. In addition, *ob* mRNA content was higher in 27 obese subjects (29.0 ± 8.7 units) than in 27 lean subjects (18.8 ± 10.9 units) and was correlated with percentage body fat (r = 0.68). Positive correlations were also observed between serum leptin and percentage body fat (r = 0.85) and BMI (r = 0.66).

Serum leptin measurements and biopsies of abdominal adipose tissue were serially repeated in 7 obese subjects who lost 10% of body weight on a controlled 800 kilocalories (kcal) per day liquid protein diet (38). Serum leptin as well as *ob* mRNA content in adipocytes decreased in these subjects immediately after losing 10% of body weight, but these measurements increased again during maintenance of the lower weight. Reduction of 10% body weight was associated with a 53% reduction in serum leptin. Although many factors influence the serum leptin concentration, obesity, more specifically, percentage body fat is very closely related. Adipocyte hypertrophy leads to increased leptin production by individual cells. Based on results of Considine's et al. (38) study, it was concluded that leptin was detectable in human serum, that SLL were correlated with percentage body fat, and that most obese persons were insensitive to endogenous leptin production.

Because obese, leptin deficient mice are known to lose weight when given exogenous leptin, scientists were curious whether the same was true for obese humans. In a randomized, controlled, dose-escalation trial, Heymsfield et al. (41) administered leptin to both obese (n = 73) and lean (n = 54) subjects to examine whether increasing doses of exogenous leptin would lead to dose-dependent weight loss. Obesity was defined as BMI $\geq$27.6 kg/m^2. Subjects injected themselves with recombinant methionyl human leptin each morning [0 (placebo), 0.01, 0.03, 0.10, or 0.30 mg/kg] for 4 weeks. After 4 weeks, only obese subjects continued to receive leptin injections since, in theory, leptin would help subjects lose weight (undesirable for the lean group). Lean subjects

consumed a eucaloric diet, but the obese group consumed a diet deficient by 500 kcal/d. Weight loss increased in all groups with increasing leptin dosages at 4 weeks and among obese subjects at 24 weeks. At 4 weeks, the mean (±SD) weight change was -0.4 (2.0) kg for placebo group (n = 36) and -1.9 (1.6) kg for 0.1 mg/kg dose group (n = 29). At 24 weeks, mean weight loss was -0.7 (5.4) kg for 0.01 mg/kg dose group (n = 6) and -7.1 (8.5) kg for 0.3 mg/kg dose group (n = 8). In addition, fat mass decreased among all subjects at week 4 and among obese subjects at 24 weeks. Furthermore, baseline serum leptin was not related to weight loss at 4 weeks or 24 weeks. No clinically significant adverse effects were reported for major organ systems, but mild-to-moderate reactions including ecchymosis, erythema, and pruritus at injection sites were reported. It is unclear whether weight loss resulted from energy deficiency or leptin supplementation. Regardless, exogenous leptin was found to be safe and effective in the short-term for weight loss.

Ruhl and Everheart (36) measured fasting SLL in males and females (n = 6303, age ≥20 y) who participated in the third National Health and Nutrition Examination Survey (NHANES III) to determine whether a relationship between leptin and demographic and anthropometric measures existed. These analyses were possible because leptin has been shown to be stable through five freeze-thaw cycles for over a period of 29 years (36). Serum leptin concentrations ranged from 0.5 to 193.5 μg/L using radioimmunoassay (RIA). Women of all ethnic groups had substantially higher fasting SLL than men. Among women, mean SLL were highest in non-Hispanic blacks followed by Mexican Americans,

then by non-Hispanic whites. In NHANES III, prevalence of obesity in women (BMI $\geq$27.3 kg/m^2) was 52% in non-Hispanic blacks, 50% in Mexican Americans, and 34% in non-Hispanic whites and corresponded to SLL in these groups. In men, the mean leptin concentration was higher in non-Hispanic whites than non-Hispanic blacks but was not significantly higher than Mexican Americans. In addition, serum leptin was positively associated with BMI in both men ($r = 0.72$) and women ($r = 0.74$) as well as with waist and hip circumference and skinfold thickness measurements. In this large, random, representative sample, serum leptin was predicted by demographic and anthropometric measures.

Because leptin plays a role in maintaining energy balance and homeostasis in the body, leptin may have an important role in obesity (36, 42). In fact, research shows that leptin increased as body fat increased (38, 41-43). Therefore, most overweight and obese individuals have higher SLL compared to lean or normal weight individuals (38, 43-46). The positive correlation between indices of body fatness and leptin held across different ethnic and gender groups. Those ethnic groups with higher rates of obesity also had higher serum leptin (36). Women of all ages and ethnic groups also had higher SLL compared to men (36, 38, 41, 45). In fact, as BMI increased, serum leptin concentration increased by more than two times in women than men (36, 38, 47). Women tend to have more body fat as a percentage of total body weight than men which suggests that the true correlation is dependent on the amount

of body fat and not gender. Interestingly, leptin was affected by weight change; when body weights decreased, SLL decreased (48, 49).

All the physiologic and metabolic functions for leptin have not yet been elucidated, but leptin receptors are found throughout the body (35), suggesting that this hormone affects the body in more ways than just energy metabolism. In fact, leptin is suspected of influencing the reproductive (39, 41-44, 50-57), hematopoietic (47, 50, 53-58), immune (39, 43, 44, 47, 54, 55, 57, 58) and central nervous systems (59), in addition to angiogenesis (47, 53-55, 57, 60), brain development (54, 55) and regulation of carbohydrate (39, 55, 58) and lipid metabolism (35, 40, 53, 58). More recently, bone metabolism has been shown to be regulated by leptin [14] but through different routes than those used to regulate energy homeostasis (60, 61).

Relationship between Leptin and Bone

The protective effect of body weight on bone density has been attributed primarily to the stress placed on the skeleton due to mechanical loading (40, 48, 50, 55, 59, 62-68). Theoretically, bone adapts by becoming denser and stronger as the demand from body weight increases. However, this association has also been found in non-weight bearing bones (69). Whether lean mass or fat mass is a stronger predictor of bone density, or if the relationship exists strictly between body weight and bone density, remains unclear (64).

Factors other than skeletal loading also influence bone density (59). For example, an increased secretion of insulin as fat mass increases may promote bone mineralization (40, 66, 70); an increased secretion of bone-building hormones (68, 70), increased secretion of sex steroids (66), and beneficial effects of estrogens produced by adipocytes also influence bone density (40, 65-67, 69-71). Finally, because leptin is related to obesity and obesity to BMD, leptin is suspected to influence bone mass.

In Vitro Studies

Bone mass and serum leptin are both increased in obesity. Furthermore, adipocytes and osteoblasts originate from a stromal cell line in bone marrow (69). Based on the theory that leptin may link adiposity and bone mass, Thomas et al. (69) investigated the action of leptin on osteoblast differentiation and function *in vitro* using a conditionally immortalized human stromal cell line (hMS2-12). This cell line has the ability to differentiate into either adipocytes or osteoblasts. While leptin did not significantly effect proliferation of hMS2-12 cells, it had a dose-dependent effect on and promoted differentiation of hMS2-12 cells into osteoblasts. At the same time, leptin inhibited differentiation of hMS2-12 cells into adipocytes. Accumulation of lipid droplets in cytoplasm often signifies adipocyte development was decreased. In addition, leptin increased matrix mineralization in osteoblasts. Although mechanisms were not elucidated in this study, researchers hypothesized that leptin may act as an autocrine/paracrine modulator of bone

marrow stromal cells. Overall, leptin exerted a positive effect on the differentiation and maturation of osteoblasts but a negative effect on these processes in adipocytes.

With the knowledge that adipocytes and osteoblasts arise from the same precursor cells in bone marrow, Reseland et al. (71) used reverse transcriptase-polymerase chain reaction (RT-PCR) and immunocytochemistry to study the relationship between leptin and primary cultures of human osteoblasts (hOBs). Human osteoblasts were obtained from four nonmalignant, osteoarthritic patients undergoing hip replacement surgery. Adipose tissue showed a high concentration of the adipocyte marker, hormone sensitive lipase (HSL), but no osteoblast characteristics, while hOBs expressed no HSL suggesting that these cells did not have characteristics of adipocytes. In the presence of leptin, hOBs displayed characteristics of osteoblasts as well as translation, transcription, and secretion of leptin and its receptor. These results in conjunction with the observation that leptin promoted bone mineralization in mature cultured osteoblasts suggests that leptin is involved in bone metabolism. Although potentially controversial, obtaining hOBs from a younger, healthier population could add tremendously to current knowledge about the influence of leptin on body cells including osteoblasts, adipocytes, and their shared precursors.

To further understand leptin's influence on bone metabolism, Gordeladze et al. (68) used iliac crest hOBs from three osteoarthritic donors without malignant bone disease to investigate the effects of leptin on hOB proliferation, differentiation and apoptosis. Human osteoblasts were grown in leptin,

calcitriol, or human PTH for 35 days. Cells grown in leptin culture showed greater cell proliferation than either of the other two conditions. Leptin also inhibited apoptosis throughout incubation. Initially, leptin inhibited calcification (weeks 1 and 2), but by weeks 4 and 5, leptin significantly enhanced mineralization. Analysis by RT-PCR showed that cells grown in culture with leptin also displayed increased mRNA expression of bone formation biomarkers [bone alkaline phosphatase (B-ALP) and osteocalcin (OC). Leptin may directly and positively impact bone osteoblasts and therefore, overall bone metabolism and mass.

In order for any cell to survive, it needs a blood supply to provide nutrients and remove waste products. To learn if leptin had any influence on osteoblasts other than their differentiation and apoptosis, Kume et al. (53) used a mouse osteoblast cell line (MC3T3-E1), a mouse chondrocyte cell line (MCC-5), and human umbilical vein endothelial cells (HUVECs) to examine the effect of leptin on bone formation with specific focus on its role in angiogenesis. Both mouse cell lines produced and secreted leptin. In 15-day old mouse embryos, leptin expression was noted before blood vessel formation suggesting that leptin affected blood vessel formation. Leptin promoted proliferation of HUVECs in a dose-dependent response and at levels of 100 ng/mL, it caused structural rearrangement that led to formation of capillary-like networks. These data suggest that leptin may stimulate angiogenesis, a precursor step to bone formation. Although these conclusions are based on mouse data, they

are an important step in further understanding how leptin impacts osteoblastic proliferation.

Bone metabolism is influenced not only by osteoblastic activity but also by osteoclast activity. While studies have shown that leptin is beneficial for osteoblast differentiation and proliferation, previous research did not address leptin's impact on osteoclasts. Holloway et al. (54) studied the influence of leptin on osteoclast formation using bone cultures of human peripheral blood mononuclear cells (PBMCs) from healthy donors for three weeks. Addition of leptin to cell cultures for the entire study period inhibited both osteoclast formation and bone resorption. Importantly, leptin showed the most dramatic effect during the first week of the experiment and less influence in the second and third weeks, leading researchers to theorize that leptin exerts more influence on bone metabolism before the osteoclasts are actually formed by inhibiting formation. Leptin had less impact once the osteoclasts were formed. Human leptin from a commercial source (Peprotech; Rocky Hill, NJ) was also tested and found to have similar results, confirming that these observations were specific to leptin and not another compound. In addition, spleen cells from mice were used to verify that leptin's inhibitory effects on osteoclast generation was not species specific. Leptin negatively affected osteoclastogenesis in mice spleen cells as well as human PBMCs. Furthermore, leptin may have promoted mRNA synthesis of the powerful osteoclastogenesis inhibitor, osteoprotegerin. Although no information regarding the medical, physical, or demographic characteristics of the donors

was given, Holloway et al. (54) suggested that these data support the argument that leptin protects bone through local reduction of osteoclast formation and subsequent bone resorption.

From recent studies, leptin appears to exert an effect on bone metabolism through both formation and resorption *in vitro*. Leptin promotes precursor cells to differentiate into osteoblasts as opposed to adipocytes (69, 71). After a cell has developed into the osteoblast, it needs to be nourished, for without this support, it will die. Leptin seems to be involved in angiogenesis, therefore playing a role in the growth and proliferation of osteoblasts (53). In addition, leptin may influence bone metabolism in favor of bone formation through the delay of apoptosis or programmed cell death (68). Finally, leptin appears to inhibit osteoclast differentiation and proliferation (54). Through the promotion of osteoblast differentiation and proliferation and delay of osteoblast apoptosis as well as the inhibition of osteoclast formation, leptin may play a pivotal role in bone metabolism and ultimately bone mass.

Evidence from Animal Studies

Often, *in vitro* observations are over-simplified versions of *in vivo* events. To better understand the role of leptin in living animals, Burguera et al. (67) studied the influence of leptin on bone mass and turnover for one month in ovariectomized Sprague Dawley rats (n = 56, age = 6 months) and controls (n = 8). Test mice were treated with estrogen (17α-ethinyl estradiol), leptin, or a combination of both (n = 8 rats/group). Cerebrospinal fluid leptin

concentrations were not significantly different between groups. The combination of estrogen and leptin decreased bone turnover more than estrogen alone. Also, leptin reduced trabecular bone loss and structural changes, increased osteoprotegerin mRNA concentrations and decreased periosteal bone formation in estrogen deficient mice. Leptin may have inhibited osteoclast activity and, therefore, bone resorption. Significant effects were seen after only one month, but more research should be conducted to determine if these benefits continue for longer periods and to learn if this effect is species dependent.

Osteocalcin, synthesized by mature osteoblasts, is considered to be a "sensitive and specific marker of osteoblastic activity and the rate of bone formation" (72). Energy restriction reduces OC secretion and can lead to osteoporosis. Goldstone et al. (72) studied *ob/ob* (leptin-deficient) and *db/db* (leptin-resistant) male mice to determine if the fall in leptin during starvation led to the observed decrease in OC secretion. During starvation, both *ob/ob* and *db/db* mice had higher OC concentrations compared to normal control mice. Leptin administered to normal mice inhibited the decline in OC during a 24 h fast and 5-day energy restriction. Leptin's observed influence on OC secretion may be the link between nutritional status and bone metabolism.

Research has shown that leptin influences energy balance and bone metabolism through separate pathways, but the relative significance of these effects and the mechanisms by which they occur are still unclear. Elefteriou et al. (73) used *ob/+* mice to compare the importance of leptin's role in energy

balance and bone metabolism as well as to assess whether leptin influences bone metabolism strictly via blood circulation or by other avenues. Similar quantities of leptin were needed to affect energy metabolism and bone turnover implying that these two functions were of equal importance in living systems. Intracerebroventricular (ICV) injections of leptin as low as 2 ng/h significantly reduced bone mass in wild-type mice. In addition, high serum leptin had an inverse relationship with osteoblastic activity and resulted in low bone mass. Likewise, decreasing free serum leptin resulted in increased bone mass. These observations that leptin, at both high and low concentrations, influences bone metabolism suggest that leptin is a regulator of bone mass.

Evidence from Human Studies

CHILDREN:

Preadolescent boys (n = 28) and girls (n = 31) of normal growth and body composition participated in a study by Roemmich et al. (74) to determine the relationship between serum leptin and total body and body region BMD and BMC adjusting for chronological age, body composition measures, and serum estradiol and IGF-1 concentrations. Children were compared on the basis of gender and stage of puberty which was defined by genital and breast development. Body composition measurements and leptin levels between the sexes showed the same differences as observed later in life. Boys had greater fat-free mass and lower percentage body fat, while girls had greater leptin concentration. Generally, older children (pubertal) had greater total body and

regional sites BMD and BMC than younger children (prepubertal). Interestingly, girls also had greater lumbar spine BMD than boys but lesser femoral neck BMC. Serum leptin was positively related to lumbar spine BMD. However, when adjusting for age, body composition, and hormonal concentrations, associations between serum leptin and any measure of BMC or BMD were not found. Data regarding these children's diets and activity levels were not collected. Therefore, further research is needed to more completely understand the role of serum leptin in this age group as well as overweight counterparts.

In another study, preadolescent, Italian children (n = 395, age = 6-13 y) were divided into normal weight (n = 163) and overweight (n = 232) groups to study leptin's effect on bone formation biomarkers including OC and carboxyterminal propeptide of type I collagen (PICP) and a bone resorption biomarker including crosslinked C-telopeptide of type I collagen (ICTP) in this stage of growth (75). Approximately one-half (n = 129, 61 males, 68 females) of the overweight children were placed on a 1 to 2 month weight loss diet of 1200 kcal/day or 20-30% energy intake reduction according to age and ideal body weight recommendations. Only 26 subjects failed to reduce excess weight on the weight loss protocol. Leptin and all bone biomarkers were measured from a fasting overnight blood sample using RIA. Mean serum leptin and OC concentrations were significantly different between the two groups, with leptin concentration higher in the overweight group in both genders and OC concentration higher in the normal weight group when children were grouped

according to gender. No differences were noted in serum PICP or ICTP concentrations in children according to weight or gender. Of the children who lost weight, OC and PICP concentrations increased, but ICTP did not show a significant change in this weight loss group. Conversely, leptin concentration dropped, in agreement with previous studies conducted with adults (48, 49). Interestingly, as body weight and leptin concentration decreased, markers of bone formation increased. However, neither BMC nor BMD were measured in this study, weakening any conclusions that could be drawn about the effect of leptin and weight loss on bone mass.

Puberty is often thought to begin when children reach a certain weight or body fat percentage. Since leptin is closely associated with body weight in humans of all ages and puberty is associated with a time of increased bone maturation and BMD, Klein et al. (76) studied obese (n = 18, 12 female, 6 male) and nonobese (n = 30, 11 female, 19 male) children to better understand the role that leptin plays in BMD through the onset of puberty. Obese children had a higher concentration of serum leptin than their normal weight counterparts, and girls had a higher concentration than boys when controlling for measures of adiposity. Interestingly, obese children were significantly younger and had a greater bone age to chronological age ratio than nonobese children. However, total body BMD (measured by DXA) did not differ significantly between obese and nonobese children. This may be explained by the significant difference in chronological and bone age of the two groups. Although leptin did not correlate with BMD in this study, it did correlate with

bone age. Thus leptin, may influence the onset of puberty and indirectly impact bone maturation and BMD.

Along with body weight, early onset of puberty seems to be protective of BMD in humans. Matkovic et al. (51) studied 343 pubertal, Caucasian females (age = 8.3-13.1 y) over a 4 year period to investigate the role of leptin in the onset of menarche and on bone indices. Serum leptin was measured via RIA (LincoResearch, St. Charles, MO) and bone via DXA (pencil beam Lunar DPX-L Machine with 1.3q software, Lunar, Madison, WI). A serum leptin concentration of 12.2 ng/mL (range 7.2-16.7 ng/mL, 95% confidence interval) corresponded to younger age at menarche, 29.7% body fat, and 16.0 kg of body fat. Each additional increase in serum leptin of 1 ng/mL further decreased the age of menarche by one month and each additional kg gain in body fat decreased menarche by 13 days. Importantly, serum leptin positively correlated with total body BMD ($r = 0.20$), bone area ($r = 0.31$) and change in bone area ($r = 0.27$). Leptin seems to act as a mediator to the timing of menarche and coincidentally has an influence on BMD.

Although studies involving children are not in agreement as to the exact role of leptin in bone metabolism, most agree that leptin may have some impact on the growing skeleton. Because this is the time when bone formation exceeds bone resorption, the bone laid down at this life stage will impact an individual's risk for osteoporosis later in life.

ADULTS:

Body weight has been positively associated with BMD, but whether fat mass or lean mass specifically affects BMD is unclear. To better understand the role of these body components and that of leptin on BMD, Blum et al. (64) measured fasting leptin and total body, hip, and lumbar spine BMD in a cross-sectional, homogeneous sample of 153 premenopausal women (age = 40-45 y, weight = 44.2-113.2 kg, BMI = 24.8 $\pm$ 5.2 kg/m^2). Although, leptin was positively associated with BMD at the hip, lumbar spine, and total body, leptin and fat mass were inversely associated with BMD at these three sites. Surprisingly, bone mass in these premenopausal women was lower with higher leptin or percentage body fat. Smoking status, physical activity, medical history, calcium supplementation, and dietary intake (including calcium) were all recorded but not analyzed in this study. Information from these records may have provided insights regarding the seeming contradiction between body fat and serum leptin measurements and bone mass. For example, some evidence links physical inactivity, certain medications, inadequate diet, and smoking with lowered bone mass (40, 77). In addition, this study sample was ethnically and socioeconomically homogeneous, weakening any ability to generalize results and conclusions to all premenopausal women.

Because leptin has been considered a regulator of bone mass during skeletal growth, Rauch et al. (50) studied healthy, premenopausal (n = 60) and postmenopausal (n = 34) (postmenopausal defined as $\geq$6 months without menstruation) German women (age = 40-60 y) to determine the relationship

between serum leptin and bone density and bone turnover. Leptin was measured by RIA and distal radius BMD by QCT, while bone metabolism was determined using bone turnover markers. Serum B-ALP, OC, and PICP were included as bone formation markers, while serum ICTP and urinary deoxypyridinoline were included as bone resorption biomarkers. None of the bone density measures were significantly associated with serum leptin. Significant differences between pre- and postmenopausal groups were found only for total body BMD (320 $\pm$ 56 vs. 284 $\pm$ 53 g/cm^3, respectively). No significant correlations between bone metabolic markers and serum leptin, either before or after adjusting for BMI, were found except between PICP and leptin in postmenoposausal (r = -0.40, after adjusting for BMI) women. However, PICP concentration may have been influenced by collagen synthesis in the skin and metabolism by the liver. Although relationships have been found between serum leptin and cortical bone at the distal radius in animals and girls with growing skeletons, no relationships between these measures in these mature skeletons were observed in this study. Ultimately, Rauch et al. (50) concluded that although leptin may influence the growing skeleton, leptin has little impact on the mature skeleton.

Leptin and its effect on bone were examined by Kontogianni et al. (78) in white premenopausal (n = 25) women and postmenopausal (n = 55, mean years since menopause 3.3 y) women, aged 42-68 years. Enrolled women were considered healthy via physical examination and were not exposed to hormone replacement therapy or contraceptives. Some (n = 13) reported use of thyroid

medications, but no other endocrine disorders were present. Dietary records of calcium and physical activity were monitored. Seventy percent of subjects were non-smokers (past or present), 6.3% were past smokers, and 23.7% were current smokers. Postmenopausal women were older, had less lean mass, and lower total body BMC and BMD than premenopausal women. Leptin was correlated with BMI (r = 0.55), percentage body fat (r = 0.56), fat-free mass (r = 0.36), lean tissue mass (r = 0.37), and total body BMC (r = -0.28) before and after adjustment for menopausal status. When subjects were further divided into normal bone mass and osteopenic/osteoporotic groups, the latter had lower BMI and were postmenopausal longer, but serum leptin did not differ between the two bone status groups. In this homogeneous, overweight population, serum leptin did not act to increase bone mass and was negatively associated with bone mass. This study was very thorough, taking the diurnal variation of leptin into account as well as recording dietary calcium intake, physical activity, and history of bone fractures in subjects. However, because smoking status is suspected of impacting bone mass, it should specifically be addressed in studies containing smokers and non-smokers to eliminate it as a variable.

In a study of 54 healthy, postmenopausal women (age = 54-79 y, BMI = 14.8-42.9 kg/m^2, percentage body fat 9-45.7%), Goulding and Taylor (63) examined the relationship between plasma leptin concentration and age-adjusted total body BMC and BMD, and markers of bone metabolism. No significant relationship between leptin and plasma OC, urinary

deoxypyridinoline/creatinine, or hydroxyproline/creatinine was observed. Leptin and all measures of body mass (body weight, BMI, percentage body fat, and fat mass) showed positive associations. Correlations between leptin and BMC were lower than those of leptin and body weight and fat mass. However, BMD was more highly correlated with leptin than with body weight or total fat mass (see Table 4). Adjusting for fat mass eliminated the significance of the correlation between BMC and leptin. Goulding and Taylor (63) concluded that leptin does not influence the bone-sparing effects consistent with obesity but acknowledged that a single measurement in time may not be the best way to explain an ongoing, dynamic process such as bone metabolism. To better explain the relationship between bone metabolism and leptin, a long-term experimental design may be necessary.

Healthy, postmenopausal women (n = 121, age = 54 $\pm$ 5 y, BMI = 19-27 kg/m^2) participated in a prospective, randomized, double-blind study of the effects of leptin on bone turnover markers and BMD (49). Urinary excretion of C-terminal crosslinking telopeptide of type I collagen (CTX) and OC were used as markers of bone resorption and formation, respectively, while BMD was measured at the spine, hip, and femoral neck. All measurements were completed at baseline and again at 6 and 24 months of treatment. Subjects were assigned to one of three treatment groups and received either 2.5 mg tibolone (n = 34), 1.25 mg tibolone (n = 45), or 2 mg estradiol plus 1 mg norethindrone (n = 42). Groups did not statistically significantly differ at baseline, and no subjects had conditions or were taking medications known to

Table 4: Bone mineral content and bone mineral density of postmenopausal women

	Total Body BMC	Total Body BMD
Body weight (kg)	r=0.643	r=0.502
Total fat mass (kg)	r=0.557	r=0.510
Plasma leptin concentration	r=0.480	r=0.551

affect bone or calcium metabolism. Subjects received their assigned treatment plus 500 mg calcium supplementation daily. At baseline, correlations between serum leptin and BMD at the spine, hip, and femoral neck were weak: (r = 0.21), (r = 0.26), and (r = 0.18), respectively. Serum leptin was negatively correlated with CTX (r = -0.38) and OC (r = -0.21). Changes in leptin and changes in bone indices were not correlated in any of the treatment groups at 6 or 24 months. Although significant changes were reported in each group in body weight, leptin, bone biomarkers and BMD, there was no control group to which to compare changes. In addition, neither dietary nor physical activity records were maintained.

Osteoporosis is the most prevalent bone remodeling disease and its prevention is of major importance. Because most research focuses on preventing or treating osteoporosis, the factors that affect bone remodeling in individuals with osteoporosis are not clear. To better understand this process in osteoporotic patients, Shaarawy et al. (77) studied leptin's role in bone remodeling in Egyptian, postmenopausal women (n = 120) with osteoporosis. Osteoporosis was defined in this study as lumbar spine BMD of >-2.5 standard

deviations below the normal mean of healthy women. None of the women in the study had history of illness or medication known to influence bone metabolism. Fasting blood samples were used to measure leptin and markers of bone formation, B-ALP and OC. Urine samples were used to measure markers of bone resorption including free deoxypyridinoline and crosslinked N-telopeptides of type I collagen (NTx). Postmenopausal osteoporotic women (age = 58.8 $\pm$ 0.51 y) were grouped according to BMI as either obese (n = 37, BMI >30 kg/m^2) or nonobese (n = 53, BMI = 20-25 kg/m^2). Both postmenopausal groups had significantly higher concentrations of bone formation biomarkers than did healthy, premenopausal controls (n = 30, age = 40.5 $\pm$ 0.49 y, BMI = 20-25 kg/m^2). The same relationship was observed with bone resorption markers in postmenopausal osteoporotic women, suggesting that overall bone turnover was higher in this group than in the healthy, normal weight, younger controls. Interestingly, no significant differences in bone turnover biomarkers or lumbar spine BMD were observed in the postmenopausal osteoporotic group when this group was classified on basis of BMI. Finally, no significant relationships between serum leptin and bone turnover biomarkers or BMD were observed. These findings suggest that serum leptin does not play an important role in bone metabolism in postmenopausal women suffering from osteoporosis. This study provided valuable information regarding the role of serum leptin in postmenopausal osteoporotic women; however, data regarding diet or physical activity were not included. Because the authors did not mention whether the control group was significantly different than the

osteoporotic group, caution should be used when interpreting the comparisons between the two groups. Using a control group closer in age, BMI, and/or menopausal status would help to increase confidence in these comparisons.

Not only are data regarding leptin's role in bone remodeling in osteoporotic patients lacking, but little is known about leptin's function in repair of recent bone fractures. Di Monaco et al. (79) measured serum leptin and BMD in Caucasian, postmenopausal women (n = 62, age = 77.7 $\pm$ 8.1 y, weight = 55.7 $\pm$ 10.5 kg, BMI = 22.18 $\pm$ 3.8 kg/m^2) within 23.4 $\pm$ 9.1 days of fracture to assess leptin's role in the relationship between fat mass and BMD. None of the women were taking osteoporosis medications; serum calcium concentration was measured for each woman. The positive association between leptin and BMD was low and only found at the intertrochanter (r = 0.259). After adjusting for fat mass, multiple regression showed that leptin was significantly and negatively associated with total (nonfractured) femur, intertrochanter, trochanter, and femoral neck BMD. Overall, leptin could not explain the link between fat mass and bone mass in this population. Because a control group (fracture-free women) was not included and this sample was racially homogeneous (specifically this sample was all Caucasian which is generally considered to be a risk factor for osteoporosis), caution should be taken when generalizing these results to a larger population of women.

Body mass is a significant predictor of bone mass as well as leptin concentration, but not all studies have found bone mass and leptin to be positively correlated. One study which failed to find a correlation between bone

mass and leptin was conducted by Sahin et al. (80). They studied 100 postmenopausal Turkish women (age = 55.1 $\pm$ 6.3 y) to investigate the association between serum leptin and BMD and to determine whether lean or fat mass was a better predictor of BMD. Women had been postmenopausal for at least 12 months and were free of bone traumas and diseases, were not taking any medications that could influence bone metabolism, and were non-smokers. Body composition was measured using skin fold thicknesses and DXA (XR-46, Norland, Fort Atkinson, WI). Total body, lumbar spine and femoral neck BMD and total body BMC were also assessed using DXA. Serum leptin was quantified by RIA (Accucyte Human Leptin, Cytimmune Science, MD). Lean body mass positively correlated with BMD at all sites as well as total body BMC, while neither fat mass nor percentage body fat correlated with BMD at any site, implying that lean mass was a stronger predictor of BMD than was fat mass or percentage body fat. Results from leptin tests showed no correlation between serum leptin and total body BMD, serum leptin and lumbar spine BMD, serum leptin and femoral neck BMD, or serum leptin and total body BMC. In addition, there were no correlations between serum leptin and markers of bone formation (osteocalcin) or resorption (type I collagen), suggesting that serum leptin was not a significant factor in bone metabolism in postmenopausal women.

Leptin receptors have been located on osteoblasts, but the extent of leptin's influence on bone metabolism is still unclear. In a study of postmenopausal women (n = 100, age = 62-97 y) and elderly men (n = 31, age =

72-92 y), leptin concentrations were determined and compared to BMD and bone metabolism biomarkers including NTx, aminoterminal procollagen extension peptides (PINP) and bone-specific alkaline phosphatase (bAP) (47). Subjects were considered healthy, and none were on medications known to influence bone metabolism, including hormone replacement therapy. Total body, femoral neck, and lumbar spine BMD were measured by DXA. The women in the study had significantly higher serum leptin and bAP, but significantly lower BMD measurements than men with similar BMI. Serum leptin was significantly correlated with spine, femoral, and total body BMD in women and lumbar spine BMD in men before adjusting for age and BMI. Although leptin was not related to NTx or PINP, leptin was significantly associated with bAP concentrations ($r = 0.28$-0.33) in both groups, both before and after adjusting for age and BMI. Alkaline phosphate activity occurs in osteoblasts during bone mineralization to catabolize orthophosphate, a potent inhibitor of mineralization. This association between leptin and alkaline phosphatase supports the hypothesis that leptin functions to promote bone formation.

In a cross-sectional study of Danish men, Morberg et al. (40) investigated the relationship between fasting serum leptin and total body BMD. Subjects were all healthy by self report and categorized as either nonobese during childhood (controls; n = 327, BMI = 26.1 $\pm$ 3.7 kg/m^2, age = 49.9 $\pm$ 6.0 y) or obese during childhood (n = 285, BMI = 35.9 $\pm$ 5.9 kg/m^2, age = 47.5 $\pm$ 5.1 y). Fasting leptin was measured by RIA (human leptin RIA, Alta Diagnostica,

Marburg, Germany), while BMD was measured at slow speed using DXA (Lunar DXA-IQ DEXA, version 4.6c, Madison, WI). Lifestyle factors such as physical activity and smoking habits were recorded, but no mention of dietary habits including calcium intake was made. After adjusting BMD for body weight, the correlation between serum leptin and BMD in the nonobese controls was r = -0.186 and the juvenile obese group was r = -0.135. Adjusting BMD for body weight strengthened the relationship between leptin and BMD. Interestingly, linear regression analysis showed no association between fasting serum leptin and BMD in either group. The subjects in this study were representative of the Danish male population and covered a range of weight histories permitting the results to be generalized. Although these data represent a specific point in time, the researchers concluded that serum leptin was inversely related to BMD and that it played a significant role in bone metabolism of both obese and nonobese Danish men.

High body weight seems protective of bone density. Specifically, fat mass positively correlates with BMD (48, 55, 59, 62, 63, 80-82). Bone density and body weight exhibit a positive correlation, at least in postmenopausal women (48, 59, 63, 81). Thomas et al. (59) found a significant correlation between bone density and fat mass in premenopausal women but not men of the same age range. In a study of 34 obese postmenopausal women, Ricci et al. (48) found that as body weight and body fat decreased so did bone density. Moreover, this change in bone density was significantly different from a weight maintenance group in the same study (48). While some studies have failed to find a

significant relationship between body weight and BMC (55), most studies agree that higher body weight is protective of bone mass and bone density, thereby reducing an individual's risk for osteoporosis (49, 62). Unfortunately, the reasons for this relationship are still debated.

To date, a number of hypotheses have been developed regarding whether this observed relationship between body weight and bone sparing could be a result of leptin. Some studies report that leptin does influence bone metabolism (69, 71, 73), while other studies report the opposite (74, 80). Some studies have found that mice with reduced SLL, because of defective receptors or a simple leptin deficiency, have higher bone density than controls (35, 60, 73). Scariano et al. (47) propose that BMI influences BMD more than leptin, but because leptin is secreted mainly by adipose tissue, fat mass may indirectly influence BMD through production of leptin. Pasco et al. (55) contend that at the whole body level, body weight and body fat mass are not significant predictors of BMC. Flier (61) offers a unique view suggesting that obesity leads to leptin resistance and that this resistance is bone sparing. In contrast, Thomas (62) believes that body weight is actually a predictor of bone status. Body weight is influenced by dietary intake and perhaps macronutrient composition. Several hormones are under dietary influence, thus leptin may also be influenced by composition of diets.

Relationship between Serum Leptin and Diet

Some *in vivo* studies have confirmed an inverse relationship between serum leptin concentration and BMD in premenopausal (64) and peri- and postmenopausal women (78). Of importance was that women with hip fractures, the definitive diagnosis of compromised bone mass, had a robust inverse correlation between serum leptin concentration and BMD at several skeletal sites (79). To date, only one published study has examined the effect of a high- versus low-carbohydrate diet on serum leptin concentration (83). Koutsari et al. (83) reported that in both fasting and post-prandial states, women who consumed a high-carbohydrate diet for three days had a higher mean serum leptin concentration compared to when they consumed a low-carbohydrate diet for three days. Findings from this study suggest that a high-carbohydrate diet may be detrimental to bone health due to a rise in serum leptin. However, this previous study did not involve weight loss or include a high-protein component of the diet. Energy deficits have been shown to decrease serum leptin (84) which would suggest a benefit to bone mass; however, considered collectively, energy restriction results in adverse changes in bone cell metabolism toward increased bone resorption (85, 86)

Although an increase in serum leptin with consumption of a high-carbohydrate diet was reported, Koutsari et al. (83) did not examine weight loss or fat mass changes. Moreover, cognitive eating restraint (CER) was not included as a variable. Defined as conscious control over what one consumes

to regulate body weight, CER has been shown to predict success with weight loss (87). Bone mineral content appears to be linked to CER and has been associated with low BMD in normal weight (88, 89) and overweight/obese premenopausal women (90). Bacon et al. (90) showed that women with higher CER scores had lower BMC. Van Loan et al. (89) also found that BMC varied significantly between women with low and high CER scores with the lowest BMC observed in women weighing <71 kg with high CER.

In a recently completed 12-week weight loss intervention study conducted in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory at Virginia Polytechnic Institute and State University (VPI&SU), 25 women were randomized to an Atkins diet group (n = 13) or a traditional diet group (n = 12). Women in the Atkins diet group consumed ≤20 g of carbohydrate per day with liberal intakes of dietary protein and fat for two weeks. At week three, women increased their dietary carbohydrate intake by 5 grams per week until urinary ketones were no longer detected or individual weight loss goals were reached. Energy intake was not restricted in the Atkins diet group. Women in the traditional diet group consumed 1300, 1500, or 1700 kcal per day, with a macronutrient composition of 60% carbohydrate, 25% fat, and 15% protein. The traditional diet was designed to induce 0.5 to 1 kg of weight loss per week. Significant differences in macronutrient intakes (i.e., dietary carbohydrate, protein, and fat) between groups were found over time; however, women in the two dieting groups consumed equivalent amounts of total energy. Women in both diet groups lost a significant proportion of body

weight and body fat mass from baseline to the end of the 12-week study, attributable to deficits in total energy but not differences in macronutrient composition of the diets. Of relevance is that women in the Atkins diet group had a significant decrease in hip BMD (1.2%), whereas women in the traditional diet group did not during this 12-week period. While serum leptin would have been expected to decrease similarly in both diet groups due to comparable body weight and fat mass losses, there may have been differential changes due to diet composition or other factors, such as eating restraint.

Data from a separate study conducted in the BONE Laboratory at VPI&SU indicated that premenopausal women with high CER differed from women with low CER only in body fat mass and percentage body fat (91). Upon further investigation, preliminary data found that mean serum leptin concentrations in these women were significantly different. Women with high CER scores (n = 5) had a higher mean serum leptin concentration (14.2 ± 3.8 ng/mL) compared to women with low CER scores (n = 5, 6.1 ± 2.5 ng/mL). Cognitive eating restraint has been posited to invoke a central stress response, perhaps in conjunction with serum leptin, to control eating behavior and energy metabolism and potentially bone metabolism. Thus, changes and differences in BMD may be explained by serum leptin shifts that occur with specific types of diets and with the presence of CER.

Summary

With many conflicting reports, further research is needed regarding the effects of leptin on bone and in relation to eating restraint and dieting for weight loss and control. Both osteoporosis and obesity are major public health concerns; if leptin is related to both diseases it could be a key factor in treatment as well as prevention. Coupled with the epidemic of overweight and obesity and the call for weight reduction, osteoporosis may present an even greater economic and societal burden in the future.

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CHAPTER III

LEPTIN IS ASSOCIATED WITH BODY FAT MEASURES IN NON-OBESE PREMENOPAUSAL WOMEN WITH HIGH OR LOW COGNITIVE EATING RESTRAINT SCORES

Abstract

Excess body weight may protect bone mineral density (BMD) and content (BMC) through mechanical loading and sex hormone synthesis in peripheral adipose tissue. The satiety hormone, leptin, produced by adipocytes, and cognitive eating restraint (CER) may also influence BMD and BMC. Premenopausal women aged 18-25 years and of healthy weight were studied to examine relationships between serum leptin concentration and bone mineral and soft tissue measures at three intervals. Women were categorized by Eating Inventory questionnaire scores into a high (score > 9, n = 20) or low (score $\leq$ 9, n = 16) CER group. Mean ($\pm$ SD) CER scores for the high and low CER groups were, 12.9 $\pm$ 2.3 and 4.2 $\pm$ 3.0, respectively, at baseline ($p \leq 0.05$). Significant differences in BMC or BMD measures between groups were not observed at any interval. Soft tissue mass measures were not significantly different between groups at any interval. However, serum leptin was significantly lower in the low CER group versus high CER group at baseline (mean $\pm$ SEM = 9.40 $\pm$ 0.96 vs. 13.22 $\pm$ 1.70 ng/mL, respectively, $p \leq 0.05$). Positive relationships between serum leptin and body fat mass and body fat percentage were found in these women of healthy weight regardless of CER status. Leptin is associated with body fat but not BMD or BMC in premenopausal women of healthy weight who have high or low CER scores.

KEYWORDS:

Bone mineral content; Bone mineral density; Cognitive eating restraint; Leptin; Women

Introduction

For all the negative health consequences associated with excess body weight, there appears to be one benefit. Overweight and obesity have been shown to protect bone mineral density (BMD) and content (BMC), thereby lowering the risk of osteoporosis (1, 2). This effect has been attributed to mechanical loading on the skeleton associated with excess body weight (3, 4). Estrogen synthesis in peripheral adipose tissue has also been identified as a contributor to high BMD (5, 6). Another possible explanation involves the hormone leptin. Leptin is a protein hormone produced primarily by adipocytes in proportion to the quantity of body fat present (7-9). Therefore, the more adipose tissue an individual possesses, the higher the concentration of serum leptin (9-12). Because leptin is related to obesity and obesity to BMD, leptin is suspected to influence BMD.

Previously, we reported greater body fat content in young women with high cognitive eating restraint (CER) scores (13). Interestingly, CER score, an indicator of the conscious control over food intake to regulate body weight, had a negative association with serum osteocalcin and with urinary N-telopeptide of type I collagen (NTx), suggesting that eating restraint may contribute to low bone mass (14). Yet, high body fat mass and leptin production from this adipose tissue may counter any impact of CER on BMD. Thus, the purpose of this study was to examine relationships between serum leptin concentration and bone mineral measures and soft tissue measures in women with high and low CER at three intervals and to evaluate changes in these variables over time.

The primary hypothesis of this study was that mean serum leptin concentration would be greater in premenopausal women with high versus low CER scores.

Materials and Methods

Study participants

Subjects for this study were drawn from a larger study (13) designed to evaluate the usefulness of the restrained eating subscale of the Eating Inventory questionnaire (15) to identify continual dieters. Subjects were recruited via paper notices, electronic-mail messages, and personal interactions. The original study and this subsequent study were approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University (VPI&SU; Blacksburg, Virginia, U.S.A.). All women provided written informed consent after they had an opportunity to have their questions and concerns answered. Women were aged 18 to 25 years.

Exclusion criteria for the primary study included parity ≥ 1; use of prescription medications for metabolic or endocrine disorders; history of metabolic or eating disorders; current chronic diseases; tobacco use; amenorrhea, oligomenorrhea, or surgically-induced menopause; cessation of menstrual cycles for > 1 month during the past year; participation in > 7 hours of intense physical activity per week; body mass index < 18 or > 25; and fluctuation in body weight of > 2.3 kg ≥ 3 times during the previous two years (13). Exclusion criteria for the current study included missing serum samples

at any one of three intervals. Data from 36 of the original 65 subjects were included in these analyses.

Procedures

Subjects entered the study on a rolling basis such that data were collected from February 2001 through October 2003. At baseline and after 6 (Time 1) and 12 months (Time 2), each subject completed an Eating Inventory questionnaire (15) and underwent anthropometric and body composition testing. Each subject also provided a fasting blood sample. Blood samples were collected between the fourth and tenth day of each subject's menstrual cycle. The study protocol was performed in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory, VPI&SU.

Cognitive eating restraint

Based on our previous work (13) and that of other researchers (16, 17), subjects were divided into two groups. Women with baseline scores on the CER subscale of the Eating Inventory of > 9 were placed in the high CER group (n = 20), and women with scores ≤ 9 were separated into the low CER group (n = 16). Possible CER scores range from 0 to 21 (15). Women were unaware of their CER categorization.

Anthropometric data

Subjects wore lightweight clothing and no shoes for body weight and height measurements. Body weight was measured (± 0.1 kg) using a calibrated electronic scale (Scaletronix, Wheaton, IL, U.S.A.). Standing height was measured (± 0.01 cm) using a stadiometer (Detecto, Webb City, MO, U.S.A.).

Body weight and standing height were used to calculate body mass index (BMI = kg/m^2) for each subject.

Bone mineral and soft tissue measurements

Dual-energy X-ray absorptiometry (DXA; QDR4500A Hologic, Hologic, Inc., Bedford, MA, U.S.A.) scans were conducted to obtain BMC (g), BMD (g/cm^2), and soft tissue mass measurements. Total body, lumbar spine (L_1-L_4), non-dominant total proximal femur (hip), including the femoral neck, and non-dominant total forearm (arm) scans were completed and analyzed with the Hologic whole body analysis software (version 8.26a:3*) and site specific software. Total body lean mass (kg), fat mass (kg), and body fat percentage (%) were obtained from total body DXA scans. All DXA scans were carried out and analyzed by one investigator. Quality control steps for BMC and BMD were performed on the morning of each testing day using a lumbar spine phantom, producing a coefficient of variation (CV) of 0.34%. Quality control steps for soft tissue mass measurements were conducted weekly using an external tissue bar. Test-retest reliability in the BONE Lab produced CV for total body, lumbar spine, hip, arm, lean mass, fat mass, and body fat % of 0.73, 0.92, 0.69, 1.09, 1.07, 1.75, and 1.79%, respectively (18).

Serum leptin concentration

At each interval, women provided fasting (12-hours) blood samples within 1.5 hours of awakening. Timing of fasting blood sample collection was monitored to reduce diurnal fluctuations in serum leptin. A certified phlebotomist collected all venous blood samples, after which serum was

separated from whole blood by centrifugation at 2500 x *g* for 10 minutes at 25°C. Serum samples were aliquotted into cryovials and stored at -80°C. A human leptin radioimmunoassay (RIA; HL-81K, Linco Research, Inc., St. Charles, MO, U.S.A.) was used to measure serum leptin (ng/mL) from stored samples. The sensitivity, specificity, and precision of this assay are 0.5 ng/mL, 100%, and ≤ 6.2% between assays, respectively. The normal fasting mean serum leptin concentration for women of healthy BMI (18 – 25 kg/m^2) is 7.4 ± 3.7 ng/mL. All samples were analyzed in duplicate, and the inter- and intra-assay CV were 8.5% and 8.8%, respectively.

Statistical analyses

Descriptive statistics were conducted to describe subject characteristics at baseline. Differences in mean BMC, BMD, soft tissue mass measures, and serum leptin concentrations between low and high CER groups were completed using student's *t*-tests at each interval. Statistical significance was set at $p \leq 0.05$. Pearson correlation coefficients were computed to examine relationships between variables of interest within the low and high CER groups. A two-tailed significance was set at $p \leq 0.05$. Analysis of variance (ANOVA) with repeated measures on the time factor was used to identify changes in measures both within and between groups over time. Statistical significance was set at $p \leq 0.05$ for all comparisons. Statistical analyses were performed using SAS (version 8.2, 1999-2001, SAS Institute Inc., Cary, NC, U.S.A.).

Results

The mean (±SD) for age, height, weight, and BMI of subjects in the low CER group were: 20.5 ± 2.6 y, 165.1 ± 6.6 cm, 57.1 ± 6.1 kg, and 21.0 ± 2.2 kg/m^2. For subjects in the high CER group, mean age, height, weight, and BMI were: 19.8 ± 1.4 y, 166.2 ± 4.6 cm, 61.2 ± 6.6 kg, and 22.1 ± 2.0 kg/m^2, respectively. Significant differences in these anthropometric measures were not found at baseline. The mean CER score of the low CER group was 4.2 ± 3.0, while the mean CER score of the high CER group was 12.9 ± 2.3 ($p \leq 0.05$) at baseline.

Bone mineral content, BMD, soft tissue measures, and serum leptin concentration of subjects in the low CER group and high CER group for baseline, six months, and twelve months are shown in Table 1. Significant differences in BMC or BMD measures between groups were not observed at any interval. Soft tissue mass measures were not significantly different between groups at any interval. Leptin was significantly lower in the low CER group versus high CER group at baseline only (mean ± SEM = 9.40 ± 0.96 ng/mL vs. 13.22 ± 1.70 ng/mL, respectively, $p \leq 0.05$).

Table 2 shows the association between serum leptin concentration and measures of BMC, BMD, and soft tissue for both low and high CER groups at baseline, six month and twelve month intervals. In the low CER group, leptin was significantly related to body weight at baseline ($p \leq 0.01$) and 12 months ($p \leq 0.01$) but not at Time 2. Serum leptin was also significantly associated with total fat mass and body fat % at each interval ($p \leq 0.05$ to $p \leq 0.0001$).

Interestingly, total lean mass was positively related to serum leptin concentration at baseline ($p \leq 0.05$) in the low CER group.

In the high CER group, leptin was not significantly associated with body weight or total lean mass at any interval. At baseline and Time 2, serum leptin was positively related to total fat mass (both $p \leq 0.001$) and body fat % (both $p \leq 0.0001$). At Time 3, body fat % was positively associated with serum leptin concentration ($p \leq 0.05$) but not with total fat mass.

At Time 2, serum leptin concentration was negatively related to total body BMD ($p \leq 0.05$) only in the low CER group. Serum leptin was not significantly associated with any other measure of BMC or BMD in either CER group at any interval. At all three intervals, serum leptin at one interval was significantly related to leptin concentration at the other two intervals in both CER groups ($p \leq 0.05$ to $p \leq 0.001$).

Table 3 displays correlation coefficients between body weight and BMC, BMD, and serum leptin measurements at baseline, Time 1, and Time 2, respectively. In the low CER group at baseline, body weight was related to all measures of BMC ($p \leq 0.05$ to $p \leq 0.01$), except for femoral neck, and only arm BMD ($p \leq 0.05$). At Time 1 and 2, body weight was associated with total body (both $p \leq 0.05$) and arm (both $p = 0.01$) BMC and arm BMD ($p \leq 0.05$ and $p \leq 0.01$, respectively) in the low CER group.

In the high CER group, body weight was significantly associated with total body BMC (all $p \leq 0.05$) at each interval. Body weight was also related to hip BMC at baseline ($p \leq 0.05$), and at Time 1 and 2 (both $p \leq 0.001$). Body

weight was associated with arm BMC at baseline and Time 1 (both $p \leq 0.05$) and with femoral neck BMC at Time 2 ($p \leq 0.05$). Body weight was positively related to hip BMD at Time 2 ($p \leq 0.05$) in the high CER group. Body weight was significantly inter-correlated at each interval for both the low and high CER groups (all $p \leq 0.0001$). Serum leptin concentrations were related to body weight at baseline ($p \leq 0.001$) and Time 2 ($p \leq 0.01$) but not at Time 1 in the low CER group. However, body weight and serum leptin were not related in the high CER group at any interval.

The mean serum leptin concentration significantly decreased from baseline to Time 2 in the high CER group but not between baseline and Time 1 or Time 1 and Time 2 (Table 4). No significant changes in the mean serum leptin concentration were observed in the low CER group over time.

Table 5 contains the changes in body weight and soft tissue mass measures over time in low CER and high CER groups. Mean ($\pm$ SD) body weight increased from baseline to Time 1 ($p \leq 0.05$) and to Time 2 ($p \leq 0.01$) in the low CER group. Total fat mass increased from baseline to Time 2 ($p \leq 0.05$) in the low CER group. No significant changes were observed in body weight, total lean mass, total fat mass, or body fat % in the high CER group.

Table 6 shows significant changes in total body BMC and specific BMC sites over time in the low and high CER groups. Total body BMC increased from baseline to Time 2 ($p \leq 0.05$) in the low CER group. Hip BMC increased (not significant) from baseline to Time 1 but decreased from baseline to Time 2 ($p \leq 0.05$) and from Time 1 to Time 2 ($p \leq 0.01$). Femoral neck BMC decreased

from baseline to Time 2 ($p \leq 0.0005$) and from Time 1 to Time 2 ($p \leq 0.05$) in the low CER group. In the high CER group, the only significant change in BMC was at the arm, where BMC increased from baseline to Time 2 ($p \leq 0.05$) and from Time 1 to Time 2 ($p \leq 0.05$).

Table 7 displays changes in total body BMD and at specific body sites for both the low and high CER groups over time. No significant increases or decreases in total body BMD, lumbar spine BMD, or arm BMD were observed through the duration of the study for either group. In the low CER group, hip BMD was significantly lower at Time 2 compared to baseline ($p \leq 0.02$) and to Time 1 ($p \leq 0.05$). In this same group, femoral neck BMD increased from Time 1 to Time 2 ($p \leq 0.02$). In the high CER group, hip deceased from baseline to Time 2 ($p \leq 0.02$), while femoral neck BMD increased from Time 1 to Time 2 ($p \leq 0.05$).

Table 1: Mean (SEM) bone mineral content and density, soft tissue mass measures, and serum leptin concentration by cognitive eating restraint (CER) group at baseline, six months, and twelve months

	BASELINE			SIX MONTHS			TWELVE MONTHS		
	Low CER (n=20)	**High CER** (n=16)	**P-value for Student's t-test**	**Low CER** (n=19)	**High CER** (n=16)	**P-value for Student's t-test**	**Low CER** (n=20)	**High CER** (n=16)	**P-value for Student's t-test**
Bone Mineral Content (g)									
Total body	2206.7 (57.0)	2193.6 (60.1)	$p = 0.8756$	2224.3 (59.3)	2192.8 (59.1)	$p = 0.7117$	2224.0 (57.1)	2194.5 (64.7) [a]	$p = 0.7354$
Lumbar spine	56.2 (2.2)	57.4 (2.3) [a]	$p = 0.7278$	56.6 (2.2)	58.6 (2.4)	$p = 0.5414$	57.5 (2.0)	58.9 (2.3)	$p = 0.6435$
Hip	34.0 (1.5)	34.3 (0.9)	$p = 0.8530$	34.4 (1.3)	34.5 (1.0)	$p = 0.9297$	32.4 (1.0)	33.9 (1.1)	$p = 0.3274$
Femoral Neck	4.5 (0.2)	4.6 (0.1)	$p = 0.6400$	4.4 (0.1)	4.5 (0.1)	$p = 0.5888$	4.3 (0.1)	4.6 (0.1)	$p = 0.1479$
Arm	12.3 (0.4)	11.8 (0.2) [a]	$p = 0.2819$	12.4 (0.4)	11.9 (0.2)	$p = 0.3011$	12.3 (0.4)	12.0 (0.2)	$p = 0.5415$
Bone Mineral Density (g/cm^2)									
Total body	1.1490 (0.0179)	1.1179 (0.0179)	$p = 0.2340$	1.1465 (0.0179)	1.1210 (0.0180)	$p = 0.3257$	1.1426 (0.0167)	1.1208 (0.0190) [a]	$p = 0.3956$
Lumbar spine	1.0073 (0.0202)	1.0183 (0.0238) [a]	$p = 0.7257$	1.0135 (0.0201)	1.0241 (0.0221)	$p = 0.7236$	1.0149 (0.0192)	1.0256 (0.0214)	$p = 0.7119$
Hip	1.0060 (0.0267)	1.0064 (0.0201)	$p = 0.9901$	1.0071 (0.0252)	0.9994 (0.0194)	$p = 0.8160$	0.9826 (0.0235)	0.9889 (0.0209)	$p = 0.8455$
Femoral neck	0.8790 (0.0259)	0.8951 (0.0211)	$p = 0.6439$	0.8821 (0.0236)	0.8937 (0.0204)	$p = 0.7184$	0.8960 (0.0206)	0.9184 (0.0210)	$p = 0.4570$
Arm	0.5748 (0.0096)	0.5661 (0.0070) [a]	$p = 0.4941$	0.5718 (0.0098)	0.5652 (0.0080)	$p = 0.6136$	0.5750 (0.0091)	0.5668 (0.0071)	$p = 0.4991$
Soft Tissue Mass									
Body weight (kg)	57.1 (1.4)	61.3 (1.7)	$p = 0.0579$	58.2 (1.2)	60.9 (1.6)	$p = 0.1822$	58.1 (1.4)	60.8 (5.8)	$p = 0.1945$
Total lean mass (kg)	40.9 (0.7)	42.8 (1.1)	$p = 0.1347$	41.3 (0.6)	42.5 (1.0)	$p = 0.2790$	41.3 (0.7)	43.0 (1.0) [a]	$p = 0.1495$
Total fat mass (kg)	14.4 (0.8)	16.8 (1.1)	$p = 0.0872$	15.0 (0.8)	16.8 (0.1)	$p = 0.1943$	15.1 (0.9)	15.8 (0.9) [a]	$p = 0.5544$
Body fat (%)	24.7 (0.9)	26.9 (1.3)	$p = 0.1694$	25.4 (1.0)	27.0 (1.3)	$p = 0.3082$	25.4 (0.9)	25.7 (1.0) [a]	$p = 0.8252$
Leptin (ng/mL)	9.40 (0.96)	13.22 (1.70)	$\mathbf{p = 0.0469}$	9.92 (1.02) [b]	11.13 (1.94)	$p = 0.5646$	9.36 (0.91)	8.39 (1.02)	$p = 0.4821$

[a] n=15, [b] n=20

Table 2: Pearson correlation coefficients for association between serum leptin concentration and bone mineral content, bone mineral density, and soft tissue mass measures by cognitive eating restraint (CER) group at baseline, six months, and twelve months

	BASELINE		SIX MONTHS		TWELVE MONTHS	
	Low CER (n=20)	**High CER** (n=16)	**Low CER** (n=19)	**High CER** (n=16)	**Low CER** (n=20)	**High CER** (n=16)
Bone Mineral Content (g)						
Total body	0.11	-0.01	-0.16	0.12	0.13	-0.28 [a]
Lumbar spine	0.18	0.02 [a]	-0.26	0.05	0.03	-0.22
Hip	0.32	0.16	0.05	0.10	0.16	0.08
Femoral Neck	0.16	0.13	-0.01	0.25	0.05	0.16
Arm	0.36	0.04 [a]	0.22	0.05	0.35	-0.24
Bone Mineral Density (g/cm^2)						
Total body	-0.19	-0.02	-0.47 *	0.16	-0.13	-0.27 [a]
Lumbar spine	0.12	0.01 [a]	-0.26	0.27	0.08	-0.20
Hip	-0.01	0.25	-0.24	0.19	0.06	0.20
Femoral Neck	0.04	0.11	-0.27	0.26	0.05	0.20
Arm	0.22	-0.01 [a]	-0.08	-0.14	0.27	-0.04
Soft Tissue Mass						
Body weight (kg)	0.71 ***	0.40	0.34	0.46	0.57 **	0.28
Total lean mass (kg)	0.51 *	-0.15	-0.02	-0.15	0.34	-0.02 [a]
Total fat mass (kg)	0.76 ****	0.77 ***	0.57 *	0.80 ***	0.67 **	0.48 [a]
Body fat (%)	0.70 ***	0.84 ****	0.65 ***	0.84 ****	0.65 **	0.53 [a*]
Leptin (ng.mL)						
Time 0: Baseline			0.63 [b] **	0.84 ****	0.63 **	0.81 ***
Time 1: 6 months	0.63 **	0.84 ****			0.46 *	0.76 ***
Time 2: 12 months	0.63 **	0.81 ***	0.46 [b] *	0.76 ***		

[a] n=15, [b] n=20

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ****$p \leq 0.0001$

Table 3: Pearson correlation coefficients for relationship between body weight and bone mineral content, bone mineral density, and serum leptin concentration by cognitive eating restraint (CER) group at baseline, six months, and twelve months

	BASELINE		SIX MONTHS		TWELVE MONTHS	
	Low CER (n=20)	**High CER** (n=16)	**Low CER** (n=19)	**High CER** (n=16)	**Low CER** (n=20)	**High CER** (n=16)
Bone Mineral Content (g)						
Total body	0.52 *	0.56 *	0.49 *	0.61 *	0.46 *	0.56 *
Lumbar spine	0.45 *	-0.04 [a]	0.33	0.10	0.40	0.08
Hip	0.48 *	0.71 **	0.39	0.76 ***	0.43	0.79 ***
Femoral Neck	0.37	0.34	0.24	0.44	0.28	0.51 *
Arm	0.65 **	0.54 [a] *	0.61 **	0.57 *	0.65 **	0.46
Bone Mineral Density (g/cm^2)						
Total body	0.12	0.32	0.13	0.31	0.02	0.32 [a]
Lumbar spine	0.30	0.11 [a]	0.19	0.29	0.26	0.26
Hip	0.14	0.37	0.01	0.43	0.08	0.51 *
Femoral Neck	0.13	0.17	0.04	0.26	0.08	0.23
Arm	0.52 *	0.16 [a]	0.59 **	-0.09	0.46 *	-0.12
Body weight (kg)						
Time 0: Baseline			0.98 ****	0.98 ****	0.96 ****	0.96 ****
Time 1: 6 months	0.98 ****	0.98 ****			0.94 ****	0.97 ****
Time 2: 12 months	0.96 ****	0.96 ****	0.94 ****	0.97 ****		
Leptin (ng.mL)	0.71 ***	0.40	0.34	0.46	0.57 **	0.28

[a] n=15, [b] n=20

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ****$p \leq 0.0001$

Table 4: Changes in serum leptin concentrations within cognitive eating restraint (CER) groups over time

	Group			
	LOW CER		**HIGH CER**	
	(n = 20)		(n = 16)	
	Serum leptin (ng/mL)		Serum leptin (ng/mL)	
	Mean (SD)	Range	Mean (SD)	Range
Baseline	9.40 (4.31)	3.54 - 19.68	13.22 (6.75) [a]	3.61 – 29.67
Time 1	9.92 (4.55)	2.88 - 21.73	11.13 (7.77)	4.00 - 32.05
Time 2	9.36 (4.07)	3.86 - 17.42	8.39 (4.07) [b]	3.80 - 19.31

[a] significantly different from low CER group, $p \leq 0.05$

[b] significantly different from baseline, $p \leq 0.05$

Table 5: Changes in body weight and soft tissue mass within cognitive eating restraint (CER) groups over time

	Group			
	LOW CER (n = 20)		HIGH CER (n = 16)	
	Mean (SD)	Range	Mean (SD)	Range
Body Weight (kg)				
Baseline	57.1 (6.1)	47.6 - 69.5	61.3 (6.6)	51.8 - 71.8
Time 1 [1]	58.2 (5.4) [a]	50.0 - 68.9	60.9 (6.5)	51.8 - 71.9
Time 2	58.1 (6.1) [b]	46.9 - 72.8	60.8 (5.8)	50.9 - 69.6
Total Lean Mass (kg)				
Baseline	40.9 (3.0)	35.8 - 46.9	42.8 (4.3)	36.9 - 50.5
Time 1 [1]	41.3 (2.5)	36.2 - 46.5	42.5 (4.1)	36.7 - 49.8
Time 2 [2]	41.3 (3.0)	34.8 - 46.8	43.0 (3.8)	37.3 - 49.1
Total Fat Mass (kg)				
Baseline	14.4 (3.7)	7.9 - 20.6	16.8 (4.5)	9.7 - 24.8
Time 1 [1]	15.0 (3.6)	8.0 - 20.5	16.8 (4.5)	9.2 - 25.9
Time 2 [2]	15.1 (3.8) [a]	8.9 - 24.4	15.8 (3.5)	10.7 - 23.7
Body fat (%)				
Baseline	24.7 (4.2)	16.4 - 31.9	26.9 (5.3)	17.8 - 38.8
Time 1 [1]	25.4 (4.2)	15.8 - 32.1	27.0 (5.3)	17.2 - 37.3
Time 2 [2]	25.4 (4.2)	18.8 - 33.1	25.7 (4.0)	19.4 - 34.0

[1] n = 19

[2] n = 15

[a] significantly different from baseline, $p \leq 0.05$

[b] significantly different from baseline, $p \leq 0.01$

Table 6: Changes in bone mineral content measures (g) within cognitive eating restraint (CER) groups over time

	Group			
	LOW CER (n = 20)		HIGH CER (n = 16)	
	Mean (SD)	Range	Mean (SD)	Range
Total Body				
Baseline	2207 (255)	1698 – 2625	2194 (240)	1892 – 2624
Time 1 [1]	2224 (259)	1721 – 2659	2193 (236)	1906 – 2593
Time 2 [2]	2224 (255) [a]	1712 – 2660	2194 (251)	1912 – 2606
Lumbar Spine				
Baseline [2]	56.2 (9.7)	37.9 - 73.6	57.4 (9.1)	49.4 - 86.7
Time 1 [1]	56.6 (9.7)	34.2 - 71.5	58.6 (9.4)	49.0 - 86.0
Time 2	57.5 (9.1)	35.1 - 72.9	58.9 (9.0)	50.2 - 84.1
Hip				
Baseline	34.0 (6.5)	21.9 - 48.7	34.3 (3.6)	27.1 - 39.5
Time 1 [1]	34.4 (5.6)	23.1 - 44.8	34.5 (3.9)	29.7 - 41.3
Time 2	32.4 (4.5) [a,c]	21.9 - 39.4	33.9 (4.3)	27.5 - 43.1
Femoral Neck				
Baseline	4.5 (0.7)	3.2 - 5.6	4.6 (0.4)	4.1 – 5.4
Time 1 [1]	4.4 (0.6)	3.2 - 5.6	4.5 (0.4)	3.9 – 5.1
Time 2	4.3 (0.6) [b,d]	3.2 - 5.3	4.6 (0.5)	3.9 – 5.4
Arm				
Baseline [2]	12.3 (1.6)	9.6 - 15.5	11.8 (0.8)	10.1 - 13.1
Time 1 [1]	12.4 (1.6)	9.5 - 15.4	11.9 (0.9)	10.1 - 13.3
Time 2	12.3 (1.6)	9.5 - 15.2	12.0 (0.8) [a,d]	10.2 - 13.4

[1] n = 19, [2] n = 15

[a] significantly different from baseline, $p \leq 0.05$; [b] significantly different from baseline, $p \leq 0.005$; [c] significantly different from Time 1, $p \leq 0.01$; [d] significantly different from Time 1, $p \leq 0.05$

Table 7: Changes in bone mineral density measures (g/cm^2) within cognitive eating restraint (CER) groups over time

	Group			
	LOW CER (n = 20)		HIGH CER (n = 16)	
	Mean (SD)	Range	Mean (SD)	Range
Total Body				
Baseline	1.1490 (0.0802)	0.9680 - 1.2860	1.1179 (0.0717)	1.0220 - 1.2720
Time 1 [1]	1.1465 (0.0781)	0.9760 - 1.2510	1.1210 (0.0721)	1.0140 - 1.2850
Time 2 [2]	1.1426 (0.0746)	0.9760 - 1.2390	1.1208 (0.0736)	1.0280 - 1.2640
Lumbar Spine				
Baseline [2]	1.0073 (0.0903)	0.8030 - 1.1570	1.0183 (0.0239)	0.9080 - 1.2720
Time 1 [1]	1.0135 (0.0876)	0.7980 - 1.1370	1.0241 (0.0885)	0.9040 -1.2360
Time 2	1.0149 (0.0858)	0.8110 - 1.1270	1.0256 (0.0857)	0.9030 - 1.2200
Hip				
Baseline	1.0060 (0.1192)	0.7680 - 1.2100	1.0064 (0.0805)	0.8550 - 1.1370
Time 1 [1]	1.0070 (0.1098)	0.8060 - 1.2340	0.9994 (0.0776)	0.8310 - 1.1470
Time 2	0.9826 (0.1050)[a,b]	0.8010 - 1.2320	0.9889 (0.0837)[a]	0.8250 - 1.1600
Femoral Neck				
Baseline	0.8790 (0.1157)	0.6770 - 1.0860	0.8951 (0.0843)	0.7570 - 1.0240
Time 1 [1]	0.8821 (0.1030)	0.6940 - 1.0900	0.8937 (0.0815)	0.7610 - 1.0260
Time 2	0.8960 (0.0923)[b]	0.7290 - 1.0500	0.9184 (0.0838)[b]	0.7830 - 1.0670
Arm				
Baseline [2]	0.5748 (0.0428)	0.4930 - 0.6590	0.5661 (0.0272)	0.5250 - 0.6140
Time 1 [1]	0.5718 (0.0426)	0.4840 - 0.6500	0.5652 (0.0320)	0.5200 - 0.6150
Time 2	0.5750 (0.0407)	0.4890 - 0.6480	0.5668 (0.0282)	0.5200 - 0.6280

[1] n = 19, [2] n = 15

[a] significantly different from baseline, $p \leq 0.02$, [b] significantly different from Time 1, $p \leq 0.05$

Discussion

As hypothesized, serum leptin was significantly higher in normal weight premenopausal women with high versus low CER scores. However, this difference in mean serum leptin concentrations between CER groups was found only at baseline. Other differences in variables of interest were not detected in these groups of women with high and low CER. This observation may be explained through weight and body fat changes in the groups through the duration of the study. Differences between the CER groups in body weight, total fat mass, and body fat % were greatest at baseline, and the magnitude of these differences decreased over time. Similarly, differences in leptin concentrations between the two CER groups also lessened and became non significant.

In general, serum leptin concentrations in the current study were positively associated with fat mass and body fat % as previously reported in other studies comparing leptin with these measures of adiposity (9-12). Serum leptin tends to change in the direction of body weight fluctuations (19, 20), and although not significant, this pattern was demonstrated in the high CER group. As mean body weight decreased through the duration of the study, so did serum leptin. In overweight preadolescent girls, a significant negative correlation between CER scores and serum leptin concentration measured 6 months later was demonstrated (21). This relationship held even after controlling for fat mass. In the current study, CER scores were inversely related with serum leptin concentration, but these observations did not reach

statistical significance. Thus, a lag between body weight or body composition changes and serum leptin may be possible and should be investigated in premenopausal women with healthy weights.

In the current study, we were able to demonstrate a significant difference in serum leptin concentrations between the low and high CER groups. The low CER group had a trend toward lower body weight and fat mass than the high CER group, but unlike previous research, no significant differences in either BMC or BMD were identified (17). Van Loan et al. (17) studied 52 premenopausal women and found that BMC was significantly different between low and high CER groups, with the low CER group having significantly lower BMC and increased risk of osteoporosis. Mechanisms to explain these observations were not investigated, but further research is warranted to identify them.

Serum leptin was inversely associated with total body BMD at 6 months in the low CER group. Serum leptin was at its highest concentration at this interval in the low CER group. Total body weight was also the highest at 6 months, contradicting previous research related to mechanical loading of body weight on bone (3, 4, 19, 22-30). These results suggest that factors other than mechanical loading, such as leptin, may influence bone mass in healthy weight, premenopausal women. Yet, these results do not support a direct association between serum leptin and BMC or BMD in this group of women.

Seasonal variation, sample characteristics, and energy consumption prior to sample collection may serve as limitations to this study. Season may

influence body weight and serum leptin. Animals may be affected by a seasonal variation in leptin. In female sheep, leptin was more effective in decreasing appetite in the spring than autumn, potentially leading to increased weight gain often observed before winter (31). In ovariectomized cows, serum leptin increased in early winter by 34% (32). In humans, evidence is inconclusive. Body weight in humans during winter is typically higher than during summer (33). Thus, leptin may follow a similar seasonal pattern. However, Donahoo et al. (33) found that leptin was not influenced by season in 18 normal weight men and women in a cross-sectional study with two study periods (summer and winter). Another cross-sectional study of 25 healthy men and women found no relationship between serum leptin concentration and season when examined once during each season for a total of 4 study periods (34). Leptin may be influenced by diet composition and quantity of energy consumed masking the true relationship between season and leptin (35, 36). Research is needed to more thoroughly elucidate any possible relationship between leptin and season or exposure to sunlight.

The sample for the current study was composed of a fairly homogeneous group of premenopausal women. Previous studies have shown that bone changes at different speeds depending on circumstances and stage in life span (37, 38). Bone formation is dominant in childhood and preadulthood, but bone resorption outpaces formation for most women following menopause. Women in the current study were in a phase where bone formation and resorption were coupled, resulting in minute changes. In addition, studies have demonstrated

that recent fractures can impact bone mass (39-42). The presence and timing of an earlier fracture may have a significant impact on bone mass measures. Although none of the women in this study suffered a fracture during the study, data regarding previous fractures in this group of women were not available.

Although subjects in both groups fasted prior to blood collection, diet composition and quantity of energy in the meal prior to blood sampling may have affected serum leptin. Leptin acts as a satiety signal in the brain which, in theory, decreases appetite and terminates nutrient consumption. In a study of 204 normal weight and obese adult men and women (age 18-80 y), Ostlund et al. (43) demonstrated that diet composition and long-term energy intake did not impact serum leptin for the group. However, serum leptin in subjects who consumed an energy deficient diet (1000 total kcal for 10 days) was 26% lower (43). In a study where subjects consumed excess energy, Kolaczynski et al. (35) found significant serum leptin increases. In the acute overfeeding group (120 kcal/kg for 12 hours prior to blood collection), serum leptin concentration was 40% higher than baseline and remained elevated until the next morning when the next meal was consumed (35). The second group, the chronically overfed group, subjects consumed excess energy to attain and maintain a 10% body weight increase for two weeks. This group displayed serum leptin concentrations that were more than three times higher than baseline measures (35). Raben and Astrup (36) found that diet composition impacted serum leptin. Specifically, fasting and postprandial leptin concentrations were higher following a sucrose-rich diet than either a starch- or fat-rich diet (36). These

studies suggest that diet composition, amount of energy, and timing of a meal can all impact fasting serum leptin concentration and any conclusions drawn regarding relationships between serum leptin and other measures.

The few studies that have examined the relationship between leptin and CER score focused on young females with unhealthy weight. Laessle et al. (21) observed a significant negative correlation between serum leptin and eating restraint score in 20 obese, preadolescent girls. Serum leptin was measured six months after eating restraint score was determined using the Eating Inventory. Similar results were found by von Prittwitz et al. (44) in a study of underweight (n = 136) and overweight (n = 49) female students.

To our knowledge, this is the first study to examine relationships between serum leptin and soft tissue measures and bone mineral measures in a healthy weight (as defined by BMI 18-25 kg/m^2), premenopausal adult population based on CER scores. In the current study, we show a positive relationship between serum leptin concentration and body fat mass and body fat % in normal weight women despite differences in CER scores. In addition, we were able to demonstrate a significant difference in serum leptin concentrations between the low and high CER groups.

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CHAPTER IV

SERUM LEPTIN DECREASES IN RESPONSE TO BODY WEIGHT REDUCTIONS INDUCED BY A LOW-CARBOHYDRATE, HIGH-PROTEIN DIET OR A LOW-FAT, HIGH CARBOHYDRATE DIET IN WOMEN

Abstract

Few studies have examined changes in serum leptin due to body weight loss induced by diets of varying macronutrient composition. Overweight and obese women, aged 32-45 years, were randomly assigned to either a low-carbohydrate, high-protein (LCHP) diet or low-fat, high-carbohydrate (LFHC) diet. Relationships between serum leptin concentration and bone mineral measures and soft tissue measures at three intervals were examined. Serum leptin concentration was significantly greater in the LCHP diet group versus the LFHC diet group at 12 weeks ($p < 0.05$). Over time, significant decreases in serum leptin concentration, BMI, body weight, total lean mass, total fat mass, and body fat % were observed in both diet groups. Serum leptin concentration was positively associated with body weight, fat mass, and body fat % regardless of diet. In the LCHP diet group, body weight was not significantly related to any measure of BMC or BMD at any interval, but in the LFHC diet group, body weight was associated with total body BMC at all intervals ($p \leq 0.05$ for all). Contrary to previous research, we observed an increase in total body BMD as body weight and serum leptin decreased over time despite differences in diet composition.

KEYWORDS:

Bone mineral content; Bone mineral density; Leptin; Low-carbohydrate, High-Protein Diet; Low-Fat, High-Carbohydrate Diet; Weight loss; Women

Introduction

With the increasing prevalence of obesity in the United States, weight loss diets are more popular than ever. The Atkins diet has regained popularity in recent years, but little is known about the long term consequences of this diet. Furthermore, few studies have been conducted to elucidate the impact of the Atkins diet composition on hormonal mediators of body composition, including bone mineral measures.

Diet composition and quantity of energy in the meal may affect serum leptin. Raben and Astrup (1) demonstrated that fasting and postprandial leptin concentrations were higher following a sucrose-rich diet than either a starch- or fat-rich diet. A separate study investigating the effect of a high- versus low-carbohydrate diet on serum leptin concentration found that in both fasting and post-prandial states, women who consumed a high-carbohydrate diet for three days had a higher mean serum leptin concentration compared to when they consumed a low-carbohydrate diet for three days (2). However, this previous study did not involve weight loss or include a high-protein component of the diet.

Energy deficits have been shown to decrease serum leptin and bone mass (3, 4). Furthermore, body weight has a protective effect on bone mass. Recent studies have demonstrated an inverse relationship between serum leptin concentration and BMD in premenopausal (5) and peri- and post-menopausal women (6), but the relationship between the macronutrients and bone mass and serum leptin is still unclear. Thus, the purpose of this study

was to examine relationships between serum leptin concentration and bone mineral measures and soft tissue measures in women following the Atkins diet, a low-carbohydrate, high-protein (LCHP) diet, or the American Heart Association's traditional low-fat, high-carbohydrate (LFHC) diet at three intervals and to evaluate changes in these variables over time. The primary hypothesis of this study was that mean serum leptin concentration would significantly differ between overweight premenopausal women who lost weight and body fat mass following the Atkins diet compared to the traditional diet over 12 weeks.

Materials and Methods

Study participants

Subjects for this study were drawn from a larger study (7) designed to evaluate changes in bone mass and BMD in overweight pre-menopausal women following either a LCHP or LFHC diet to induce weight loss. Subjects were recruited via paper notices, electronic-mail messages, and personal interactions. The original study and this subsequent study were approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University (VPI&SU; Blacksburg, Virginia, U.S.A.). All women provided written informed consent after they had an opportunity to have their questions and concerns answered. Women were aged 32 to 45 years, were eumenorrheic, had stable body weight for $\geq$ 1 year, and had body mass index (BMI, kg/m^2) BMI $\geq$ 25 to $\leq$ 43.

Exclusion criteria for the primary study included being or trying to become pregnant; lactation; use of prescription medications for metabolic or endocrine disorders known to affect bone metabolism or acid-base balance; current chronic diseases known to affect bone health; impaired renal function; metabolic acidosis or alkalosis; hysterectomy or ovariectomy without hormone replacement therapy; > 7 hours of intense physical activity per week; consumption of >2 alcoholic beverages per day on more then 2 days per week; body mass index < 25; and participation in weight loss intervention within the past year (7). Exclusion criteria for the current study included missing serum samples at any one of three intervals. Data from 25 of the original subjects were included in these analyses.

Procedures

Subjects were randomly assigned to either the LCHP or LFHC diet group and followed the intervention program for 12 weeks between August and December 2003. At baseline and after 6 (Time 1) and 12 weeks (Time 2), subjects underwent anthropometric and body composition testing. Each subject also provided fasting blood samples at these intervals. The study protocol was performed in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation (BONE) Laboratory, VPI&SU.

Anthropometric data

Subjects wore lightweight clothing and no shoes for body weight and height measurements. Body weight was measured (± 0.1 kg) using a calibrated electronic scale (Scaletronix, Wheaton, IL, U.S.A.). Standing height was

measured (± 0.01 cm) using a digital stadiometer (Heightronic, Measurement Concepts, North Bend, WA, U.S.A.). Body weight and standing height were used to calculate body mass index (BMI = kg/m^2) for each subject.

Bone mineral and soft tissue measurements

Dual-energy X-ray absorptiometry (DXA; QDR4500A Hologic, Hologic, Inc., Bedford, MA, U.S.A.) scans were conducted to obtain BMC (g), BMD (g/cm^2), and soft tissue mass measurements. Total body, lumbar spine (L_1-L_4), non-dominant total proximal femur (hip), including the femoral neck, and non-dominant total forearm (arm) scans were completed and analyzed with the Hologic whole body analysis software (version 8.26a:3*) and site specific software. Lumbar spine was analyzed using the standard protocol; hip using version 8.23; and arm using version 8.25. All women were tested for pregnancy before each DXA scan. Total body lean mass (kg), fat mass (kg), and percentage body fat (%) were obtained from total body DXA scans. All DXA scans were carried out and analyzed by one investigator. Quality control steps for BMC and BMD were performed on the morning of each testing day using a lumbar spine phantom, producing a coefficient of variation (CV) of 0.36%. Quality control steps for soft tissue mass measurements were conducted weekly using an external tissue bar of aluminum and lucite calibrated against stearic acid and water (Hologic, Bedford, MA, U.S.A). Test-retest reliability in the BONE Lab produced CV for total body, lumbar spine, hip, arm, lean mass, fat mass, and body fat % of 0.73, < 2.00, 0.69, 1.09, 1.07, 1.75, and 1.79%, respectively (8).

Serum leptin concentration

At each interval, women provided fasting (12-hours) blood samples between 0700 – 0930. Timing of fasting blood sample collection was monitored to reduce diurnal fluctuations in serum leptin. A certified phlebotomist collected all venous blood samples, after which serum was separated from whole blood by centrifugation at 2500 x *g* for 10 minutes at 25°C. Serum samples were aliquotted into cryovials and stored at -80°C. A human leptin radioimmunoassay (RIA; HL-81K, Linco Research, Inc., St. Charles, MO, U.S.A.) was used to measure serum leptin (ng/mL) from stored samples. The sensitivity, specificity, and precision of this assay are 0.5 ng/mL, 100%, and ≤ 6.2% between assays, respectively. The normal fasting mean serum leptin concentration for overweight women (BMI 30.9 $\pm$ 5.5 kg/m^2) is 23.4 ± 13.7 ng/mL (9). All samples were analyzed in duplicate, and the inter- and intra-assay CV were 8.5% and 8.8%, respectively.

Statistical analyses

Descriptive statistics were conducted to describe subject characteristics at baseline. Differences in mean BMC, BMD, soft tissue mass measures, and serum leptin concentrations between LCHP and LFHC diet groups were completed using student's *t*-tests at each interval. Statistical significance was set at $p \leq 0.05$. Pearson correlation coefficients were computed to examine relationships between variables of interest within the LCHP and LFHC diet groups. A two-tailed significance was set at $p \leq 0.05$. Analysis of variance (ANOVA) with repeated measures on the time factor was used to identify

changes in measures both within and between groups over time. Statistical significance was set at $p \leq 0.05$ for all comparisons. Statistical analyses were performed using SAS (version 8.2, 1999-2001, SAS Institute Inc., Cary, NC, U.S.A.).

Results

The mean (±SD) for age, height, weight, and BMI of subjects in the LCHP diet group were: 39.2 ± 3.7 y, 165.5 ± 4.3 cm, 85.6 ± 12.8 kg, and 31.3 ± 4.9 kg/m^2. For subjects in the LFHC diet group, mean age, height, weight, and BMI were: 39.8 ± 3.1 y, 161.6 ± 6.9 cm, 78.0 ± 16.6 kg, and 29.7 ± 5.4 kg/m^2, respectively. Significant differences in these anthropometric measures were not found at baseline.

Bone mineral content, BMD, soft tissue measures, and serum leptin concentrations of subjects in the LCHP diet group (n = 13) and LFHC diet group (n = 12) for baseline, six weeks, and twelve weeks are shown in Table 1. Significant differences in BMC or BMD measures between groups were not observed at any interval. Soft tissue mass measures were not significantly different between groups at any interval. Serum leptin was significantly higher in the LCHP diet group versus LFHC diet group at twelve weeks only (mean ± SEM = 14.54 ± 1.51 ng/mL vs. 10.34 ± 1.09 ng/mL, respectively, $p \leq 0.05$).

Table 2 shows the association between serum leptin concentration and measures of BMC, BMD, and soft tissue for both LCHP and LFHC diet groups at baseline, six week (Time 1), and twelve week (Time 2) intervals. In the LCHP group, leptin was significantly related to body weight at baseline ($p \leq 0.05$), Time 1 ($p \leq 0.01$), and Time 2 ($p \leq 0.01$). Serum leptin was also significantly associated with total fat mass and body fat % at each interval ($p \leq 0.05$ to $p \leq 0.0001$). At baseline, serum leptin was not significantly associated with BMI but was significantly associated at Time 1 and Time 2 (both $p \leq 0.001$).

Interestingly, total lean mass was positively related to serum leptin concentration at Time 1 and Time 2 (both $p \leq 0.05$).

In the LFHC diet group, leptin was significantly associated with body weight at baseline and Time 1 (both $p \leq 0.001$) but not at Time 2. Serum leptin was not significantly associated with total lean mass at any interval but was associated with total fat mass at baseline, Time 1, and Time 2 ($p \leq 0.001$, $p \leq 0.0001$, and $p \leq 0.05$, respectively). Body fat % and serum leptin were positively associated at all intervals ($p \leq 0.01$ to $p \leq 0.0001$). At baseline and Time 1, BMI was positively associated with serum leptin concentration ($p \leq 0.01$ and $p \leq 0.001$, respectively) but was not significantly associated at Time 2.

Table 3 displays correlation coefficients between body weight and BMC, BMD, and serum leptin measurements at baseline, Time 1, and Time 2, respectively. In the LCHP diet group, body weight was not significantly related to any measure of BMC or BMD at baseline. In the LFHC diet group, body weight was associated with total body BMC at all intervals ($p \leq 0.05$ for all). Hip BMC and body weight were positively related at baseline and Time 2 (both $p \leq 0.05$). Arm BMC and body weight were significantly associated at Time 1 ($p \leq 0.01$). Lumbar spine BMD and body weight were positively associated at Time 1 and Time 2 ($p \leq 0.05$). No other significant associations between body weight and BMD were observed at any interval.

Table 4 shows significant associations between BMI and BMC and BMD measures were not found at any interval. However, BMI and serum leptin was

significantly related at Time 1 ($p \leq 0.001$) and Time 2 ($p \leq 0.001$) in the LCHP group and at baseline ($p \leq 0.01$) and Time 1 ($p \leq 0.001$) in the LFHC group.

Table 5 shows changes in mean serum leptin concentrations over time in both diet groups. Mean (± SD) serum leptin significantly decreased from baseline to Time 1 in both the LCHP and LFHC diet groups (both $p \leq 0.001$). Also, in the LCHP and LFHC diet groups, the mean serum leptin concentration significantly decreased from baseline to Time 2 ($p \leq 0.001$, respectively).

Table 6 contains the changes in body weight and soft tissue mass measures over time in LCHP and LFHC diet groups. Mean (± SD) body weight and total fat mass decreased from baseline to Time 1, Time 1 to Time 2, and baseline to Time 2 ($p \leq 0.0001$ for all time intervals) in the LCHP diet group. Total lean mass and body fat % decreased from baseline to Time 1, Time 1 to Time 2, and baseline to Time 2 ($p \leq 0.05$ to $p \leq 0.0001$) in the LCHP diet group. In the LFHC diet group, body weight and body fat % decreased from baseline to Time 1 ($p \leq 0.0001$), Time 1 to Time 2 ($p \leq 0.0005$), and baseline to Time 2 ($p \leq 0.0001$). Total lean mass was significantly greater at baseline than either Time 1 or Time 2 ($p \leq 0.005$). Total fat mass decreased from baseline to Time 1, Time 1 to Time 2, and baseline to Time 2 ($p \leq 0.0001$ for all time intervals).

Table 7 shows significant changes in total body BMC and specific BMC sites over time in the LCHP and LFHC diet groups. In the LCHP diet group, total body BMC decreased from baseline to Time 1 and baseline to Time 2 (both $p \leq 0.05$). Arm BMC increased from baseline to Time 1 ($p \leq 0.02$) and baseline

to Time 2 ($p \leq 0.01$) for LFHC group. No other significant changes in BMC were observed in either group.

Table 8 displays changes in total body BMD and at specific body sites for both the LCHP and LFHC diet groups over time. In the LCHP diet group, total body BMD significantly increased from baseline to Time 1 and baseline to Time 2 (both $p \leq 0.05$). In the LFHC diet group, total body BMD increased from baseline to Time 1 ($p \leq 0.05$) and baseline to Time 2 ($p \leq 0.01$). No significant changes were observed at any specific body sites in either group.

Table 9 shows significant changes in BMI within the LCHP and LFHC diet groups over time. Both diet groups showed significant decreases in BMI between baseline and Time 1 and baseline and Time 2 ($p \leq 0.0001$ for all comparisons). From Time 1 to Time 2 BMI in both the LCHP and LFHC groups decreased ($p \leq 0.0001$ and $p = 0.0003$, respectively).

Table 1: Mean (SEM) bone mineral content and density, soft tissue mass measures, and serum leptin concentration by diet group at baseline, six weeks, and twelve weeks

	BASELINE			SIX WEEKS			TWELVE WEEKS		
	LCHP [1]	**LFHC** [2]	**P-value for Student's t-test**	**LCHP** [1]	**LFHC** [2]	**P-value for Student's t-test**	**LCHP** [1]	**LFHC** [2]	**P-value for Student's t-test**
Bone Mineral Content (g)									
Total body	2314.2 (47.8)	2350.8 (100.9)	$p = 0.7401$	2310.1 (43.1)	2361.3 (103.4)	$p = 0.6427$	2310.1 (47.4)	2343.5 (99.1)	$p = 0.7581$
Lumbar spine	64.4 (2.7)	62.3 (3.7)	$p = 0.6445$	64.4 (2.7)	62.8 (3.5)	$p = 0.7150$	64.1 (2.7)	62.0 (3.5)	$p = 0.6336$
Hip	30.6 (1.1)	31.6 (1.9)	$p = 0.6536$	30.2 (1.2)	30.6 (2.1)	$p = 0.8896$	30.4 (1.2)	31.6 (2.0)	$p = 0.5888$
Arm	12.5 (0.2)	12.0 (0.5)	$p = 0.3725$	12.7 (0.2)	12.4 (0.6)	$p = 0.6434$	12.7 (0.2)	13.1 (0.8)	$p = 0.5995$
Bone Mineral Density (g/cm^2)									
Total body	1.1234 (0.0208)	1.1799 (0.0263)	$p = 0.1027$	1.1419 (0.0192)	1.1920 (0.0249)	$p = 0.1220$	1.1438 (0.0207)	1.1938 (0.0267)	$p = 0.1502$
Lumbar spine	1.0745 (0.0299)	1.0970 (0.0366)	$p = 0.6370$	1.0718 (0.0283)	1.1068 (0.0355)	$p = 0.4551$	1.0749 (0.0263)	1.1043 (0.0371)	$p = 0.5200$
Hip	0.9403 (0.0327)	1.0078 (0.0326)	$p = 0.1578$	0.9362 (0.0338)	1.0059 (0.0349)	$p = 0.1651$	0.9334 (0.0318)	1.0018 (0.0357)	$p = 0.1647$
Arm	0.5662 (0.0110)	0.5843 (0.0167)	$p = 0.3708$	0.5685 (0.0116)	0.5854 (0.0157)	$p = 0.3921$	0.5688 (0.0112)	0.5843 (0.0175)	$p = 0.4588$
Soft Tissue Mass									
Body weight (kg)	85.6 (3.5)	78.0 (4.8)	$p = 0.2117$	80.5 (3.3)	73.9 (4.5)	$p = 0.2421$	78.3 (3.2)	71.9 (4.2)	$p = 0.2315$
Total lean mass (kg)	48.0 (1.5)	46.2 (2.0)	$p = 0.4782$	46.4 (1.4)	44.9 (1.9)	$p = 0.5308$	45.8 (1.3)	44.9 (1.9)	$p = 0.6919$
Total fat mass (kg)	35.3 (2.3)	29.5 (3.0)	$p = 0.1319$	32.0 (2.2)	26.7 (2.8)	$p = 0.1486$	30.2 (2.1)	24.6 (2.5)	$p = 0.1056$
Body fat (%)	40.8 (1.3)	36.9 (1.7)	$p = 0.0804$	38.9 (1.5)	35.2 (1.8)	$p = 0.1246$	37.9 (1.5)	33.4 (1.6)	$p = 0.0566$
Body mass index (kg/m^2)	31.3 (1.3)	29.7 (1.5)	$p = 0.4537$	29.4 (1.2)	28.2 (1.5)	$p = 0.5198$	28.6 (1.2)	27.4 (1.4)	*$p = 0.5172$*
Leptin (ng/mL)	24.95 (2.71)	18.53 (2.31)	$p = 0.0868$	13.59 (1.18)	11.89 (1.77)	$p = 0.4249$	14.54 (1.51)	10.34 (1.09)	**$p = 0.0361$**

[1] n=13, LCHP = low-carbohydrate, high-protein; [2] n=12, LFHC = low-fat, high carbohydrate

Table 2: Pearson correlation coefficients for association between serum leptin concentration and bone mineral content, bone mineral density, and soft tissue mass measures by diet group at baseline, six weeks, and twelve weeks

	BASELINE		SIX WEEKS		TWELVE WEEKS	
	LCHP [1]	LFHC [2]	LCHP [1]	LFHC [2]	LCHP [1]	LFHC [2]
Bone Mineral Content (g)						
Total body	-0.15	0.31	0.18	0.27	-0.18	-0.02
Lumbar spine	-0.35	0.18	0.07	0.15	-0.38	-0.15
Hip	0.01	0.34	0.22	-0.17	0.07	0.07
Arm	0.34	0.24	0.32	0.29	0.18	0.23
Bone Mineral Density (g/cm^2)						
Total body	-0.37	-0.08	-0.26	-0.01	-0.43	-0.07
Lumbar spine	-0.26	0.26	0.19	0.35	-0.06	0.05
Hip	-0.13	0.25	0.13	0.21	0.13	0.06
Arm	0.17	0.04	0.18	0.03	0.04	-0.07
Soft Tissue Mass						
Body weight (kg)	0.57 *	0.79 **	0.79 **	0.78 **	0.79 **	0.45
Total lean mass (kg)	0.26	0.55	0.61 *	0.49	0.61 *	0.15
Total fat mass (kg)	0.71 **	0.88 ***	0.80 ***	0.89 ****	0.81 ***	0.64 *
Body fat (%)	0.84 ***	0.90 ****	0.73 **	0.85 ***	0.74 ***	0.79 **
Body mass index (kg/m^2)	0.45	0.75 **	0.81 ***	0.87 ***	0.84 ***	0.54
Leptin (ng.mL)						
Time 0: Baseline			0.66 *	0.84 ***	0.59 *	0.63 *
Time 1: 6 months	0.66 *	0.84 ***			0.67 *	0.81 **
Time 2: 12 months	0.59 *	0.63 *	0.67 *	0.81 **		

[1] n=13, LCHP = low-carbohydrate, high-protein; [2] n=12, LFHC = low-fat, high carbohydrate

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ****$p \leq 0.0001$

Table 3: Pearson correlation coefficients for relationship between body weight and bone mineral content, bone mineral density, and soft tissue mass measures by diet group at baseline, six weeks, and twelve weeks

	BASELINE		SIX WEEKS		TWELVE WEEKS	
	LCHP [1]	**LFHC** [2]	**LCHP** [1]	**LFHC** [2]	**LCHP** [1]	**LFHC** [2]
Bone Mineral Content (g)						
Total body	0.38	0.68 *	0.30	0.67 *	0.24	0.70 *
Lumbar spine	0.06	0.48	0.06	0.51	0.04	0.50
Hip	0.46	0.60 *	0.41	0.28	0.41	0.59 *
Arm	-0.05	0.15	0.17	0.78 **	0.50	0.42
Bone Mineral Density (g/cm^2)						
Total body	-0.24	0.30	-0.25	0.24	-0.31	0.31
Lumbar spine	0.23	0.54	0.16	0.59 *	0.19	0.59 *
Hip	0.32	0.44	0.27	0.46	0.26	0.52
Arm	-0.14	0.37	-0.07	0.36	-0.05	0.42
Body weight (kg)						
Time 0: Baseline			0.99 ***	0.99 ***	0.98 ***	0.99 ***
Time 1: 6 weeks	0.99 ***	0.99 ***			0.99 ***	0.99 ***
Time 2: 12 weeks	0.98 ***	0.99 ***	0.99 ***	0.99 ***		
Leptin (ng.mL)	0.57 *	0.79 **	0.79 **	0.78 **	0.79 **	0.45

[1] n=13, LCHP = low-carbohydrate, high-protein; [2] n=12, LFHC = low-fat, high carbohydrate

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.0001$

Table 4: Pearson correlation coefficients for relationship between body mass index and bone mineral content, bone mineral density, and soft tissue mass measures by diet group at baseline, six weeks, and twelve weeks

	BASELINE		SIX WEEKS		TWELVE WEEKS	
	LCHP [1]	**LFHC** [2]	**LCHP** [1]	**LFHC** [2]	**LCHP** [1]	**LFHC** [2]
Bone Mineral Content (g)						
Total body	0.35	0.49	0.29	0.49	0.23	0.50
Lumbar spine	0.08	0.29	0.07	0.31	0.03	0.32
Hip	0.41	0.45	0.42	-0.04	0.37	0.44
Arm	-0.03	0.26	0.22	0.57	0.55	0.20
Bone Mineral Density (g/cm^2)						
Total body	-0.16	0.28	-0.13	0.21	-0.20	0.31
Lumbar spine	0.34	0.46	0.30	0.52	0.30	0.54
Hip	0.42	0.44	0.38	0.44	0.35	0.50
Arm	-0.02	0.37	0.08	0.32	0.08	0.42
Body mass index (kg/m^2)						
Time 0: Baseline			0.99 **	0.99 **	0.98 **	0.99 **
Time 1: 6 weeks	0.99 **	0.99 **			0.99 **	0.99 **
Time 2: 12 weeks	0.98 **	0.99 **	0.99 **	0.99 **		
Leptin (ng.mL)	0.45	0.75 *	0.81 *	0.87 *	0.84 *	0.54

[1] n=13, LCHP = low-carbohydrate, high-protein; [2] n=12, LFHC = low-fat, high carbohydrate

* $p \leq 0.05$; ** $p \leq 0.0001$

Table 5: Changes in serum leptin concentrations within diet groups over time

	Group			
	Low-Carbohydrate, High-Protein (n=13)		**Low-Fat, High-Carbohydrate** (n=12)	
	Serum leptin (ng/mL)		Serum leptin (ng/mL)	
	Mean (SD)	Range	Mean (SD)	Range
Baseline	24.95 (9.76)	8.33 – 38.64	18.53 (8.00)	6.51 – 30.57
Time 1	13.59 (4.25) [a]	5.03 – 20.15	11.89 (6.12) [a]	4.60 – 25.53
Time 2	14.54 (5.43) [a]	4.96 – 26.35	10.34 (3.79) [a,b]	4.56 – 16.98

[a] significantly different from baseline, $p \leq 0.001$
[b] significantly different from LCHP group, $p \leq 0.05$

Table 6: Changes in body weight and soft tissue mass within diet groups over time

	Group			
	Low-Carbohydrate, High-Protein (n=13)		Low-Fat, High-Carbohydrate (n=12)	
	Mean (SD)	Range	Mean (SD)	Range
Body Weight (kg)				
Baseline	85.58 (12.77)	61.6 – 113.1	78.01 (16.59)	56.1 -109.6
Time 1	80.54 (11.98) [a]	56.3 – 104.1	73.91 (15.51) [a]	52.4 – 103.6
Time 2	78.31 (11.49) [a,b]	55.1 – 100.3	71.86 (14.67) [a,f]	52.1 – 99.3
Total Lean Mass (kg)				
Baseline	47.95 (5.38)	40.1 – 61.2	46.17 (6.96)	33.8 – 61.0
Time 1	46.40 (5.02) [a]	38.2 – 58.8	44.90 (6.75) [g]	33.2 – 60.6
Time 2	45.83 (4.70) [a,c]	38.7 – 57.7	44.92 (6.56) [g]	34.4 – 59.9
Total Fat Mass (kg)				
Baseline	35.31 (8.22)	19.2 – 49.1	29.49 (10.36)	14.5 – 48.4
Time 1	31.95 (7.95) [a]	15.7 – 42.6	26.66 (9.73) [a]	13.7 – 45.4
Time 2	30.17 (7.73) [a,b]	14.1 – 39.8	24.59 (8.81) [a,b]	13.3 – 42.6
Body fat (%)				
Baseline	40.79 (4.66)	31.2 – 46.2	36.93 (5.87)	25.7 – 47.4
Time 1	38.86 (5.31) [d]	27.9 – 45.4	35.17 (6.27) [a]	24.2 – 46.4
Time 2	37.93 (5.57) [d,e]	25.6 – 45.2	33.42 (5.67) [a,f]	23.7 – 44.8

[a] significantly different from baseline, $p \leq 0.0001$
[b] significantly different from Time 1, $p \leq 0.0001$
[c] significantly different from Time 1, $p \leq 0.05$
[d] significantly different from baseline, $p \leq 0.001$
[e] significantly different from Time 1, $p \leq 0.005$
[f] significantly different from Time 1, $p \leq 0.0005$
[g] significantly different from baseline, $p \leq 0.005$

Table 7: Changes in bone mineral content measures (g) within diet groups over time

	Group			
	Low-Carbohydrate, High-Protein (n=13)		**Low-Fat, High-Carbohydrate** (n=12)	
	Mean (SD)	Range	Mean (SD)	Range
Total Body				
Baseline	2314 (172)	2037 – 2776	2351 (349)	1754 – 3017
Time 1	2310 (155) [a]	2105 – 2716	2361 (358)	1700 – 3037
Time 2	2310 (171) [a]	2037 – 2757	2344 (343)	1679 – 2989
Lumbar Spine				
Baseline	64.4 (9.7)	50.7 – 87.4	62.3 (12.9)	39.4 – 85.1
Time 1	64.4 (9.6)	52.7 – 88.4	62.8 (12.1)	40.5 – 85.2
Time 2	64.1 (9.7)	51.6 – 88.0	62.0 (12.3)	39.9 – 84.0
Hip				
Baseline	30.6 (3.8)	26.3 – 40.1	31.6 (6.7)	19.2 – 46.7
Time 1	30.2 (4.4)	23.5 – 41.5	30.6 (7.3)	20.2 – 45.3
Time 2	30.4 (4.3)	24.1 – 40.4	31.6 (6.8)	20.7 – 44.9
Arm				
Baseline	12.5 (0.6)	11.6 – 13.4	12.0 (1.6)	7.8 – 14.0
Time 1	12.7 (0.7)	11.6 – 13.5	12.4 (2.0) [b]	7.8 – 15.7
Time 2	12.7 (0.7)	11.6 – 13.8	13.1 (2.9) [c]	8.1 – 20.1

[a] significantly different from baseline, $p \leq 0.05$
[b] significantly different from baseline, $p \leq 0.02$
[c] significantly different from baseline, $p \leq 0.01$

Table 8: Changes in of bone mineral density measures (g/cm^2) within diet groups over time

	Group			
	Low-Carbohydrate, High-Protein (n=13)		**Low-Fat, High-Carbohydrate** (n=12)	
	Mean (SD)	Range	Mean (SD)	Range
Total Body				
Baseline	1.1234 (0.0748)	1.0090 – 1.2480	1.1799 (0.0912)	0.9910 – 1.3060
Time 1	1.1419 (0.0692) [a]	1.0300 – 1.2660	1.1920 (0.0864) [a]	1.0070 – 1.3180
Time 2	1.1438 (0.0747) [a]	1.0010 – 1.2560	1.1938 (0.0926) [b]	1.0020 – 1.3320
Lumbar Spine				
Baseline	1.0745 (0.1079)	0.9090 – 1.3660	1.0970 (0.1268)	0.8520 – 1.2350
Time 1	1.0718 (0.1021)	0.9530 – 1.3540	1.1068 (0.1228)	0.8970 – 1.2600
Time 2	1.0749 (0.0948)	0.9640 – 1.3390	1.1043 (0.1284)	0.8710 – 1.2710
Hip				
Baseline	0.9403 (0.1177)	0.7930 – 1.2330	1.0078 (0.1130)	0.8370 – 1.2610
Time 1	0.9362 (0.1220)	0.7760 – 1.2390	1.0059 (0.1207)	0.7730 – 1.2280
Time 2	0.9334 (0.1148)	0.7820 – 1.2200	1.0018 (0.1237)	0.7980 – 1.2390
Arm				
Baseline	0.5662 (0.0397)	0.4850 – 0.6370	0.5843 (0.0580)	0.5090 – 0.6980
Time 1	0.5685 (0.0419)	0.4810 – 0.6530	0.5854 (0.0545)	0.5220 – 0.6980
Time 2	0.5688 (0.0404)	0.4780 – 0.6360	0.5843 (0.0606)	0.5140 – 0.7190

[a] significantly different from baseline, $p < 0.05$
[b] significantly different from baseline, $p < 0.01$

Table 9: Changes in body mass index within diet groups over time

	Group			
	Low-Carbohydrate, High-Protein (n=13)		**Low-Fat, High-Carbohydrate** (n=12)	
	Mean (SD)	Range	Mean (SD)	Range
Baseline	31.3 (4.9)	24.1 – 42.6	29.7 (5.4)	24.5 – 42.5
Time 1	29.4 (4.5) [a]	22.0 – 39.2	28.2 (5.1) [a]	23.3 – 40.7
Time 2	28.6 (4.3) [a,c]	21.5 – 37.7	27.4 (4.8) [a,b]	23.0 – 39.6

[a] significantly different from baseline, $p < 0.0001$
[b] significantly different from Time 1, $p = 0.0003$
[c] significantly different from Time 1, $p < 0.0001$

Discussion

Serum leptin was positively associated with body weight, fat mass, and body fat % as previously reported in other studies comparing leptin with these measures of adiposity (10-13). Serum leptin tends to change in the direction of body weight fluctuations (3, 4), and this pattern was observed in the current study regardless of which diet was followed. As mean body weight decreased through the duration of the study, so did serum leptin. This was also observed with changes in total fat mass and body fat % in the current study.

Women in the current study significantly reduced body weight and body fat regardless of diet regime followed. Both diet groups also displayed significant decreases in lean mass; however, the LCHP diet group had a 41.0% larger lean mass decrease than the LFHC diet group and a 4.7% lower decrease in fat mass. In addition, the LFHC diet group showed a larger decrease in body fat %. These results suggest that the LFHC group had greater lean mass retention than the LCHP diet group.

Serum leptin concentration in the LCHP diet group was significantly greater than in the LFHC group at Time 2. This was the only significant measure between diet groups throughout the study. Although the difference in body fat % between diet groups at Time 2 neared significance, body weight, total fat mass, and body fat % were not significantly different between diet groups. This may be partially explained through the observation that both diet groups showed body mass and body composition changes in the same

direction. Another possible explanation may be the differences in the macronutrient composition characteristics of the diets.

The current study shows that serum leptin concentrations in overweight, premenopausal women following a LCHP are significantly higher than concentrations in those following LFHC diet. Our results contradict previous research. In a study of 204 normal weight and obese adult men and women (age 18-80 y), Ostlund et al.(14) demonstrated that diet composition and long-term energy intake did not impact serum leptin for the group. In contrast, Raben and Astrup (1) found that diet composition impacted serum leptin. Specifically, fasting and postprandial leptin concentrations were higher following a sucrose-rich diet than either a starch- or fat-rich diet (1). Koutsari et al. (2) also demonstrated that diet impacts leptin but found that women who consumed a high carbohydrate for 3 days had higher fasting and postprandial serum leptin concentrations than those who consumed a low carbohydrate diet for 3 days. While the current study shows that diet composition appears to influence serum leptin concentration, women consuming a low carbohydrate diet had significantly higher serum leptin concentrations than those consuming a high carbohydrate diet which is in contrast to what other researchers have found.

Another important finding is that body weight was positively associated with total body BMC at all intervals in the LFHC group. In this group, body weight was also positively associated with hip BMC at baseline and Time 2 and arm BMC at Time 1. Bone mineral density and body weight were positively

associated at Time 1 and Time 2. These data support previous research related to mechanical loading of body weight on bone (3, 5, 15-24). However, the arm is not a weight-bearing bone suggesting that factors other than mechanical loading, such as leptin, may influence bone mass in overweight, premenopausal women. Yet, these results do not support a direct association between serum leptin and BMC or BMD in women consuming a diet high in carbohydrates but low in fat.

Over time, significant decreases in serum leptin concentration, BMI, body weight, total lean mass, total fat mass, total fat mass, and body fat % were observed in both groups. In the LCHP diet group total body BMC also decreased versus the LFHC diet group where arm BMC increased. Interestingly, both groups displayed increased total body BMD over time. This increase in BMD while serum leptin and body mass and composition measurements decreased suggests another factor influences bone mass. Diet composition was different between the two groups and may have played an influential role.

To our knowledge, this is the first study to examine relationships between serum leptin and soft tissue measures and bone mineral measures in overweight and obese (as defined by BMI >25 kg/m^2), premenopausal adult population consuming a LCHP versus LFHC diet. In the current study, we show that leptin was significantly higher in the LCHP diet group than in the LFHC diet group regardless of body weight and body composition changes. In addition, we were able to demonstrate a relative conservation of lean body

mass in the LFHC group as compared to the LCHP group over 12 weeks. Also, contradictory to previous research, we observed an increase in total body BMD as body weight and serum leptin decreased over time despite differences in diet composition.

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CHAPTER V

SUMMARY AND FUTURE RESEARCH

While increasing waistlines have many negative health risks, they do have one positive aspect. Excess body weight seems to be protective of bone mass and is preventive of osteoporosis, a growing health concern as individuals are living longer. Understanding the influencing factors and mechanisms of osteoporosis is integral to prevention and treatment of this debilitating disease.

Premenopausal women of healthy weight (Chapter 3) demonstrated a positive relationship between serum leptin concentration and body fat mass and body fat % despite differences in cognitive eating restraint (CER) scores. Serum leptin was significantly higher in women with high versus low CER scores at baseline. The low CER group had a trend toward lower body weight and fat mass than the high CER group, but no significant differences in either BMC or BMD were identified. In conclusion, these results do not support a positive association between serum leptin and BMC or BMD in women based on eating restraint.

Overweight and obese, premenopausal women (Chapter 4) significantly reduced body weight and body fat regardless of diet regime followed. The low-fat, high-carbohydrate (LFHC) diet group had significantly lower serum leptin concentrations than the low-carbohydrate, high-protein (LCHP) diet group. In addition, body weight was positively associated with total body BMC at all intervals in the LFHC group. Interestingly, both groups had increased total body BMD over time. Diet composition was different between the two groups

and may have played an influential role. Again, these results do not support a direct association between serum leptin and BMC or BMD in women consuming either LFHC or LCHP diets.

Both studies presented focused on fairly homogenous populations and reported data contradicting previous research. More research is required in these areas to resolve these conflicting results. First, whether CER and BMC or BMD are related needs to be explored further in both males and females. In addition, subjects in different stages of life should be examined because bone turnover changes throughout life and if a link between CER and bone mass can be established then this may imply a psychological influence on a physical attribute. Large sample sizes over multiple decades of life can help to determine whether statistically and clinically significant relationship exists.

Another important topic needing further study is the influence of diet composition on bone mineral mass over time. Health professionals are likely going to continue to prescribe weight loss in an attempt to curb obesity rates and the effect of diet composition on bone mass needs clarification so that effective precautions can be taken when necessary. Well controlled, dietary interventions with detailed macronutrient composition and regular BMC and BMD measures are needed. Again, data for both males and females at different stages of life should be collected.

Although vitamins and minerals do not make up as large a proportion of our diet as macronutrients do, monitoring their dietary intake may provide valuable information. Data for calcium could be especially useful. A link

between serum leptin and dietary calcium intake could be especially important in the battle against both obesity and osteoporosis. Dietary records, controlled feeding studies, and biochemical analysis could all be used to collect data.

Finally, because studies measuring leptin are influenced by quantity of fat mass, subjects should be grouped not only by gender and/or stage of life but also by body fat percent. Adipose tissue is metabolically and biologically active and its impact on hormones and other compounds in the body has been largely unstudied.

VITA

Joanne J. Volpe, daughter of William and Donna Jackson, was born on August 5, 1974 in Toms River, NJ. Joanne received her Bachelor of Science degree in Environmental Science from University of Mary Washington (formerly Mary Washington College), Fredericksburg, VA in May 1996. After graduation, Joanne moved back to New Jersey and worked in environmental remediation. In 1998, Joanne moved to Virginia where she continued to work in the environmental field through a private, government contractor and then through the United States Census Bureau. In 2001, Joanne decided to return to school and received a Bachelor of Science degree in Human Nutrition, Foods, and Exercise with the options of Dietetics and Exercise and Health Promotion from Virginia Polytechnic Institute and State University (VPI&SU) in May 2004. During the 2003-2004 academic year, Joanne enrolled as a dual student working on her graduate degree while completing her undergraduate degree. Joanne was supported by Graduate Teaching Assistantship from 2003 to 2005. During her time at VPI&SU, Joanne also became a certified American College of Sports Medicine (ACSM) Health/Fitness Instructor. Joanne will remain at VPI&SU where she will participate in the Virginia Tech Dietetic Internship.