Effect of a Low-Carbohydrate, High-Protein Diet on Bone Mineral Density, Biomarkers of Bone Turnover, and Calcium Metabolism in Healthy Pre-Menopausal Females

by

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ABSTRACT

EFFECT OF A LOW-CARBOHYDRATE, HIGH-PROTEIN DIET ON BONE MINERAL DENSITY, BONE TURNOVER MARKERS, AND CALCIUM METABOLISM IN PRE-MENOPAUSAL WOMEN

Mary Dean Coleman

Low-carbohydrate, high-protein (LCHP) diets have been shown to induce weight loss and beneficial changes in blood lipids that suggest cardiovascular disease risk reduction; however, LCHP diets have not been adequately investigated for health effects on the skeleton. A randomized trial to determine the effects of a LCHP diet on bone mineral status, biomarkers of bone turnover, indicators of acid-base balance, calcium homeostasis and fasting lipids in healthy pre-menopausal women was conducted. Women, aged 32 - 45 y, with a body mass index between 25 – 41 kg/m² were randomized into one of two diet groups: LCHP (n = 13) or high-carbohydrate, low-fat (HCLF) (n = 12). Anthropometric (body weight, lean mass, fat mass) and bone mineral density (BMD) and content (BMC) measures and markers of lipid metabolism were taken at weeks 0, 6, and 12. Measures of acid-base balance, protein metabolism, and calcium homeostasis were conducted at weeks 0, 1-4, 6, and 12. Serum osteocalcin was analyzed at weeks 0, 1, 2, 6, and 12, while urinary NTx was analyzed at weeks 0, 1 and 2. Weight loss was significant at the end of 12 weeks in both diet groups (P < 0.05) but there was no Diet x Time interaction. Total proximal femur BMD was lower in the LCHP group (P < 0.05) compared to the HCLF group by week 12. Femoral neck BMC decreased in the LCHP diet group (P < 0.05), whereas total forearm BMC increased (P < 0.05) in the HCLF diet group by week 12 of the study. Serum osteocalcin showed significant main effects of diet (P < 0.05) and time (P < 0.0001), but a Diet x Time interaction was not observed. Urinary NTx exhibited no main diet effect, time effect or Diet x Time interaction at weeks 1 or 2. Urinary pH was lower in the LCHP group compared to the HCLF group throughout the study (P < 0.0001). Urinary calcium excretion was higher in the LCHP group and lower in the HCLF group (P < 0.0001) compared to baseline values at all intervals of the study. Urinary phosphorus excretion exhibited a significant diet effect (P < 0.001) and time effect (P < 0.002), while no Diet x Time interaction was observed. Total cholesterol, high-density and low-density lipoprotein cholesterol, and triacylglycerol concentrations did not differ between diets during the study. In conclusion, a LCHP diet appears to stimulate bone loss, while a HCLF diet appears to attenuate bone loss in healthy pre-menopausal women undergoing 12 weeks of weight loss.
Dedication

The completion of this degree would not have been possible without the ever present strength of God and Jesus Christ in my life. Because of this, I dedicate the greatest accomplishment in my life thus far to God and Jesus.

I can do all things through Him who strengthens me – Philippians 4:13
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Chapter I

Introduction

The Atkins New Diet Revolution (1), a low-carbohydrate, high-protein (LCHP) diet, has been the center of debate among health care professionals in recent years. Proponents of the diet claim that it stimulates weight loss and reduces chronic disease risk. Conversely, critics suggest that the disproportionate amount of fat negatively impacts blood lipids (cholesterol and triacylglycerols) and increases risk for cardiovascular disease. Further, the excess protein may stimulate bone loss and increase the risk for osteoporosis (2), a debilitating disease defined by reduced bone mass and breakdown of the microarchitecture of the bone (3).

In recent years, a plethora of independent research studies were conducted to determine the effectiveness of LCHP diets, modeled after the Atkins diet plan, on weight loss and risk factors for cardiovascular disease compared to traditional high-carbohydrate, low-fat (HCLF) diets. Surprisingly, after six months, these studies showed that the LCHP diet did not negatively affect blood cholesterol or triacylglycerol concentrations (4-7). Moreover, participants following the LCHP diet lost more weight and exhibited greater decreases in triacylglycerol concentrations compared to individuals consuming a traditional, HCLF diet (5-7). While these initial results are positive in the short-term, little is known about the long-term effects of this diet on cardiovascular disease risk. Furthermore, these studies did not evaluate the impact of this diet on risk factors for other chronic diseases, such as osteoporosis. The purpose of this research study was to evaluate the accuracy of the nitroprusside test to assess ketone status by comparing results with serum \(\beta\)-hydroxybutyrate concentration and to determine whether ketones associated with weight loss (Chapter 3); and to comprehensively examine the effect of a LCHP diet on bone status by analyzing changes in calcium metabolism, biomarkers of bone turnover, and bone mineral status over 12 weeks (Chapter 4).

Osteoporosis is predominant in women because less bone is accrued during the developmental years and bone loss begins earlier in life. Furthermore, dietary intake of key micronutrients, such as calcium, tends to be inadequate in women, particularly in women who are dieting. The prevalence of obesity is greater among women (28.4%) compared to men (23.7%) (8), which may lead to more frequent dieting behaviors among women. In fact, data show that 45% of women are dieting at any given time, compared to 30% of men (9). Because women are more likely to exhibit dieting behavior, they are at greater risk for participating in dietary interventions that may stimulate bone loss. Careful examination of the influence of
weight loss diets on bone health is vital for ensuring individuals are not placing themselves at risk for osteoporosis.

The claim that LCHP diets promote bone loss was spurred from previous investigations that demonstrated high dietary protein intake induced hypercalciuria (10, 11). The sulfur-amino acids released from protein metabolism reduce systemic pH by simultaneously increasing extracellular hydrogen ion concentrations and reducing bicarbonate concentrations. Systemic acidosis is corrected by increased renal acid excretion and bicarbonate reabsorption, which is accompanied by reduced renal calcium reabsorption (12). Because the majority of body calcium is stored in bone and suppressed urinary calcium reabsorption stimulates hyperparathyroidism, the source of the excess urinary calcium is believed to come from bone. However, human studies that have examined the effect of high dietary protein intake on bone status have found discrepant findings.

Cross-sectional studies using self reported dietary protein intake data, have found dietary protein was positively correlated (13-15), negatively correlated (16), and had no effect (17) on bone mineral density or fracture risk. Several epidemiological studies have found that high-dietary protein consumption was protective against bone loss (18, 19), while low-protein consumption stimulated bone loss in the elderly population. Discrepant results in controlled trials suggest that bone metabolism is related to the duration of high-protein intake. Urinary calcium excretion and bone resorption markers were higher when women had high dietary protein intake compared to low dietary protein intake for 4 days (20) whereas high dietary protein intake at 3, 5, and 8 weeks did not negatively affect bone resorption markers or urinary calcium excretion (21).

Little is known about the effects of an Atkins-type diet on bone health. In addition to the acidosis incurred from the breakdown of dietary protein, systemic pH may be further reduced as a result of additional hydrogen ions that are released during ketone formation (22). Furthermore, the lack of alkaline forming fruits and vegetables in this diet may further reduce pH levels as the excess base production from fruits and vegetables is thought to buffer metabolic acid produced from protein-rich diets, thus attenuating bone loss (23). To date, only two published studies have examined the impact of a LCHP diet similar to the “Atkins Nutritional Approach™” on bone mineral status and bone metabolism. In the first study, an acute adverse change in bone metabolism was observed; however, at week 6 of the study, the body had adjusted to conserve bone (24). In the second study, total body (TB) bone mineral content (BMC) did not differ within or between pre-menopausal women assigned to follow either a LCHP diet or a traditional HCLF diet (4). Metabolic adaptations that occur during the induction phase
of this diet suggest that it could be detrimental to bone health; however, studies that comprehensively evaluate the impact of this diet on bone by assessing calcium metabolism, acid-base balance, bone turnover biomarkers, and bone mineral status are lacking.

Ketones, produced when fatty acid production exceeds the body’s ability to metabolize them, are desired because they confirm lipolysis, indicating the loss of body fat and are suggested to promote feelings of satiety and fullness which will result in weight loss (1). The Atkins program recommends daily urinary ketone testing with dipsticks or tablets to monitor fat metabolism and to determine the optimal level of carbohydrate intake that will promote weight loss (1). Presence of urinary ketones confirms adherence to the LCHP diet. However, these are semi-quantitative tests and are specific to acetoacetate. Because of this, these tests may not accurately assess ketone production. Therefore, the association between urinary ketones and weight loss would be inherently inaccurate with use of a nitroprusside test.

The methodology paper (Chapter 3) found that the nitroprusside test correlated with serum β-hydroxybutyrate concentrations, thus rendering it an accurate method for determining ketone status in healthy individuals. However, weight loss was not associated with β-hydroxybutyrate concentration, indicating that the presence of ketones may not predict weight loss.

In this group of healthy pre-menopausal women, the LCHP diet had an adverse effect on total proximal femur bone mineral density and femoral neck BMC, whereas the HCLF diet attenuated bone mineral losses at these sites and promoted bone mineralization at the total forearm (Chapter 4). The observed decreased urinary pH and corresponding increase in urinary calcium excretion in the LCHP diet group suggest that the imposed acid load from excess dietary protein intake and ketone production may stimulate bone mineral dissolution, whereas alkaline salts abundant in fruits and vegetables (in the HCLF group) reduce urinary calcium excretion and protect against bone losses. However, these results were not confirmed with the measured biomarkers of bone turnover. Future directions are discussed (Chapter 5).
References Cited


CHAPTER II

Literature Review

Obesity and osteoporosis are major public health threats in the United States. An estimated 44 million Americans have osteoporosis and/or low bone mass (1), while obesity is reaching epidemic proportions. Recent survey data estimates indicate that two-thirds of the American population is overweight or obese [defined as a body mass index (BMI) \( \geq 25 \text{ kg/m}^2 \)] (2). Overweight and obesity increase risk for chronic disease (cardiovascular disease, cancer, diabetes) (3) and recently, investigators have identified a link between cardiovascular disease and osteoporosis. Modest weight loss, as low as 10%, has been shown to reduce the risk for chronic disease (4); therefore, weight loss is recommended for overweight and obese individuals to reduce both morbidity and mortality risks. Ironically, weight loss and certain weight loss plans, particularly low-carbohydrate, high-protein (LCHP) diets, may be detrimental for bone health. This review will briefly discuss the link between osteoporosis and cardiovascular disease, present the mechanisms and factors involved with normal bone function, and then discuss the current literature that has examined the effect of LCHP diets on bone health.

Osteoporosis

Osteoporosis is a debilitating disease characterized by low bone mass and weakened bone structure (5). Osteoporosis is also referred as the “silent” disease because there are no early warning signs that signal its onset; therefore, once diagnosed, individuals are typically at an age where lifestyle changes can only prevent the loss of further bone rather than replace lost bone. Osteoporosis is becoming a major public health threat in America with an estimated 44 million Americans diagnosed with osteoporosis or low bone mass. The largest proportion of Americans diagnosed with osteoporosis or low bone mass is women. In fact, recent estimates suggest that 68% of Americans with low bone mass or osteoporosis are women (1). Each year, osteoporosis is responsible for more than 1.5 million fractures resulting in an economic burden of $14 billion in hospital and nursing home costs (6). While advances in medicine have produced medications that reverse bone loss in osteoporotic women, incorporating lifestyle habits that maximize peak bone mass and prevent bone loss is desirable.
Osteoporosis and the Link to Cardiovascular Disease

Osteoporosis and cardiovascular disease are chronic diseases that represent two separate systems in the body. Recently, researchers have begun to identify common etiologies shared by these two diseases. At least one epidemiological study has shown a positive association between osteoporosis and cardiovascular disease independent of age (7), and low bone mineral density (BMD) has been associated with cardiovascular disease mortality, atherosclerosis, and high lipid levels (8-10). Two apparent commonalities exist between these diseases in that risk for both increases with age and sedentary lifestyles; however, the pathophysiological mechanisms relating the two are just beginning to be explored.

Arterial calcification is positively correlated with cardiovascular disease and increased osteoporosis (11). Arterial walls contain many cells and regulatory factors seen in bone tissue such as osteoblast-like cells, macrophages, monocytes, and lymphocytes. Furthermore, paracrine factors associated with bone metabolism have been found to regulate vascular mineralization such as matrix Gla protein, osteocalcin, and osteoprotegerin, receptor-activated nuclear factor-kappa B ligand (RANKL), vascular smooth muscle cells (VSMC) and inflammatory cytokines (12). Thus, it appears mineralization in the vascular system is regulated in a manner similar to bone, although the consequences of mineralization in this system can be fatal.

Osteoprotegerin (OPG) is a soluble decoy receptor that binds to RANKL, a receptor located in osteoclasts and inhibits their activity. Osteoprotegerin is produced in both bone (by osteoblasts and bone marrow stromal cells) and vascular walls (from VSMC and endothelial cells) (13). OPG-deficient mice exhibited medial arterial calcification and severe osteoporosis (14), and these symptoms were reversed upon restoration of the gene (15). A study with 201 human subjects found significant increases in serum OPG concentrations as the severity of coronary artery disease (CAD) increased (12). In addition, multiple logistic regression analysis showed a 1% increase in serum OPG concentration was significantly associated with the presence of CAD (odds ratio 5.2, 95% CI, 1.7 to 16.0; \( P < 0.01 \)) (16). These results suggest OPG may serve as a marker for both CAD and osteoporosis (12).

Lipids and oxidative stress are theorized to be one of several uniting factors linking osteoporosis and cardiovascular disease. Dyslipidemia and increased risk of atherosclerosis and cardiovascular disease have been well established in epidemiological studies (7, 17) and controlled trials (12). Investigators have speculated that oxidized low-density lipoproteins (Ox-LDL) play an integral role in this association. Parhami and colleagues (18) determined the
effects of a high-fat atherosclerotic diet on bone mineral content (BMC) and BMD and osteoblast activity in mice that differed in their susceptibility for atherosclerosis. One mouse strain was resistant to atherosclerosis (C3h/HeJ), while the other strain was susceptible for atherosclerosis (C57BL/6). Mice were fed either an atherogenic diet known to cause hypercholesterolemia or regular chow. The atherosclerotic sensitive mice fed the high-fat diet showed a 43% and 15% decrease in femoral BMC and BMD compared to mice on the chow diet. In contrast, the atherosclerotic resistant mice exhibited no significant changes in femoral BMC or BMD compared to control mice. There was a significant reduction in osteocalcin expression by bone marrow cells of the atherosclerotic sensitive mice consuming the high-fat diet. These results suggest that a high-fat diet reduces bone mineralization by inhibiting osteoblast differentiation. In vitro cell models have provided evidence that oxidative stress stimulates bone dissolution while promoting arterial calcification (19). However, the underlying mechanisms involved were unclear. Mody and associates (20) used an in vitro model to determine the action of oxidative stress on osteoblastic calcifying vascular cell (CVC) and bone osteoblastic cell differentiation and mineralization. Calcifying vascular cells and osteoblastic cells were treated with compounds that induced oxidative stress by producing reactive oxygen species. Alkaline phosphatase and calcium incorporation was significantly increased \( (P < 0.01) \) in the vascular cells whereas the opposite was found in osteoblastic bone cells \( (P < 0.01) \). Minimally ox-LDL (MM-LDL) was added to the cells to determine if oxidative stress was increased in its presence. The addition of MM-LDL enhanced oxidative stress and the provision of antioxidants suppressed the effects of MM-LDL. These results suggest that oxidative stress regulates osteoblastic differentiation oppositely in vascular cells compared to bone cells, which may explain the concomitant increase in calcification with bone mineral dissolution.

Elevated homocysteine concentrations have been associated as a risk factor for cardiovascular disease (21). Previous work in individuals with homocysteineuria have shown early onset of osteoporosis (22) and the suggested mechanism behind the loss in bone mass is interference of collagen cross-linking by homocysteine (23). Two epidemiological studies were recently published that found an association between homocysteine levels and risk for osteoporotic fracture (24, 25). Van Muers and colleagues (25) examined the association between homocysteine levels and risk of incidence of osteoporotic fracture using two prospective, independent population-based studies. A total of 2,406 adults aged 55 years and older were included in the study. Multivariate regression analysis of continuous data found the risk of fracture was 1.4 (95% CI, 1.2 to 1.6) for each increase in 1 SD of the natural log-transformed homocysteine level using pooled data. In women only, risk of fracture was 1.3
(95% CI, 1.1 to 1.5) for each increase in 1 SD of homocysteine level. When categorized into quartiles, risk for fracture was two times higher in the highest quartile versus the lowest quartile. No association was shown for homocysteine concentrations and BMD; however, the calculation population attributable risk for these measures was 19%—a value that is similar to population attributable risks for well-known risk factors for fracture such as low BMD. These results provide compelling evidence that elevated homocysteine levels are independent risk factors for osteoporotic fracture in older adults; however, it is important to note that fractures at peripheral and axial skeletal sites were reported, some of which are not common to osteoporotic fracture. Furthermore, different methods were used to analyze homocysteine concentrations for each cohort resulting in wide variation in the values between each cohort. Because of this, the authors did not assign a cutoff for the highest homocysteine value. Another investigation was conducted with participants from the prospective population-based Framingham study to evaluate the association of homocysteine levels and risk of hip fracture (24). Similar to van Muers results, the age-adjusted incidence rates for hip fracture was highest (16.57; 95% CI, 11.8 to 21.3; \( P < 0.01 \)) in the women classified into the highest quartile of total homocysteine concentration (18.6 ± 6.4 \( \mu \)mol/L) compared to the lowest quartile (7.6 ± 6.8 \( \mu \)mol/L). Furthermore, the risk of hip fracture was increased by 26% for each 1 SD in the log-transformed total homocysteine concentration. Two primary strengths to this study include: 1) the same method was used to measure homocysteine levels; and 2) the authors focused on hip fracture, which is the site that shows the greatest association with mortality in older adults. Similar to the previous study, the authors used non-fasting blood samples (homocysteine levels tend to be lower in a non-fasted state), and they did not examine other factors that could contribute to risk of fracture (e.g., dietary factors). Controlled trials need to be conducted before definite causality can be established.

The prospect of a link between cardiovascular disease and osteoporosis is exciting and the possibilities for modifying diet to prevent the onset of both diseases are enormous. It is important to note, however, that the evidence presented is far from conclusive and a vast body of research is needed to further confirm the current findings.

**Normal Bone Function**

Bone is primarily recognized for its structural properties that provide protection for internal organs and locomotion via its attachment to large and small muscle groups. In addition to these characteristics, bone protects the network of blood vessels that transport nutrients to
and from the bone marrow and bone tissue, protects the blood-forming marrow, and acts as a reservoir for the ions: calcium, magnesium and phosphorus (26). Contrary to the belief that bone is a static tissue, bone is dynamic with a continuous flux of nutrients, cytokines, growth factors and other compounds that serve to aid in bone formation, breakdown, and repair (27).

There are periods of the life cycle during which bone undergoes modeling, a process defined as “formation where bone has not been before” (pp. 154) (28). Modeling occurs during developmental periods, including childhood, adolescence and pregnancy. Even as formation occurs, bone constantly undergoes remodeling, a coupled process of removing old structurally unstable bone and replacing it with new stronger bone (26, 28). The reported length of time to complete a full remodeling cycle is variable. Some investigators have reported that one cycle takes 3 to 6 months (26), while other researchers have reported up to three months with resorption lasting 7-10 days and formation 2-3 months (29). Remodeling occurs throughout life but proceeds without modeling during the young adult, adult, and elder years.

Bone is classified as cortical (or compact bone) or trabecular (cancellous or spongy bone). Cortical bone forms the external portion of bone that functions to protect the hematopoietic bone marrow and to provide locomotion. Trabecular bone comprises 80% of bone surface area and contains spaces between the mineralized tissue that serves to enclose hematopoietic bone marrow (26, 29). This bone is located at the ends of long bone and in the central portion of vertebrae (26). Of the two bone types, trabecular bone is more metabolically active. In fact, 25% of trabecular bone, compared to 2-3% of cortical bone, is remodeled each year (29). Because of this, the exchange of nutrients and other compounds occurs more readily in trabecular bone, and as a result, sites composed of primarily trabecular bone (i.e., spine, femoral neck) are first to lose bone mineral during nutrient deficiency or metabolic disease states.

Mineral, collagen, water, and non-collagenous proteins are the primary constituents that make up the composite material of bone (28). Collagen is the second most abundant constituent of bone and is predominantly in the form of Type I collagen. These collagen fibers are woven together to form the bone matrix. The minerals calcium and phosphorus along with hydroxide form crystals known as hydroxyapatite \([3\text{Ca}_3(\text{PO}_4)_2\cdot(\text{OH})_2]\). Hydroxyapatite binds to the collagen fibers of the bone matrix and aids in the mineralization process (26). Non-collagenous proteins help with mineralization and the formation of the bone matrix (26, 28). Bone also contains a variety of cells that support the structural and functional aspects of bone and include: osteoblasts, osteocytes, and osteoclasts.
Osteoblasts are the bone building cells that form both bone matrix and bone surfaces. These cells secrete Type I collagen which, in most instances, is laid down in alternating layers called a lamellar structure. Once the matrix is formed, it becomes calcified and the osteoblasts mature into osteocytes, which function to provide structure and support to the mineralized bone matrix. Osteoclasts are the demolition cells that break down bone. These cells tend to work alone or in pairs and begin the resorption process by attaching to the bone and forming a seal called the “ruffled border”. Once attached, the osteoclast creates a highly acidic environment that dissolves the bone crystals; once the matrix is exposed, lysozomal enzymes are released and digest the matrix. The matrix residues are then packaged into transport vesicles and taken to the basolateral membrane (26). Osteoblasts are generally located immediately behind osteoclasts, and the resorbed bone is immediately replaced with new bone. Both osteoblasts and osteoclasts work to maintain serum calcium and phosphorus homeostasis by incorporating these minerals into bone or releasing them from bone. Because of this function, bone cell activity is regulated by hormones and cell proteins that respond to changes in serum calcium and phosphorus. Advances in technology have added to current knowledge of the bone mineralization process, however, despite these technological advances, the mechanisms orchestrating bone mineralization are poorly understood. More research is needed to fully understand the communication network involved in mineralization.

**Mineral Ion Homeostasis**

Mineral ion homeostasis is maintained by an intricate communication network between the intestine, kidney, and skeleton that is mediated primarily by the hormones, parathyroid hormone (PTH), calcitriol or vitamin D₃ [1,25- (OH)₂D], and calcitonin. Each organ has a specific action that contributes to maintaining the balance of calcium, phosphorus, and magnesium ions. The intestine introduces these ions into the circulation via intestinal absorption, the kidney is responsible for mineral ion excretion and reabsorption; and the skeleton is the reservoir for these ions (30-33).

Mineral ion homeostasis occurs when the entry of the ions calcium, magnesium, and phosphorus equals the excretion of these minerals. Of the three, serum calcium is the most tightly regulated, and thus, slight fluctuations of this ion activate both PTH and 1,25- (OH)₂D. The parathyroid gland contains calcium sensitive chief cells that produce PTH, the hormone primarily responsible for serum calcium regulation when the serum calcium concentration is depressed. Parathyroid hormone directly activates bone resorption and kidney reabsorption of calcium, and indirectly activates intestinal calcium absorption by catalyzing the synthesis of
active vitamin D by the kidney (30, 31). Initial exposure to an elevated PTH level stimulates the release of calcium from a calcium pool located near the bone surface. Prolonged exposure to elevated PTH increases osteoclast cell number and activity, which stimulates the release of calcium, phosphate, and collagen fragments from bone matrix. In the kidney, PTH maintains calcium homeostasis by inhibiting and enhancing renal phosphorus and calcium reabsorption, respectively. Specifically, PTH depresses the formation of renal tubule transporters that carry phosphorus across the cell membrane. While most calcium reabsorption occurs in the proximal tubule, PTH acts on the distal tubule to increase calcium reabsorption.

Vitamin D is synthesized by the body when the skin is exposed to ultra-violet radiation. Upon exposure to sunlight, 7-dehydrocholesterol or provitamin D is transformed to vitamin D$_3$ in the skin. Vitamin D$_3$ then enters the circulation and is immediately bound to vitamin D-binding protein and is shuttled to the liver. In the liver, vitamin D$_3$ is hydroxylated by D-25-hydroxylase to form 25-OH-D$_3$ or calcidiol, an inert form of vitamin D. Calcidiol is transformed into the active form of vitamin D via vitamin D 1α-hydroxylase, an enzyme located in the proximal tubules in the kidney (33). Parathyroid hormone stimulates this enzyme to increase production of active vitamin D or calcitriol.

Calcitriol elevates the serum calcium concentration by acting on both the intestine and skeleton. In the intestine, active vitamin D binds to nuclear vitamin D receptor (VDR) cells located along the intestinal villi. Once bound, the efficiency by which calcium enters, crosses, and exits the enterocyte into circulation is enhanced; however, the exact mechanism by which this occurs is unknown (33). Calcitriol also enhances intestinal absorption of phosphorus. Unlike calcium, phosphorus absorption occurs primarily in the jejunum and ileum rather than the duodenum. Similar to PTH, 1,25- (OH)$_2$D stimulates osteoclast formation; however, once these cells are mature, they no longer are sensitive to 1,25- (OH)$_2$D. Calcitriol also provides a negative feedback signal to reduce PTH by increasing the serum calcium level and by binding to VDR receptors located on the parathyroid gland. Once calcitriol has bound to the VDR receptor, PTH gene expression is decreased, thereby decreasing the production and release of PTH (33).

Calcitonin is a peptide hormone produced by the C-cells of the thyroid gland. The primary function of calcitonin is to inhibit osteoclastic bone resorption and to reverse hypocalcemia and hypophosphatemia. Calcitonin secretion is directly proportional to increases and decreases in the serum calcium concentration, thereby making circulating calcium the main regulator of this peptide. Gender and age influence calcitonin secretion. Secretion is greater in
women compared to men, and is highest in newborns. After adulthood, some forms of calcitonin appear to progressively decrease with age (34).

Magnesium homeostasis is not regulated by systemic or hormonal influences, rather it appears to be primarily regulated by circulating magnesium and other dietary nutrients (30). Dietary phosphorus regulates intestinal magnesium absorption by forming a complex with magnesium, thereby limiting its absorption. Parathyroid hormone alone increases magnesium reabsorption; however, in the presence of PTH-induced hypercalcemia, renal reabsorption of magnesium is reduced (31).

**Peak Bone Mass**

Peak bone mass is defined as the maximal amount of bone mineral accrued before bone growth ceases (35). The attainment of peak bone mass is generally accepted to occur during adolescence, is site specific, and is a strong determinant for reducing risk for skeletal fractures during the elder years. In fact, it is estimated that 90-95% of bone mass accretion occurs near the termination of longitudinal growth and an additional 5-10% of bone mass is tacked on after maximal height is attained (36). Although extensively studied, the age and site in which peak bone mass is attained is unclear. Most studies concede that the majority of bone mass accrual occurs by late adolescence (37) and peaks during the second decade (35, 36); however, a few studies have suggested that bone mineral continues to accumulate and peaks into the third (36, 38) and fourth decades of life (36) and that the timing of peak bone mass differs between cortical and trabecular bone (39).

There are many factors that influence longitudinal bone growth, the accumulation of bone density after growth ceases, and the rate of bone loss. These factors have been categorized into non-modifiable and modifiable risk factors. Non-modifiable risk factors include (40):

- **Gender**: Women are at higher risk for low bone mass than males;
- **Age**: Post-menopausal women and men ≥ 65 years are at greater risk for low bone mass;
- **Race**: Caucasians and Asians tend to have lower peak bone mass than Hispanics and African Americans;
- **Genetics**: Twin and family studies suggest genetic factors have an important role in bone mineral density (BMD);
- **Physical characteristics**: Individuals with a small skeletal frame size are at greater risk for low bone mass compared to those with a large skeletal frame size.
Modifiable risk factors include (40):

**Diet:** Diets deficient in key nutrients (e.g., calcium, phosphorus, and vitamin D, among others) reduce bone mass accretion and increase risk of skeletal fractures at any age;

**Smoking:** Individuals who smoke are at greater risk for low bone mass;

**Exercise:** Sedentary lifestyle increases the risk for low bone mass, particularly during the elder years;

**Weight cycling:** Repeated weight loss and gain has been associated with low BMD;

**Lack of sun exposure:** Individuals who live in northern geographic locations, spend the daylight hours indoors, or do not expose the skin to sunlight are at risk for low bone mass;

While all individuals are encouraged to adopt positive lifestyle behaviors that will maximize peak bone mass and reduce age related bone loss, those persons with non-modifiable risk factors, in particular, are encouraged to adopt positive lifestyle behaviors as early as possible. Screening for osteoporosis, particularly for individuals who have non-modifiable characteristics is important so that positive lifestyle changes can be adopted that will prevent bone loss. There are several techniques available to analyze bone health status.

### Analysis of Bone Health

Bone health can be determined by examining the mineral status in skeletal sites that are readily mobilized during nutritional deficiency or metabolic disease or by biochemical analysis of markers specific to by-products of bone metabolism. Bone mineral status is typically measured with the use of X-ray, while markers of bone turnover are analyzed using assays that are specific to proteins secreted by bone cells or found on fragments of bone mineral, which are present in the circulation or in urine.

**Dual Energy X-Ray Absorptiometry**

Dual-energy X-Ray absorptiometry (DXA) is a method that measures the BMD of specific sites known to predict risk of osteoporotic fracture. These sites include the total proximal femur or hip, lumbar spine (L1-L4 or L2-L4), total body, and total forearm. Total proximal femur and lumbar spine, however, are the sites typically measured because they are the strongest predictors of fracture risk (40). During a DXA scan, two X-rays are passed (one high-energy and one low-energy) through the body. Mineral in the bone absorbs more X-ray energy than fat tissue or lean tissue, and the attenuated X-rays are measured and used to
calculate bone mineral content (BMC) and BMD. Bone mineral content indicates the amount of X-ray that is absorbed by bone mineral, whereas BMD is the average amount of BMC per unit volume of a specific unit of bone in either two or three dimensions (41). Specifically, BMD is expressed as surface area density (grams of bone mineral per square centimeter, g/cm²) or as volumetric density (grams of bone mineral per cubic centimeter, g/cm³), known as areal BMD (aBMD) or volumetric BMD (vBMD), respectively (42). Dual-energy X-ray absorptiometry expresses BMD as areal density or in g/cm².

Dual-energy X-ray absorptiometry provides many advantages that make it an ideal technique to use in the research setting over other techniques that measure BMD. These advantages include:

- **Multiple measurement sites**: This instrument can measure BMD at multiple sites that are common for fracture risk such as the hip and spine. Other techniques such as quantitative computed tomography (QCT), are limited to one site.

- **High precision**: Dual-energy X-ray absorptiometry has a percent precision error of 1-2%, which means serial measurements over time have minimal deviation from one another. This is advantageous because it eliminates error introduced by the machine, thereby increasing the confidence that the change in values are from the experimental variable.

- **Low radiation exposure**: Four body site measurements by DXA expose an individual to radiation doses of ≤ 20 mrem compared to a dental bite-wing film (334 mrem) or environmental background (4 mrem per week).

- **Rapid measurement**: A four-site scan takes ~20 minutes, whereas other measures such as QCT require 10-20 minutes per site.

- **Minimal subject inconvenience**: Dual-energy X-ray absorptiometry requires little preparation to obtain the scan and is a noninvasive procedure (41, 42).

While DXA has many advantages, it is not without its limitations. Two dimensional measurements may show a false elevation or reduction in BMD in persons with large and small bones, respectively. Because of the greater surface area of the large bone, the DXA will assess the BMD to be greater when compared to a smaller bone containing an equal BMC (41, 42). Dual-energy X-ray absorptiometry does not differentiate between cortical and trabecular bone. The inability to distinguish losses of bone mass in cortical versus trabecular bone by DXA is disadvantageous because once losses in cortical bone are realized, the severity of this BMD loss is often irreversible and devastating with increased fracture risk. Trabecular bone is the more metabolically active portion of bone, and alterations are readily seen as a result of changes in lifestyle such as nutrition or exercise habits or to added pharmacotherapy (43).
Using methods that can detect changes in these bone types are ideal; however, current available methods are expensive, time-consuming, and are limited to one site.

It is important to consider these factors when using BMD as a method to assess changes in bone status. Because of these limitations, other methods to monitor changes in bone metabolism are beneficial to provide a comprehensive analysis of alterations in bone status in relation to changes in dietary habits, physical activity, or other interventions or therapeutic treatments.

**Biomarkers of Bone Turnover**

Bone is a dynamic tissue, and at any given time, specific sites of the skeleton undergo remodeling. Less structurally sound bone is continually removed and replaced with new stronger bone. As previously mentioned, certain life stages reflect periods of bone building (i.e., childhood, adolescence, and pregnancy) while others reflect a steady state (i.e., young adulthood, mid-life and pre-menopausal years) and a degradative state (i.e., elder years or disease that stimulates bone loss). Biochemical markers of bone turnover is a general term that refers to proteins present in the blood and/or urine that reflect bone formation or degradation. Breakdown products of bone resorption and by-products of bone formation can be measured from serum and/or urine; however, by-products of bone formation are usually measured from serum (44). Due to the rapid response of these biomarkers to alterations in bone remodeling, they are ideal measures for tracking acute changes in bone in response to diet, drug, or exercise therapies. This discussion is limited to the biochemical markers, osteocalcin and N-telopeptide of Type I collagen cross-links (NTx).

Osteocalcin, also known as bone GLA-protein (BGP) is a noncollagenous protein secreted by osteoblasts and is characterized by three vitamin K dependent gamma carboxylated acid residues that function to bind calcium to the protein. Although it is accepted as a marker for bone formation, its precise function is unknown. Osteocalcin is the most abundant noncollagenous protein in bone with proportions of ≥ 90% and 70% incorporated into the bone matrix during childhood and adulthood, respectively (29). During bone formation, a small amount of intact osteocalcin is released into the circulation and can be measured by radioimmunoassay (RIA) (29, 45). Serum osteocalcin has been correlated with histomorphometric measures of bone formation, is considered a highly sensitive marker of bone formation, and is elevated during conditions of high bone turnover (29). Because the majority of osteocalcin is embedded in the matrix, some researchers have indicated it is also present in circulation during
bone resorption; hence, it may be more reflective of bone turnover rather than bone formation (46).

Type I collagen is joined together in a fibrous weave to form the bone matrix. Sites of intersection are known as “crosslinks” and N-terminal and C-terminal peptides are located at these sites. During resorption, collagen is broken down, and N-terminal and C-terminal peptide fragments are released into the circulation and cleared by the kidneys (47). An enzyme linked immunosorbent assay (ELISA) is available that can measure NTx fragments in urine. The antibody identifies the epitope within the \( \alpha \)-2 chain of the NTx fragment. N-telopeptide is typically measured from a second-void morning urine or a 24-hour urine collection and is reported in nM bone collagen equivalents (BCE). To account for variations in urine dilution, NTx is corrected with urine creatinine (Cr) (47). Sixty percent of the crosslinks released during resorption are in the form of N- and C-terminal peptides, and a few studies have reported this assay to be a more sensitive indicator in detecting changes in bone resorption than other markers of bone resorption such as deoxypyridinoline (DPD) (29). Because this assay measures total NTx and Type I collagen is found in several tissues, the specificity of this assay to bone collagen has been questioned (29).

**Factors Influencing Bone Function**

Bone formation and bone homeostasis are regulated by a constellation of factors including those produced by the endocrine system among others. These factors are regulated by dietary intake of nutrients known to directly and indirectly affect bone. Because a description of all the factors involved with bone metabolism would be quite extensive, only the endocrine factors insulin-like growth factor-1, estrogen, progesterone, leptin, and nutritional factors specific to bone function will be discussed.

**Endocrine Factors**

Factors of the endocrine system are regulators of bone mineral metabolism and are mediated by environmental influences, such as dietary intake and body weight. While these factors have multiple pathophysiological roles in the body involving growth and development, this section will focus on their effects on the skeletal system.
**Insulin-Like Growth Factor-1**

Insulin-like growth factor-1 (IGF-1) is a peptide hormone synthesized primarily by hepatic cells and by other non-hepatic tissues including bone. The skeleton is considered the reservoir for IGF-1, and the majority of skeletal IGF-1 is produced via *de novo* synthesis by osteoblasts. Insulin-like growth factor from the liver acts in an endocrine fashion, whereas skeletal IGF-1 functions in an autocrine/paracrine manner. Both are regulated by growth hormone; however, skeletal IGF-1 is also regulated by cytokines. Insulin-like growth factor-1 is found in the circulation in free form or bound to a family of binding proteins (IGFBP-1-6); however, the majority of circulating IGF-1 is bound to IGFBP-3 (48, 49). Circulating IGF-1 has been shown to be a major contributor to bone acquisition in mice. Double knockout mice for the liver deficient IGF-1 gene (LID) and the acid labile subunit gene (ALSKO, a binding protein for IGF-1) were compared to mice with a single knockout of the LID and ALSKO gene and control mice. Serum IGF-1 concentrations were significantly lower in the LID-ALSKO mice compared to all groups, with the largest reductions (65%) when compared to controls (*P* < 0.01). Significant decreases in bone length, bone size, bone mass and the growth plate were shown in the ALSKO-LID mice compared to all groups (*P* < 0.01). The administration of IGF-1 restored bone height and the growth plate in LID-ALSKO mice (49). These results suggest that circulating IGF-1 plays a role in the accumulation of peak bone mass and supports current reports for its relationship with BMD and strength in humans. Langlois and colleagues (50) examined the association between serum IGF-1 concentration and BMD in a cross-sectional study of 425 women (mean age: 72.7 ± 4.6 y) included in the Framingham Osteoporosis Study. Serum IGF-1 concentrations were significantly and positively associated with BMD at Ward’s triangle, femoral neck, trochanter, radius, and lumbar spine (*P* ≤ 0.01) when adjusted for confounding variables. Additional research must to be conducted to more fully evaluate the role of IGF-1 in peak bone mass accretion and osteoporosis prevention in humans.

**Estrogen**

Estrogen deficiency is a major determinant of post-menopausal bone loss. Estrogen mediates its effect through direct and indirect mechanisms. Estrogen receptors have been identified on both osteoblasts and osteoclasts; therefore, estrogen is believed to directly mediate bone mineralization by stimulating osteoblastic activity and inhibiting osteoclastic activity. Estrogen has been shown to exert its action on bone indirectly by regulating the production of cytokines by osteoclasts (51). In addition to modulating cytokine release by osteoclasts, estrogen also regulates cytokine production released from other cells. Cytokines
such as interleukin-6 (IL-6), IL-1, tumor necrosis factor-α (TNF-α), and macrophage-colony stimulating factor promote bone resorption during estrogen deficiency. The effect of estrogen on osteoblast activity is less clear, but it is believed to directly stimulate osteoblastic differentiation and proliferation (52).

A positive relationship between estrogen and BMD in women has been shown in several epidemiologic studies with post-menopausal women but not in pre- or peri-menopausal women (53-55). Greendale and associates (54) examined the effects of endogenous sex steroids on BMD in 457 post-menopausal women (mean age: 72.1 ± 8 y) participating in the Rancho Bernardo Study. Bone mineral density was measured at the distal radius, lumbar spine, and total hip by DXA. Estrogen showed a statistically significant and positive association with all measured sites (P < 0.001) in these women. Studies comparing post-menopausal women using estrogen therapy versus nonusers have also found a positive association between estrogen and BMD. Elderly women (mean age: 74 ± 4.5 y) enrolled in the Framingham Osteoporosis Study participated in a four-year longitudinal study that examined the relationship to BMD loss in women using estrogen therapy and nonusers. At the end of the study, women without estrogen therapy lost 2.7% more BMD at the femoral neck compared to those using estrogen. Estrogen’s effect on BMD has been confirmed in randomized clinical control trials as well. The effect of estrogen therapy on BMD and bone biomarkers of turnover was examined in 13 pre-menopausal women (mean age 45 ± 5 y) one year following ovariectomy (56). Bone mineral density was measured by DXA at the proximal femoral neck, total body, and the spinal region of the total body. Additional analysis of BMD of the spine was measured using QCT. Despite a 5 kg weight gain and 600 mg calcium supplementation, cancellous spine loss was significantly reduced by 8% (P < 0.0007). In contrast, BMD sites measured with DXA showed a slight, non-significant decrease in BMD. Furthermore, the biomarkers of bone resorption decreased to normal values within one year of estrogen therapy. It is possible that high rates of bone remodeling were initiated before therapy had begun and that DXA analysis was unable to detect these changes. These results correspond with a similar study by Genant and colleagues (57) who found two years of estrogen therapy in six women resulted in a -3.0 ± 10% decrease (although non-significant) in spinal BMD as measured by QCT.

Studies examining the association between estrogen and BMD in pre-menopausal women have shown discrepant results. Sowers and colleagues (55) conducted a cross-sectional study in a cohort of 2,336 women aged 42-52 years who were either pre- or peri-menopausal. Unadjusted Pearson’s correlations found a non-significant negative relationship between estrogen and BMD at the lumbar spine and femoral neck in women with pre- or peri-
menopausal status. Multiple regression analysis, controlling for confounding variables, found similar results. In contrast, Hui and associates (58) reported estrogen levels were positively associated with bone loss. One hundred thirty women (mean age: 40.4 ± 4.2 y) participated in this four-year longitudinal study. Mean femoral neck BMD significantly decreased by -0.43% ($P < 0.001$) and was positively correlated with estrogen levels. The women were then separated into three categories based on femoral neck BMD loss: fast losers (< -1.0%), slow losers (-1.0 – 0.0%), and nonlosers (> 0.0%). A significant linear trend was shown between estrogen concentrations and fast losers to nonlosers ($P < 0.05$). These results suggest that lower levels of endogenous estrogen are associated with bone loss.

**Progesterone**

Progesterone, like estrogen, is a steroid hormone synthesized from its cholesterol precursor and is released from the ovary. When released into circulation, the majority of progesterone is bound to corticosteroid-binding globulin and a small portion is bound to albumin. Contrary to estrogen, the effect of progesterone on bone metabolism is unclear. In vitro cell culture studies have identified progesterone receptors on human osteoblastic cells, human osteosarcoma cells, and fetal osteoblast cells; however, the presence of estrogen is needed to activate progesterone receptors in these cells (52). A small number of human studies have evaluated the effect of progesterone on bone metabolism and have shown mixed findings. Progesterone was reported to prevent cortical bone loss in post-menopausal women in one study (59) while another study showed bone mineral loss at several sites (56). The use of more sensitive and accurate techniques to measure bone mineral status in the latter study may account for the discordant results. Prior and colleagues (56) conducted a randomized, double-blind clinical control trial and measured BMD with QCT and DXA and biomarkers of bone turnover to assess the effects of progesterone therapy on bone health. Thirty-three ovariectomized women, aged 30 to 55 years, were randomly assigned to receive either medroxyprogesterone or estrogen therapy for one year post-ovariectomy. Progesterone therapy resulted in significantly greater decreases of total body and femoral neck BMD (measured by DXA) and spinal BMD (measured by QCT) ($P < 0.04$) compared to estrogen therapy. N-telopeptide and serum osteocalcin concentrations were significantly increased with progesterone therapy ($P < 0.003$) when compared to mean pre-menopausal reference values, while no significant changes were seen with the estrogen therapy. These results suggest that the effects of progesterone differ from those of estrogen.


**Leptin**

Leptin is a polypeptide synthesized and released by white adipose tissue (60). Leptin is primarily known for its role as a regulator of body weight, and recent evidence suggests that it may regulate bone mineral status as well. However, studies have not shown consistent results. Leptin is a product of the Ob gene (61), which is located in adipose tissue. *In vitro* studies have been conducted to determine if a relationship between leptin and bone metabolism exists. High-affinity leptin receptors are expressed in bone marrow stromal cells. The addition of leptin to these cells directs their differentiation toward the osteoblast lineage (62).

Epidemiological studies examining the relationship with leptin and BMD have shown a positive relationship between leptin and BMD (63, 64). Thomas and colleagues (63) conducted a cross-sectional study in 137 pre-menopausal women (age range: 21 to 54 y) and 165 post-menopausal women (age range: 34 to 93 y) that examined the role of leptin on BMD at the total hip, mid-lateral spine, and mid-distal radius in women. Leptin was significantly correlated with BMD in the total hip, but not spine or radius when adjusted for lean mass and age (r = 0.31; *P* < 0.001) in pre-menopausal women. In post-menopausal women, however, leptin was significantly correlated with BMD at the total hip (r = 0.42; *P* < 0.001), spine (r = 0.18; *P* < 0.05), and radius (r = 0.27; *P* < 0.001). In effect, leptin explained 10%, 0.3%, and 5% of the variance of the total hip, spine, and radius, respectively, in pre-menopausal women and 19%, 6%, and 10% in post-menopausal women. Leptin levels were significantly and negatively correlated with osteocalcin (r = -0.20; *P* < 0.05) and NTx (r = -0.21; *P* < 0.05) in pre-menopausal women. In post-menopausal women, significant negative correlations were only observed with NTx (r = -0.24; *P* < 0.01) (63). Because fat mass has been positively associated with BMD in women and it is believed to be mediated by leptin (65), investigators analyzed the effect of both leptin and fat mass on BMD in a separate study. Fat mass was positively correlated with BMD at the total hip and radius in both pre- and post-menopausal women (*P* < 0.05). When adjusted for leptin, the association between fat mass and BMD remained positive, but was weakened. In a similar study, Martini and colleagues (64) showed a significant positive correlation (r = 0.29; *P* < 0.01) between leptin concentration and total body BMD measured by DXA in 123 post-menopausal women (age range: 39-82 y). When leptin values were adjusted for fat mass and BMI, however, the association with BMD was eliminated. Furthermore, markers of turnover were not correlated with leptin when adjusted for BMI. These two studies suggest that leptin is associated with BMD; however, this association is not independent of fat mass and thereby is a potential mediator of the protective effect of fat mass and BMD.
Nutritional Factors

Endocrine factors and dietary nutrients work together to regulate bone metabolism as the presence of a nutrient often stimulates the release of a hormone and, in turn, the hormone regulates nutrient concentration in the circulation and its entry into cells. It is important to understand the role of various nutrients involved in bone mineral status. Calcium and phosphorus are primary constituents of the bone matrix and have been extensively studied for their roles in bone mineralization during the developmental and elder years. Dietary calcium is the nutrient believed to have the most impact on bone mineralization. Recent evidence, however, suggests that calcium alone is not the primary determinant of bone mineralization—rather it involves a complex interaction between several nutrients. The interaction between calcium, phosphorus, and protein has been studied extensively, and a new wave of research implies that similar interactions exist with other nutrients. Nutrients important for bone growth and maintenance are presented.

Calcium

Calcium is the most abundant mineral in the body with 99% of it stored as hydroxyapatite in bone while the remaining 1% is found in the blood and in extracellular and intracellular spaces (30). Skeletal calcium is released into the circulation when serum calcium levels are low, a situation that may occur from a variety of factors such as metabolic disease, impaired renal function, or metabolic acidosis. Dietary calcium is an important player in bone mineralization—especially during periods of longitudinal bone growth and mineralization. In fact, calcium requirements were recently increased to 1,500 mg, 1,000 mg, and 1,300 mg per day during adolescence, adulthood, and the elder years, respectively, to better meet the demand for bone development and maintenance (66). Despite these recommendations, the literature examining the optimal calcium intake to maximize and sustain peak bone mass is controversial.

Some, but not all, studies have found a significant association between calcium intake and BMD (38, 67), (68). Cross-sectional studies have shown significant positive associations in calcium intake and BMD in the radius (38, 67) and lumbar spine (38) of young adult females. However, no relationship was found between calcium intake and spinal, femoral neck, or radial BMD in a two-year longitudinal study of 200-300 young women aged 20-39 years. Furthermore, calcium intake was not a significant predictor of BMD in these women (68). These differences may be due to the type of study design (cross-sectional vs. longitudinal), although a recent
follow-up study of four years found that higher calcium intakes enhanced BMC in pre-menopausal women (69). Ninety-two pre-menopausal women aged 25-30 years were categorized by their typical calcium intakes into a high-calcium group (mean intake: ~1,000 mg/day) or a low-calcium group (644 mg/day); BMC was measured four years later. The radius showed significantly higher increases (1.2% vs. -1.2% change; \( P < 0.019 \)) in BMC, while the trochanter showed lower, but not significant, decreases (-4.6% vs. -7.3% change) in BMC between women in the high-calcium and low-calcium intake groups, respectively.

Adequate calcium intake during the pubertal years is essential to ensure maximal bone mass accretion (70), and the need for adequate calcium intake has been shown to be greater immediately following menarche. Rozen and colleagues (71) examined the impact of 1,000 mg of a calcium supplement in 100 adolescent girls with a currently low dietary calcium intake (~800 mg per day) in a randomized double-blind placebo-controlled trial. Forty-nine girls (mean age: 14.8 ± 0.1 y) were given a 1,000 mg calcium supplement, while the other 51 girls (mean age: 14.9 ± 0.1 y) were received placebo for one year. Girls consuming the calcium supplement showed greater accretion of total body and lumbar spine BMD \( (P < 0.05) \), but not BMC, compared to girls in the placebo group. Moreover, biochemical markers of bone turnover showed significantly greater decreases in the calcium supplemented compared to placebo group \( (P < 0.001) \). Interestingly, greater accretion in bone mass was seen in girls two years post-menarche. These results show that calcium is needed to maximize bone mineral accrual and that two years post-menarche is a crucial time frame in which the greatest gains can be realized. While the importance of calcium to bone mineralization has been established, this nutrient does not act alone. In fact, several studies have found a complex interaction between calcium and several nutrients to promote bone mineralization.

**Phosphorus**

Phosphorus, like calcium is found primarily in bones. It is estimated that 85% of the body’s total phosphorus is located in the skeletal tissue bound with calcium to form hydroxyapatite (30). The other 15% is found in extracellular fluids and soft tissue. The serum phosphorus concentration, unlike calcium, is not tightly regulated and demonstrates wide fluctuations throughout the day. Phosphorus is regulated via renal reabsorption and appears to have a threshold within the proximal renal tubule. This setpoint regulates serum phosphorus concentrations and PTH (30).

There are few prospective studies that have examined the influence of a low-calcium, high-phosphorus diet on bone mass. High phosphorus diets have no effect on serum
phosphorus levels, but stimulate hormonal changes known to cause bone loss. Moreover, high-phosphorus diets have shown mixed results in biomarkers of bone turnover in studies lasting 4-7 weeks. Biomarkers of bone formation have decreased (72, 73) and have shown no changes (74, 75), while biomarkers of bone resorption have consistently shown no response to high-phosphorus diets despite increases in PTH (72-75). It is important to note that all but one of these studies used adequate amounts of dietary calcium, albeit the study with low-calcium intake demonstrated larger increases in PTH. These studies provide insight into the potential effects of a high-phosphorus diet on BMD; they suggest that the calcium:phosphorus ratio may strongly influence bone mineralization.

While both calcium and phosphorus are essential for bone mineralization, imbalances of these nutrients can reduce the degree of mineralization or even stimulate bone loss in extreme circumstances. Since these two minerals are tightly regulated, the new Dietary Reference Intakes (DRIs) were altered such that the optimal ratio for dietary intake increased from 1:1 to 1.2:1 (1,500 mg calcium to 1,250 mg phosphorus for adolescents) and 1.4:1 (1,000 mg calcium to 700 mg phosphorus for adults) (66) to maximize bone accretion and to minimize bone loss. Despite these guidelines, few studies have been conducted that have examined the relationship of varied calcium:phosphorus ratios on BMD. Teegarden and associates (38) predicted significant increases in total body and lumbar spine BMD and BMC when the calcium:phosphorus ratio was 0.8, 1.2, and 1.4 (calcium intake 800 mg, 1,200 mg, and 1,400 mg, respectively to 1,000 mg phosphorus) (66) in women aged 18-31 years. For example, total body BMD was predicted to increase by 2.6%, 5% and 7.4%, at the 0.8, 1.2, and 1.4 proportions, respectively. In contrast, higher phosphorus intakes (800 mg calcium to 1,400 or 1,800 mg phosphorus) predicted increases in total body BMD of only 2%. When the calcium to phosphorus ratio was maintained at a 1:1 ratio at levels of 1,400 mg, prediction equations estimated a 0.6% loss of total body BMD. The accuracy of dietary phosphorus intake is questionable because food frequency questionnaires (FFQ) were used to determine dietary phosphorus intake. Food frequency questionnaires report phosphorus intakes from natural food sources such as dairy and meat and do not distinguish whether phosphorus is from natural foods or processed foods. Processed foods contain higher amounts of phosphorus (76). Because of this, phosphorus intake may have been underestimated. Brot and colleagues (77) used food records to estimate dietary calcium and phosphorus intakes to examine the effect of the calcium to phosphorus ratio on serum levels of 1,25- (OH)\textsubscript{2}D, PTH, and its association with BMD in 510 peri-menopausal women (mean age: 50.6 ± 2.8 y). A significant inverse relationship between serum 1,25- (OH)\textsubscript{2}D and BMD was found at the total body, spine, and
femoral neck. In addition, there was a positive association between serum 1,25-(OH)_{2}D levels and biomarkers of bone turnover; however, there was no relationship with concentrations of PTH, serum calcium or phosphorus. Furthermore, the dietary calcium:phosphorus ratio was inversely related to 1,25-(OH)_{2}D but positively associated with BMD ($P < 0.0005$). These results suggest that a low-calcium:phosphorus ratio can negatively influence BMD and that this is mediated via serum 1,25-(OH)_{2}D. These studies provide support for a dietary calcium:phosphorus ratio intake $\geq 1.2$ to maintain BMD.

**Magnesium**
Magnesium is the third most abundant mineral in the bone with 66% of total body magnesium present in the skeleton (30). Magnesium is one of the minerals that form the bone matrix. Epidemiological studies have suggested a link between low serum magnesium and low bone mass (78-80). Magnesium intake showed no correlation with lumbar spine or hip BMD and a close, but non-significant association with forearm BMD and cortical BMD in a cross-sectional study with pre-menopausal women (78). However, when grouped into quartiles of magnesium intakes, greater intake was significantly associated with higher BMD and cortical BMD in the forearm. In another cross-sectional study with pre-menopausal women, Houtkooper et al. (79) showed that magnesium intake was a significant predictor of total body BMD. Markers of bone resorption but not formation are influenced by magnesium intake. New et al. (78) showed that magnesium intake was a strong predictor of pyridinoline and deoxypyridinoline excretion accounting for 12.3% and 12% of the variation in each marker, respectively. In addition, women with the highest intake of magnesium showed significantly lower pyridinoline and deoxypyridinoline excretion compare to women with the lowest intake. These results suggest that low magnesium intake promotes bone loss by stimulating bone resorption rather than depressing bone formation.

**Sodium**
The relationship between dietary sodium intake and bone health has received much attention in the past decade (44). Earlier studies first associated dietary sodium intake with increased urinary calcium excretion (81, 82) and theorized that this increase was a result of bone loss. Although a mechanism explaining the etiology of hypercalciuria has been proposed, (44) recent studies have yet to confirm the impact of high dietary sodium intake on BMD and bone metabolism.

Sodium and calcium appear to share similar transport mechanisms for reabsorption in the renal tubule. In the presence of excess dietary sodium, reabsorption of sodium declines,
which leads to a similar reduction in calcium reabsorption by the renal tubule. It is estimated that for every 0.5 – 1 mmol/100 mmol of sodium ingested, approximately 100 mmol of calcium is excreted. This results in a decrease in the serum calcium concentration and consequently increases serum PTH levels (44). Parathyroid hormone in turn increases the serum calcium concentration. It is estimated that over two decades, a 1 mmol calcium loss per day in a woman with a skeletal store of 900 g of calcium would result in a loss of one-third of her skeletal calcium, provided no compensatory response occurred via intestinal absorption (83).

Several studies have examined the influence of a high-sodium diet on bone health, and while population-based cohort studies show no association, (84, 85) cross-sectional studies and controlled trials have found mixed results. Controlled intervention studies have consistently shown an increased urinary calcium in response to a high-sodium diet in both pre-menopausal and post-menopausal women consuming a sodium load between 180 – 250 mmol/day compared to a low-sodium diet of 50 - 87 mmol/day (83, 86). These studies have examined the effect of sodium on markers of bone turnover and have found conflicting results; however, this discrepancy appears to be related to age and menopausal status. Bone resorption markers were increased in post-menopausal women, (78, 80, 83) whereas no change was seen in pre-menopausal women (86).

Twenty-six post-menopausal women (mean age: 63 ± 8 y) were adapted to a low-sodium diet (87 mmol per day) for three weeks and then placed on a high-sodium diet (225 mmol per day) with calcium supplementation of 500 mg/day for four weeks. Urine calcium excretion increased by 42 ± 12 mg/day ($P < 0.002$), while urine NTx increased by 6.4 ± 1.4 nmol BCE/mmol Cr ($P < 0.001$), and serum osteocalcin decreased by 0.57 ± 21 ng/dl ($P < 0.01$) after consuming the high-sodium diet (83). Similar results were shown in a randomized crossover study of 11 post-menopausal women (mean age: 57 y, calcium intake: ~740 mg per day). In this trial, Evans and associates (86) instructed the women to follow either a high-sodium diet (250 mmol per day) or a low-sodium diet (50 mmol per day) for one week. Urine calcium excretion increased by 48% ($P = 0.005$), urine DPD increased by 27% ($P = 0.02$), and serum osteocalcin did not change between the two dietary treatments. Serum PTH concentrations did not differ between treatments in either study, indicating a lack of hormonal adaptation to compensate for these increases in urine calcium excretion.

In the same trial described above, Evans and associates (86) randomized 12 pre-menopausal women (mean age: 32 ± 8.9 y, calcium intake: 741 ± 172 mg/day) to follow either a high-sodium diet or a low-sodium diet for one week. Urinary calcium excretion increased by 37% ($P = 0.005$) in women following the high-sodium diet. Deoxypyridinoline was not
significantly different between dietary treatments, while osteocalcin decreased by 8% ($P < 0.02$) during the high-sodium diet period. The lack of change in the bone resorption marker DPD, indicated that the serum calcium concentration was not maintained by skeletal calcium. These combined studies suggest that there is a compensatory response possibly by increased intestinal calcium absorption to prevent calcium loss from bone. To substantiate this claim, evidence from a study by Breslau and associates (87) showed intestinal calcium absorption increased by 26% in young men and women (mean age: 27 y) given sodium supplementation of 250 mmol per day compared to 10 mmol per day.

Together, these findings suggest that increasing dietary sodium intake can stimulate urinary calcium loss which increases bone resorption in post-menopausal women, suggesting a loss of calcium from bone in this population (83, 86). Young adults appear to have a protective mechanism to attenuate bone resorption via increased intestinal calcium absorption (83, 86). While these studies provide compelling evidence that a high-sodium diet has potential detrimental effects to bone, these studies were short-term [less than the time needed for one bone remodeling cycle, (i.e., at least 6 months)], and therefore may not have allowed bone to reach homeostasis in response to the dietary treatment (44). Furthermore, these studies compared the bone response to a very low-sodium diet compared to a high-sodium diet. Current statistics indicate that the average American consumes ~3,400 mg of sodium per day (88), although the recommended intake by the American Heart Association is 2,400 mg per day (89). Therefore, in the American population, bone may have already undergone an adaptive response to the high-sodium diet. In addition, these studies did not examine the impact of high-sodium diets when combined with other dietary factors known to stimulate calciuria (such as dietary protein) or dietary factors that serve to protect bone (such as potassium and other basic compounds).

**Potassium**

Potassium, like sodium, is another cation that impacts bone status. In contrast to sodium, potassium serves to protect bone by reducing hypercalciuria and bone resorption induced by excessive sodium consumption (83). In addition, potassium salts have been shown to prevent acid induced hypercalciuria and bone dissolution when given alone (90) or when combined with either citrate (91) or bicarbonate (92). Sellmeyer and associates (83) examined the effect of potassium citrate supplementation on urine calcium excretion and bone resorption in 52 post-menopausal women following a low-salt diet (~2,000 mg) for three weeks and then a high-salt (~5,200 mg) diet for seven weeks. Women were randomized to receive either a
placebo (n=26, mean age: 63 ± 8 y) or potassium citrate (n=26, mean age: 65 ± 5 y) throughout the study. Potassium citrate supplementation blunted the rise in urinary calcium excretion and NTx seen in the placebo group when sodium intake was increased. Urinary calcium excretion increased 33% and decreased 8% in the placebo and potassium supplemented groups, respectively, (P = 0.008). Urine NTx concentrations increased by 23% and 7% in the placebo and potassium citrate supplemented groups, respectively (P = 0.49). Bone formation markers, however, were not affected by potassium citrate supplementation. These results indicate that potassium citrate can reverse the negative effects of dietary factors known to stimulate bone resorption in post-menopausal women with reportedly normal bone status. Potassium citrate also appears to reduce bone resorption biomarkers and urinary calcium excretion in post-menopausal women diagnosed with low BMD (91), indicating that it may be useful for preventing post-menopausal osteoporosis. These results provide provocative evidence for the benefit of potassium citrate in preventing osteoporosis in post-menopausal women.

In lieu of the evidence found in post-menopausal women, few studies have examined the effect of potassium salts on bone health in this young population. Lemann and colleagues (90) evaluated the effect of potassium deprivation and supplementation in the form of KCl or KHCO₃ on urinary calcium excretion in ten healthy young adult men and women (mean age: 37 ± 2 y). During the deprivation period, urinary calcium excretion was significantly higher (P < 0.0005) compared to controls and was restored to control values when KCl and KHCO₃ were added back to the diet. Supplementation with 90 mmol per day of KHCO₃, but not KCl reduced urinary calcium excretion compared to control values. These studies support previous reports that showed potassium, particularly combined with HCO₃⁻ protects against urinary calcium losses.

These combined results suggest that the inclusion of an alkaline salt, such as potassium citrate in normal and high salt diets, can indeed reduce urinary calcium excretion and markers of bone resorption, thus reducing the negative impact on bone. Fruits and vegetables are naturally rich in alkaline salts of potassium. The current recommendation of greater than 5 servings of fruits and vegetables per day (89) could potentially provide protection against bone loss and negate the effect of diets containing large amounts of salt or acid producing foods on bone.

Iron
Dietary iron intake and BMD have not been typically associated; however, the role of iron in bone mineralization has recently been explored with promising results demonstrating the importance of this nutrient to BMD (93-95). The association of iron overload and iron deficiency
with low bone mass have been established; however, these studies have primarily used animal models (96) or subjects with genetic alterations in iron metabolism (97, 98). A few epidemiological studies in pre-menopausal and post-menopausal women have evaluated the association of dietary iron intake with bone mineral status (93-95). Iron intake was positively associated with forearm BMC (95), total body, lumbar spine, and femoral neck BMD (93, 94), and femoral trochanter and neck BMD (93). Michealsson and colleagues (94), however, found no associations with iron intake and BMD of the total body, lumbar spine, or femur with multivariate analysis controlling for covariates. Smaller sample size (n = 175) may have contributed to these nonsignificant findings. Harris and associates (93) introduced provocative evidence that an interaction between dietary calcium and iron exists to enhance bone mineralization. In this cross-sectional study of 272 post-menopausal women (aged 40-66 y), greater intake of iron (>20 mg) was significantly associated with higher BMD at several bone sites when calcium intake was between 800-1,200 mg. Women with higher or lower intake of calcium or iron intake (<14 mg) showed lower BMD. From these findings, the relationship of dietary iron and BMD appears not to be limited to this nutrient alone. Rather, they suggest a complex interaction exists between iron and calcium to promote bone mineralization.

**Zinc**

Zinc plays an important role in maintaining bone mineral status by stimulating osteoblast activity, collagen synthesis, and alkaline phosphatase activity and inhibiting osteoclast activity (99, 100). Specifically, zinc is an essential cofactor for enzymes that participate in the formation of constituents of the bone matrix (100). Zinc forms a complex with fluoride in the hydroxyapatite of bone and, therefore, is thought to play a functional and structural role in hydroxyapatite (101). The relationship between zinc deficiency and loss of bone mineral has been examined in animal studies. Decreased cancellous bone mass and marked deterioration of trabecular bone was shown in one study of rats deficient in zinc (102). Another study found reduced femur zinc concentration in rats with zinc deficiency (100). These results suggest a potential relationship between zinc status and osteoporosis; however, little data exist examining this relationship in humans.

Epidemiological studies have shown a positive effect of zinc intake on bone mineral status in the forearm. A significant inverse relationship between zinc intake and radial bone loss ($r = 0.37; P = 0.03$) in post-menopausal women, but not in pre-menopausal women participating in a longitudinal study of four years was observed (103). In contrast, Angus et al. (95) reported a weak positive correlation ($r = 0.26; P < 0.05$) between zinc intake and forearm BMD in pre-
menopausal women, but not post-menopausal women, in a cross-sectional study. Furthermore, no association existed between zinc intake and the lumbar spine or proximal femur in either group of women. Because both studies used similar techniques to measure BMD, these discordant data are most likely due to different methods used to obtain dietary intake and study design. The latter study obtained dietary intake data using 24-hr recall records, whereas the former study used 4-day food diet records. Assessment of biochemical indicators of zinc status would provide a better indicator of zinc deficiency and its relationship with low bone mass. However, specific and sensitive biochemical indicators of zinc status are lacking (100). More clinical trials using reliable indicators for zinc status are warranted before definite conclusions can be drawn about the role of zinc on bone mineral status in humans.

**Vitamin D and Vitamin A**

In addition to synthesis from ultraviolet radiation, vitamin D is also available from limited dietary sources such as fatty fish, fish oils, fortified foods, and vitamin supplements. Survey data indicate that vitamin D concentration and dietary intake are deficient in the elderly population and in adult women of childbearing age (104, 105). Vitamin D deficiency has been associated with increased fracture risk in post-menopausal women (106); however, the effects of vitamin D supplementation and hip fracture risk have been mixed. Hip fracture incidence was reduced when vitamin D was provided as an injection (107); other studies using elderly populations, however, found no fracture-preventing effect with supplementation of vitamin D in the form of fish oil (108) or tablets (109). Studies evaluating the effects of vitamin D supplementation and bone mineral status in post-menopausal women showed attenuated bone loss at the spine (110) and femoral neck (111). The effect of vitamin D deficiency in the elderly population has raised concern that vitamin D deficiency in young adult women may induce similar skeletal losses; however data examining the relationship of this deficiency and bone health in the young adult population are limited. In a cross-sectional cohort of pre-menopausal women in Mexico (n = 1,888, mean age: 38.6 ± 6.4 y), greater vitamin D intake was a strong predictor of forearm BMD in women (r = 0.07; P < 0.01) (112). Vitamin D intake was assessed via self-administered dietary questionnaires and reported intake may not be representative of actual vitamin D intake. Therefore, these results should be interpreted cautiously. Serum vitamin D and PTH would provide a more accurate assessment of the association between vitamin D status and bone mineral status.

A renewed interest in the relationship between vitamin A and bone health has sparked among investigators in the past decade. The detrimental effects of hypervitaminosis A on bone
Health was first identified in animal and in vitro models in the early 1900s. Researchers demonstrated that excessive intake of vitamin A in the form of retinol caused spontaneous fractures, premature closing of the epiphyses, growth retardation, increased osteoclast number and bone resorption, and decreased osteoblast density and bone formation in laboratory animals. Furthermore, in vitro models showed shrinkage and disappearance of cartilage matrix, reduced collagen synthesis, and augmented bone resorption in calvarie and embryos of mice and chicks (113). These data imply excessive vitamin A consumption can increase the risk for osteoporosis; yet, a paucity of studies has addressed this important issue in humans. In a prospective longitudinal study, a cohort of women aged 34-77 years provided data on their dietary intakes of vitamin A, retinol, and beta carotene using FFQ. Relative risk for hip fracture was highest in women consuming the highest intakes of retinol (> 3,250 µg/day, RR: 1.48; \( P = 0.003 \)) compared to those with the lowest intakes (<1250 µg/day). Of the two forms of vitamin A, retinol was the greatest contributor to this risk (RR: 1.89; \( P < 0.001 \)), while beta carotene intake exhibited no risk for hip fracture (114). Similar results were shown in a study by Melhaus et al. (115) who reported that risk for hip fracture doubled in women consuming >1,500 µg/day of retinol compared to women who consumed < 500 µg/day. Furthermore, proximal femur BMD was reduced by 10% in those consuming >1,500 µg/day of vitamin A. While these studies indicate that excessive vitamin A intake, particularly the retinol form, is detrimental to bone health, none examined blood indices of vitamin A and the risk for hip fracture. Michaelsson and colleagues (116) conducted a longitudinal population based study using 2,322 men aged 49-52 years. Baseline serum retinol and beta carotene concentrations were measured and fractures were documented for 30 years thereafter. Men in the highest quintile of serum retinol concentration (>2.64 µmol/L) exhibited higher relative risk for any fracture (1.64) and hip fracture (2.47) compared to those in the middle quintile of serum retinol concentration (2.17-2.36 µmol/L). The level of serum beta carotene showed no related risk to fracture. The authors concluded that serum retinol concentration >3 µmol/L are detrimental to bone health and that the use of vitamin A supplements and foods fortified with vitamin A should be reevaluated in Western societies.

**Polyunsaturated Fatty Acids (PUFAs)**

The association between PUFA intake and BMD has been recently studied. Epidemiological studies have found a significant negative correlation between PUFA intake and BMD (94, 117, 118). In a recent study, MacDonald and colleagues (118) reported that a PUFA intake was significantly and negatively correlated with the change in BMD of the femoral neck in
both pre-menopausal and post-menopausal women \( (r = -0.069; \ P < 0.01) \) and in a subcategory of women who were either pre- or peri-menopausal \( (r = -0.193; \ P < 0.05) \). Furthermore, BMD was further reduced when high PUFA intake was accompanied by low dietary calcium intake. It is important to note, however, that the PUFA profile was not taken into consideration with these findings. Studies using animal and in vitro cell culture models have found a positive and negative association of the essential PUFAs, \( \alpha \)-linolenic acid (n-3) and linoleic acid (n-6), respectively, with bone mineral status (119-121). Eicosapentanoic acid (EPA), a member of the n-3 family, prevented bone loss of bone weight and strength in ovariectomized rats consuming inadequate dietary calcium (120) and was negatively correlated, although not significantly, to the bone resorption marker, DPD (122). Fish oil, which is rich in EPA, was shown to prevent bone loss seen in ovariectomized rats given corn oil, which is rich in n-6 fatty acids (119). Omega-3 fatty acids appear to protect bone in humans as well. Kruger and colleagues (121) supplemented 60 post-menopausal women (mean age: \( 79.5 \pm 5.8 \) y) previously diagnosed with low bone mass or osteoporosis with 600 mg of calcium and either a fish oil/primrose oil mixture rich in omega-3 fatty acids \( (n = 29) \) or a placebo of coconut oil \( (n = 31) \) for 18 months. Significant reductions in serum osteocalcin \(-0.39\% \) and \(-0.34\% \) change) and deoxypyridinoline \(-0.67\% \) and \(-0.69\% \) change; \( P < 0.05 \) from baseline values were seen in both groups. Although group differences were not analyzed, changes in bone turnover markers were similar in both groups. These results imply that the attenuated bone turnover rate occurred in response to calcium supplementation. After 18 months of treatment, the treatment group exhibited no changes in lumbar spine BMD and femoral neck BMD increased by 1.3\%, while the placebo group showed reductions of 3.2\% and 2.1\%, respectively. For 18 additional months, all patients were supplemented with omega-3 fatty acids. Lumbar spine BMD increased by 3.1\% and 2.3\% while femoral neck BMD increased by 2.3\% and 4.7\% in patients who remained on active treatment and who began active treatment, respectively. These results indicate that n-3 fatty acids increase BMD; however, it was unclear whether they act on markers of bone turnover in this study (121). Additional controlled human trials need to be conducted before specific recommendations can be made.

**Protein**

High-protein diets have been cited as a risk factor for osteoporosis (123, 124). Researchers propose that excessive intake of dietary protein increases sulfur-amino acid concentration, which increases extracellular hydrogen ion concentration and reduces the concentration of extracellular bicarbonate, thus reducing systemic pH. This marked decrease in
pH is accompanied by an increase in urinary calcium excretion with no change in net calcium absorption (125). The majority of body calcium is stored in bone, suggesting that the urinary calcium source comes from the mineral phase of bone (126). Factors that may contribute to hypercalciuria are enhanced glomerular filtration rate (127, 128) and reduced renal reabsorption of calcium, which induces secondary hyperthyroidism and stimulation of bone resorption. This process is a concern in individuals who consume acid-forming foods over a long period of time because the gradual loss of calcium from bone, over time, may lead to osteoporosis.

There is a wide body of literature that has examined the role of dietary protein intake in relation to bone metabolism. The relationship between high dietary protein intake [defined as an intake greater than the Recommended Dietary Allowance (RDA) (0.8 g/kg bw)] and bone health, however, has shown inconsistent results. Cross-sectional studies, using self-reported dietary protein intake data, have found dietary protein was positively correlated (129, 130), negatively correlated (131), and had no effect (67) on bone mineral density or fracture risk. The type of protein consumed may influence bone mineralization. A prospective study in post-menopausal women found attenuated femoral bone mineral loss and reduced risk for hip fracture in women who consumed a diet with a low ratio of animal to vegetable protein intake compared to women consuming a high ratio of animal to vegetable protein diet (132).

The impact of high-protein diets on bone metabolism appears to be related to the duration of high-protein consumption. Pre-menopausal women (n = 16, mean age: 26.7 ± 1.3 y, mean BMI: 22.3 ± 0.6 kg/cm²) were placed on a cyclic experimental diet that contained one of three levels of protein [high-protein diet (2.0 g/kg bw), moderate protein diet (1.0 g/kg bw), and low-protein diet (0.7 g/kg bw)] for four days. Two weeks before each experimental diet, the women consumed a well-balance diet containing moderate amounts of calcium, sodium, and protein. The high-protein intake increased urinary calcium excretion (high, 196 ± 19 mg/day; moderate, 129 ± 14 mg/day; P < 0.0005) and the low-protein intake decreased urinary calcium excretion (low, 108 ± 14 mg/day; moderate 129 ± 14 mg/day; P < 0.05) compared to the moderate protein diet. N-telopeptide was significantly increased during the high-protein intake compared to the low-protein intake (48.2 ± 7.2 vs. 32.7 ± 5.3 nM BCE/mM Cr; P < 0.05), and osteocalcin concentration did not differ between the three levels of protein consumption (133, 134). These results suggest that the hypercalciuric effect following the acute ingestion of a high-protein diet will promote the loss of bone; however, whether these effects would remain over a sustained period of time is not clear. Recent evidence suggests physiological adaptations may occur after the acute ingestion of a high-protein diet that reduces the hypercalciuric effect of a high-protein diet. Post-menopausal women (n = 15, mean age: 60.5 ± 7.8 y, mean BMI: 26.5 ± 4.0 kg/cm²)
were placed on a high-protein (1.6 g/kg) or lower-protein (0.9 g/kg bw) diet for 8-weeks in a randomized crossover study. At four weeks, calcium retention was not significantly different during the high- or lower-protein dietary treatment periods. Initial urinary acid excretion was greater (5.68 vs. 6.02; \( P < 0.05 \)) in the high-protein group compared to the lower protein group, but declined to similar levels of the lower protein group (5.88 vs. 5.90; \( P > 0.05 \)) by week five. Urinary calcium excretion and bone markers of formation or resorption were not significantly different between the two dietary treatment groups at any time point in the study (135).

It is clear that the consumption of the correct balance of bone building nutrients is needed to optimize bone health during all life stages. As these studies show, a severe deficit or excess of any of these nutrients, particularly during the developmental years in which bone accretion is maximal, could lead to osteoporosis. Dietary intake of key micronutrients such as calcium tends to be inadequate in women, particularly in women who are dieting (136). Furthermore, reduced bone mass has been shown to accompany weight loss, which is discussed in the following sections.

**Dieting and Bone Health**

One benefit to being overweight is the impact it has on bone health. It is well established that body weight is a strong determinant of BMD and BMC (38, 103, 137). Weight loss is encouraged in overweight individuals because it reduces risks for many chronic diseases; ironically, weight loss may, in fact, impose greater risk for osteoporosis. Animal studies have found reductions in bone mass accompany weight loss (138, 139), while human studies have shown mixed results (140-143). Rats aged 3 and 10 months were placed on a 40% energy-restricted diet for 9 weeks. Diets were isonitrogenous and contained equal amounts of nutrients while carbohydrate content was adjusted to create the caloric deficit. Indices of bone turnover and BMD were measured in the energy-restricted group and compared to controls. Serum osteocalcin increased by 10-20% and urinary \(^{3}\text{H}\)TC excretion (a marker of bone resorption) increased by 20-40% compared to controls in both age groups (\( P < 0.05 \)). Final BMD did not change in the younger rats as result of dietary restriction (+0.024 g/cm\(^2\)) compared to controls; however, rats with restricted calcium intake (+0.14 g/cm\(^2\)) and restricted calcium and energy intake gained less BMD (+0.14 and +0.01 g/cm\(^2\), respectively) compared with controls (+0.22 g/cm\(^2\); \( P < 0.01 \)). Older rats exhibited no change in BMD in all dietary groups, while the control group saw a 3.3% gain in BMD (\( P < 0.01 \)) (138).

Human studies have shown mixed results, and may be attributable to differences in study design and methods used to measure bone mineral status. Many studies have found
weight reduction of 3 to 22 kg is accompanied by a 1-2% loss in BMC and BMD in the total body
(143, 144), and BMD in the lumbar spine and radius (140), while other studies have found non-
significant differences in total body BMD (141) and BMC (140, 142) with weight reduction. Van
Loan et al. (140) placed 14 obese women (mean age: 25-42 y, mean fat mass%: 44.9 ± 8.1) on
an energy-restricted diet (50% proportional reduction of current energy intake) for 15 weeks
followed by a 3 week weight maintenance diet. Body weight was significantly decreased by ~15
kg (-21%) during the energy-restriction phase of the study. A significant decline in total body
BMD was shown (-0.017 ± 1.4 g/cm²; P = 0.02), but no changes were seen in BMC or
bone area following the 15-week energy restriction. Because of the discrepancy in BMD and
BMC measures, the authors speculated that the decrease in BMD may have been a result of
artifacts from the densitometer, although a larger sample size may have resolved this
discrepancy. Analysis of other sites containing a greater proportion of metabolic bone tissue
(i.e., spine and femur) may provide more conclusive answers. Fogelholm and colleagues (142)
measured changes in BMD, BMC and bone area in five different sites in a 3-month weight loss
intervention with 74 pre-menopausal women (mean age: 40 ± 4 y, mean BMI: 34 ± 3.6 kg/m²).
Women were placed on a low-energy diet for one week, a very low-energy diet for 8 weeks, and
then returned to the low-energy diet for two weeks. Women then followed a weight
maintenance program for the remainder of the study. Only results from the end of the energy
restriction phase are discussed. The women lost 14.3% of their initial body weight during the 3-
month weight loss intervention with the greatest proportion from fat mass as compared to lean
mass. Bone mineral density of the total body, spine, trochanter, and distal radius were
significantly reduced by -0.01 ± 0.03, -0.02 ± 0.04, -0.01 ± 0.2, and 0.005 ± 0.02 g/cm²,
respectively (P < 0.05). Femoral neck BMD did not change during the weight loss intervention.
Only the spine (-1.02 ± 2.22 g), femoral neck (-0.47 ± 1.0 g), and trochanter (-0.01 ± 0.02 g)
BMC and the bone area of the femoral neck (-0.43 ± 0.96 cm²) and trochanter (-0.33 ± 0.77
cm²) were significantly reduced during the weight reduction phase (P < 0.05). The other
measured sites showed no difference from baseline values. Neither study included information
about the caloric deficit or nutrient profile of the weight reducing diets; furthermore, biomarkers
of bone turnover were not measured. Differences in intake of key nutrients that influence bone
metabolism may be a factor in the discordant results; however, similar to the previous study,
artifact from the densitometer may have influenced the bone mineral status measures.
Increasing dietary intake of nutrients that promote bone mineralization may alleviate losses in
bone mineral during weight reducing diets. Shapses et al. (141) examined the impact of 1,000 g
of supplemental calcium on bone mineral status and markers of bone turnover in pre-
menopausal women consuming an energy-restricted diet for six months. Thirty-eight women (mean age: 40.4 ± 5.8 y; BMI: 35 ± 4.0 g/kg²) were randomly assigned to one of three diet groups. Two groups were to follow an energy-restricted diet while the third maintained normal dietary intake and served as the control. Within the two energy-restricted groups, one was provided with 1,000 g of supplemental calcium (n = 14) while the other was energy-restricted with no calcium supplementation (placebo; n = 14). Women in both groups had similar weight loss at the end of the study (7.9 ± 4.1% vs. 7.1 ± 2.5%, respectively). Total body BMD did not change significantly in either weight loss group compared to the control while lumbar spine BMD increased significantly (P < 0.05) in the calcium-supplemented group compared to the placebo and control. No difference was seen between the placebo versus control group. No significant differences were seen in serum osteocalcin, urinary pyridinolamine, or serum NTx between any of the dietary treatments. However, change in urinary DPD values in the placebo group were significantly higher (34.7 ± 35.8%) compared to the calcium-supplemented group (4.2 ± 53.2%) and the control (5.1 ± 24.5%). These results suggest that calcium supplementation is protective against bone mineral losses in the BMD of the lumbar spine and that energy restriction stimulates bone mineral loss. Although losses in bone mineral were not seen in the energy-restricted diet group, the increase in DPD indicates energy-restriction without calcium supplementation stimulates bone turnover, which may lead to future loss of bone mineral. These authors did not measure BMC or bone area as was done in previous studies that have shown discordant results between BMC, bone area, and BMD. Furthermore, it is possible that the calorie deficit was not great enough to induce losses in BMD or BMC.

Energy-restricted diets lacking essential nutrients needed to promote bone health may lead to irreversible bone loss if energy restriction is prolonged. There is much pressure placed on overweight and obese individuals to lose weight by both society (to meet aesthetic expectations) and by health professionals (to reduce risk for disease and promote good health). A more popular diet plan on the market today is the LCHP diet. Nutrition experts have criticized these diets citing the excess fat and protein would increase the risk for cardiovascular disease and osteoporosis. A new wave of research has emerged that has evaluated the effect of these diets on risk factors for cardiovascular disease and osteoporosis and the findings show LCHP diets may not be as detrimental as previously believed.
Low-carbohydrate, High-protein Diets

The Atkins diet has re-emerged and is rapidly gaining popularity as a successful weight loss plan. The diet plan limits the intake of carbohydrate containing foods while allowing unlimited consumption of protein-rich and high-fat foods. Specifically, individuals are instructed to limit carbohydrate intake to ≤20 g per day for two weeks. Each week thereafter, carbohydrate intake can increase by 5 g per week until weight loss stops or ketones are not present in the urine (145). The theory behind this diet plan is that restricting carbohydrate intake to very low levels will change the body’s metabolism by increasing lipolysis and decreasing fat synthesis induced by elevated insulin levels triggered by excessive carbohydrate intake. The presence of ketones is desired for two reasons: 1) they confirm lipolysis; and 2) they are suggested to contribute to weight loss by promoting feelings of satiety and fullness. These metabolic adaptations will result in weight loss (145).

Nutritionists have expressed concern that the excessive amounts of total fat, saturated fat, and protein will increase risk for chronic diseases such as cardiovascular disease, cancer, and osteoporosis. In the past two years, a plethora of studies have been published examining the effects of LCHP diets on cardiovascular risk factors; however, a dearth of studies exist that examine the impact of this diet and its high-protein content on bone health. The rest of this review will examine the current findings on the effect of LCHP diets on weight loss, risk factors for cardiovascular disease, and bone mass.

Weight Loss

The popularity of LCHP diet plans have increased due to testimonials reporting rapid weight loss. Health professionals argue that the initial weight loss seen in response to this diet is from water lost due to depletion of glycogen stores rather than body fat. Recent studies have evaluated the rapidity of weight loss and changes in body composition in individuals following LCHP diets, and most studies agree that these diets are, in fact, effective for weight loss (146-148); however, long-term adherence to this diet and maintenance of weight loss is questionable (148). A short-term study of ten healthy adults (mean BMI: 29.4 kg/m²) reported significant weight loss from baseline values (81.3 ± 18.5 kg) at week two (78.4 ± 18.1 kg; P < 0.001) and week six (77.2 ± 17.5 kg; P < 0.001) (146). Subjects were given extensive instruction on the diet protocol and provided constant metabolic meals during each of the testing weeks, indicating they were compliant with the LCHP dietary guidelines. Caloric intake was decreased by ~500 kcal per day during the initial two weeks; however, there was no comparison to individuals
following another type of diet, such as a low-fat calorie restricted diet, to determine if macronutrient composition, rather than energy reduction, was the factor responsible for weight loss. Two separate, but similarly designed, randomized control trials reported significant weight loss in individuals following a LCHP diet versus a high-carbohydrate, low-fat diet for six months (147, 149). Samaha and associates (147) randomly assigned 132 severely obese adults to either a LCHP diet (< 30 g carbohydrate/day) (n = 64, mean age: 53 ± 9 y, BMI: 42.9 ± 6.6 kg/m²) or a low-fat diet (< 30% fat) (n = 68, mean age: 54 ± 9 y, BMI: 42.9 ± 7.7 kg/m²).

Participants were provided with detailed instructions for both dietary protocols and met weekly during the first four weeks and then monthly for the remaining five months of the study. Weight loss was greater in the subjects following the LCHP diet (-5.8 ± 8.6 kg) compared to the low-fat diet (-1.9 ± 4.2 kg; \( P = 0.002 \)). In a similarly designed study, Brehm et al. (149) reported comparable results in 42 obese females randomized to follow either a LCHP diet (20 g/day for two weeks then 40-60 g/day thereafter) (n = 22, mean age: 44.2 ± 6.8, BMI: 33.2 ± 1.8 kg/m²) or a low-fat diet (< 30% fat; 500 kilocalorie reduction) (n = 20, mean age: 43.1 ± 8.6, mean BMI: 34.0 ± 1.83 kg/m²). Women following the LCHP diet lost 7.6 ± 0.7 kg and 8.5 ± 1.0 kg at three and six months, respectively, while women on the low-fat diet lost 4.2 ± 0.8 and 3.9 ± 1.0 kg at three and six months, respectively. Weight loss in the LCHP diet group was significantly greater at both three and six months (\( P < 0.001 \)) compared to the low-fat diet group. Diet records showed equal reductions in energy intake between the two groups, implying that macronutrient composition was responsible for the differing weight loss. Body fat was also significantly decreased in the LCHP diet group compared to the low-fat diet group at three and six months, suggesting that fat mass losses accompany a LCHP diet.

Because the majority of weight is lost during the initiation of a LCHP diet, diuresis has often been cited as the reason for the decreased weight as opposed to fat loss. Recently, the source of weight loss from the initiation to six months of a LCHP diet was investigated (150). One-hundred twenty healthy adults were randomly assigned to follow either a LCHP diet (n=45, mean age: 45.3 ± 9.5, mean BMI: 34.6 ± 5.2 kg/m²) or low-fat diet (n=34, mean age: 44.1 ± 8.7, mean BMI: 33.9 ± 5.3 kg/m²). Both dietary protocols were similar to those described by Brehm et al. (149) mentioned above. Weight loss was significantly greater in the LCHP group; however, the percentage of total weight loss from fat mass was similar for both groups (78% in the LCHP group vs. 74% in the low-fat group). A trend toward greater losses in lean mass in the LCHP group (-3.3 kg) versus the low-fat group (-2.4 kg; \( P = 0.054 \)) was seen, coinciding with changes in total body water. The LCHP diet group saw a decrease of -2.4 kg while the low-
fat diet group had -1.8 kg loss in body water. Interestingly, the LCHP diet group lost more body water (-1.1 kg) than the low-fat diet group (-0.5 kg) during the first two weeks of the study. These results confirm reports that the rapid weight loss typically seen at the initiation of a LCHP diet is due to diuresis. Energy and macronutrient intakes were assessed by self-reported 24-hour recall records and were not reported in this study; therefore, it is difficult to determine whether the weight loss observed in this study was more likely reflective of a caloric deficit rather than macronutrient composition.

While these studies show promise for short-term weight loss, long-term adherence to the LCHP diet and weight maintenance have yet to be explored. Foster and colleagues (148) conducted a multi-center trial that examined the weight loss patterns of participants following a LCHP diet or a low-fat diet for one year. Dietary protocols were similar to those described by Samaha et al. (147) except that participants were instructed one time and were self-directed thereafter. Weight loss was significantly greater in participants following the LCHP diet versus the low-fat diet at three ($P = 0.001$) and six months ($P = 0.02$); however, at one year, there was no difference between groups. In fact, men and women following the LCHP diet regained more weight (although not significant) than individuals following the low-fat diet. These results suggest adherence to a LCHP diet for the long-term is difficult.

The evidence that LCHP diets are successful at inducing weight loss is compelling. While weight loss is known to reduce risk factors for many chronic diseases, the large proportion of kilocalories from total fat, saturated fat, and protein found in LCHP diets has raised concern because of their known risks for chronic diseases such as cardiovascular disease and osteoporosis. Before recommending LCHP diets to the general public, impact of these diets on risk factors for cardiovascular disease risk and bone health need to be carefully examined.

**Cardiovascular Risk Factors**

In an effort to dispel the claims of Dr. Atkins that his “nutritional approach” was safe and reduced risk for cardiovascular disease, a flurry of studies were recently conducted by independent researchers. Surprisingly, after six months, these studies showed that a LCHP diet did not negatively affect blood cholesterol or triglyceride levels (147, 148). In a one-year multi-center trial, Foster and colleagues (148) assessed the changes in body weight and lipid parameters in 63 obese adults (20 men and 43 women, mean age: 44.1 ± 8.2 y, mean BMI: 34 kg/m$^2$) randomized to follow a traditional high-carbohydrate weight loss diet ($n = 33$) or the LCHP diet ($n = 30$). The traditional diet group had greater decreases in total cholesterol (-5.4 ± 10.1 vs. 1.7 ± 15.0 % change; $P < 0.03$) and low-density lipoprotein (LDL) cholesterol
concentrations (-7.4 ± 16.6 vs. 5.4 ± 19.2 % change; \( P < 0.007 \)) compared to the LCHP diet group at 3 months. No differences were shown at 6 months or one year between the two diet groups. Triglyceride concentrations showed greater decreases in the LCHP diet group (-18.7 ± 25.7 % change) versus the traditional diet group (1.1 ± 34.6 % change; \( P < 0.01 \)) while high-density lipoprotein (HDL) concentrations were significantly higher in the LCHP group (9.6 ± 19.1, 14.7 ± 20.5, 11.0 ± 19.4 % change) vs. the traditional diet group (1.4 ± 16.1, 2.5 ± 12.0, 1.6 ± 11.1 % change) at 3 months, 6 months, and one year, respectively. Samaha and colleagues (147) compared the effects of the LCHP diet versus a traditional diet on risk factors for atherosclerosis in 79 adults (64 men and 15 women, mean age: 53 ± 9 y, mean BMI: 43 kg/m^2) at 6 months. The LCHP diet group had greater decreases (-20 ± 43%) in triglyceride concentrations compared to the traditional diet group (-4 ± 31%; \( P < 0.001 \)) after six months. Total cholesterol, HDL and LDL-cholesterol concentrations, however, did not differ between the two diet groups at six months. While these initial results showed positive results in the short-term, little is known about the long-term effect of this diet on risk factors for cardiovascular disease risk. Furthermore, these studies did not evaluate the impact of this diet on risk factors for other chronic diseases, such as osteoporosis.

**Bone Health**

Little is known about the effects of an “Atkins-type” diet on bone health. The LCHP diet promotes weight loss by inducing ketosis by severe dietary carbohydrate restriction and liberal ingestion of dietary fat and protein (145). The high acid load produced from excessive protein consumption is suggested to stimulate dissolution of calcium from bone as a compensatory response to the acid induced hypercalciuria. Furthermore, the hydrogen ions released as a result of ketosis may further reduce declining pH levels induced by the high-protein level of this diet, thus resulting in greater losses of skeletal calcium.

Another factor that may contribute to the acid load is the lack of fruits and vegetables in this diet. Fruits and vegetables produce basic compounds when metabolized. This excess base production is thought to buffer metabolic acid produced from protein-rich diets, thus reducing bone loss (151). Muhlbauer and associates (152) proposed that the protective effect of vegetables, onions and herbs was mediated from pharmacologically active compounds found in these plant foods rather than their base excess. A recent retrospective cross-sectional study was conducted in 62 healthy women (age range: 44-45 y) to determine whether childhood intake of fruits and vegetables and nutrients abundant in fruits and vegetables was associated with bone mineral status (78). Retrospective nutrient intake was assessed by FFQ and past
dietary habits during stages of the lifecycle where skeletal accretion is considered crucial: childhood (< 12 y) and early adulthood (20 – 30 y). After controlling for energy intake, Pearsons correlations showed a significant positive relationship between higher potassium and magnesium intakes and total bone mass ($P < 0.005$). Women who consumed large amounts of fruits and vegetables during childhood (1-4 times/day ≥5 days/week) had higher femoral neck BMD compared to those who consumed medium or low amounts (1-4 times/day <5 days/week). This study provides evidence that high intake of fruits and vegetables and the nutrients abundant in these foods promote bone mineralization. The “Atkins diet” recommends that individuals consume 2-3 cups of “salad vegetables” per day to limit carbohydrate intake to 20 grams per day during the induction phase. While not a complete list, examples of these salad vegetables include: alfalfa sprouts, celery, radishes, mushrooms, cucumber, peppers, and lettuce (145). The limited intake of fruit and vegetables, excessive intake of animal protein, and excess ketone production may cause a greater production of metabolic acid with this diet than is seen with high-protein, low-fat diets that provide adequate carbohydrate through grains, fruits, and vegetables. It is likely that these cumulative factors may be more detrimental to bone health than a high-protein diet alone.

There is a paucity of data that comprehensively examine the impact of short-term and long-term adherence to a LCHP diet on bone health. Reddy and colleagues (146) placed ten healthy adults (3 men, 7 women, mean age: 38.4 y, mean BMI: 29.4 kg/m$^2$) on an “Atkins-type” diet for six weeks. The participants were placed on 20 grams of carbohydrate per day for two weeks (induction phase) and then allowed to increase the amount of carbohydrate by 5 grams per week until the completion of the study (maintenance phase). Subjects were allowed to consume liberal amounts of protein and fat. Urinary acid and calcium excretion was significantly increased during the induction phase compared to the pre-study diet. These urinary acid and calcium concentrations decreased slightly at week six but remained significantly higher than the pre-study diet [urinary calcium excretion, 160 ± 75 mg/day (pre-study), 258 ± 88 mg/day; $P < 0.001$ (induction), 248 ± 106 mg/day; $P < 0.01$ (maintenance)]. Bone resorption markers were increased, but not significantly, during the induction phase or maintenance phase of the “Atkins-type diet” compared to the pre-study diet. Bone formation markers were significantly decreased during the induction phase of the diet compared to the usual diet [5.7 ± 2.2 ng/mL (pre-study) vs. 4.8 ± 1.6 ng/mL (induction); $P < 0.01$], but showed no difference compared to the usual diet by week six of the study. The lack of change in these biomarkers of bone turnover suggests that long-term indicators of bone status such as BMD or BMC would not be affected. Bone mineral density and BMC typically require a minimum of four to six months before significant
changes are seen. A positive argument for high carbohydrate diets are their inclusion of fruits and vegetables, foods that are believed to be protective for bone since they produce basic compounds when metabolized. Inclusion of a traditional diet group in this study would have provided evidence for its affects of bone metabolism compared to the “Atkins diet”.

Brehm and associates (149) randomly assigned 42 women to follow either a LCHP diet (n = 22, mean age: 44.2 ± 6.8 y, mean weight: 91.2 ± 8.4 kg) or low-fat traditional calorie-restricted, high carbohydrate diet (n = 22, mean age: 44.2 ± 6.8 y, mean weight: 92.3 ± 6.0 kg) for six months. The women on the LCHP diet were instructed to consume ≤ 20 g carbohydrate per day for two weeks and then add 5 g of carbohydrate per week up to 40 to 60 g carbohydrate per day or until urine ketones were no longer present. The women on the low-fat diet were instructed to follow a low-calorie diet (mean ~1,200-1,300 calories) with a macronutrient distribution of 55% carbohydrate, 15% protein, and 30% fat. Both groups were monitored for three months and were unmonitored for the additional three months. Total BMC was measured by DXA at baseline and at months three and six. Bone mineral content was not significantly different between or within the diet groups at either three or six months. The lack of response may be due to the site measured. Total body BMC contains both trabecular and cortical bone and of the two bone types, trabecular bone is more metabolically active and responds more rapidly to therapeutic changes such as diet. Therefore, sites containing primarily trabecular bone such as the femur and lumbar spine tend to respond more rapidly to dietary changes than cortical bone. Acute indices of bone turnover were not measured, so alterations that may have occurred during the induction phase and maintenance phase were not evaluated.

The Atkins Nutritional Approach™ encourages individuals to remain on the induction phase to continue rapid weight loss (145); therefore, obese individuals may continue the induction phase beyond past two weeks to reach their weight loss goals. The long-term impact on bone health when individuals maintain carbohydrate levels at <10% of total kilocalories is unknown. Clearly, more studies are warranted to evaluate the acute and long-term changes to bone that occur when following a LCHP diet, particularly in individuals who remain in the induction phase for more than two weeks. Furthermore, more comprehensive studies that evaluate all factors related to alterations in bone such, including calcium metabolism, biomarkers of bone turnover, and BMD are needed before definite conclusions can be drawn concerning the impact of a LCHP diet on bone status.
Summary

The re-emergence of the Atkins Nutritional Approach™ to weight loss has been fueled by several recent studies suggesting that a LCHP diet is effective in reducing body weight and lowering total cholesterol and triglycerides (147, 148). Yet concern regarding the level of protein included in the LCHP diet will stimulate bone loss and increase osteoporosis risk has been expressed. However, there is little scientific foundation to support this contention. In one recent study, an acute adverse change in bone metabolism was observed; however, at week 6 of the study, the body had adjusted to conserve bone (146). Metabolic adaptations that occur during the induction phase of this diet suggest that it could be detrimental to bone health; however, more research must be conducted to comprehensively evaluate the full effects in the short- and long-term.

The purpose of this study was to evaluate the short-term (induction phase) and long-term (maintenance phase) changes in bone metabolism and BMD in overweight pre-menopausal women following a LCHP (Atkins) diet compared to a high-carbohydrate, low-fat (traditional, endorsed by the American Heart Association) diet designed to induce weight loss. Specifically, the following objectives were met:

**Objective 1:** To assess the change in total body, lumbar spine, proximal femur, and forearm BMD following 12-weeks of dietary intervention.

**Objective 2:** To analyze change in biomarkers of bone turnover at weeks 1-4, 6 and 12 following 12-weeks of dietary intervention.

**Objective 3:** To analyze changes in calcium homeostasis at weeks 1-4, 6, and 12 following 12-weeks of dietary intervention.

**Objective 4:** To analyze changes in acid-base balance at weeks 1-4, 6, and 12 following 12-weeks of dietary intervention.

The *primary null hypotheses* related to the stated objectives include:

Significant differences in total body and site-specific BMD will not be observed within or between diet groups during a 12-week period of dieting.

Significant differences in bone metabolism (i.e., serum osteocalcin and urinary NTx) will not be observed within or between diet groups during a 12-week period of dieting.

Significant differences in calcium metabolism (i.e., serum and urinary calcium, urinary phosphorus) will not be observed within or between diet groups during a 12-week period of dieting.
Significant differences in acid-base balance (i.e., urinary pH, serum β-hydroxybutyrate, and urinary acetoacetate) will not be observed within or between diet groups during a 12-week period of dieting.
References Cited


growth factor-1 and leptin on bone mass in healthy postmenopausal women. Bone

65. Khosla S, Atkinson EJ, Riggs BL, Melton LJ, 3rd. Relationship between body

66. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium,
phosphorus, magnesium, vitamin D, and fluoride. Washington DC: National Academy

67. Metz JA, Anderson JJ, Gallagher PN, Jr. Intakes of calcium, phosphorus, and protein,
and physical-activity level are related to radial bone mass in young adult women. Am J
Clin Nutr 1993;58:537-42.

68. Mazess RB, Barden HS. Bone density in premenopausal women: effects of age, dietary

69. Uusi-Rasi K, Sievanen H, Pasanen M, Oja P, Vuori I. Association of physical activity and
calcium intake with the maintenance of bone mass in premenopausal women.

70. Kerstetter JE. Do dairy products improve bone density in adolescent girls? Nutr Rev

71. Rozen GS, Rennert G, Dodiuk-Gad RP, et al. Calcium supplementation provides an
extended window of opportunity for bone mass accretion after menarche. Am J Clin Nutr

72. Karkkainen M, Lamberg-Allardt C. An acute intake of phosphate increases parathyroid
hormone secretion and inhibits bone formation in young women. J Bone Miner Res

73. Grimm M, Muller A, Hein G, Funfstuck R, Jahreis G. High phosphorus intake only slightly
affects serum minerals, urinary pyridinium crosslinks and renal function in young women.

74. Brixen K, Nielsen HK, Charles P, Mosekilde L. Effects of a short course of oral
phosphate treatment on serum parathyroid hormone(1-84) and biochemical markers of

75. Calvo MS, Kumar R, Heath H. Persistently elevated parathyroid hormone secretion and
action in young women after four weeks of ingesting high phosphorus, low calcium diets.
J Clin Endocrinol Metab 1990;70:1334-40.


CHAPTER III

URINARY KETONES REFLECT SERUM KETONE CONCENTRATION BUT DO NOT RELATE TO WEIGHT LOSS IN OVERWEIGHT PRE-MENOPAUSAL WOMEN FOLLOWING A LOW-CARBOHYDRATE/HIGH-PROTEIN DIET

1Coleman MD, Nickols-Richardson SM. Submitted to the Journal of the American Dietetic Association, In Press.
ABSTRACT

This study examined the effect of a low-carbohydrate/high-protein diet on serum and urine ketone body production. Thirteen overweight pre-menopausal women aged 32-45 years consumed ≤20 grams of carbohydrate/day with liberal intakes of protein and fat for two weeks; thereafter, carbohydrate intake increased 5 grams/week for 10 weeks. Women were weighed and provided fasting urine and blood samples to detect urinary ketones and quantify serum ketone concentrations, respectively, at baseline, weeks 1-4, 6 and 12. Women lost 7.0±2.9% of initial body weight by week 12. Serum β-hydroxybutyrate production was highest at week 1 and declined weekly, with all values higher than baseline (P<0.05). Each week, serum β-hydroxybutyrate was correlated with the presence of urinary ketones (P<0.05), but no relationship was found between weekly weight change and serum ketone production. Urinary ketones are detected in pre-menopausal women complying with a low-carbohydrate/high-protein diet and are associated with serum ketone concentration. However, serum ketones do not reflect weight loss.

Keywords: High-protein diet; Ketones; Low-carbohydrate diet; Weight loss
INTRODUCTION

The Atkins New Diet Revolution™ has re-emerged as a weight loss plan that allows liberal intakes of dietary protein and fat while restricting carbohydrate consumption. The severe reduction in carbohydrate intake stimulates lipolysis and production of ketone bodies, including \(\beta\)-hydroxybutyrate (3HB), acetoacetate, and acetone. Of these three ketone bodies, 3HB and acetoacetate are the most abundant in serum and urine (1). The Atkins program recommends daily urinary ketone testing to monitor fat metabolism and to determine the optimal level of carbohydrate intake that will promote weight loss (2).

In previous studies, the presence of urinary ketones has been measured with nitroprusside tests (dipsticks or tablets) to ensure dietary compliance by study subjects (3, 4). Nitroprusside tests are only specific for acetoacetate, however, and are semi-quantitative and known to produce false-negative results when urine specimens are highly acidic (1). Due to these limitations, the nitroprusside test may not provide an accurate assessment of ketone production in individuals following a low-carbohydrate diet. Therefore, the association between urinary ketones and weight loss would be inherently inaccurate with use of a nitroprusside test.

Intravenous infusion of 3HB reduced food intake and increased energy expenditure in rats (5). Moreover, ketones have been shown to be elevated in humans consuming high fat diets (6); thus, investigators have theorized that greater ketone body production should equate to greater weight loss. Yet, a dearth of studies has measured serum ketone bodies which are primarily found as 3HB rather than acetoacetate. One study reported no correlation between weight loss and serum 3HB (5), questioning the role of serum ketones as a satiety factor. While urinary ketone testing may indicate compliance with a low-carbohydrate diet, such testing may not forecast weight loss success. The purpose of this study was, therefore, to examine the effect of a low-carbohydrate/high-protein diet on serum and urine ketone body production and to explore the relationship between serum ketone concentration and weight loss in overweight pre-menopausal women.

METHODS

Thirteen healthy, but overweight, pre-menopausal women (age = 39.2±3.7 years, height = 165.5±0.04 cm, weight = 84.8±12.8 kg) were recruited by advertisement to participate in this 12-week dietary intervention. Inclusion criteria included: body mass index (BMI) ≥25 or ≤43 kg/m²; eumenorrhea; <7 hours of planned physical activity per week; and stable weight for
12 months. Exclusion criteria included: smoking; pregnancy; lactation; metabolic/endocrine disorders; use of medications affecting acid-base balance; and consumption of >2 alcoholic beverages per day more than twice per week. All subjects acquired medical clearance from their primary care physicians and provided written informed consent prior to participation. This study was approved by the Institutional Review Board for Human Subjects Testing at Virginia Tech.

Women were educated on a low-carbohydrate/high-protein diet using the Atkins New Diet Revolution™ plan; each subject was given a copy of Dr. Atkins’ New Diet Revolution (2). Subjects were instructed to consume ≤20 grams of carbohydrate per day with liberal intakes of dietary protein and fat for the first two weeks of the study. Beginning in week 3, women increased their daily carbohydrate intakes by 5 grams each week while maintaining liberal consumption of protein and fat. The weekly increase in dietary carbohydrate stopped when a subject no longer produced urinary ketones, achieved her weight goal, or reached the end of 12 weeks. Each week throughout the study, a Registered Dietitian provided a comprehensive nutrition education and motivation session in a group setting. These sessions also facilitated discussion about the diet, assisted with problem-solving, and established a support network for dietary compliance among subjects.

For data collection, each subject arrived in the laboratory at the same time and on the same day each week. Baseline measures were conducted prior to the initiation of the dietary intervention with follow-up measures completed at the end of weeks 1, 2, 3, 4, 6, and 12 of dietary treatment, unless otherwise noted. Body height was measured with a calibrated wall-mounted, digital stadiometer (Heightronic, Measurement Concepts, North Bend, WA) to the nearest 0.1 cm at the baseline testing session only. Body weight was measured on a calibrated electronic scale (ScaleTronix, Wheaton, IL) to the nearest 0.1 kg.

Subjects were thoroughly trained in completion of four-day diet records. Verbal and written instructions for home completion were provided, and each subject recorded foods, beverages, and dietary supplements consumed, preparation methods, and portion sizes for three weekdays and one weekend day during weeks 1-4, 6, and 12. Diet records were analyzed with The Food Processor® (version 8.1 for Windows, 2003, ESHA Research, Salem, OR) by a Registered Dietitian, who added dietary supplements and low-carbohydrate/high-protein food items to the database as needed. Estimated mean daily energy and macronutrient intakes were examined.

Fasting urine and venous blood samples were collected between 0700-0930 hours. Serum was separated from whole blood by centrifugation at 1070 x g for 10 minutes at 25°C;
serum samples were stored at -80°C for later analyses. Urine ketone production was detected by the presence of acetoacetate using the Acetest Reagent Tablets (Bayer Corporation, Elkhart, IN). The Acetest is a semi-quantitative method in which a test tablet changes color when acetoacetate reacts with nitroprusside. Specifically, acetoacetate of ~20 mg/dL, ~30-40 mg/dL, and ~80-100 mg/dL is detected by a change in tablet color from tan to light purple, moderate purple, and dark purple, respectively. Serum 3HB concentration was quantified by use of a colorimetric bioassay (Stanbio Labs, Boerne, TX). All serum samples were analyzed in duplicate. Inter- and intra-assay CVs for our lab were 2.3% and 3.2%, respectively.

Descriptive statistics were computed for variables of interest and reported as mean ± standard deviation (SD). Repeated measures analysis of variance was used to detect significant changes in body weight and serum 3HB concentration within subjects across time. Pearson’s correlation coefficient was used to examine the relationship between serum 3HB concentration and both weight change and urinary ketones at each time point. Statistical analyses were performed using the Statistical Analysis System (SAS, version 8.2 for Windows, 2001, SAS Institute, Inc., Cary, NC), with statistical significance set at P<0.05.

RESULTS AND DISCUSSION

Mean body weight decreased from 84.8±12.8 kg to 80.0±12.1 kg and to 77.8±11.6 kg at week 6 and 12, respectively. By week 12, the average weight loss of ~7.0 kg was statistically significant (P < 0.05). Results from the current study are consistent with other published reports that have shown similar weight loss patterns in individuals consuming low-carbohydrate/high-protein diets (3, 7, 8). These studies suggest that these diets are effective in inducing short-term weight loss. Estimated average intakes of energy (kcal/day) and carbohydrate (grams/day) were significantly different from baseline (P<0.05) at all time points. Dietary fat intake (grams/day) was higher only at week 12 compared to baseline (P<0.05), while protein intake (grams/day) was higher at all time points (P<0.05) compared to baseline, except at week 12. Dietary intake analyses suggest that subjects were conscientious in complying with the dietary protocol, as average energy intake decreased from 2,025±645 kcal/day (49% CHO, 15% PRO, 36% FAT) at baseline to 1,290±340 kcal/day (10% CHO, 32% PRO, 57% FAT) at week 1 and to 1,535±345 kcal/day (15% CHO, 24% PRO, 61% FAT) at week 12.

Urinary ketone testing indicated that the majority of women (8 of 13, 61.5%) were in ketosis (acetoacetate = ≥80 mg/dL) during the initial week of the study. As dietary carbohydrate increased, urinary ketones were estimated at ≤30 mg/dL, denoting that ketone production was
reduced by week 3 of the study. Because interpretation of urine ketone production was limited by the semi-quantitative nature of nitroprusside tablets, serum 3HB concentration was also measured to quantitatively examine ketone body production. Mean serum 3HB concentration was greatest at week 1 compared to baseline (1.3±1.1 vs. 0.8±0.2 mM, P<0.002), and although average serum 3HB concentrations declined incrementally during the remaining weeks of the study, all concentrations were significantly higher at each time point compared to baseline (P<0.05; Figure). The normal value for serum total ketones (3HB + acetoacetate) is <0.5 mM, with hyperketonemia ranging from 1.0-3.0 mM and ketoacidosis at ≥3.0 mM (9).
Figure. Serum β-Hydroxybutyrate (3HB) concentrations for weeks 1-4, 6 and 12 in overweight pre-menopausal women following a low-carbohydrate/high-protein diet

Values (means ± standard deviation) significantly higher compared to baseline; *P< 0.05; **P< 0.001.
Following a mixed meal, the ratio of 3HB to acetoacetate is 1:1. This ratio increases to ≥3-10:1 during diabetic ketoacidosis (DKA) and ≥3-6:1 during prolonged starvation (9, 10). Serum acetoacetate was not measured in these women; however, it was likely that the 3HB:acetoacetate ratio was 1:1 due to the normal health status of these women. Thus, estimated total serum ketone concentration fell from 2.5 to 1.2 mM from week 1 to 4, respectively. Our estimates suggest that subjects had mildly elevated serum ketones, but below that observed with DKA or prolonged starvation. Consistent with urinary ketones, serum 3HB concentration declined in accordance with the addition of carbohydrate to the diet, confirming a metabolic response to dietary treatment.

Urinary ketones were positively correlated with serum 3HB concentrations at all time points ($P<0.05$; Table). During DKA, nitroprusside tests are inaccurate indicators of improved ketone status. As 3HB declines, acetoacetate increases; therefore, urine ketone tests suggest worsening DKA when, in fact, ketone status is improving (9). In contrast, the current study showed that the degree of elevation of serum 3HB with the low-carbohydrate/high-protein diet was not high enough to show false positive results with urinary ketone tests. Thus, urinary ketones may be used to accurately estimate the degree of ketosis in individuals following a low-carbohydrate/high-protein diet.
Table. Relationship between urinary ketone production and serum ketone concentration in overweight pre-menopausal women following a low-carbohydrate/high-protein diet

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<th>Week</th>
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<tr>
<td>1</td>
<td>0.72</td>
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<tr>
<td>2</td>
<td>0.79</td>
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<td>3</td>
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<td>4</td>
<td>0.87</td>
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<tr>
<td>6</td>
<td>0.75</td>
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<tr>
<td>12</td>
<td>0.83</td>
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It has been proposed that increased circulating ketones suppress appetite and increase satiety in individuals following a low-carbohydrate diet, thereby resulting in a restricted energy intake and weight loss (2). Krotkiewski (11) reported that plasma ketone production paralleled increased feelings of satiety and decreased hunger in obese women consuming a very-low-calorie, low-carbohydrate (30 grams/day) diet supplemented with medium chain triglycerides. However, the study was designed as a feeding trial such that women were not allowed to independently select foods; thus, the application to self-selection of food and impact on 3HB production, satiety, and weight loss is uncertain. Consistent with Brehm et al. (7), findings from the current study do not support a relationship between serum 3HB concentration and body weight change, suggesting that ketone production does not play a role in appetite suppression in pre-menopausal overweight women following a low-carbohydrate/high-protein diet. Rather, components of the high-protein, and subsequently high-fat, portion of the diet may trigger satiety and suppress hunger.

**CONCLUSIONS**

Urine and serum ketone production were positively correlated at all time points, confirming that urinary ketone testing may serve as an accurate and inexpensive method of estimating ketone production and dietary compliance in overweight pre-menopausal women following a low-carbohydrate/high-protein diet.

- Elevated serum 3HB concentrations were measured, although not to the degree found in DKA or starvation.

- Serum 3HB production was not positively associated with weight loss, suggesting that ketone production may not play a role in appetite suppression in women following a low-carbohydrate/high-protein diet.

- Advanced research must be conducted to further clarify the role of ketone body production on satiety and hunger and the relationships to energy intake and body weight loss.
REFERENCES


4. Volek JS, Sharman MJ, Gomez AL, Scheett TP, Kraemer WJ. An isoenergetic very low carbohydrate diet improves serum HDL cholesterol and triacylglycerol concentrations, the total cholesterol to HDL cholesterol ratio and postprandial lipemic responses compared with a low fat diet in normal weight, normolipidemic women. *J Nutr*. 2003;133:2756-2761.


CHAPTER IV

EFFECT OF A LOW-CARBOHYDRATE, HIGH-PROTEIN DIET ON BONE MINERAL DENSITY, BONE TURNOVER MARKERS, AND CALCIUM METABOLISM IN PRE-MENOPAUSAL WOMEN

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Abstract

Low-carbohydrate, high-protein diet (LCHP) plans are hypothesized to reduce bone mass, thereby increasing risk for osteoporosis, yet few studies have assessed the impact of this diet on bone health. This randomized trial was designed to determine the effects of a LCHP diet on body composition, bone mineral density (BMD), bone mineral content (BMC), biomarkers of bone turnover, and calcium metabolism in healthy pre-menopausal women. Thirty-two healthy overweight pre-menopausal women (aged 32 - 45 y) were randomized to 12 wks of either a LCHP diet or a HCLF diet with 25% of total calories as fat. Total body (TB), lumbar spine (LS), total proximal femur (TPF), including the femoral neck region (FN), and total forearm (TF) BMD and BMC were assessed at baseline and week 12. Serum osteocalcin was assessed at baseline, weeks 1, 2, 6, and 12, while indices of calcium homeostasis were measured at baseline, weeks 1-4, 6, and 12. Twenty-seven women completed the study. Women on both diets consumed comparable amounts of energy at all data collection points. Each group lost comparable amounts of weight, fat mass, lean mass and % fat by week 12 of the study. There was a significant main effect of diet on urinary calcium excretion with higher excretion in the LCHP group and lower excretion in the HCLF group ($P < 0.0001$) at all intervals of the study; however, there were no differences between the two groups. Serum osteocalcin showed significant main effects of diet and time with concentrations declining at week 2 and rising by week 12 of the study. The LCHP group had significantly lower TPF BMD ($P < 0.05$) compared to the HCLF group by week 12 of the study. Femoral neck BMC significantly decreased in the LCHP diet group ($P < 0.05$), whereas TF BMC significantly increased ($P < 0.05$) in the HCLF diet group by week 12 of the study. These results suggest that the increased urinary calcium excretion shown in the LCHP diet group may be from bone loss at the TPF and FN. In summary, a LCHP diet appears to stimulate bone loss, while a HCLF diet appears to attenuate against bone loss in healthy pre-menopausal women.
Introduction

Overweight and obesity are reaching epidemic proportions in America. In fact, current estimates indicate that in the past decade, the prevalence of overweight has risen from 55.9% to 64.5% of the adult population (1). Overweight and obesity increase risk for chronic disease (2) and all cause mortality (3)—creating an economic burden of 9.1% of the total US health expenditure in 2002 (4). Modest weight loss, as low as 10%, has been shown to reduce the risk for chronic disease (5); therefore, weight loss is recommended for overweight and obese individuals to reduce both morbidity and mortality risks.

A plethora of weight loss plans exist on the market today and among them is the Atkins Nutritional Approach™—a low-carbohydrate, high-protein (LCHP) diet plan (6). Concern has been raised that the disproportionate levels of fat and protein in this diet will increase risk for chronic conditions such as cardiovascular disease and osteoporosis. While several independent studies have shown that LCHP diets have had no adverse effects on lipid parameters (7-9), few studies have examined the impact of such a diet on bone health.

Osteoporosis, like obesity, is becoming a public health threat. Current estimates indicate that 44 million Americans either have osteoporosis or low bone mass, and of those, 68% are women (10). Women are more likely to follow weight loss plans (11), and should they choose to follow a LCHP diet, the acid load produced from the excessive protein and lack of alkaline forming foods in LCHP diets may stimulate bone mineral dissolution, thereby increasing the risk for osteoporosis. Diets high in dietary protein have been shown to acutely increase urinary calcium excretion (12-14) as a direct result of excess acid production from protein metabolism (15). However, the effect of high-protein intake on bone mass is unclear. Cross-sectional studies, using self-reported dietary protein intake data, have found that dietary protein was positively correlated (16, 17), negatively correlated (18), and had no effect (19) on bone mineral density (BMD) or fracture risk. Controlled trials have also reported discordant results. Kerstetter and colleagues (14) reported that pre-menopausal women consuming 2.0 g protein per kg bw protein for four days had significantly higher urinary calcium excretion, bone resorption, and no effect on bone formation compared to moderate protein (1.0 g protein per kg bw protein) and low-protein (0.7 g/kg bw) diets. However, in another study using a similar diet, urinary calcium excretion and markers of bone turnover were not affected at weeks 4 or 8, suggesting positive physiological adaptations in response to a high-protein diet in the long-term (20).
The aforementioned studies provide compelling evidence that high-protein diets may, in fact, be detrimental to bone health. However, these diets contained moderate amounts of carbohydrate. Little is known about the effects of an “Atkins-type” or LCHP diet on bone health. In addition to the acidosis incurred from the breakdown of dietary protein, systemic pH may be further reduced as a result of additional hydrogen ions that are released during ketone formation (21). Furthermore, the lack of alkaline forming fruits and vegetables in this diet may further reduce pH levels as the greater base production from fruits and vegetables is thought to buffer metabolic acid produced from protein-rich diets, thus attenuating bone loss (22). To date, only two studies have examined the impact of a LCHP diet similar to the “Atkins Nutritional Approach™” on bone mineral status and bone metabolism. In the first study, an acute adverse change in bone metabolism was observed; however, at week 6 of the study, the body had adjusted to conserve bone (23). In the second study, total body (TB) bone mineral content (BMC) did not differ within or between pre-menopausal women assigned to follow either a LCHP diet or a traditional low-fat, high-carbohydrate diet (9). Metabolic adaptations that occur during the induction phase of this diet suggest that it could be detrimental to bone health; however, more research must be conducted to comprehensively evaluate the full effects in the short- and long-term. Thus, the purpose of this study was to evaluate the short-term (induction phase) and long-term (maintenance phase) changes in bone metabolism and BMD in overweight pre-menopausal women following a LCHP (Atkins) diet compared to a high-carbohydrate, low-fat (HCLF, traditional, low-fat diet, endorsed by the American Heart Association) diet designed to induce weight loss.

Subjects and methods

Ninety-eight healthy pre-menopausal women, aged 32 - 45 years, were recruited by advertisement to participate in this 12-week dietary intervention study. Subjects were included if they had a body mass index (BMI, kg/m²) BMI ≥ 25 to ≤ 43, were eumenorrheic, participated in < 7 hours of planned physical activity per week, and had stable body weight within the past year. Exclusion criteria included smoking, pregnant or attempting to become pregnant, lactation, use of medications known to affect bone metabolism and/or acid-base balance (i.e., steroid or thyroid hormones, bisphosphonates, anticonvulsives, glucocorticoids); metabolic or health conditions known to affect bone (e.g., Crohn’s disease, diabetes, thyroid disorders, hirsutism); impaired renal function; metabolic acidosis or alkalosis; hysterectomy or ovariectomy without hormone replacement therapy; participation in a weight loss intervention within the past year; and consumption of > 2 alcoholic beverages per day more than twice per week.
All subjects attended detailed information sessions, acquired medical clearance from their primary care physicians and gave written informed consent prior to participation in the study. All procedures and the protocol used in this study were approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University.

Of these 98 screened applicants, 36 met the inclusion criteria for the study. Thirty-two subjects began the study and 27 completed the study. After the study began, 2 subjects withdrew within in the second week due to the inability to adhere to the dietary program, while 1 withdrew on day one due to the reoccurrence of a pre-existing medical complication. These 3 women agreed to serve as controls and to maintain their current dietary intake habits. The investigators removed two subjects from the study, one due to an undisclosed pre-existing medical complication that became known after the initiation of the study and the second due to unexpected pregnancy after week 6. Data from three other subjects were excluded due to total lack of dietary compliance as indicated by their 4-day food records. All subjects participated in < 5 hours of planned physical activity and maintained their pre-study physical activity throughout the intervention. Weekly physical activity was monitored using a 7-day physical activity recall.

**Diet and nutrition education**

Women were randomized to follow either the LCHP diet (n = 13) or the traditional, HCLF diet (n = 14). Briefly, the women were randomized into the dietary intervention groups using a random number generator. After randomization, a paired t-test was conducted to ensure that no significant differences in mean BMI existed between the two dietary groups prior to initiation of the diets. Participants in the LCHP dietary intervention group were asked to follow the guidelines of the Atkins diet plan (24). In this plan, participants were instructed to consume no more than 20 grams of carbohydrate per day with liberal amounts of protein and fat for the first 2 weeks of the study (“induction phase”). After two weeks, participants entered the “maintenance phase,” during which the amount of carbohydrate increased by 5 grams per week while protein and fat intake was unrestricted. Carbohydrate intake increased until participants stopped producing ketones or had met their goal weights. Urinary ketones were measured using Acetest Tabs (Bayer, Elkart, IN) to ensure dietary compliance during the study. Participants were instructed to consume a calcium supplement that provided no more than 250 mg calcium per tablet to meet the guidelines for calcium intake indicated in the Atkins New Diet Revolution™ (6). Energy intake was not restricted throughout the 12-week study period.
Subjects in the HCLF group were assigned to follow a calorie-restricted low-fat, high-carbohydrate diet designed to induce 0.5 to 1 kilogram(s) of weight loss per week. Energy intake levels ranged from 1,300-1,800 kcal/day, with a macronutrient composition of 60% carbohydrate, 25% fat, 15% protein and <300 mg of dietary cholesterol. Assigned energy intake for each woman was determined by using current body weight and the Harris Benedict equation (25).

Women attended weekly nutrition education sessions with peers assigned to the same diet. During these meetings, participants received extensive instruction about the dietary patterns of their respective diets by a Registered Dietitian. The women following the LCHP diet were given a copy of Dr. Atkins’ New Diet Revolution™ (6), which provided the guidelines for the dietary intervention, sample menus, and lists of acceptable and non-acceptable foods for the LCHP dietary intervention. The HCLF group received a dietary intervention plan based on the food exchange system to ensure that they met the prescribed caloric and macronutrient amounts in their daily diets. Furthermore, recipes, sample menus, and a packet containing written instruction for the exchange system were provided. These weekly nutrition education sessions were designed to facilitate participant discussion concerning the diet, to establish a support network, and to promote dietary compliance. Sessions were specific to each dietary intervention, although session topics were the same for both groups.

**Dietary intake analyses**

Each participant was extensively trained to complete a 4-day (3 weekdays + 1 weekend day) dietary food record for the baseline period prior to the intervention and for weeks 1, 2, 3, 4, 6, and 12 during the intervention. Four-day food record forms and handouts that contained examples of typical serving sizes of different foods and beverages were provided to each participant to reduce error in estimating portion size. Participants recorded all food, beverage, and food and nutritional supplement intake and brought labels of any new food items that were not listed in the food nutrient database. Food records were analyzed for energy, macro- and micro-nutrient content using Food Processor® dietary analysis software (ESHA Research, Version 8.1; 2003, Salem, OR). Total energy, carbohydrate, protein, fat, and micronutrient intake were averaged over the 4-days to ensure dietary compliance. Any unlisted food, beverage, or nutritional supplement was added to the database by the investigator to ensure accurate results.
Protocol

Both dietary groups followed the dietary intervention for 12 weeks. Body height was measured at baseline with a calibrated wall-mounted, digital stadiometer (Heightronic, Measurement Concepts, North Bend, WA) to the nearest 0.1 cm. Body weight was measured on a calibrated electronic scale (ScaleTronix, Wheaton, IL) to the nearest 0.1 kg. Participants were asked to remove their shoes and to wear similar clothing at each weigh-in. Each participant arrived at the lab at the same time and on the same day each week for each data collection session. Baseline body weight, fasting venous blood samples, and urine collections were taken prior to the initiation of the diets and during the dietary intervention at weeks 1, 2, 3, 4, 6, and 12. Samples were taken between 0700 – 0930 to reduce the effects of diurnal variation on all measurements. After obtaining serum the samples were separated from whole blood by centrifugation at 2500 x g for 10 minutes at 25°C. Serum samples were either analyzed immediately or aliquotted into cryovials and stored at -80°C for later analysis. These serum samples were analyzed for markers of lipid metabolism, blood urea nitrogen (BUN), markers of calcium metabolism and markers of bone formation. Second-void urine was collected to test for the presence of ketones, a marker of bone resorption, and to perform a pregnancy test. Twenty-four hour urine samples were collected to measure creatinine, pH, and calcium and phosphorus excretion. One 4-L urine collection container was provided to each participant prior to baseline data collection and at end of each data collection session during the study. Each participant was instructed to collect all voided urine 24-hours prior to their scheduled appointment, for the data collection sessions at baseline and weeks 1, 2, 3, 4, 6, and 12. Urine was prepared according to the instructions given for each urinary assay and was analyzed immediately or stored at -80°C for later analysis. Body composition (lean mass, fat mass, and percent body fat) and bone mineral measures were measured at baseline and weeks 6 and 12 by dual-energy X-ray absorptiometry (DXA; QDR 4500A, Hologic, Inc., Bedford, MA).

Blood lipid concentrations

Fasting serum samples were analyzed for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triacylglycerols (TG) at baseline, week 6, and week 12. Total cholesterol was analyzed using the Stanbio Enzymatic Cholesterol procedure (Stanbio Labs, Boerne, TX). All samples were prepared in duplicate. The inter- and intra-assay coefficient of variation (CV) was 2.4% and 2.6%, respectively. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were precipitated using the magnesium
chloride/dextran sulfate reagent. The HDL-C in the remaining supernatant was determined using the Stanbio Enzymatic Cholesterol procedure described above (Stanbio Labs, Boerne, TX). Unknowns were calculated using the 55 mg/dl HDL-C standard, and all samples were prepared in duplicate. Inter- and intra-assay CVs were 0.6% and 1.5%, respectively. Triglyceride concentration was analyzed using the Liquicolor triglyceride kit (Stanbio Labs, Boerne, TX). Duplicate samples were mixed with triglyceride reagent and measured with the spectrophotometer at 500 nm. The intra-assay CV was 2.1%. Low-density lipoprotein cholesterol was calculated using the Friedwald equation: LDL cholesterol = total cholesterol – HDL cholesterol – (TG/5) (26).

Renal status

Serum blood urea nitrogen concentration was analyzed immediately after blood collection using the BUN kit (Stanbio Labs, Boerne, TX). Duplicate samples were mixed with BUN color reagent and BUN acid reagent and read at 520 nm on the spectrophotometer. Inter- and intra-assay CVs were 0.4% and 1.7%, respectively.

Urinary pH

Urinary pH was measured at each data collection point from 24-hour urine samples to determine the acidity or alkalinity of the urine specimen using the Accumet 900 pH meter (Fisher Scientific, Suwannee, GA). The pH meter was calibrated prior to the reading of each urine sample.

Calcium and phosphorus metabolism

Serum and urinary calcium were analyzed at baseline and weeks 1, 2, 3, 4, 6, and 12 of the study using the Calcium Liquicolor kit (Stanbio Labs, Boerne, TX). Serum samples were mixed with the calcium color reagent and calcium base reagent and read at an absorbance of 550 nm by spectrophotometry. Serum calcium concentration was calculated using a standard of 10 mg/dl. Inter- and intra-assay CVs were 0.9% and 2.9%, respectively. Quantification of urinary calcium followed the methods previously described. All samples were analyzed in duplicate. Urinary calcium inter- and intra-assay CVs were 1.0% and 2.4%, respectively. Urinary phosphorus was analyzed in duplicate using the Phosphorus Liqui-UV kit (Stanbio Labs, Boerne, TX). Urine was adjusted to a pH 5.0 with HCl to ensure solution of all phosphates and
diluted 10-fold with water. Samples were mixed with Phosphorus Liqui-UV reagent and read at an absorbance of 340 nm with the spectrophotometer. Urinary phosphorus concentration was calculated using a standard of 10 mg/dl. Inter- and intra-assay CVs were 0.5% and 1.6%, respectively.

**Insulin-like growth factor-1**

Serum insulin-like growth factor-1 (IGF-1) concentrations were measured via radioimmunoassay (RIA) according to methods established by Weber et al. (27). Samples containing undetectable concentrations (n = 10 at baseline; n = 19 at week 1; n = 19 at week 2; n = 16 at week 3; n = 14 at week 4; n = 8 at week 6; and n = 6 at week 12) were assigned a value of 23.3 ng/mL. Intra- and inter-assay CVs were 15.6% and 4.8%, respectively.

**Bone mineral density**

Dual-energy X-ray absorptiometry was performed to measure BMD and BMC of the total body (TB), lumbar spine (L₁-L₄) (LS), non-dominant total proximal femur (TPF), including the femoral neck (FN) region, and non-dominant total forearm (TF). Standard protocols for TB, LS, TPF, and TF were used to measure BMD and BMC of these body sites. Total body (version 8.25), LS (standard protocol), TPF (version 8.23), and TF (version 8.25) scans were analyzed with Hologic software, using the compare function for reference to baseline scans. All DXA scans were conducted by one investigator (a Radiologic Technologist - Limited in the Commonwealth of Virginia) in the Bone Metabolism, Osteoporosis, and Nutrition Evaluation (BONE) laboratory. Prior to each scan, all women provided a 2nd void urine sample, which was used to conduct a pregnancy test to ensure no woman was pregnant during the scan. Briefly, a drop of urine was placed on the QuPID (Stanbio Labs, Boerne, TX) pregnancy test strip. After a few seconds, either one or two pink lines would appear. One pink line indicated a negative pregnancy test, while two pink lines indicated a positive pregnancy test. All pregnancy tests conducted during the study were negative. The same investigator analyzed all scans to eliminate inter-tester variation. Quality control for BMD was ensured by daily scans of an anthropomorphic phantom lumbar spine prior to any participant testing. The CV for phantom spine BMD scanning is 0.36%. Test-retest reliabilities for TB, LS, TPF, and TF are 0.73%, < 2.0%, 0.69%, and 1.09%, respectively. Lean mass (LM), fat mass (FM), and percent fat (% fat) were measured from the TB scans. Reliability testing for soft tissue composition in the BONE laboratory has produced CVs of 1.07%, 1.75%, and 1.79% for LM, FM, and % fat, respectively.
Quality control for soft tissue mass was ensured by weekly scans of an external soft tissue bar comprised of aluminum and lucite calibrated against stearic acid and water (Hologic, Bedford, MA).

**Biochemical markers of bone turnover**

Bone mineral density measurements are useful to provide information about the mineral content of bone; however, the time frame of this study (12 weeks) may not have allowed changes to be detected. Therefore, biomarkers of bone formation and resorption, factors that assess acute changes in bone turnover, were measured. Serum osteocalcin, a biochemical marker of bone formation, was measured by RIA (Human Osteocalcin RIA I125, Biomedical Technologies, Stoughton, MA) and analyzed in duplicate. The intra-assay CV for our lab was 11.6%. Crosslinked N-telopeptide of type I collagen (NTx), a biochemical marker of bone resorption, was measured in duplicate by enzyme-linked immunosorbent assay (ELISA, Wampole Labs, Seattle, WA) from second-void urine samples. The intra- and inter-assay CVs for NTx assays completed in our laboratory were 6.5% and 2.9%, respectively. Urinary NTx concentration may be affected by renal function, and NTx must be normalized to creatinine; thus, urinary creatinine was measured by quantitative spectrophotometry with the urine collected from the 24-hour urine sample (Stanbio, Boerne, TX). Samples were analyzed in duplicate. The intra- and inter-assay CVs were 1.3% and 1.5%, respectively.

**Statistical Analysis**

Descriptive statistics were performed to describe general characteristics of the total sample and each group of women. A general linear model analysis of covariance (PROC GLM ANCOVA), using baseline value as the covariates, for repeated measures was used to determine significant effects of diet, time, and Diet x Time interactions for the continuous variables: weight (kg), BMI (kg/m²), % fat, FM (kg), LM (kg), and TB, TPF, LS, and TF BMD (g/cm²) and BMC (g). A mixed model ANCOVA for repeated measures was used to determine significant effects of diet, time, and Diet x Time interactions for the remaining continuous variables: serum osteocalcin (ng/ml), urinary NTx (nM BCE/mM Cr), serum calcium (mg/dl), urinary calcium (mg/dl), urinary phosphorus (mg/dl), IGF-1 (ng/ml), and all dietary intake variables. Tukey’s HSD test was used to make *post hoc* comparisons within groups if a time effect was demonstrated. All statistical analyses were conducted using SAS® software v.8.2 (for PC, SAS Institute, Cary, NC). Statistical significant was set at a *P* < 0.05.
Results

A total of 27 women completed the study, were compliant with diets, and attended all data collection sessions. Fourteen women followed the LCHP diet until week 4, while 13 women in the LCHP diet group, and 12 women in the HCLF diet group, and the three women in the control group completed the study. Data collection for the control group occurred at baseline, week 6 and week 12. Baseline characteristics of women assigned to each group are found in Table 1. There were no significant differences in baseline age, height, weight or BMI between the three groups. Weekly planned exercise remained at < 5 hours per week for all participants.
Table 1. Subjects characteristics at baseline screening

<table>
<thead>
<tr>
<th></th>
<th>LCHP diet (n = 14)</th>
<th>HCLF diet (n = 12)</th>
<th>Control (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.2 ± 3.7</td>
<td>40.1 ± 3.2</td>
<td>40.0 ± 4.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.8 ± 12.8</td>
<td>77.3 ± 16.6</td>
<td>79.4 ± 21.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.5 ± 0.05</td>
<td>162.0 ± 0.07</td>
<td>161.0 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.8 ± 5.4</td>
<td>29.5 ± 5.4</td>
<td>30.4 ± 6.1</td>
</tr>
</tbody>
</table>

¹Mean ± SD. LCHP, low-carbohydrate, high-protein diet; HCLF, high-carbohydrate, low-fat diet. There were no significant differences between the LCHP, HCLF, and control groups.
Body composition

Each diet was equally effective in promoting weight loss as each group lost ~6.5 kg at week 12 of the study (Figure 1). There was a significant time effect for weight loss within both diet groups ($P < 0.0001$) at week 12; however, no Diet x Time interaction or main effect of diet was shown.

Baseline BMI was not different between the women following the LCHP diet (31.1 ± 4.9), the HCLF diet (29.5 ± 5.4), or the controls (30.4 ± 6.0) at the initiation of the diet intervention. Overall, there was a significant main effect of diet on BMI as both diet groups had a lowering of BMI compared to the control group (28.3 ± 3.9, LCHP, 27.2 ± 4.9, HCLF vs. 30.2 ± 6.0, control; $P < 0.003$). Body mass index was significantly reduced over time in both groups ($P = 0.01$) but no Diet x Time interaction was found (Table 2).

Lean mass (kg), FM (kg), % fat, and the ratio of FM loss to LM loss were compared between the LCHP and HCLF diet groups at baseline, week 6, and week 12 of the study. Lean and fat mass in all groups exhibited no significant changes throughout the study; however, there was a significant main effect of diet and a Diet x Time interaction on % fat loss in the diet groups compared to the control. Percent fat was significantly lower in both the LCHP group (37.9 ± 5.6%) and HCLF group (33.4 ± 5.7%) compared to controls (39.3 ± 3.9%) at week 12 ($P < 0.05$). There was no time effect shown with these variables. Although not significant, the proportion of FM:LM loss was greater in the LCHP diet group compared to the HCLF diet group at week 6 (3.7:1 vs. 2.6:1). However, the proportion of FM:LM loss was not different at the end of 12-weeks in both diet groups (2.3:1 vs. 2.9:1). There were no significant diet, time or Diet x Time differences in FM:LM loss in women following the LCHP or HCLF diets or in women assigned to the control group.
Figure 1. Weekly changes in body mass during the study for subjects following either a low-carbohydrate, high-protein (LCHP, n = 13) or high-carbohydrate (HCLF, n = 12) low-fat, energy restricted diet for 12 wk. Values are means ± SD. * Significantly different from week 0 for both diet groups ($P < 0.0001$).
Table 2. Body composition data for week 0, week 6, and week 12 of the study.\\n
|                     | LCHP² (n = 13) | HCLF² (n = 12) | Control (n = 3) |
|---------------------|---------------|----------------|----------------
| **Body Mass Index (BMI)**³,⁴,⁵ |
| Week 0              | 31.1 ± 4.9    | 29.5 ± 5.4     | 30.4 ± 6.0     |
| Week 6              | 29.3 ± 4.6    | 27.9 ± 5.1     | 30.2 ± 6.1     |
| Week 12             | 28.3 ± 3.9    | 27.2 ± 4.9     | 30.2 ± 6.0     |
| Net Change          | -2.8 ± 1.8    | -2.3 ± 0.9     | -0.2 ± 0.8     |
| **Lean mass (kg)**  |
| Week 0              | 47.9 ± 5.4    | 46.1 ± 7.1     | 45.4 ± 8.1     |
| Week 6              | 46.4 ± 5.1    | 45.1 ± 6.4     | 45.0 ± 9.1     |
| Week 12             | 45.2 ± 5.4    | 44.9 ± 6.6     | 45.9 ± 9.5     |
| Net change          | -2.8 ± 3.2    | -1.2 ± 1.3     | +0.5 ± 2.6     |
| **Fat mass (kg)**   |
| Week 0              | 35.3 ± 8.2    | 29.5 ± 1.0     | 32.2 ± 1.3     |
| Week 6              | 32.0 ± 8.0    | 26.7 ± 9.7     | 31.5 ± 1.1     |
| Week 12             | 31.0 ± 9.1    | 24.6 ± 8.8     | 31.2 ± 1.1     |
| Net Change          | -4.3 ± 3.9    | -4.9 ± 2.1     | -1.0 ± 1.3     |
| **Percent Fat Mass (%)**³,⁴,⁵ |
| Week 0              | 40.8 ± 4.7    | 36.9 ± 5.9     | 39.3 ± 5.2     |
| Week 6              | 38.9 ± 5.3    | 35.1 ± 6.3     | 39.2 ± 3.9     |
| Week 12             | 37.9 ± 5.6    | 33.4 ± 5.7     | 39.3 ± 3.9     |
| Net Change          | -2.9 ± 1.9    | -3.5 ± 1.2     | 0.0 ± 0.0      |
| **FM:LM loss**¹     |
| Week 6              | 3.7 ± 3.9     | 2.6 ± 4.3      | -0.5 ± 1.2     |
| Week 12             | 2.3 ± 3.5     | 2.9 ± 3.6      | 0.2 ± 0.8      |
| Net Change          | -1.4 ± 3.4    | +0.35 ± 3.4    | +0.7 ± 0.7     |

¹ Means ± SD. Fat Mass: FM, Lean Mass: LM.
² Low-carbohydrate, high-protein diet: LCHP, High-carbohydrate, low-protein diet: HCLF.
³ BMI calculated as kg/m²
⁴ Significant main effect of diet, P < 0.05.
⁵ Significant effect of time, P < 0.05.
**Nutrient intake**

Estimated energy, macro- and micro-nutrient intake was determined from 4-day diet records collected at baseline, weeks 1, 2, 4, 6, and 12 of the study to ensure participants adhered to their prescribed dietary protocols.

Subjects randomized to the LCHP diet (n = 13) and the HCLF diet (n = 12) consumed similar intakes of total energy (2024.9 ± 645.2 vs. 2340.0 ± 1235.7 kcal, respectively), carbohydrate (250.8 ± 97.5 vs. 289.9 ± 131.6 g, respectively), protein (76.0 ± 21.7 vs. 90.4 ± 19.8 g respectively), and total fat (83.4 ± 25.8 vs. 97.0 ± 77.9 g, respectively) at the beginning of the diet intervention (Table 3). Mean energy intake did not differ between dietary treatments at any interval of the study. As prescribed, carbohydrate intake was lower (9-14% vs. 49-60% of total kcal, respectively), while protein (24-32% vs. 18-19% of total kcal, respectively) and total fat (56-61% vs. 23-25% of total kcal, respectively) were higher in women following the LCHP diet compared to women following the HCLF diet (effect of diet *P* < 0.0001). Results from food records indicate that the women complied with their assigned diets. Along with the increase in total fat, percent saturated fatty acid (SFA) increased and was higher in women following the LCHP diet at all intervals (22-26%) compared to the HCLF diet (7-8%; *P* < 0.05). In addition, dietary cholesterol was significantly higher in the women following the LCHP diet (>300 mg/d) compared to women complying with the HCLF diet (<200 mg/d; *P* < 0.0001) at each food record collection period. There were no significant diet or time effects of polyunsaturated (PUFA) or monounsaturated fatty acid (MUFA) intakes between the LCHP and HCLF diets; however, there was a significant Diet x Time interaction for dietary MUFA intake (*P* = 0.02). Post hoc comparisons showed a greater proportion of energy from MUFA was consumed by the women following the LCHP diet (18.6%) compared to women on the HCLF diet (6.5%; *P* = 0.03) at week 12 only.

The emphasis on fruits and vegetables in the HCLF diet and lack of fruits and vegetables in the LCHP diet was evident as estimated daily dietary fiber, vitamin C, vitamin A, potassium, and magnesium intakes (Table 3) were significantly higher in the HCLF group compared to the LCHP group at all intervals (*P* < 0.05). The emphasis in meat products in the LCHP diet was evident as there was a significant main effect of diet for estimated daily zinc intake (Table 3) with LCHP showing greater values compared to the HCLF diet group (*P* < 0.05). A significant main effect for dietary iron intake was shown between the dietary groups with the HCLF group consuming greater amounts of iron compared to the LCHP group. There were no differences in calcium and sodium intakes at any interval between the two dietary...
interventions. Actual values for total energy and reported nutrients for baseline, and weeks 1, 2, 4, 6, and 12 are reported in Table 3.
Table 3. Nutrient composition of the study diets based on 4-day dietary intake record by women on the LCHP diet and HCLF diet at baseline, weeks 1, 2, 4, 6 and 12.  

<table>
<thead>
<tr>
<th></th>
<th>LCHP diet (n = 12)</th>
<th>HCLF diet (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Energy (kcal/d)³,⁴</td>
<td>2024.9 ± 645.2</td>
<td>1288.1 ± 340.8</td>
</tr>
<tr>
<td>Carbohydrate (g/d)³,⁴</td>
<td>250.8 ± 97.5</td>
<td>28.4 ± 10.0</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>49.0 ± 5.9</td>
<td>9.2 ± 3.2</td>
</tr>
<tr>
<td>Protein (g/d)³,⁴</td>
<td>76.0 ± 21.7</td>
<td>101.1 ± 29.4⁷</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>15.3 ± 3.0</td>
<td>71.6 ± 13.9</td>
</tr>
<tr>
<td>Total fat (g/d)³,⁴</td>
<td>83.4 ± 25.8</td>
<td>79.5 ± 18.5⁶</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>37.2 ± 2.4</td>
<td>56.0 ± 6.8</td>
</tr>
<tr>
<td>SFA (% of energy)³,⁴</td>
<td>13.0 ± 1.7</td>
<td>22.2 ± 4.7⁵</td>
</tr>
<tr>
<td>MUFA (% of energy)</td>
<td>9.1 ± 3.6</td>
<td>16.7 ± 6.8</td>
</tr>
<tr>
<td>PUFA (% of energy)</td>
<td>3.2 ± 1.5</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>Dietary Fiber (g/d)³,⁴</td>
<td>16.1 ± 5.9</td>
<td>5.6 ± 3.3⁶</td>
</tr>
<tr>
<td>Cholesterol (mg/d)³,⁴</td>
<td>101.2 ± 74.5</td>
<td>554.6 ± 173.6⁷</td>
</tr>
</tbody>
</table>

³ Mean ± SD, low-carbohydrate, high-protein diet: LCHP, high-carbohydrate, low-fat diet: HCLF, SFA: saturated fatty acid, monounsaturated fatty acid: MUFA, polyunsaturated fatty acid: PUFA.  
⁴ 4-day diet records were missing from one subject in the LCHP group reducing the total by one.  
⁵ Significant main effect of diet, P < 0.05.  
⁶ Significant effect of time, P < 0.05.  
⁷ Significant Diet x Time interaction, P < 0.05, ⁸ P < 0.01; ⁹ P < 0.0001.
### Table 3 continued. Nutrient composition of the study diets based on 4-day dietary intake record by women on the LCHP diet and HCLF diet at baseline, weeks 1, 2, 4, 6 and 12.

<table>
<thead>
<tr>
<th></th>
<th>LCHP diet (n = 12)</th>
<th>HCLF diet (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Energy (kcal/d)4</td>
<td>1457.4 ± 317.4</td>
<td>1420.8 ± 374.0</td>
</tr>
<tr>
<td>Carbohydrate (g/d)3,4</td>
<td>39.1 ± 16.0</td>
<td>43.4 ± 21.8</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>11.2 ± 5.6</td>
<td>12.5 ± 5.7</td>
</tr>
<tr>
<td>Protein (g/d)3,4</td>
<td>106.2 ± 28.57</td>
<td>94.2 ± 29.07</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>29.2 ± 4.8</td>
<td>26.5 ± 5.4</td>
</tr>
<tr>
<td>Total fat (g/d)3,4</td>
<td>98.6 ± 24.76</td>
<td>97.1 ± 26.16</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>60.8 ± 3.4</td>
<td>61.6 ± 3.5</td>
</tr>
<tr>
<td>SFA (% of energy)3,4</td>
<td>24.3 ± 3.85</td>
<td>22.4 ± 3.45</td>
</tr>
<tr>
<td>MUFA (% of energy)</td>
<td>17.6 ± 3.9</td>
<td>18.1 ± 18.6</td>
</tr>
<tr>
<td>PUFA (% of energy)</td>
<td>6.4 ± 2.4</td>
<td>6.7 ± 2.1</td>
</tr>
<tr>
<td>Dietary Fiber (g/d)3,4</td>
<td>7.3 ± 3.47</td>
<td>9.6 ± 3.67</td>
</tr>
<tr>
<td>Cholesterol (mg/d)3,4</td>
<td>670.1 ± 214.67</td>
<td>507.0 ± 210.47</td>
</tr>
</tbody>
</table>

1Mean ± SD, low-carbohydrate, high-protein diet: LCHP, high-carbohydrate, low-fat diet: HCLF, SFA: saturated fatty acid, monounsaturated fatty acid: MUFA, polyunsaturated fatty acid: PUFA.
24-day diet records were missing from one subject in the LCHP group reducing the total by one.
3 Significant main effect of the diet, \( P < 0.05 \).
4 Significant effect of time, \( P < 0.05 \).
5, 6, 7 Significant Diet x Time interaction, \( P < 0.05, 6 P < 0.01; 7 P < 0.0001 \).
Table 3 continued. Nutrient composition of the study diets based on 4-day dietary intake record by women on the LCHP diet and HCLF diet at baseline, weeks 1, 2, 4, 6 and 12.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>LCHP diet (n = 12)</th>
<th>HCLF diet (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>799.8 ± 303.3</td>
<td>618.3 ± 319.6</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>889.0 ± 340.8</td>
<td>1064.7 ± 380.8</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>3419.7 ± 933.2</td>
<td>2552.9 ± 898.1</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>1587.1 ± 3707.9</td>
<td>1758.3 ± 572.2</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>799.3 ± 409.0</td>
<td>793.3 ± 386.5</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>61.4 ± 35.8</td>
<td>59.8 ± 46.7</td>
</tr>
<tr>
<td>Vitamin D (µg/d)</td>
<td>1.98 ± 1.8</td>
<td>3.39 ± 1.8</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>194.5 ± 59.7</td>
<td>144.6 ± 39.3</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>15.3 ± 5.6</td>
<td>9.2 ± 3.1</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>9.8 ± 5.0</td>
<td>10.2 ± 3.9</td>
</tr>
</tbody>
</table>

1 Mean ± SD, low-carbohydrate, high-protein diet: LCHP, high-carbohydrate, low-fat diet: HCLF.
24-day diet records were missing from one subject in the LCHP group reducing the total by one.
3 Significant main effect of the diet, \( P < 0.05 \).
4 Significant effect of time, \( P < 0.05 \).
5, 6, 7 Significant Diet x Time interaction, \( 5P < 0.05, 6P < 0.01; 7P < 0.0001 \).
Table 3 continued. Nutrient composition of the study diets based on 4-day dietary intake record by women on the LCHP diet and HCLF diet at baseline, weeks 1, 2, 4, 6 and 12.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>LCHP diet (n = 12)</th>
<th>HCLF diet (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>706.5 ± 266.2</td>
<td>551.4 ± 215.0</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1157.9 ± 359.2</td>
<td>069.3 ± 344.1</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>2523.14 ± 1122.6</td>
<td>2188.3 ± 561.7</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>1814.41 ± 544.5</td>
<td>1818.9 ± 518.9</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>828.4 ± 433.8</td>
<td>1763.7 ± 465.8</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>60.1 ± 44.1</td>
<td>118.8 ± 35.5</td>
</tr>
<tr>
<td>Vitamin D (µg/d)</td>
<td>4.34 ± 3.5</td>
<td>2.89 ± 2.0</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>156.2 ± 55.0</td>
<td>278.6 ± 80.4</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>8.7 ± 1.8</td>
<td>27.0 ± 3.1</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>10.9 ± 3.7</td>
<td>25.0 ± 4.3</td>
</tr>
</tbody>
</table>

1 Mean ± SD, low-carbohydrate, high-protein diet: LCHP, high-carbohydrate, low-fat diet:
2 4-day diet records were missing from one subject in the LCHP group reducing the total by one.
3 Significant main effect of the diet, P < 0.05.
4 Significant effect of time, P < 0.05.
5, 6, 7 Significant Diet x Time interaction, 5 P < 0.05, 6 P < 0.01; 7 P < 0.0001.
8 Women in both diet groups took a standard multivitamin every day after week 4. Micronutrient values at weeks 6 and 12 reflect the amount consumed from the multivitamin supplement in addition to dietary intake.
Lipid analysis

A lipid panel for TC, HDL-C, LDL-C, and TG was analyzed to compare the effect of a LCHP diet and HCLF diet on risk factors for cardiovascular disease (Table 4). Diets were ineffective in lowering TC concentration during this 12-week period, although the HCLF diet lowered TC concentration slightly more than the LCHP diet (-8.1 ± 15.8% vs. -6.4 ± 14.8%), respectively. Total cholesterol concentration in the control group increased by 20.8% at the completion of the 12-week study.

A significant main effect of diet was observed ($P = 0.04$) between the control and two dietary groups. Fasting serum TG decreased by 24.4% and 24.5% in the LCHP and HCLF diet groups, respectively, and increased in the control group by 27% at week 12. However, there was no time effect or Diet x Time interaction observed in TG concentration in these groups.

Fasting HDL-C concentration showed a significant main effect of diet ($P < 0.05$), with decreases observed in the HCLF (-21.5%) and LCHP diet (-16.8%) groups. Low-density lipoprotein concentration showed no time effect or Diet by Group interaction at the end of the 12-week study (Table 4). A significant main effect of diet was observed for both the LDL-C:HDL-C and TC:HDL-C ratio with increases shown in the control compared to either diet intervention ($P < 0.05$).
Table 4. Fasting lipid concentrations in the two diet groups and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LCHP Diet&lt;sup&gt;2&lt;/sup&gt; (n=13)</th>
<th>HCLF Diet&lt;sup&gt;2&lt;/sup&gt; (n=12)</th>
<th>Control (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>202.6 ± 29.7</td>
<td>189.9 ± 33.8</td>
<td>179.0 ± 41.5</td>
</tr>
<tr>
<td>Week 6</td>
<td>221.9 ± 38.4</td>
<td>190.4 ± 38.8</td>
<td>214.7 ± 45.0</td>
</tr>
<tr>
<td>Week 12</td>
<td>183.1 ± 24.4</td>
<td>177.4 ± 36.0</td>
<td>214.7 ± 45.0</td>
</tr>
<tr>
<td>% Change</td>
<td>-8.1 ± 15.8</td>
<td>-6.4 ± 14.8</td>
<td>20.8 ± 10.6</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)&lt;sup&gt;3&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>97.3 ± 32.9</td>
<td>102.3 ± 40.6</td>
<td>75.2 ± 36.9</td>
</tr>
<tr>
<td>Week 6</td>
<td>75.2 ± 40.3</td>
<td>67.1 ± 30.5</td>
<td>92.8 ± 29.9</td>
</tr>
<tr>
<td>Week 12</td>
<td>69.2 ± 20.2</td>
<td>73.7 ± 22.4</td>
<td>87.0 ± 14.5</td>
</tr>
<tr>
<td>% Change</td>
<td>-24.4 ± 23.6</td>
<td>-24.5 ± 19.0</td>
<td>27.0 ± 34.3</td>
</tr>
<tr>
<td><strong>HDL-Cholesterol (mg/dl)&lt;sup&gt;3&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>56.3 ± 15.7</td>
<td>56.9 ± 12.7</td>
<td>57.7 ± 16.8</td>
</tr>
<tr>
<td>Week 6</td>
<td>41.0 ± 9.0</td>
<td>40.2 ± 9.2</td>
<td>47.7 ± 14.1</td>
</tr>
<tr>
<td>Week 12</td>
<td>45.6 ± 9.6</td>
<td>44.3 ± 9.9</td>
<td>52.9 ± 12.6</td>
</tr>
<tr>
<td>% Change</td>
<td>-16.8 ± 12.4</td>
<td>-21.5 ± 10.7</td>
<td>-7.4 ± 4.8</td>
</tr>
<tr>
<td><strong>LDL-Cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>126.9 ± 23.3</td>
<td>118.4 ± 31.5</td>
<td>106.2 ± 27.2</td>
</tr>
<tr>
<td>Week 6</td>
<td>165.9 ± 39.4</td>
<td>140.3 ± 37.7</td>
<td>137.2 ± 30.8</td>
</tr>
<tr>
<td>Week 12</td>
<td>123.2 ± 22.9</td>
<td>117.1 ± 31.5</td>
<td>144.6 ± 32.3</td>
</tr>
<tr>
<td>% Change</td>
<td>-0.53 ± 20.8</td>
<td>-0.71 ± 4.8</td>
<td>37.8 ± 15.4</td>
</tr>
<tr>
<td><strong>TC:HDL-C ratio&lt;sup&gt;4&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>3.8 ± 1.0</td>
<td>3.5 ± 0.7</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>Week 6</td>
<td>5.7 ± 1.7</td>
<td>5.0 ± 1.3</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Week 12</td>
<td>4.1 ± 0.8</td>
<td>4.1 ± 0.7</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>% Change</td>
<td>11.1 ± 0.1</td>
<td>17.3 ± 0.2</td>
<td>40.3 ± 0.5</td>
</tr>
<tr>
<td><strong>LDL-C:HDL-C ratio&lt;sup&gt;4&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>2.4 ± 0.8</td>
<td>2.1 ± 0.6</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Week 6</td>
<td>4.3 ± 1.6</td>
<td>3.6 ± 1.1</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.8 ± 0.8</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>% Change</td>
<td>20.3 ± 21.5</td>
<td>29.8 ± 27.4</td>
<td>50.1 ± 21.4</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are expressed as means ± SD.
<sup>2</sup> Low-carbohydrate, high-protein diet: LCHP, High-carbohydrate, low-fat diet: HCLF.
<sup>3</sup> Significant main effect of the diet, *P* < 0.05.
<sup>4</sup> Significant effect of time, *P* < 0.05.
Biomarkers of protein metabolism and urinary pH

Blood urea nitrogen was analyzed to monitor kidney function during the diet intervention. At baseline, individual BUN concentrations were within normal limits (5-25 mg/dL), indicating that all participants had normal kidney function. Significant main effects of diet and time were observed for BUN, but there was no Diet x Time interaction within the three groups (Table 5).

Urinary creatinine, a measure of muscle metabolism, is typically measured to assess kidney function; however, it can also be used to confirm changes in dietary protein intake. Similar to BUN, baseline values were within normal limits (0.6-1.6 g/24 hr) for both dietary groups, confirming normal kidney function in these women prior to the study. At week 12 of the study, there was a significant main effect of diet ($P = 0.03$). Creatinine decreased in both the LCHP ($1.08 \pm 0.47$ to $0.98 \pm 0.26$ g/24h) and HCLF diet ($1.15 \pm 0.96$ to $1.02 \pm 0.37$ g/24h) groups while creatinine concentration was increased in the control group ($0.62 \pm 0.88$ to $0.98 \pm 0.19$ g/24h). No Diet x Time interaction existed (Table 5).

Urinary pH was measured to analyze the effects of the diet intervention on urinary acid excretion. As expected, urinary pH was significantly lower in the LCHP (range: $5.6 \pm 0.21$ to $5.8 \pm 0.44$) diet group compared to the HCLF group (range: $6.3 \pm 0.47$ to $6.1 \pm 0.21$; $P < 0.0001$) at all time intervals (Table 5). A Diet x Time interaction was not found. Urinary ketone tablets indicated that ketones were present in the majority of women following the LCHP diet (see Chapter 3), while urinary ketones were not present in women in the HCLF diet group at any time interval.
Table 5. Biochemical markers of protein metabolism and urinary pH\(^1\).  

<table>
<thead>
<tr>
<th></th>
<th>LCHP Diet(^2) (n=13)</th>
<th>HCLF Diet(^2) (n=12)</th>
<th>Control (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum BUN (mg/dl)(^4,5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>15.5 ± 3.7</td>
<td>13.8 ± 2.2</td>
<td>16.5 ± 2.0</td>
</tr>
<tr>
<td>Week 1</td>
<td>15.5 ± 4.4</td>
<td>10.6 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>16.3 ± 4.4</td>
<td>11.4 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>14.4 ± 4.8</td>
<td>9.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>14.9 ± 2.9</td>
<td>11.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>13.2 ± 3.1</td>
<td>9.4 ± 2.1</td>
<td>12.2 ± 2.4</td>
</tr>
<tr>
<td>Week 12</td>
<td>14.9 ± 5.7</td>
<td>13.7 ± 3.0</td>
<td>12.3 ± 2.1</td>
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<tr>
<td><strong>Urinary Creatinine (g/24h)(^5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>1.08 ± 0.47</td>
<td>1.15 ± 0.96</td>
<td>0.62 ± 0.88</td>
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<td>Week 13</td>
<td></td>
<td>1.26 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>1.11 ± 0.36</td>
<td>1.21 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>1.24 ± 0.26</td>
<td>1.15 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>1.32 ± 0.34</td>
<td>1.38 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>1.07 ± 0.30</td>
<td>0.93 ± 0.35</td>
<td>0.63 ± 0.26</td>
</tr>
<tr>
<td>Week 12(^3)</td>
<td>0.98 ± 0.26</td>
<td>1.02 ± 0.37</td>
<td>0.98 ± 0.19</td>
</tr>
<tr>
<td><strong>Urinary pH(^4)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Week 0</td>
<td>5.8 ± 0.32</td>
<td>6.1 ± 0.51</td>
<td>5.8 ± 0.36</td>
</tr>
<tr>
<td>Week 1</td>
<td>5.7 ± 0.28</td>
<td>6.1 ± 0.39</td>
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</tr>
<tr>
<td>Week 2</td>
<td>5.6 ± 0.21</td>
<td>6.1 ± 0.21</td>
<td></td>
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<tr>
<td>Week 3</td>
<td>5.6 ± 0.28</td>
<td>6.1 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>5.6 ± 0.26</td>
<td>6.1 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>5.7 ± 0.48</td>
<td>6.2 ± 0.37</td>
<td>6.1 ± 0.64</td>
</tr>
<tr>
<td>Week 12</td>
<td>5.8 ± 0.44</td>
<td>6.3 ± 0.47</td>
<td>6.1 ± 0.64</td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as means ± SD.
\(^2\) Low-carbohydrate, high-protein diet: LCHP, High-carbohydrate, low-fat diet: HCLF.
\(^3\) N=12 in LCHP diet group as one individual did not provide a 24-hr urine sample for that time point.
\(^4\) Significant main effect of the diet, \(P < 0.05\).
\(^5\) Significant effect of time, \(P < 0.05\).
Biomarkers of calcium metabolism

A significant main effect of diet on serum calcium concentration was observed during this study \((P < 0.03)\). A significant time effect was observed in serum calcium concentration as a decrease in serum calcium was observed in the LCHP diet group (-7.4%), the HCLF diet group (-8.3%), and the controls (-9.6%) by week 12 \((P = < 0.001)\). This main effect of time may be due to the low concentration observed in the LCHP group at week 4. No Diet x Time interaction was demonstrated, although a trend toward a significantly lower serum calcium concentration was observed in the LCHP diet group (-7.8 ± 0.3 mg/dl) compared to the HCLF diet group (-8.2 ± 0.4 mg/dL; \(P = 0.08\)) at week 4.

As expected, there was a significant main effect of diet on urinary calcium excretion at week 12 \((P < 0.0001)\). Urinary calcium excretion was approximately two-fold higher in the LCHP diet group compared to the HCLF diet group at all data collection points except week 12 (Table 6). Interestingly, in the LCHP diet group, urinary calcium excretion was greater than baseline values during the first six weeks of the study but declined to about baseline by week 12. There was no effect of time on urinary calcium excretion.

Urinary phosphorus excretion exhibited a significant main effect of diet \((P < 0.0001)\) during the 12-week study. A significant effect of time was observed with the greatest decrease in the LCHP diet group (-22.1%) compared to the HCLF group (-3.7%) and control group, which increased by 25.2% \((P = 0.002)\) at week 12. No Diet x Time interaction was demonstrated.
<table>
<thead>
<tr>
<th></th>
<th>LCHP Diet&lt;sup&gt;2&lt;/sup&gt; (n=13)</th>
<th>HCLF Diet&lt;sup&gt;2&lt;/sup&gt; (n=12)</th>
<th>Control (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Calcium (mg/dl)&lt;sup&gt;4,5&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>9.5 ± 0.7</td>
<td>9.6 ± 0.7</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>Week 1</td>
<td>9.2 ± 0.7</td>
<td>9.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>8.8 ± 0.6</td>
<td>9.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>8.4 ± 1.0</td>
<td>8.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>7.8 ± 0.3</td>
<td>8.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>8.8 ± 0.4</td>
<td>8.9 ± 0.3</td>
<td>9.0 ± 0.1</td>
</tr>
<tr>
<td>Week 12</td>
<td>8.8 ± 0.4</td>
<td>8.8 ± 0.4</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td><strong>Urinary Calcium (mg/24h)&lt;sup&gt;4&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>161.5 ± 75.0</td>
<td>162.1 ± 124.4</td>
<td>107.8 ± 68.2</td>
</tr>
<tr>
<td>Week 1&lt;sup&gt;3&lt;/sup&gt;</td>
<td>206.6 ± 103.6</td>
<td>102.4 ± 50.4</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>206.7 ± 107.4</td>
<td>114.4 ± 53.9</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>214.0 ± 85.1</td>
<td>110.0 ± 57.4</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>219.4 ± 114.5</td>
<td>123.1 ± 86.4</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>195.5 ± 134.8</td>
<td>101.8 ± 69.3</td>
<td>136.5 ± 4.0</td>
</tr>
<tr>
<td>Week 12&lt;sup&gt;3&lt;/sup&gt;</td>
<td>155.8 ± 119.5</td>
<td>120.1 ± 49.2</td>
<td>118.9 ± 28.7</td>
</tr>
<tr>
<td><strong>Urinary Phosphorus (mg/dl)&lt;sup&gt;4,5&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>75.1 ± 32.6</td>
<td>51.2 ± 32.0</td>
<td>61.4 ± 46.7</td>
</tr>
<tr>
<td>Week 1&lt;sup&gt;3&lt;/sup&gt;</td>
<td>51.5 ± 32.8</td>
<td>36.9 ± 24.1</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>77.5 ± 32.8</td>
<td>54.3 ± 41.8</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>74.9 ± 37.0</td>
<td>32.1 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>71.1 ± 25.6</td>
<td>39.0 ± 18.0</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>77.6 ± 31.7</td>
<td>29.0 ± 17.3</td>
<td>40.5 ± 18.3</td>
</tr>
<tr>
<td>Week 12&lt;sup&gt;3&lt;/sup&gt;</td>
<td>58.5 ± 35.4</td>
<td>49.3 ± 23.2</td>
<td>76.9 ± 62.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are expressed as means ± SD.
<sup>2</sup> Low-carbohydrate, high-protein diet: LCHP; High-carbohydrate, low-fat diet: HCLF.
<sup>3</sup> N=12 in LCHP diet group as one individual did not provide a 24-hr urine sample for that time point.
<sup>4</sup> Significant main effect of the diet, \( P < 0.05 \).
<sup>5</sup> Significant effect of time, \( P < 0.05 \).
Insulin-like Growth factor-1

There was a main effect of diet on serum IGF-1 concentration with greater mean concentration observed in the HCLF diet group compared to the LCHP diet group ($P = 0.01$). A significant time effect for IGF-1 concentration ($P = 0.004$) was shown. There was an increase of 51.7% and 7.3% in the HCLF and LCHP diet groups, respectively, at week 12 with the greatest increase in IGF-1 concentration demonstrated at week 6 in the HCLF group (130%; $P < 0.002$). There was no Diet x Time interaction for IGF-1 concentration.
Figure 2. Weekly changes in insulin-like growth factor-1 during the study for subjects following either a low-carbohydrate, high-protein diet (LCHP, n = 10) or high-carbohydrate low-fat, energy restricted diet (HCLF, n = 12) for 12 wk. Subject number dropped in each group because the subject was unable to provide enough serum to conduct the analysis. Values are expressed as group means ± SD. Significant main effect of diet (P < 0.05). *Significant time effect on IGF-1 concentrations (P < 0.01) with the greatest increase observed at week 6 (P = 0.002).
Biomarkers of bone turnover

Serum osteocalcin was analyzed at baseline and at weeks 1 and 2 to monitor the acute changes in bone turnover during the induction phase of the LCHP diet and again at weeks 6 and 12 to assess physiological adaptations that occurred as carbohydrate was added to the diet. The covariate controlled for different baseline concentrations between the two diet groups. There was a significant main effect of diet on osteocalcin concentration ($P = 0.05$). A significant time effect was shown in both groups ($P < 0.0001$), with a sharp decline in osteocalcin concentration observed at weeks 1 and 2 and a sharp rise from week 6 to week 12 in the LCHP diet group (Table 7). In contrast, osteocalcin concentration remained steady at weeks 1 and 2 and a sharp rise was observed in the HCLF diet group at week 6, which was maintained through week 12 (Figure 3). By week 12, osteocalcin concentration was not significantly different between groups. There was no Diet x Time interaction observed.

Average urinary NTx concentrations in the LCHP and HCLF diet groups are presented in Table 7. Urinary NTx began to rise in the LCHP diet group and remained steady at week 2, while it declined at week 1 and rose by week 1 in the HCLF diet group. However, there was no significant main effect of diet or time.
Table 7. Biochemical markers of bone turnover.

<table>
<thead>
<tr>
<th></th>
<th>LCHP Diet(^2) (n=13)</th>
<th>HCLF Diet(^2) (n=12)</th>
<th>Control (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Osteocalcin (ng/ml)(^4,5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>6.9 ± 1.01</td>
<td>7.4 ± 1.57</td>
<td>6.7 ± 2.65</td>
</tr>
<tr>
<td>Week 1</td>
<td>5.7 ± 1.03</td>
<td>7.1 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>6.3 ± 1.06</td>
<td>7.4 ± 1.64</td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>6.7 ± 0.93</td>
<td>8.4 ± 1.81</td>
<td>7.7 ± 2.40</td>
</tr>
<tr>
<td>Week 12</td>
<td>8.0 ± 2.16</td>
<td>8.1 ± 1.51</td>
<td>8.0 ± 3.33</td>
</tr>
<tr>
<td>Urinary NTx (nM BCE/mM Cr)(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>50.5 ± 33.6</td>
<td>75.8 ± 61.8</td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>67.7 ± 35.0</td>
<td>66.6 ± 35.0</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>61.4 ± 49.5</td>
<td>99.8 ± 79.3</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as means ± SD.
\(^2\) Low-carbohydrate, high-protein diet: LCHP, High-carbohydrate, low-fat diet: HCLF.
\(^3\) Controls were not included for urinary NTx due to limited space on the ELISA.
\(^4\) Significant main effect of the diet, \(P < 0.05\).

Figure 3. Changes in serum osteocalcin concentration from week 0 to week 1, 2, 6, and 12 for subjects following either a low-carbohydrate, high-protein (LCHP, \(n = 13\)) or high-carbohydrate low-fat, energy restricted diet (HCLF, \(n = 12\)) for 12 weeks. Average values are presented.
Bone mineral analysis

Bone mineral density and BMC were measured and analyzed at five sites: TB, LS, TPF, FN, and TF. A Diet x Time interaction was observed for TPF BMD (Table 8). The LCHP diet had significantly lower TPF BMD compared to the HCLF diet and control group ($P = 0.02$) at week 12, while no differences were observed between the HCLF and control groups. Total proximal femur BMC, however, did not differ between groups (Table 8). There was no main effect of diet or time for TPF BMD or BMC. There was a significant time effect for FN ($P = 0.006$) and TF BMC ($P < 0.0001$). At week 12, FN BMC decreased by -6.3%, -0.92%, and -9.6% in the LCHP, HCLF, and control groups, respectively. In contrast, TF BMC increased by 1.4% and 8.8% in the LCHP and HCLF diet groups, respectively, while TF BMC decreased by -4.2% in the control group. Femoral neck and TF BMC and BMD were unaffected by diet. Total body and LS BMD and BMC were unaffected by diet, time, and did not exhibit a Diet x Time interaction.
Table 8. Bone mineral measures of women following a LCHP or HCLF diet at Week 0 and Week 12.$^1$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Body BMD (g/cm$^2$)$^2$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>1.1234 ± 0.07</td>
<td>1.1438 ± 0.07</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>1.1799 ± 0.00</td>
<td>1.1938 ± 0.09</td>
</tr>
<tr>
<td>Control</td>
<td>1.2433 ± 0.09</td>
<td>1.2410 ± 0.09</td>
</tr>
<tr>
<td><strong>Total Body BMC (g)$^2$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>2314.2 ± 172.4</td>
<td>2310.1 ± 170.8</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>2350.8 ± 349.5</td>
<td>2343.5 ± 343.3</td>
</tr>
<tr>
<td>Control</td>
<td>2464.4 ± 342.9</td>
<td>2469.0 ± 331.6</td>
</tr>
<tr>
<td><strong>Lumbar Spine BMD (g/cm$^2$)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>1.0745 ± 0.11</td>
<td>1.0749 ± 0.09</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>1.0970 ± 0.13</td>
<td>1.1043 ± 0.13</td>
</tr>
<tr>
<td>Control</td>
<td>1.2467 ± 0.11</td>
<td>1.2280 ± 0.15</td>
</tr>
<tr>
<td><strong>Lumbar Spine BMC (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>64.4 ± 9.7</td>
<td>64.1 ± 9.7</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>62.3 ± 12.9</td>
<td>62.0 ± 12.3</td>
</tr>
<tr>
<td>Control</td>
<td>72.9 ± 10.1</td>
<td>72.9 ± 10.6</td>
</tr>
</tbody>
</table>

$^1$ Means ± SD. Low-carbohydrate, high-protein diet: LCHP, (n = 13), High-carbohydrate, low-fat diet: HCLF, (n = 12). There was no significant main effect of diet.

$^2$ Bone mineral density: BMD, Bone mineral content: BMC.

$^3$ Significant time effect from Wk 0 within the diet group, $P < 0.05$.

$^4$ Significant Diet x Time interaction, $P < 0.05$. 

Table 8 continued. Bone mineral measures of women following a LCHP or HCLF diet at Week 0 and Week 12.1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Proximal Femur BMD (g/cm²)²⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>0.9467 ± 0.12</td>
<td>0.9382 ± 0.11</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>1.001 ± 0.11</td>
<td>0.9967 ± 0.13</td>
</tr>
<tr>
<td>Control</td>
<td>1.002 ± 0.11</td>
<td>1.0333 ± 0.10</td>
</tr>
<tr>
<td>Total Proximal Femur BMC (g)²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>30.6 ± 3.8</td>
<td>31.4 ± 4.3</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>31.6 ± 6.7</td>
<td>31.6 ± 6.8</td>
</tr>
<tr>
<td>Control</td>
<td>30.1 ± 6.0</td>
<td>30.9 ± 6.1</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>0.8253 ± 0.11</td>
<td>0.8125 ± 0.09</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>0.8608 ± 0.10</td>
<td>0.8489 ± 0.11</td>
</tr>
<tr>
<td>Control</td>
<td>0.8750 ± 0.11</td>
<td>0.8710 ± 0.11</td>
</tr>
<tr>
<td>Femoral Neck BMC (g)³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>4.44 ± 0.83</td>
<td>4.16 ± 0.29</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>4.37 ± 0.76</td>
<td>4.33 ± 0.63</td>
</tr>
<tr>
<td>Control</td>
<td>4.33 ± 0.63</td>
<td>4.16 ± 1.04</td>
</tr>
<tr>
<td>Total Forearm BMD (g/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>0.5662 ± 0.04</td>
<td>0.5688 ± 0.04</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>0.5842 ± 0.06</td>
<td>0.5843 ± 0.06</td>
</tr>
<tr>
<td>Control</td>
<td>0.6130 ± 0.04</td>
<td>0.6130 ± 0.04</td>
</tr>
<tr>
<td>Total Forearm BMC (g)³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCHP diet</td>
<td>12.5 ± 0.63</td>
<td>12.7 ± 0.67</td>
</tr>
<tr>
<td>HCLF diet</td>
<td>12.0 ± 1.61</td>
<td>13.1 ± 2.91</td>
</tr>
<tr>
<td>Control</td>
<td>13.6 ± 1.06</td>
<td>13.0 ± 1.84</td>
</tr>
</tbody>
</table>

1 Means ± SD. Low-carbohydrate, high-protein diet: LCHP, (n = 13), High-carbohydrate, low-fat diet: HCLF, (n = 12). There was no significant main effect of diet.
2 Bone mineral density: BMD, Bone mineral content: BMC.
3 Significant time effect from Wk 0 within the diet group, \( P < 0.05 \).
4 Significant Diet x Time interaction, \( P < 0.05 \).
Discussion

Results of this study showed that a LCHP diet (modeled after the Atkins Nutritional Approach™) and HCLF diet were equally effective in inducing weight loss over a 12-week period. These findings contradict previous studies that reported greater weight loss in subjects following a LCHP diet compared to a traditional HCLF energy-restricted diet (8, 9, 29). Although energy intake was not restricted in the LCHP group, 4-day food records indicated that these women reduced their intake to levels similar to those energy levels prescribed in the HCLF diet during the weeks that food record data were collected. These findings suggest that caloric reduction, rather than macronutrient distribution was the primary mediator of weight loss in these women. The voluntary reduction in energy intake among participants in the LCHP diet has been seen in other studies using the LCHP diet plan (9, 29) and further research to determine the impetus for this reduction is warranted. In the present study, the women in the LCHP diet group reported less perceived hunger compared to women in the HCLF diet at week 6 (30). Diet composition may have influenced this perception as diets with higher proportions of fat and protein have been reported to induce satiety (31, 32). Limited food choices and boredom with the diet are other factors that may have promoted this voluntary reduction in energy intake.

Changes in body composition were consistent with the weight loss shown in both diet groups. Although not significant, both groups reduced FM and LM during the weight loss study. Other studies have reported that high-protein diets preserve lean body mass (32); however, no differences were observed in the ratio of FM:LM in this study, indicating that the higher intake of protein in the LCHP diet provided no advantage over the HCLP diet in preserving LM. When expressed as % fat, however, both groups significantly reduced % fat mass by week 12, indicating health risk-reducing benefits.

A primary concern with LCHP diets is that the high-fat and saturated fat content of the diet will adversely affects lipid concentrations (33). It is well established that excess lipids and saturated fats are related to the onset of cardiovascular disease (33), and recent evidence has shown a causal relationship between dietary lipids and saturated fatty acids with atherosclerosis (34). Consistent with other findings, we found that the LCHP diet did not negatively affect TC or LDL-C at week 12 (7, 9, 29). Total cholesterol and LDL-C declined by 8.1% and 0.53%, respectively, in the LCHP group and by 6.4% and 0.71%, respectively, in the HCLF group. Triacylglycerol concentrations were improved by ~24% in both diet groups, although statistical significance was not reached. These findings support other studies with greater subject numbers (n = 22 - 68) showing significant reductions in TG ranging from 20-47% (8, 9, 29). The small
sample size in this study may explain the lack of significance. While changes in HDL-C concentration were not significant, this study showed HDL-C declined by 16.8% and 21.5% in the LCHP and HCLF groups, respectively. In contrast, other studies using the same diet protocol report significantly higher HDL-C concentrations (29) or no change in HDL-C concentrations (8, 9). Differences in study length (6 months vs. 12 weeks) or subject number may also explain these discrepant findings.

The effect of high-protein diets on renal function has been widely debated. Dietary protein intake increases glomerular filtration rate (35), and in persons with compromised renal function, reducing dietary protein can delay the onset of renal failure. These observations have been extrapolated to adults with normal kidney function and recommendations have been made to avoid high dietary protein intake. However, there is no documented evidence that high-protein diets lead to impaired renal function in the long term. A recent study found that long-term protein intake ranging from 1.2 to 2.0 g of protein/kg bw had no negative effect on renal function (36). This study found serum BUN remained within normal limits in the LCHP diet and suggest LCHP diets do not impair kidney function in healthy, but overweight, adult women.

Low-carbohydrate, high-protein diets have been criticized because of concern for adverse affects on bone mineral status; yet, no published study examining high-protein diets with <15% calories of carbohydrate has confirmed this contention (9, 23). High dietary protein intake is reported to induce hypercalciuria (13, 35). This excess calcium is believed to come from bone mineral dissolution as a result of the hydrogen load presented from the metabolic breakdown of proteins that contain sulfur-amino acids (15, 37). As reported in other studies, urinary pH was significantly lower in the LCHP diet group compared to the HCLF diet group indicating increased acid production (20, 23). The acidosis caused by this diet is likely due to several factors known to reduce urinary pH including: the presence of urinary ketones; higher dietary protein intake; and lower intake of alkaline-forming foods (20-22). Consistent with other studies, women in the LCHP diet had higher urinary calcium excretion compared to women following the HCLF diet (14, 20, 23); however, by week 12, urinary calcium excretion had fallen to levels below baseline values. Surprisingly, serum calcium concentration decreased in both diet groups by week 12. Other studies have reported constant serum calcium concentrations with high dietary protein consumption (20, 23, 40). The effect of diet we observed may be due to the low concentration shown at week 4 in the LCHP group.

Insulin-like growth factor-1 is one of several endocrine factors positively associated with bone mineral status (41). Energy and dietary protein restriction has been shown to reduce serum IGF-1 concentrations (42), and this reduction is positively correlated with reductions in bone
formation. In this study, circulating IGF-1 concentrations did not differ between diet groups and were affected by time only at week 6 in the HCLP diet. The values obtained at each interval of this study were markedly lower than those reported using a similar healthy population (42), which may be due to inadequate binding of the radioisotope. Most samples were given a minimum value of 23.3 ng/ml and therefore the results were skewed to the left for all data collection points.

In this study, the LCHP diet appeared to induce an acute reduction in a biomarker of bone formation, which was resolved as carbohydrate was added back into the diet. Serum osteocalcin concentration was lowered during the initial 2 weeks of the LCHP and increased by week 6 of the study. A similar pattern was shown in the HCLF diet group; however, changes were not as great. The findings observed in the LCHP diet group correspond with results reported by Reddy et al. (23) who demonstrated a significant decrease in osteocalcin concentration at the end of the first 2 weeks of a high-protein diet and thereafter, rose toward baseline concentrations. While in the present study we were only able to measure urinary NTx at weeks 1 and 2, no change was found in this biomarker of bone resorption. Reddy et al. (23) reported no significant change in urinary NTx or deoxypyridinoline following the induction phase but noted they trended upward during the maintenance phase of a LCHP diet. A high-protein diet study showed no differences in urinary NTx or hydroxyproline in women following a high-protein (1.6 g/kg bw) compared to a low-protein (0.7 g/kg bw) diet at 3, 5, and 6 weeks; however, this diet was not a ketogenic diet and included 50-60% of calories from carbohydrate (20). Because only one biomarker of bone turnover was measured at multiple weeks, it is difficult to determine whether bone turnover is directly influenced by LCHP diets.

This is the first study to evaluate the effect of a LCHP diet modeled after the Atkins Nutritional Approach™ (6) on BMD and BMC at several sites. Femoral neck BMC showed the greatest decreases in the LCHP diet group, while TF BMC demonstrated the greatest increases in the HCLF diet group by week 12 of the study, although no Diet x Time interaction existed. Total proximal femur BMD, however, showed significantly greater decreases in the LCHP diet (-0.96%) compared to the HCLF (-0.42%) diet by week 12. Total proximal femur, FN, and TF contain greater proportions of trabecular bone which respond more readily to environmental changes such as diet. Dietary calcium intake was not different between the two diet groups, suggesting that other nutrients may have been responsible for the observed changes. Dietary protein intake was significantly higher in the LCHP diet, while the nutrients known to protect bone mineral status, potassium, magnesium, and iron were significantly higher in the HCLF diet group. Thus, the higher protein intake and lack of alkaline forming foods in the LCHP diet may have stimulated bone loss at these sites. The protective effect of fruits and vegetables and the nutrients potassium, iron, and
magnesium on bone mineral status have been observed in previous studies (41-44). There were no changes observed in TB or LS BMD or BMC during the 12 week study. Study duration or limitations with DXA are possible reasons for this lack of change. Overweight individuals have greater tissue thickness in all areas in the body, particularly the abdominal region. In vitro studies that examined tissue thickness on the precision of BMD and BMC measured by DXA showed increased tissue thickness resulted in decreased precision (45). Although mean BMI decreased by week 12, the BMI recorded for the women in both groups at the end of the study classified them as overweight. The greater tissue thickness in these women may have prevented detecting changes in BMD and BMC to be detected at these sites.

While this study has shown that a LCHP diet reduces bone mineral at certain sites, these results are far from showing that LCHP diets lead to osteoporosis in the long-term and should not be used to make recommendations to the public. However, the fact that significance was shown with this small sample size, the magnitude of change in bone mineral at the TPF, FN, and TF would be potentially be greater with a larger sample size. Other limitations of this study include the relatively homogenous sample of healthy women with no known pre-existing medical conditions, except for overweight, and the short-term study period. More research that examines BMD and BMC at specific sites, using a larger, more diverse subject pool for longer periods of time must be conducted.

In summary, this study showed that a LCHP diet negatively impacted bone mineral status at the FN and TPF, whereas a HCLF diet positively affected bone mineral status of the TF. The observed decreased urinary pH and corresponding increase in urinary calcium excretion suggest that the imposed acid load from excess dietary protein intake and ketone production may stimulate bone mineral dissolution and that the alkaline salts abundant in fruits and vegetables are protective for bone. Finally, there was not an adverse effect on markers of lipid metabolism or significant differences in weight loss in subjects following a LCHP diet compared to a HCLF diet.
References Cited


30. Nickols-Richardson SM, Coleman MD, Volpe JJ, Hosig KW. Perceived hunger is lower and weight loss is greater in overweight premenopausal women consuming a low-carbohydrate/high-protein versus high-carbohydrate/low-fat diet. JADA 2004:In review.


Chapter V

Summary and Future Directions

A low-carbohydrate, high-protein (LCHP) diet did not result in greater weight loss, fat mass loss, or higher indices of cardiovascular risk compared to the traditional high-carbohydrate, low-fat (HCLF) diet (Chapter 4). Contrary to the claim that ketones promote weight loss in LCHP dieters, no association between serum ketone production and weight loss was observed in this study (Chapter 3). Total proximal femur (TPF) bone mineral density (BMD) was significantly lower in the LCHP group compared to the HCLF group at 12 weeks, while the greatest decreases in femoral neck (FN) bone mineral content (BMC) were seen in the LCHP group, although no Diet x Time interaction existed. The HCLF diet appeared to be protective against bone loss and even promoted bone mineralization as total forearm (TF) BMC was increased by the end of this 12-week study. Other measured sites of BMD and BMC (total body, lumbar spine) were not affected by either diet. However, study duration may not have allowed adequate time for changes in these sites to be observed. The observed loss in FN BMC in the LCHP may have been stimulated by the acid load generated from protein metabolism and ketone production. This assumption is based on the observed decrease in urinary pH, increased serum and urinary ketone production, and increased excretion of urinary calcium and phosphorus. Serum osteocalcin concentrations were depressed during the initial two weeks of the study and increased beyond baseline values indicating bone turnover was influenced by both weight loss interventions. Because osteocalcin levels were similar at the end of week 12, these changes may be reflective of weight loss rather than diet composition. The human body is a complex system and it is rare that clear cut findings are observed when assessing physiological adaptations in response to alterations in diet. Because of this, we were unable to confirm each of our hypotheses; however, these results suggest that LCHP diets may negatively impact bone health in the short-term.

Surprisingly, the women in the LCHP did not lose more weight than the women on the HCLF diet during any week of the study. This finding suggests that caloric restriction rather than alterations in macronutrient composition may facilitate weight loss. At the beginning of this study, there was a surge in the availability of low-carbohydrate products in the Blacksburg area. During week two, women on the LCHP diet began including these low carbohydrate items in their diet. After that time, we observed that in some women, weight loss slowed and even stopped when these products were introduced into their diets, even though reported caloric and carbohydrate was similar at weeks 2, and 4 (~1400 kcal). When these products were removed from the diet, the women began to lose weight. Because the carbohydrate content in these products is labeled in
terms of “net” carbs rather total carbohydrate, the amount of carbohydrate reported by the women was lower than what was actually consumed and because caloric intake was not controlled, it is possible that the women consumed more calories with these products than they would had they limited their food consumption to those foods specified in the Atkins Nutritional Approach™. In fact, we estimated that the mean caloric contribution from these items ranged from 74 to 98 calories per day. This equates to an additional 518 to 686 calories per week—enough to retard weight loss. The surge in low-carb food items in supermarkets may redefine the success of this diet plan. Future research should focus on the impact of LCHP diet plans when processed low-carb food products are a part of the diet to determine how they influence weight loss, vitamin and mineral intake, portion control, and risk for chronic diseases.

In this study, we instructed our participants to refrain from adding an exercise program to their current level of activity in order to control the effects of exercise on bone mineralization. Because the inclusion of exercise is recommended in sound exercise programs and in the Atkins Nutritional Approach™, future study examining the affects of an exercise program with LCHP diets is warranted.

While the sample treatment in this study was small and the length of the study relatively short, significant decreases in TPF BMD and FN BMC were shown in the LCHP group. It is possible that a larger sample size would magnify these differences. Clearly longer-term studies with a larger sample size need to be conducted in order to detect changes in bone mineral status associated with this diet plan. Because obese individuals have more weight to lose, they are more likely to remain in the induction phase for longer periods in order to meet their weight loss goals. Long-term studies with a prolonged induction phase would provide insight into the impact of the LCHP diet on bone mineral status in this population. Furthermore, biomarkers of bone turnover need to be assessed to provide information concerning the acute changes in bone health. Studies evaluating the response of endocrine factors (e.g., parathyroid hormone, IGF-1, GH) that regulate bone metabolism to LCHP diets in the short- and long-term are recommended.
APPENDIX A: Repeated Measures of Analysis of Covariance Tables
Repeated Measures Analysis of Covariance for BMD measures.

<table>
<thead>
<tr>
<th>DXA Measurement BMD (g/cm²)</th>
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<th>Mean Squares</th>
<th>F</th>
<th>p &gt; F</th>
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Repeated Measures Analysis of Covariance for BMC measures.

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## Repeated Measures Analysis of Covariance for Lipid measures

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<tr>
<td><strong>Triacylglycerols (mg/dl)</strong></td>
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<tr>
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<td><strong>HDL-C (mg/dl)</strong></td>
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<tr>
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<td><strong>LDL-C (mg/dl)</strong></td>
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</table>
APPENDIX B: Screening Form and Health History Questionnaire
VIRGINIA TECH
BONE METABOLISM, OSTEOPOROSIS, AND NUTRITION EVALUATION LABORATORY
Medical and Health History Form for Study Candidates

Name: __________________________ Age: ______ yr Date of Birth: ________________
Address: ___________________________
Telephone Number (work): ___________ Email Address: ________________________
Daytime Telephone (home): ___________ Gender: FEMALE MALE
Primary Care Physician __________________________ Phone: _________________________

Medical History
Please indicate any current or previous conditions or problems you have experienced or have been told by a physician that you have had:

Heart disease or heart problems: ______ Yes ______ No
Circulation problems: ______
Kidney disease or problems: ______
Urinary problems: ______
Reproductive problems: ______
Muscle problems: ______
Skeletal problems: ______
Neurological problems/disorders: ______
High blood pressure: ______
Low blood pressure: ______
High blood cholesterol ______
Diabetes: ______
Hypoglycemia: ______
Thyroid problems: ______
Eating disorders (bulimia, anorexia): ______
Crohn's disease: ______
Excessive hair growth on atypical areas of the body: ______

Please list any hospitalizations/operations/recent illnesses (Type/Date):

__________________________________________
__________________________________________
__________________________________________
Family Health History

Has anyone in your family (blood relatives only) been diagnosed or treated for any of the following?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Relationship</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Crohn's disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Thyroid disorders</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Osteoporosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Osteopenia</td>
<td></td>
<td></td>
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</tbody>
</table>

Health Habits

Are you on any special type of diet? Yes ____ No ____

If “yes” please describe ____________________________________________________________

Do you consume a vitamin or mineral supplement? Yes ____ No ____

If “yes”, list the supplement(s) you are taking?

__________________________________________________________

How long have you been taking the above supplements?

__________________________________________________________

Do you drink caffeinated beverages? Yes ____ No ____ Cups per day? ____ Per week? ____

Do you drink alcoholic beverages? Yes ____ No ____ Drinks per day? ____ Per week? ____

Do you smoke cigarettes? Yes ____ No ____ Packs per day? ____ Packs per week? ____

Exercise Habits

Do you engage in regular exercise? Yes ____ No ____

If “yes” please list:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (times per week)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Are there any orthopedic limitations you have that may restrict your ability to exercise? YES ____ NO ____

If “yes” please explain: ____________________________________________________________

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Questions Related to Reproductive Function
Do you use an oral contraceptive?  Yes ____  No ____
If "yes" what brand and dose of oral contraceptive? ____________________________________________
If "yes," for how long have you used this oral contraceptive? ______________________________________
When was the first day of your last menses? ____________________________________________
Have you had any abnormal menses or absence of menses in the last 12 months?  Yes ____  No ____
If "yes", describe: ________________________________________________________________

Are you pregnant or do you think that you may be pregnant?  YES  NO

**If you are pregnant or think that you may be pregnant, you should not participate in this study. This study involves DXA scans and exposure to radiation. Radiation exposure from DXA scans may cause harm to your unborn fetus; therefore, if you are pregnant or think that you may be pregnant, you should not participate in this study. A pregnancy test will be completed by all participants prior to any DXA scan.

How many menstrual cycles do you have per year?
   a)  12 to 14 per year
   b)  9 to 11 per year
   c)  6 to 8 per year
   d)  3 to 5 per year
   e)  < 3 per year

Do you have children?  YES  NO
If "yes" how many children do you have? ________________________

Has your physician or OB/GYN diagnosed you with menopause or perimenopause?  YES  NO
Have you been experiencing symptoms of menopause or “perimenopause” such as hot flashes, irregular menstrual cycles, spotting etc?  YES  NO
Have you had a hysterectomy or had your ovary’s removed?  YES  NO
If "yes", have you been placed on hormone replacement therapy (HRT)?  YES  NO
If you are on HRT, please list the medications you are currently taking:

____________________________________________________________________

Medications
Please indicate any current medications that you are taking:

                    Yes          No
Steroids (such as Prednisone): ________________________
Thyroid medications (such as Synthroid): ________________________
Biphosphonates (such as Fosamax): ________________________
Anticonvulsants (such as Dilantin): ________________________
Glucocorticoids (such as Dexamethasone): ________________________
Other bone medications (such as Miacalcin): ________________________
Please list any other medications (prescription and over-the-counter) you are currently taking or have taken in the past month: ____________________________________________________________

Weight History
What is your current weight? __________
How much did you weigh six months ago? ______________
How much did you weigh one year ago? ______________
Do you have a desire to lose weight? YES NO
What is your height? __________

BMI: _______________ (For investigator use, please leave blank)

Please sign to indicate the above information is correct:

__________________________  ________________________  __________
Print Name                                           Signature                                           Date

Results of Screening: Make certain that all questions on this form are properly completed. Query candidate, immediately after they complete this questionnaire, about any items left blank or for which clear answers are not provided. If no unusual problems are disclosed that may affect the candidate's safety or eligibility for the study, note this finding below and submit to the investigator. THIS CANDIDATE QUALIFIES FOR PARTICIPATION IN THE STUDY, SUBJECT TO VERIFICATION BY THE INVESTIGATOR. Yes: ___ No: ___.
If No, complete next section, below.

The candidate was disqualified due to:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Virginia Polytechnic Institute and State University
Informed Consent for Participants of Investigative Projects

Title of Project: Effects of a Low-Carbohydrate/High-Protein diet on Acid-Base Balance, Calcium metabolism, and Oxidative stress in premenopausal women.

Investigators: Mary Dean Coleman, M.S. R.D., Abigail D.Turypn, B.S., Sharon M. Nickols-Richardson, Ph.D., R.D., and Janet W. Rankin, Ph.D.

I. Purpose: The purpose of this project is to determine the effects of long-term adherence to a several weight loss diets on bone mineral density, markers of bone changes, acid/base metabolism, oxidative stress, and indicators of inflammation in a group of premenopausal women ages 32-45 over a 12-week period of time.

II. Procedures: Prior to being included in this research study, you will complete a Screening Questionnaire that will help determine if there are reasons why you should not participate in this study. If results of this Screening Questionnaire indicate that you are an appropriate participant for this study, asked to read, review, and sign this Informed Consent Form and to obtain authorized medical clearance from your primary care physician.

Following your acceptance into the study, you will be randomized into one of three dietary treatment groups. The investigators will then contact you to inform you of your assigned dietary intervention protocol. You will be scheduled to attend an informational session held in a classroom in Wallace Hall on the Virginia Tech campus. Other members selected to participate in the dietary intervention protocol will be asked to attend the same meeting. During this session, the dietary regimen that the investigators will expect you to follow will be explained in further detail. If you are selected to be in Group 1 (wait-list control group), you will follow your regular dietary eating habits and asked to maintain your current weight for 12-weeks. If you are assigned to Group 2, you will follow a low-carbohydrate/high-protein Atkins Diet in which carbohydrate intake will be limited to 20 grams per day for a period of 4-weeks. After the 4-week period, you will increase your carbohydrate intake by 5 grams per week until the end the study at 12-weeks. You will be asked to consume a calcium supplement that contains no more than 250 mg of calcium per tablet. Group 3 will be asked to follow a moderately energy restricted, low fat diet (similar to a Weight Watchers approach). Books and handouts that explain the requirements of the assigned dietary protocol, and that include recipes and lists of acceptable and non-acceptable foods will be provided during this time. Once the dietary protocol has been explained, an education session will be given to explain the proper method for filling out a 4-day dietary food record. You will be asked to take the 4-day dietary food record home and to fill it out four days prior to coming in for the first scheduled testing date. You will also be given a 24-hour urine specimen container and instructions explaining how to collect your urine by the investigators.

If you are selected to be in this study, you will be scheduled for the first (baseline) testing date. You will do the listed procedures, in order, during one, 2-hour (± 30 minute) session at 7 time points (baseline, 1-week, 2-weeks, 3-weeks, 4-weeks, 6-weeks, and 12-weeks after baseline). You will be asked to do the following during each testing session:

1. arrive in Room 229 Wallace Hall on the Virginia Tech campus at your scheduled day/time after having fasted overnight (minimum 12 hour fast; not having consumed foods or beverages except water);
2. provide investigators with a 24-hour urine specimen collected during the 24-hours prior to appointment (collected in containers provided by investigators);
DXA scans will be conducted in the BONE Laboratory, Room 229 Wallace Hall, on the Virginia Tech campus by one of the Principal Investigators who are both Licensed Radiologic Technologists – Limited in the Commonwealth of Virginia.

IV. Benefits of this Project: The researchers do not guarantee any specific benefit to you as a result of following the dietary intervention or from your participation in the study, however, you will receive the following tests and results during your participation in the study: (1) analysis of your dietary intake, (2) measurement of your body composition; (3) analysis of your bone formation and resorption status; (4) measurement of your bone mineral density of your whole body, lumbar spine, hip, and forearm (5) analysis of your blood lipid profile; (6) analysis of your acid/base metabolism; (7) analysis of your calcium metabolism; and (8) analysis of indicators of oxidative stress. At completion of this study, you will be provided with your individual results from every procedure at each time point and may contact the investigator at a later date for a summary of the overall research results. You will be referred to an appropriate health care professional, if necessary, based on your individual results. Any and all costs related to such referral will be borne by you and not by Virginia Tech. The general public will benefit from your participation in this research as new understandings of links among dietary practices and chronic disease identified from this study will extend osteoporosis prevention knowledge and applications. Information concerning the effects of the diets on oxidative stress and inflammatory indicators will provide insight into the health risks or benefits of various dietary approaches to weight loss.

V. Extent of Anonymity and Confidentiality: Due to the inability to assure anonymity, you understand that confidentiality of your results will be preserved. You understand that this means that all of your answers to questions that you are asked, measurement and laboratory values, and DXA scans results will be kept confidential. A three-digit code number will be assigned to you. All questionnaires, data collection sheets, data analysis sheets, blood and urine collection and storage containers, and DXA scan sheets will be identified by code number only and not by your name. You understand that a master list of participants' code numbers will be kept in a locked filing cabinet separate from completed data which will also be maintained in a locked filing cabinet. You further understand that only the investigators of this study will be allowed access to any data.

VI. Compensation: You will not be compensated or paid to be in this research project. However, you will receive your individual results from each procedure that you complete at the end of this study.

VII. Freedom to Withdraw: You understand that you can withdraw from this study at any time without penalty. You are free to not answer any questions or to not participate in any procedure included in this study without penalty. You understand that there may be circumstances under which the investigator may determine that you should not continue to participate in this project.

VIII. Emergency Procedure: If a minor emergency arises during your participation in this study, you will discontinue your participation and seek care from your personal physician. If a major emergency arises during your participation in this study, emergency personnel will be called (911), and they will care for you.

IX. Approval of Research: This research project has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods and Exercise.

X. Subject's Responsibilities: You voluntarily agree to participate in this study. You have the following responsibilities:
(3) provide a 4-day dietary record which was kept during the week prior to your appointment. The investigator will review the 4-day recall with each participant to clarify any questions the investigator may have regarding the recorded information (30 minutes);

(4) provide a second-void urine sample for pregnancy testing (all participants) and to detect the presence urinary Ketones (Atkins diet group only; 5 minutes);

(5) in an interview fashion, complete the Food Frequency Questionnaire (20 minutes); and KIHD Leisure-Time Activity History (5 minutes);—baseline testing only;

(6) have 44 mL (~4 Tablespoons) of whole blood drawn from your arm by a Licensed Medical Technician (10 minutes);

(7) provided with breakfast foods and beverages if desired;

(8) have your height and weight measured by an investigator (to determine your body mass index; 5 minutes);

(9) in an interview fashion, complete the 7-Day Physical Activity Recall (5 minutes);

You will be asked to have a DXA scan taken 3 times throughout the study. The initial scan will be taken during the baseline testing session, while the other two scans will be taken at week 6 and at week 12 following baseline testing. To complete the scan, you will be asked to:

(10) lie on or sit next to the dual-energy X-ray absorptiometer (DXA) as directed by a Licensed Radiologic Technologist – Limited who will conduct DXA scans of your whole body, lumbar spine, hip, and forearm for bone mineral density testing (15 minutes).

If you are selected for this study, you understand that your participation will require approximately 2-hours (± 30 minutes) of your time at each of seven testing times. You also understand that you may require more or less time than estimated to complete each procedure and that you will be given ample opportunity to complete all procedures in an unhurried manner.

You will be expected to discontinue the use of any vitamin or mineral supplements unless the investigator asks you to take a supplement as part of the dietary regimen. If at anytime during the study there are any changes to your present health or medical status, or you experience any unusual symptoms, you understand you need to inform the investigator immediately.

Throughout the study, you will participate in weekly group sessions that will last approximately 1 hour (± 30 minutes). During this time, the investigators will offer motivational support and additional educational materials related to the dietary intervention being followed by the low-carbohydrate/high-protein and low-fat diet group. These sessions will be offered several times during the week to ensure each participant can attend. The investigators will also review the proper techniques for filling out the 4-day food record to ensure the participants are recording their intake as accurately as possible. The investigator will be available to answer any questions the participants may have regarding the diets during the weekly group sessions and individual testing sessions.

At the end of this study, the participants selected to be in the wait-list control group, will be given the option to follow the weight loss diet for a period of 12 weeks. The investigators will assign you to either the low-carbohydrate/high-protein diet (Atkins diet) or the conventional weight loss, low-fat diet with the supervision of the investigators of this study. If you choose to follow the weight loss diet, you will be scheduled for the first (baseline) testing date and you will complete the listed procedures (as explained above), in order, during one, 2-hour (± 30 minute) session at 7 time points (baseline, 1-week, 2-weeks, 3-weeks, 4-weeks, 6-weeks, and 12-weeks after baseline).

**III. Risks:** The researchers are not aware of any specific risks associated with following either of the weight loss interventions proposed in this study. You understand that potential risks while participating in this study exist during blood draws and DXA scans. You understand that there is
minimal risk involved in blood draws. A bruise may result from blood collection procedures with no known detrimental effects to your health or well being. You understand that to avoid or minimize bruising, a certified medical laboratory technician will draw your blood. Additionally, you will be allowed to sit or recline in the most comfortable position for yourself during your blood draw. You may rest for as long as needed after your blood is drawn and will be provided with breakfast foods and beverages after your blood is collected. Two attempts to draw your blood (or two needle sticks) will be allowed. If a second attempt is unsuccessful, no further tries for collection of your blood will be performed. You understand that all personnel involved in drawing and handling blood have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech. You understand that precautions will be taken by research personnel during handling of your blood (and urine) samples. You further understand that the standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood or urine exposure occurs and includes HIV and hepatitis testing of your blood.

You understand that exposure to radiation will occur during DXA scans for measurement of your bone mineral density. Radiation exposure will occur from the DXA scans because the DXA machine uses x-ray technology. Radiation exposure is measured in millirads (or mR). Your total amount of exposure is 20 mR (whole body = 1mR, lumbar spine = 7 mR, hip = 7 mR; forearm = 5 mR) during each testing time and your cumulative total exposure is 60 mR if you complete all DXA scans throughout the 12 weeks of this study. Because your combined total exposure for the entire study represents 6% of the estimated exposure to increase cancer risk in only 0.03% or the population, you understand that this dose is very small, and poses minimal risk compared to radiation doses from dental bite-wing films (334 mR) and environmental background exposure (100 to 400 mR per year) expected to occur in one 12-week period. The following table lists the radiation limits for an adult research participant according to the National Institutes of Health, Office for Protection from Research Risk (NIH-OPRR), compared to your exposure during this study.

<table>
<thead>
<tr>
<th>NIH-ORPP Radiation Limits for an Adult Research Participant</th>
<th>My Exposure During Participation in this Research Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body (single dose) = 3,000 mR</td>
<td>Whole body (single dose) = 1 mR</td>
</tr>
<tr>
<td>Whole body (annual cumulative dose) = 5,000 mR</td>
<td>Whole body (annual cumulative dose) = 3 mR</td>
</tr>
<tr>
<td>Lumbar spine (single dose) = 5,000 mR</td>
<td>Lumbar spine (single dose) = 7 mR</td>
</tr>
<tr>
<td>Lumbar spine (annual cumulative dose) = 15,000 mR</td>
<td>Lumbar spine (annual cumulative dose) = 21 mR</td>
</tr>
<tr>
<td>Hip (single dose) = 5,000 mR</td>
<td>Hip (single dose) = 7 mR</td>
</tr>
<tr>
<td>Hip (annual cumulative dose) = 15,000 mR</td>
<td>Hip (annual cumulative dose) = 21 mR</td>
</tr>
<tr>
<td>Forearm (single dose) = 5,000 mR</td>
<td>Forearm (single dose) = 5 mR</td>
</tr>
<tr>
<td>Forearm (annual cumulative dose) = 15,000 mR</td>
<td>Forearm (annual cumulative dose) = 15 mR</td>
</tr>
<tr>
<td>ANNUAL CUMULATIVE EXPOSURE (whole body + lumbar spine + hip + forearm) = 60 mR during 12 weeks</td>
<td></td>
</tr>
</tbody>
</table>

You have been informed of the risks and may choose to not complete any one, combination, or all of these DXA scans. If in the event that any scan is unreadable or unusable, a replacement scan will not be conducted to avoid further exposure. **You further understand that if you are pregnant or think that you may be pregnant that you should not undergo DXA scans because radiation exposure from DXA scans may cause harm to your unborn fetus.** These
For each dietary protocol:
(1) provide consent to complete procedures for this study;
(2) provide authorized medical clearance from your primary care physician (baseline testing only);
(3) follow the guidelines established by the investigators for the dietary protocol assigned to you in this study;
(4) attend the scheduled weekly group support and educational meetings;
(5) arrive at Wallace Hall on the scheduled day after having fasted overnight for a minimum of 12 hours;
(6) provide a 24-hour urine sample;
(7) provide a 4-day dietary intake record;
(8) provide a second-void urine sample;
(9) honestly, and to the best of your knowledge, answer questions from the Food Frequency Questionnaire and KHID 12-month Leisure-Time Physical Activity History (baseline testing only);
(10) have 44 mL (~ 4 Tbsp) of venous blood drawn by a Certified Medical Technician;
(11) consume breakfast foods and beverage if desired;
(12) have your height and weight measured by an investigator;
(13) honestly, and to the best of your knowledge, answer questions from the 7-day Physical Activity Recall;
(14) have your bone mineral density of the whole body, lumbar spine, hip, and forearm measured by DXA;
(14) follow directions of the investigator as related to this project.

VI. Subject's Permission: You have read and understand the Informed Consent and conditions of this project. You have had all of your questions answered. You hereby acknowledge the above and give your voluntary consent for participation in this project. If you participate, you may withdraw at any time without penalty. You agree to abide by the rules of this project.

__________________________________________  ________________________________________
Participant's Signature                       Date

__________________________________________  ________________________________________
Investigator's Signature                      Date

Should you have any questions about this research or its conduct, you may contact:

Sharon M. (Shelly) Nickols-Richardson, Investigator
(540) 231-5104
Janet W. Rankin, Investigator
(540) 231-6355

OR

Dr. David M. Moore, IRB Chair
(540) 231-4991
APPENDIX D: 4-Day Food Record Forms
4-Day Diet Record (3 Week Days + 1 Weekend Day)

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time of Day</th>
<th>Food Eaten</th>
<th>Amount</th>
<th># of Carbs</th>
<th>Cooking Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
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# 4-Day Diet Record (3 Week Days + 1 Weekend Day)

Day of week Taken: M T W Th F Sat Sun (Circle)

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<th>Amount</th>
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APPENDIX E: 7-Day Physical Activity Records
7-Day Physical Activity Recall Questionnaire

1. On the average, how many hours did you sleep each night during the last 5 weekday nights (Sunday - Thursday)? Record to the nearest quarter-hour.
   Hours: _____________ Minutes: _____________

2. On the average, how many hours did you sleep each night last Friday and Saturday nights?
   Hours: _____________ Minutes: _____________

3. First let's consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour.
   Hours: _____________ Minutes: _____________

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)
   Hours: _____________ Minutes: _____________

5. Now let's look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.
   Hours: _____________ Minutes: _____________

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)
   Hours: _____________ Minutes: _____________

7. Now let's look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.
   Hours: _____________ Minutes: _____________

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)
   Hours: _____________ Minutes: _____________

9. Compared with your physical activity over the past 3 months, was last week's physical activity more, less, or about the same? (Circle One)
   More
   Less
   About the same
APPENDIX F: Institutional Review Board for Research Involving Human Subjects Approval
July 2, 2003

MEMORANDUM

TO: Sharon Nikols-Richardson HNFE 0430

FROM: David M. Moore

SUBJECT: IRB APPROVAL – “Effects of a Low-Carbohydrate High-Protein diet on Acid-Base Balance, Calcium Metabolism, and Oxidative Stress in Premenopausal Women” – IRB # 03-286 FR

The above referenced protocol was submitted for full review and approval by the IRB at the June 9, 2003 meeting. The board had voted approval of this proposal contingent upon receipt of responses to questions raised during its deliberation. Following receipt and review of your responses, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective June 9, 2003.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. It is your responsibility to report to the IRB any adverse reactions that can be attributed to this study.

To continue the project past the 12 month approval period, a continuing review application must be submitted (30) days prior to the anniversary of the original approval date and a summary of the project to date must be provided. Our office will send you a reminder of this (60) days prior to the anniversary date.

cc: File
Vita

Mary Dean Coleman, Ph.D., R.D., daughter of Dr. Jack and Carla Coleman, was raised in Monroe City, Missouri. She plans to stay at Virginia Tech working as the Senior Program Manager for the Macromolecular Interfaces with Life Sciences Program (MILES).

Education

Master of Science Degree in Human Nutrition
Department of Food Science and Human Nutrition
University of Illinois, C-U
Urbana, Illinois

Saint Louis University Dietetic Internship Program
Saint Louis University
Saint Louis, Missouri

Bachelor of Science (Dietetics) and Minor (Exercise Science)
Southwest Missouri State University
Springfield, Missouri

Professional Experience

Graduate Teaching Assistant
Virginia Polytechnic Institute and State University

Adjunct Professor for Nutrition Throughout the Lifecycle (HNFE 2014)
Virginia Polytechnic Institute and State University

Instructor Dietetics/Sports Nutrition
Mansfield University, Mansfield, PA

Graduate Teaching Assistant
University of Illinois- C-U

Clinical Dietitian
Provena Covenant Medical Center, Urbana, Illinois

Awards and Scholarships
Jewell L. Taylor Teaching Scholarship, AAFCS
Cunningham Research Scholarship, Virginia Polytechnic Institute and State University
Mansfield University Academic Mentor Award
Jonathan Baldwin Turner Fellowship, University of Illinois, C-U
Missouri Public Health Department Scholarship
Regents Scholarship, Southwest Missouri State University
Peer-reviewed publications


Coleman MD, Nickols-Richardson SM. Urinary ketones reflect serum ketone production but do not relate to weight loss in overweight premenopausal women following a low-carbohydrate/high-protein diet. Journal of the American Dietetic Association, In Press.


Professional Presentations

Premenopausal women following a low-carbohydrate/high-protein diet experience greater weight loss and less hunger compared to a high-carbohydrate/low-fat diet. Poster Presentation. Experimental Biology, Washington DC, April 2004.
