THE EFFECTS OF HIGH- AND LOW-CARBOHYDRATE WEIGHT LOSS DIETS ON BONE HEALTH IN OVERWEIGHT AND OBESE ADULTS

By

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A THESIS

Presented to the Department of
Dietetics and Nutrition and the
Oregon Health & Science University
School of Medicine
in partial fulfillment of the
requirements for the degree of
Master of Science in
Clinical Nutrition

April 2008

School of Medicine

Oregon Health & Science University

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COMMONLY USED ABBREVIATIONS

ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BSAP	Bone-Specific Alkaline Phosphatase
CHO	Carbohydrate
DASH	Dietary Approaches to Stop Hypertension
DEXA	Dual-Energy X-ray Absorptiometry
GFR	Glomerular Filtration Rate
GLM	General Linear Model
HIPAA	Health Insurance Portability and Accountability Act
IGF	Insulin-like Growth Factor
KPCHR	Kaiser Permanente Center for Health Research
MANOVA	Multivariate Analysis of Variance
NTx	N-telopeptide
OCTRI	Oregon Center for Translational and Research Institute
OHSU	Oregon Health & Science University
PTH	Parathyroid Hormone
RANKL	Receptor Activator of NF-κB Ligand
RCT	Randomized Controlled Trial
RR	Relative Risk or Risk Ratio

ACKNOWLEDGMENTS

I never thought that I would go to graduate school, much less ever write a thesis, so I'll forever be grateful to the people that have helped me along the way.

First and foremost, Dr. Diane Stadler has been the most wonderful mentor. She's guided me and inspired me to do the best I can.

My wonderful husband Graham has supported me and kept me going when I doubted my abilities.

My parents and family believed in me and supported me from a distance.

I'm grateful for the guidance from my committee members and their willingness to take time out of their busy schedules to serve on my committee.

Many thanks go to KPCHR and OHSU for conducting the Insight study, and all of the staff members that have helped me with data entry and analysis.

Thanks to the OCTRI Core Lab and staff who lent a helping hand when I had no idea what I was doing.

And, I'm thankful for the friends I've made at OHSU and all that they've given me—the help, the good memories, and the laughs.

ABSTRACT

Low-carbohydrate (CHO) diets are popular in the United States because of the promises of quick and easy weight loss, but the health effects of these diets on bone have yet to be described. Low-CHO diets severely restrict the amount of carbohydrate a person can eat in one day; consequently, several foods are excluded from the diet, including milk and yogurt, fruits, starchy vegetables and grains. Restricting these foods may result in low consumption of the bone-related nutrients including calcium, potassium, and magnesium. Also, restricting carbohydrate intake greatly increases protein and fat intake, a pattern that may negatively affect skeletal health. While previous studies report that low-CHO diets result in increased urinary calcium excretion, the impact of low-CHO diets on bone turnover and bone quality have not been established. We hypothesized that individuals consuming a low-CHO weight loss diet would have increased urinary calcium excretion and bone turnover, subsequently resulting in decreased bone quality compared to those consuming a high-CHO weight loss diet after six months.

This prospective, parallel cohort, randomized, interventional study compared the effects of following a low-CHO or high-CHO weight loss diet on bone health after six months. Bone health was assessed in 115 overweight and obese adults by measuring calcium intake, urinary calcium excretion, bone turnover markers, and bone mineral content (BMC) and bone mineral density (BMD). Differences in absolute values at six-months and changes from baseline were compared using independent sample t-tests,

correlational analysis, repeated measures analysis of variance (ANOVA), multivariate and univariate linear regression models, and post-hoc analysis.

Energy intake decreased in both groups (-343 ± 87 kcal/d in high-CHO vs -447 ± 107 kcal/d in low-CHO), but the differences were not significantly different between groups. Dietary intake of calcium was significantly lower at six-months in the low-CHO group compared to the high-CHO group whether reported as absolute intake (613 ± 216 mg/d vs 793 ± 522 mg/d; $p<0.05$) or as dietary calcium density (402 ± 164 mg/1000 kcal vs 512 ± 276 mg/1000 kcal; $p<0.05$). The low-CHO group lost more weight (-13 ± 0.9 kg) than the high CHO group (-8 ± 1 kg; $p<0.01$). The mean change from baseline in urinary calcium excretion was higher in the low-CHO group than the high-CHO group (0.099 ± 0.03 mmol/mmol creatinine vs 0.014 ± 0.23 mmol/mmol creatinine; $p<0.05$). The mean change in urinary calcium excretion rate was higher in the low-CHO group than the high-CHO group (46 ± 17 mg/24 hr vs -3.5 ± 11 mg/24 hr; $p<0.01$). There were no significant changes in bone turnover markers within or between groups. Whole body BMC increased in the low-CHO group ($p<0.05$); however, the changes in whole body BMC were not significantly different between groups. Spine BMC increased significantly in both groups ($p<0.01$ and $p<0.05$, respectively), but the changes were not significantly different between groups at six-months. No other significant differences or changes in bone quality were found.

These results provide evidence that low-CHO diets followed to promote weight loss in an overweight and obese population increase urinary calcium excretion compared to a high-CHO diet but do not change bone turnover markers or bone quality over six months.

SIGNIFICANCE

There is a rising epidemic of obesity in the United States, accompanied by a general effort by the populace to lose weight (1). As a result, obese and overweight people alike have adopted diets to lose weight and lower their risk of current and future health problems. Low-carbohydrate (CHO) diets are popular, in part because they promise rapid weight loss without hunger or adverse effects on health. While research of the efficacy and safety of low-CHO diets has focused primarily on weight loss and effects on cardiovascular disease risk markers, there is only a minimum amount of research on the impact on bone health: short-term impact (e.g. two days to two months) and long-term impact on bone quality. Bone quality, however, is an important outcome variable to measure as diets low in carbohydrate are by nature high in fat and protein and low in bone-related micronutrients, such as calcium, potassium, and magnesium. A low-CHO diet pattern may be detrimental to bone health, and may promote increased urinary calcium excretion and higher rates of bone turnover, and in the long run, lower bone quality and increased fracture risk.

This analysis is important because the long-term (i.e. more than six months) effects of a low-CHO, high-protein diet on bone quality are not conclusive, and because of the lack of randomized controlled trials performed to determine the long-term effects of low-CHO diets on urinary calcium excretion, bone turnover markers, and bone quality. This study's results are significant because of the six-month duration of the low-carbohydrate diet, and it provides a new perspective of the impact of low-CHO diets on bone health of free-living, overweight and obese subjects.

SPECIFIC AIMS

The primary aim of this study was to measure the impact of two different weight loss diets with different macronutrient composition on urinary calcium excretion. We hypothesized that the high-protein, high-fat dietary pattern of a low-carbohydrate diet would increase urinary calcium excretion compared to a high-carbohydrate diet.

The secondary aim of this study was to compare the changes in bone turnover markers after the high-carbohydrate and low-carbohydrate diets by measuring serum bone-specific alkaline phosphatase (BSAP) and urinary N-telopeptide (NTx). We hypothesized that the low-carbohydrate diet would result in higher rates of bone turnover than the high-carbohydrate diet, measured by higher concentrations of the bone resorption marker NTx and lower concentrations of the bone formation marker BSAP.

The tertiary aim of this study was to measure the impact of the two six-month behavioral weight loss interventions on bone quality (assessed by dual-energy X-ray absorptiometry) by measuring whole body, left hip and lumbar spine bone mineral content (BMC) and bone mineral density (BMD), and hip and spine BMD t- and z-scores. We hypothesized that these bone quality measures would be lower after the low-carbohydrate diet than the high-carbohydrate diet.

BACKGROUND

CHAPTER ONE: OBESITY AND OSTEOPOROSIS

OBESITY

Obesity by definition is an excess accumulation of body fat; however, since it can be difficult to measure body fat, Body Mass Index (BMI) is used as an indicator of body composition in the general population (2). A BMI of less than 25 kg/m^2 is considered "healthy" (3). Although a value of 25 kg/m^2 is the cutoff point for a "healthy" BMI, more than half of adults in the United States have a BMI over 25kg/m^2 and are categorized as overweight; also, more than one-third of American adults have a BMI greater than 30 kg/m^2 and are considered obese (4). Expert panels estimate that at least 60% of Americans are overweight or obese based on BMI calculations (5). The high prevalence of overweight in the U.S. is comparable to other industrialized countries' growing obesity rates, but the current U.S. rate of overweight and obesity is one of the highest in the world (4). This illustrates the point that obesity needs to be prevented and treated to avoid the problems associated with excess body weight and fat.

Obesity is a serious problem in the United States due in part to its common and costly co-morbidities and complications: diabetes, hypertension, dyslipidemia, cardiovascular disease, cerebrovascular disease, respiratory disease, gastrointestinal dysfunction, chronic kidney disease, and certain types of cancer (6). The costs of obesity and its related problems are staggering. One study estimated that approximately 27% of national health care costs are spent on problems associated with physical inactivity, overweight, and obesity (7). A different study concluded that in the U.S., an estimated 96.2 billion dollars is spent annually on obesity-related conditions (8). This shows the

magnitude of the problem; it also shows that obesity prevention, including weight loss, is important to avoid costly complications associated with increased body weight.

OSTEOPOROSIS

Along with the increasing rate of obesity, there is a corresponding high prevalence of osteoporosis in the United States population. This is a paradox because osteoporosis is normally thought of as a disease among elderly frail women. Osteoporosis is a metabolic bone disease characterized by abnormal bone remodeling. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increased susceptibility to bone fractures (9). Currently 10 million Americans are estimated to have the disease and 34 million have low bone mass (osteopenia), which makes them at a higher risk of developing osteoporosis (10). Over the age of 50, four in ten women will experience a forearm, hip, or vertebral fracture, and are more likely to die from fracture-related complications than breast cancer (12). The costs of osteoporosis and osteoporosis-related fractures are estimated to be $10-20 billion annually in the U.S. alone (12). Hip fractures related to osteoporosis can be debilitating and may result in incapacitation, long-term nursing care, and death (13). The high costs of osteoporosis and related injuries demonstrate a need for the population to prevent low bone mass by avoiding lifestyles and dietary choices that negatively impact bone quality.

Osteoporosis, like obesity, is related to dietary factors and the risk of developing osteoporosis can be minimized by consuming a diet high in calcium, vitamin D, and other related nutrients such as potassium, vitamins A and K, and magnesium, which may

be consumed in lower amounts in low-carbohydrate dietary patterns compared to high-carbohydrate dietary patterns. Lifestyle and risk factors that may increase the incidence of low bone mass in the population include low calcium intake, physical inactivity, Vitamin D deficiency, cigarette smoking, and low or high dietary protein intake (10). Osteoporosis is a complicated condition that is affected by many factors, but should be prevented to avoid the high costs, related injuries and fractures, and reduced quality of life related to the injuries.

BMI AND BONE

Genetics, lifestyle, and diet all play important roles in bone quality, as does a person's body weight. In the general population, obesity and a higher BMI are associated with a positive relationship with bone mass (14). In a cross-sectional study of 958 women between the ages of 50 and 84, the odds ratio of being osteoporotic or osteopenic was significantly lower in women with a higher BMI. Also, the odds of bone loss decreased by 12% for each unit increase in BMI (15). A prospective cohort study of 2025 women in Finland found that women with a BMI of 25-30 kg/m^2 had a higher BMD than those with a lower BMI (16). These studies demonstrate that there is a positive relationship between BMI and BMD. One study suggested that BMI is a better predictor of bone density than weight alone, as BMI may more accurately represent body composition (15). The positive association between BMI and BMD may be due to the greater mass and pressure exerted on the weight-bearing joints and skeleton, therefore increasing the amount of bone mass necessary to support the extra weight (14).

Therefore, it is often observed that those with a higher body mass and weight have increased bone mass.

Fat Mass and Bone

Greater fat mass, as well as increased fat-free mass, has been observed in obese people compared to non-overweight people; this increase in fat mass may contribute to the increased BMD observed in obesity (17). Leptin, a hormone mainly secreted by adipose tissue, may be associated with the protective effect that increased fat mass has on bone. A cross-sectional study of 204 women, aged 18-40 years, found that fasting leptin concentration was significantly related to BMC and femoral neck and lumbar spine BMD, but not after adjusting for body mass and fat mass (18). This suggests that there may be a relationship between leptin and bone quality; however, the relationship may be confounded by fat mass and body size. Body fat may also be an indirect protector of bone mass as it provides a source and warehouse of peripheral conversion of the pro-hormone androstenedione to the metabolically active form of estrogen, estrone (19,20), which is protective against bone loss. These studies suggest that the relationship between fat mass and bone quality is complex and dependent on many biological variables.

Body Weight and Fracture Risk

Although increased body mass and fat mass may be positively associated with BMD, the relationship between obesity and fracture risk is not conclusive. In a large prospective study of 16,047 European men and women, those women with a higher BMI

had a decreased risk of vertebral deformities (21). Body weight was examined in a study comparing hip fracture patients with controls; Hassager et al found that the patients were 3.5 kg lighter than the controls (22). For the most part, evidence shows that increased body weight is related to a lower fracture risk, but some studies have concluded differently. A prospective cohort study of 11,798 women found that those with a BMI of 25-30 kg/m^2 had a significantly higher hazard ratio of ankle fracture than those with a BMI of less than 25 kg/m^2 (23). Another prospective study of 9559 women found that women with a higher BMI had higher rates of ankle fractures (24), possibly due to the greater mass and pressure exerted on the weight-bearing joint. Obesity may not be the only factor that predisposes a person to increased risk for fractures, but also the co-morbidities associated with obesity. A prospective study of 3078 women found that obesity, when coupled with hypertension, increased the hazard ratio of fracture to 2.0 compared to non-obese women with hypertension (25). Although increased body weight may be associated with decreased risk of fracture, certain types of fractures may be more prevalent in the obese population. These observations suggest that fractures are not solely confined to individuals with low body mass.

CHAPTER TWO: BONE HEALTH

BONE COMPOSITION AND PHYSIOLOGY

Bone is composed of collagen fibers, non-collagenous proteins, and deposited minerals, mostly the inorganic mineral hydroxyapatite, $Ca_5(PO_4)_3(OH)_2$. Bone is divided into two types: cortical (compact) bone and trabecular (spongy) bone. Cortical bone composes 80% of the total bone mass, making up the thick shafts of arms and legs (26). Trabecular bone composes 20% of the total bone mass in the axial skeleton. Trabecular bone is more important in calcium regulation and bone remodeling due to the fivefold increase in surface area compared to cortical bone (26). Bone remodeling, which can maintain and regulate extracellular calcium, is mediated by two main bone cell types: osteoblasts and osteoclasts.

Calcium is an important physiological component of bone. Since the main two minerals deposited in bone are calcium and phosphorus, the body uses bone as a reservoir for those minerals. Extracellular calcium concentration is tightly regulated; so when the serum calcium concentration is high, calcium is deposited into bone, and when the serum calcium concentration is low, calcium is released from bone. These two processes are mediated by three different hormones: calcitonin, parathyroid hormone (PTH), and 1,25-dihydroxycholecalciferol (vitamin D_3). Calcitonin is released from the thyroid gland in response to hypercalcemia and inhibits osteoclast activity and opposes PTH actions. PTH is released from the parathyroid gland in response to hypocalcemia and acts on kidney and bone to increase extracellular calcium concentration. PTH increases resorption by stimulating expression of Receptor Activator of NF-κB Ligand (RANKL), which promotes osteoclast differentiation and activation; also, PTH increases

the activity of the enzyme (1α-hydroxylase) that converts 25-hydroxyvitamin D to its active form, vitamin D_3. Active vitamin D increases intestinal calcium absorption by increasing the expression of a membrane calcium channel, CaT1, and calbindin (a channel protein that transports calcium across enterocytes in intestines). Osteoblasts also have vitamin D receptors and produce vitamin D-regulated proteins, such as osteocalcin. Thus, it can be observed that the bone formation and resorption processes are complex and involve several factors, including hormones and dietary intake.

BONE REMODELING

Bone is constantly changing and undergoing a metabolic process called remodeling (27), which consists of two interrelated phases: bone formation and bone resorption. This coupled process is necessary for normal development and skeletal maintenance; it is tightly regulated and is considered to be in balance when rates of resorption and formation are equal. Bone formation takes about three months to complete, and resorption takes about 45 days (28). Bone formation is mediated by osteoblasts. Osteoblasts, which have receptors for PTH, estrogen, and vitamin D, mediate the deposition of hydroxyapatite in bone tissue (28). Osteoblasts synthesize and deposit calcium and phosphorus (in the form of phosphate, PO_4) into the bone matrix and secrete Type I collagen on bone surface (26). Bone resorption is mediated by osteoclasts, which dissolve minerals from the matrix and release them into the blood. Osteoclasts release lysosomal enzymes and collagenase, as well as protons, to acidify the environment. The enzymes break down the bone matrix, and the acid dissolves the hydroxyapatite crystals. Osteoclast regulation is complex because of the large number

of receptors and other physiological factors that impact osteoclast activity. Osteoclasts have calcitonin receptors, which decrease their activity; also, osteoclasts respond to other regulatory signals, such as PTH, vitamin D, and prostaglandin E_2, via osteoblast recruitment of osteoclasts (28).

There are several biomarkers used clinically to approximate osteoclastic (resorption) and osteoblastic (formation) activity. Urinary calcium excretion may approximate resorption, although it is not technically a specific marker of bone resorption. There are three proposed physiological mechanisms by which excessive urinary calcium excretion can occur: 1) a renal leak of calcium due to decreased renal reabsorption, 2) increased bone resorption generating an increased filtered load of calcium, and 3) hyperabsorption of calcium in the gut which also may result in an increased filtered load of calcium (29). The second mechanism is the reason why increased urinary calcium excretion and hypercalciuria are considered indicators of excessive bone resorption.

Other biomarkers of bone resorption are utilized to provide a more specific measure of osteoclast activity. A commonly used biomarker of bone resorption is cross-linked N-telopeptides (NTx) of Type I Collagen in urine. Approximately 90% of the organic matrix of bone tissue is type I collagen. Type I collagen, a helical protein that is cross-linked at the N-terminal and C-terminal ends of the molecule, forms the basic fabric and tensile strength of bone tissue. Generation of the NTx molecule is mediated by osteoclasts on bone, and is found in the urine as a stable end-product of degradation (30). A bone formation marker commonly used is bone-specific alkaline phosphatase (abbreviated BSAP and BAP). The skeletal isoform (bone-specific) of alkaline

phosphatase is a tetrameric glycoprotein found on the cell surface of osteoblasts (31). The exact function of this substance is not known, but its role in skeletal mineralization and formation has been well-documented (31,32). Additional bone formation and resorption markers are listed in Table 1. Bone turnover markers are useful tools in clinical settings and research to estimate osteoblast and osteoclast activity. As an adult, it is hazardous when bone resorption rates exceed formation rates because it can lead to osteoporosis and increased risk of fracture, so it is beneficial to measure both markers of bone formation and resorption to obtain the best perspective of a person's level of bone remodeling.

***Table 1**: Bone formation and bone resorption markers, their functions, and their advantages and disadvantages of use*

Bone Turnover	Marker	Function	Advantages	Disadvantages
Resorption	*Cross-linked telopeptides of Type I collagen (NTx and CTx)*	Type I collagen; stable end product of degradation	See greater changes in shorter amount of time; most commonly used; ease of analysis	Greater variation; circadian rhythm and variation
	Deoxypyridinonline (DPD)	Type I collagen released in bone resorption	Clear role in bone resorption	Not commonly used
	Hydroxyproline (OHP)	Degradation product from helical portion of Type I collagen	Commonly used as a bone resorption marker	Use has been superseded by newer markers
Formation	*Serum Bone-Specific Alkaline Phosphatase (BSAP)*	Membrane-bound osteoblast enzyme	Ease of analysis; comparison to other studies	Difficult to see changes early on
	Osteocalcin (OC)	Most abundant non-collagenous protein in bone	Ease of analysis; strong role in bone turnover	Role in metabolism; can be used as a bone resorption marker (33)
	Procollagen Type I propeptide (PINP)	Released from osteoblasts in formation	Clear role in bone formation	Not totally bone-specific (34); limited number of assays

BONE QUALITY

Bone quality is a quantitative parameter measured by bone mass, content, and density, as well as a comparative parameter measured by t-scores and z-scores. All of this data can be measured by utilizing a technology that measures bone mass and body composition. In research, a commonly used method for measuring bone mass is dual-energy X-ray absorptiometry (DEXA). DEXA scanners measure bone quality by scanning an individual's body with an x-ray and relaying the information to a computer software program to analyze the image. The computer imaging software displays the individual's body and distinguishes between the fat mass, lean mass, and bone mass in grams by the amount of the x-ray absorbed by the different tissues. The computer software then measures the bone area in cm, bone mineral content in grams, and bone mineral density in gm/cm^2; also, the t- and z-scores are calculated by comparing to population averages of bone mineral density. T- and z-scores are statistical measures that demonstrate how many standard deviations a certain value is from the mean. T-scores for BMD are based on "young-matched" (meaning a healthy 30-year old person) population averages that compare a person's bone mineral density (for whole body or regional areas) to people of the same gender and ethnicity. T-scores are often used clinically to diagnose low bone mass: a t-score between -1 and -2.5 indicates osteopenia, and a t-score below -2.5 indicates osteoporosis. Z-scores are used to compare a person's bone mineral density to people of the same gender, age, and ethnicity.

URINARY CALCIUM EXCRETION AND BONE QUALITY

Urinary calcium excretion must be linked with low BMD, osteoporosis, or increased fracture risk to strengthen the argument that increased excretion signifies an increased loss of calcium from the bone matrix. A cross-sectional study found that, among Chinese women 65-75 years old, urinary calcium excretion was negatively associated with size-adjusted BMD of femoral neck and lumbar spine (35). Giannini et al found in a retrospective chart-review study that urinary calcium was significantly higher in women with the worst degree of osteoporosis (36). Also, Vezzoli et al found that Italian men with the highest levels of urinary calcium excretion had significantly lower trabecular vBMD (volumetric bone mineral density) z-scores (37). On the contrary, Giannini also found that the presence of hypercalciuria was not related to bone density t-scores (36). Another study concluded that 24-hour urinary calcium excretion was not significantly related to BMD (38). In people with idiopathic hypercalciuria, urine calcium excretion significantly predicted bone loss in the femoral neck and spine over a three-year period (39). However, in the same study, bone turnover markers did not significantly predict bone loss in the hypercalciuric subjects, showing that there is not always a corresponding change between urinary calcium excretion and bone turnover markers. In a study of postmenopausal women, fasting urinary calcium correlated with bone loss and BMD over a five-year period (40). It also significantly predicted bone loss over the time period, but was not the best predictor of bone loss of the bone resorption markers. These studies strengthen the view that excessive urinary calcium excretion can be used as a bone resorption marker.

CHAPTER THREE: DIET AND BONE HEALTH

MINERALS AND BONE

Dietary macronutrient composition has been linked to bone quality, as well as many minerals including calcium, phosphorus, sodium, zinc, and magnesium. A relationship between sodium intake and bone quality has been observed in many studies, although the magnitude of the relationship is still controversial. An increased sodium intake is related to increased urinary sodium and calcium excretion (41), and also related to increased concentrations of bone turnover markers (42). Increased phosphorus intake theoretically could be linked with increased bone turnover due to PTH's response to hyperphosphatemia. However, only a few studies have demonstrated a negative association with bone quality (43), and a solid relationship between bone and phosphorus intake has been difficult to prove (41). Magnesium supplementation has shown to increase bone mineral density and decrease fracture risk in menopausal women (40), but its role in bone quality from diet alone has not been established. However, in a different study about fruit and vegetables, nutrients associated with an increased fruit and vegetable intake (including magnesium, potassium, and fiber) were related to an increase in bone mineral density in an elderly population (45). Zinc's relationship to bone has also been examined, but the relationship is still not established. Zinc supplementation has been linked to an increase in circulating insulin-like growth factor 1 (IGF-1) concentrations (46), which is related to bone growth; however, other studies have found no connections between zinc intake or zinc supplementation on bone turnover (41). Mineral intake can affect bone, but many of the relationships still remain unclear.

PROTEIN AND URINARY CALCIUM EXCRETION

Protein intake has been linked to increased urinary calcium excretion, and furthermore a theorized increased loss of calcium from bone. This is based on the theory that high-protein foods and certain nutrients (such as anions of chloride, phosphorus, and sulfur) can contribute to the acid-forming potential of the urine (47). In a crossover study comparing diets similar in protein, calcium, phosphorus, and sodium composition that were high in either acid ash-forming foods or base-forming foods found that the diet high in acid ash significantly increased urinary calcium excretion, increased acidity of blood and urine, and increased urinary C-telopeptide excretion (48). In 1959, Lemann and Relman developed a theory from results of several experiments and studies that sulfur-containing amino acids (e.g. cysteine and methionine) increase urinary calcium excretion, as well as the net acid excretion (49). In an acidic environment, PTH indirectly stimulates the release of calcium and phosphate from bone by signaling osteoblasts to recruit osteoclasts to buffer the blood's pH and bring it back to a normal level. Also, high-protein diets increase blood flow to the kidneys, increase glomerular filtration rate, thereby increasing the intrarenal blood pressure (50). Allpern et al suggest that a high-protein, high-meat diet may induce chronic metabolic acidosis, consequently leading to a continuous leaching of minerals (i.e. calcium and phosphorus) from the bone matrix (51). Controlled trials have found that a higher protein intake resulted in increased urinary calcium excretion (52-57). Also, a moderate restriction of dietary protein intake in hypercalciruic stone-forming subjects resulted in significantly decreased urinary calcium excretion (58). These studies all demonstrate a strong

relationship between protein and urinary calcium excretion as well as an association with acid excretion.

Although urinary calcium excretion may increase with a higher protein intake, it can also be due to increased intestinal calcium absorption (59). Some studies have focused on specific amino acids as the source of increased urine excretion of calcium via increased intestinal absorption. In a study by Dawson-Hughes et al, subjects with a usual protein intake (<0.75 g/kg/d) were administered diets either high in branched-chain amino acids (leucine and isoleucine) or aromatic acids (histidine and phenylalanine) to gauge the effects on urinary calcium excretion (60). Aromatic amino acids significantly increased urinary calcium excretion compared to the branched-chain amino acids. Bone turnover changes were not significantly different between the two groups; therefore, the increased calcium excretion appears to have been from increased intestinal absorption, due to the hypothesized aromatic amino acids' interaction with the calcium-sensing receptor in the intestine. This demonstrates that increased urinary calcium excretion caused by increased protein intake may not be due to increased bone resorption, but possibly from increased absorption of calcium from diet.

HIGH-PROTEIN DIETS AND BONE TURNOVER MARKERS

The impact of high-protein diets on bone turnover markers has been studied in relation to bone formation and resorption. Osteocalcin and BSAP are used in research to estimate the amount of bone formation occurring at a certain time. One randomized cross-over study with three, four-day dietary interventions, found that BSAP was higher in individuals consuming a low-protein diet (0.7 g/kg body weight) compared to a

medium-protein diet (1.0 g/kg) (52). A different study found that a high-protein, low-carbohydrate diet, when consumed for six weeks, resulted in a lower osteocalcin concentration (61). However, other studies found that increased protein intake did not affect bone formation markers (53,62-64).

Bone resorption markers, like serum PTH and urinary and serum NTx, have also been studied in response to a high-protein diet. N-telopeptide did not change as a result of higher protein intake in several studies (54,61,62,65), and no effect on PTH was found in these studies (54,61,64,65). Conversely, the cross-over study of three dietary protein levels (low, medium, and high) over four days found that urinary NTx concentration was significantly higher after consumption of the high-protein diet compared to the low-protein intervention ($p<.05$) (52). Harrington et al also found in a crossover trial of a high-protein, high-sodium diet and a normal-protein, low-sodium diet, that the high-protein, high-sodium diet significantly increased urinary NTx concentration ($p<.05$) (53). A study of subjects with idiopathic hypercalciuria and nephrolithiasis found that lowering dietary protein intake to 0.8 g/kg resulted in lower bone resorption rates, as measured by urinary hydroxyproline excretion (58). This coincides with the result from a feeding study by Ince et al, which demonstrated that when healthy, premenopausal women lowered their protein intake to the Dietary Reference Intake of 0.8 g/kg, urinary NTx concentration was decreased by 17% ($p<.001$) (55). Although there is some evidence that implies a diet high in protein suppresses bone formation and stimulates bone resorption, most of the evidence suggests that there is no significant change in the concentration of bone turnover markers and the rate of bone turnover.

PROTEIN, BMD, AND FRACTURE RISK

The relationship between bone quality and diet is complicated; various nutrients, including protein, fat, calcium, phosphorus, magnesium and others (66) can affect bone health. Currently, protein's role in bone health is being questioned. A large number of epidemiological studies have investigated the effects of protein on long-term bone health as measured by bone mineral density, fracture rates or risk of fracture, and cases of osteoporosis. The results of high-protein vs. low-protein on bone quality remain inconsistent. Promislow et al concluded from a prospective study of 960 men and women aged 55-92 years that total protein intake was positively correlated with BMD in women (67). Although protein was positively correlated with BMD in the previous study, a cross-sectional study in 1986 found that increased dietary protein intake was positively correlated with hip fracture rates (68). A prospective study of 85,900 female nurses found that, after adjusting for age, those in the highest quintile of total protein intake had an increased relative risk (RR) of forearm fracture (RR = 1.29) versus the women in the lowest quintile of protein intake (69). Conversely, the same set of data found that a higher protein intake was associated with a lower risk of hip fracture (RR = 0.64). A relationship may also exist between dietary protein and calcium intakes and bone health. In a Norwegian prospective study, Meyer et al found that a diet high in protein and low in calcium was associated with an increased risk of fracture in women (RR = 1.96) (70). These results demonstrate the complexity of the relationship between protein and long-term bone health.

Animal Protein and Bone

Another complicating factor of protein's relationship with bone is that the source of protein may be more important than the total amount of protein. A prospective cohort study of 1035 women >65 years found that those with a high animal to vegetable protein ratio had increased annual femoral bone loss compared to those with a low ratio (0.78% and 0.21%, respectively) (71). The same study also concluded that the women with the highest ratio of animal to vegetable protein intake had an increased risk of hip fracture (RR = 3.7) compared to those with a low ratio. The Nurses Health Study also concluded that high animal protein intake is related to fracture risk; a high animal protein intake was associated with an increased risk of forearm fracture (RR = 1.21) (69). This demonstrates that the sources of dietary protein, as well as the ratio between the protein sources, are important considerations in bone health.

Randomized Controlled Trials of Protein Intake and BMD

There is a need for more randomized, controlled trials (RCT) that examine the effects of high-protein or low-carbohydrate intake on BMD. In a 12-week RCT of 100 women on either a high-protein diet or a high-carbohydrate weight loss diet, no difference in BMD between the two interventions was observed (62). However, interpretation of this study's results is limited by the length of the intervention, and the diet being high-protein instead of a low-carbohydrate diet. The obvious lack of intervention studies of high-protein or low-carbohydrate diets that examine bone mineral density show that no convincing conclusions have been made, and that changes

in bone mineral density may be too slow-moving or too complex to be captured in a short-term study.

FAT INTAKE AND CALCIUM ABSORPTION

Calcium absorption and dietary macronutrient intake are inherently linked due to the interrelationships in nutrient digestion, absorption, and metabolism. The relationship between calcium absorption and lipid metabolism has been studied for years with inconclusive results. In 1977, Gacs et al found that calcium soap formation in the intestines was related to saturated fatty acid length (72). This soap formation indicated that increased consumption of saturated fatty acids of certain lengths can inhibit calcium absorption. However, this study was performed in rats, the form of calcium studied and delivered to the rats was in soap form, and the calcium and fatty acid soaps were delivered to the duodenum directly (not ingested from food). Therefore, the conclusions drawn from this study can only be interpreted for the limited conditions studied. Other studies have examined the relationship between calcium intake via supplementation and the formation of micelles in the intestines, thereby inhibiting lipid absorption (73). With short-term dietary calcium supplementation and food fortification (~2200 mg/day), fecal saturated fat content was increased and serum lipids were decreased. This study, however, did not evaluate calcium absorption in relation to lipid intake. Most studies have focused on increasing calcium intake and measuring serum lipids and lipid absorption (as measured by fecal fat content) as the main outcome variables, so the results from these studies may not be completely applicable to the current study which is examining the effects of a high-fat diet on bone.

To examine the effects of a high-fat diet on bone health, studies have mainly used animals as subjects. One study on roosters found that roosters fed a high-fat diet had similar bone properties as those fed a low-fat diet, but the roosters fed a low-fat diet had greater bone mineral content and cancellous bone measures (74). This study concluded that cancellous bone is more sensitive than cortical bone to the high-fat diet; also, adult cortical bone is not as sensitive to diet as are growing animals. Although this study was not performed in humans, it brings up an interesting point that bone growth may be affected by a high-fat diet. In addition to the rooster study, a different study found that mice fed a high-fat atherogenic diet had lower BMD after four months and lower BMC after seven months as measured by peripheral quantitative computed tomographic scans (75). The authors hypothesized that lipids interfere with osteoblast maturation and osteoclast expression. An opposite relationship was found in humans; a study of pre- and peri-menopausal women found that dietary fat was positively linked with fractional calcium absorption (76). This result was explained by fat increasing transit time in the gastrointestinal tract, thereby increasing the amount of time the absorptive surface has contact with the ingested calcium. The participants of this study had a lower fat intake than US averages, so the positive association between fat and calcium absorption may peak at a certain percentage of energy from fat. The association between high-fat intake and bone needs to be further examined to fully explore the relationship between fat and calcium balance.

WEIGHT LOSS AND BONE

When a person is trying to lose weight, he or she will often change composition of the diet. This may mean that the person will consume less of the bone-related nutrients and put themselves at risk for bone loss, but weight loss in itself may cause bone loss. Weight loss is beneficial to obese and overweight people because it is often accompanied by a corresponding decline in insulin resistance, cardiovascular disease markers, and decreased risk for other weight-related chronic conditions. However, weight loss does not benefit bone mass; when weight loss occurs, bone resorption increases (77) and bone mass is lost (78). Consequently, weight loss is associated with bone loss and lower bone density. One randomized controlled trial found a weight loss method that eliminated a decline in bone mass. In two weight loss groups, one group followed a energy-restricted diet while the other group exercised to match the 16% energy deficit in the first group. The energy-restricted group had a significant decrease in BMD, while the exercise group did not have a significant change in BMD (79). This suggests that exercise-induced weight loss can offset the expected decrease in BMD. Another determinant in bone loss from weight loss is an individual's weight before a weight loss intervention. When comparing normal weight individuals and obese individuals during weight loss, obese individuals lost a lower percentage of bone mass (78). These studies suggest that bone loss occurs in energy-restricted diets, but the amount of bone lost may be mitigated by physical activity and pre-loss body weight.

CHAPTER FOUR: HIGH- AND LOW-CARBOHYDRATE DIETS AND BONE HEALTH

DIETARY APPROACHES TO STOP HYPERTENSION (DASH)

The DASH diet is a dietary pattern designed to lower blood pressure by emphasizing consumption of whole grains, fruits and vegetables, low- and non-fat dairy, nuts and seeds, lean meats, and avoidance of foods high in saturated fat, concentrated sugars and sodium (80). Consequently, it is high in calcium, magnesium, potassium, and fiber (81). Additionally, a cross-sectional study concluded that a diet high in fruits and vegetables (like the DASH diet) was correlated with higher bone mineral density (82). The diet's high levels of these bone-related minerals and potential benefits make the DASH diet a potential model dietary pattern for improving bone quality, especially in hypertensive populations.

DASH DIET AND BONE

The DASH diet was initially designed to lower blood pressure; consequently, the diet's effects on bone are limited. A clinical trial of 186 men and women comparing the bone turnover of participants on the DASH diet and the "typical" American diet found that the DASH diet significantly decreased both circulating osteocalcin and C-terminal telopeptide of Type I collagen concentrations compared to the control diet, thereby suggesting a decreased rate of bone turnover (83). This study suggests that a diet high in whole grains, low-fat dairy products, and fruits and vegetables may be beneficial in decreasing bone turnover, leading to improved bone quality.

LOW-CARBOHYDRATE DIETS

Although the popularity of low-carbohydrate diets may have waned in the general population in recent years, the diet was once widespread in the United States population. During the low-CHO diet's rise in 2002, a telephone survey showed that approximately 12.5% of people surveyed reported ever using a low-CHO diet for weight loss, and 3.4% of the respondents reported a current low-CHO diet regimen (84). Among users, 40% of males and 30% of females reported long-term use of a low-CHO diet for weight maintenance. The safety of long-term use of a low-CHO diet on bone has yet to be determined because a diet that is high in protein and fat may affect bone quality. The popular Atkins and South Beach diets are promoted as low in carbohydrate and high in fat and protein (85). An article in Consumer Reports recounts each diet's composition: the "induction" phase of the Atkins diet has an approximate macronutrient distribution of 8% of calories from carbohydrate, 31% from protein, and 61% from fat. Phase I in the South Beach Diet is similar in distribution: 15% of energy from carbohydrate, 34% from protein, and 51% from fat. Low-CHO diets are markedly different than the Dietary Reference Intake recommendations of 45-60% from CHO, 20-35% from fat, and 10-35% from protein (86). The imbalance of macronutrients in a low-CHO diet compared to recommended amounts may cause disturbances in a body's normal homeostatic functions.

LOW-CARBOHYDRATE DIETS AND BONE

A low-CHO diet and its effects on bone health have been studied in only a few studies, with contrasting results and various short-term study lengths (from six weeks to

three months). A metabolic study of 10 people found that an Atkins-type diet increased calcium excretion without compensating with increased intestinal calcium absorption (61). Calcium balance, as measured by urinary excretion and fractional intestinal absorption, decreased by 130 mg/day during the "induction" phase (at <20 grams of CHO/day for two weeks) and 90 mg/day for four weeks in the "maintenance" diet. Consumption of the diet also significantly decreased serum osteocalcin concentrations. The authors suggest that a theoretical increased acid load from the low-CHO diet, both from increased protein intake and the increased production and utilization of ketones, may be deleterious to bone health.

On the opposite side of the debate, a case-control study of a low-carbohydrate diet had the case group (15 people) follow an Atkins-type diet of less than 20 grams of carbohydrates for one month, and less than 40 grams of carbohydrates for two months. The controls were matched to the case group by age and weight, and were instructed to consume their usual diet. The case group did not experience a significant change in urinary NTx or BSAP, nor was there a significant difference between the case and control groups in other bone turnover markers (63). Even though both of these studies concluded differently about the impact on bone health, the small sample sizes of the study and the absence of a true control group make it difficult to formulate any definite conclusions. In addition to these studies, a study that compares the effects of a ketogenic versus non-ketogenic low-carbohydrate, high-protein diet found that the ketogenic diet (5% of energy as carbohydrates) limited potassium-rich fruits and vegetables, and consequently disturbed the acid-base balance and caused increased urinary calcium excretion (87). This suggests that the presence and utilization of ketones

in a low-carbohydrate diet is deleterious to bone by possibly increasing bone resorption or increasing urinary excretion of calcium.

Research Study Methods

Experimental Design

This study used a prospective, thirty-month behavioral, parallel cohort interventional design. Overweight and obese participants (n=260) were enrolled in "Metabolic Consequences of Low and High Carbohydrate Diets Study" (a.k.a. the "Insight Weight Loss Study"). Balanced allocation to one of two dietary counseling interventions was performed before baseline measurements were taken to minimize assignment bias and differences between groups. The two comparison diets were a low-carbohydrate diet and a high-carbohydrate diet. Experienced weight loss counselors and interventionists conducted weekly group sessions for six-months at the Kaiser Permanente Center for Health Research (KPCHR) to facilitate compliance with the diet interventions.

A sub-analysis of 115 participants was conducted to analyze the effects on bone health over six-month duration. The data used to assess the effects of diet on bone quality is derived solely from the participants that had hip and spine DEXA scans. 58 people were assigned to the low-carbohydrate diet group and 57 to the high-carbohydrate diet group. Differences in dietary and biological parameters at six months were compared to baseline values within diet groups and changes from baseline between the two diet groups.

SUBJECTS

Participants included in the sub-analysis were overweight and obese (BMI 27-50 kg/m^2) men and non-pregnant and non-lactating women enrolled in the Insight study. Inclusion and exclusion criteria for participation in the Insight study are provided in Table 2. Furthermore, subjects were excluded from this sub-analysis of bone mineral analysis if the DEXA scan showed any abnormalities, such as metal pins or prostheses. Subjects were excluded from dietary analysis if energy levels were above 5000 kcal/d or below 800 kcal/d. Also, subjects were excluded from urine analysis if they did not successfully complete a 24-hour collection.

***Table 2**: Inclusion and exclusion criteria for the Insight Weight Loss Study*

INCLUSION CRITERIA	• Individuals 21 years of age or older • BMI of 27-50 kg/m^2 • Normal blood pressure, or stable high blood pressure (taking three or fewer hypertensive medications) • Fasting glucose of <126 mg/dl • Total fasting cholesterol of <260 mg/dl • Total fasting triglycerides of <300 mg/dl • Normal liver and kidney function • Willing to modify diet and other health behaviors • Able to give consent
EXCLUSION CRITERIA	• Contraindication for weight loss (e.g., malignancy or other serious condition) • Renal insufficiency (Glomeular Filtration Rate <60 ml/min as assessed by Cockroft-Gault equation) • Cardiovascular disease event in past year • Cancer diagnosis or treatment in the past two years • Psychiatric hospitalization within preceding two years • Consumption of more than three alcoholic drinks a day • Diagnosed type I or II diabetes or fasting blood sugar ≥126 mg/dl • Medication use including, but not limited to, hypolipidemics, anti-psychotics, hypoglycemics, or antidepressants • Other miscellaneous reasons—planning to move before study ends, pregnant, breastfeeding, or planning pregnancy before the end of study participation, current participation in another clinical trial, investigator discretion for safety, or compliance reasons.

BEHAVIORAL DIETARY INTERVENTION

An environment conducive to weight loss with a peer support network was created by scheduled group meetings with lifestyle change interventionists and other participants. The intervention strategy was based on the transtheoretical, or stages-of-change model (87), in combination with the motivational interviewing technique (88). The participants had weekly group counseling sessions at KPCHR to facilitate weight loss, encourage physical activity, and promote dietary compliance. At the sessions, participants were able to taste foods, watch cooking demonstrations, learn how to exercise, and discuss health and medical issues related to the diets.

The two diet groups used different dietary approaches, but had similar long-term goals: at least 10% weight loss from baseline and moderate-intensity exercise for at least 30 minutes most days of the week. The low-carbohydrate diet group completed two specific phases: induction phase in which the participants were counseled to limit carbohydrate intake to no more than 20 grams per day, and the gradual re-reintroduction of carbohydrates to the diet. During the second phase, participants were instructed to add carbohydrates back into the diet until weight loss stopped, and then to titrate carbohydrate intake back down to find a balance between carbohydrate intake and weight loss. The high-carbohydrate diet group were provided several recommendations: to eat eight to twelve servings of fruits and vegetables per day, to consume two to three servings of low- or non-fat dairy products each day, to reduce fat to 25% of total energy per day, to reduce portion sizes, and to reduce the consumption of sweets and sweetened beverages. With these very different approaches to weight

loss, it was expected that significant differences in dietary intake would be observed at six-months.

MEASUREMENTS

Participants' appointments were scheduled within a two month "window" of the baseline and six-month visits. Participants arrived for their scheduled morning appointments at the Oregon Clinical and Translational Research Institute (OCTRI) at the Oregon Health & Science University (OHSU) in Portland, Oregon between 7:00 and 8:30 AM after a 12-hour overnight fast.

24-hour urine collections for creatinine, mineral and NTx excretion were collected and recorded by Insight study staff. Blood pressure and blood samples for serum creatinine and BSAP were taken by the OCTRI nursing staff. Female participants provided a spot urine sample to verify non-pregnant status (Acceava human chorionic gonadotropin Combo test kit, Thermo BioStar, Boulder, CO). All participants were asked to change from street clothing to a hospital gown and to remove all metal-containing objects. Height, weight and DEXA measurements were obtained by trained and licensed technicians in the OCTRI's Body Energy and Composition Core (BECC) facility. After all measurements were completed, participants changed back into their street clothes and were provided a complimentary continental breakfast.

ANTHROPOMETRIC MEASUREMENTS

Participants' weight in a hospital gown & scrub pants was measured using a Scale-Tronix (Model 5002) stand-on-scale with digital display (Model 5002, Wheaton,

IL). Weight was measured in duplicate and each measurement was recorded to the nearest 0.01 kg. If the two measurements differed more than 0.5 kg, a third weight measurement was taken. Height (without shoes) was measured using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Ltd, Crymych, UK), and was recorded to the nearest 0.01 cm. Height was only measured at baseline and measurements were carried forward to six-months.

FASTING BLOOD SAMPLES

OCTRI nursing staff collected fasting blood samples by venipuncture using sterile technique. Blood samples were collected into pre-chilled red-top tubes and sent on ice to the OCTRI Core Laboratory for processing and storage at or below -70°C for batched analysis at the end of the study. Bone-Specific Alkaline Phosphatase (BSAP) was measured from fasting serum samples in the OCTRI Core Lab using an enzyme-linked immunoabsorbent assay commercial kit by Quidel Corporation (Metra BAP, San Diego, CA).

URINE COLLECTION AND ANALYSIS

Participants were given two non-reactive and light-protective plastic jugs for the 24-hour urine collection. Participants were instructed to collect urine for 24 hours after the first void in the morning of the collection period and to keep the contents refrigerated during the collection period. Confirmation of a complete 24-hour urine collection was obtained from participants by asking the dates and start/stop times of the urine collection and whether any urine was not collected (voided into the toilet by

mistake) or spilled during the collection or transport of the sample. Adequate volume of the 24-hour urine collection was considered to be >800 ml. If total volume was < 800 ml, if any voids were missed, or if any of the urine spilled, the participant was asked to repeat the 24-hour urine collection. Total volume of the urine collection was measured using graduated cylinders and recorded by the OCTRI nursing staff. Aliquots derived from the total volume were transferred to the OCTRI Core Lab for storage at or below -70°C for batched analysis at the end of the study.

Urinary mineral (calcium, potassium, sodium, and phosphate) and creatinine concentration was measured at the Kaiser Permanente Northwest Regional Laboratory (Portland, OR). Urinary calcium and creatinine concentrations were measured spectrophotometrically, phosphorus concentration was measured by colorimetric assay, urea nitrogen was measured by enzymatic assay, and sodium and potassium were measured by ion-selective electrodes. Urine N-telopeptide was measured at the OCTRI Core Lab with an enzyme-linked immunoabsorbant assay (EIA) commercial kit (Osteomark NTx Urine by Wampole Laboratories, Princeton, NJ) and referenced to urinary creatinine excretion to account for differences in urine volume.

BONE QUALITY ANALYSIS

Bone mineral content and density were measured by whole body, left hip, and lumbar spine DEXA scans. Measurements were made using a Discovery Series Densitometer (Hologic, Inc., QDR Discovery A, Bedford, MA). The DEXA machine was calibrated each morning with a phantom spine calibrator before any measurements were performed. The left hip measurement was taken by positioning the legs of the

participant at a 90° angle with a square pillow and focusing the scanner on the left hip region. The lumbar spine measurement was taken by focusing the scanner on the lumbar region. DEXA scans were only performed on individuals who weighed ≤340 pounds and who were ≤74" tall. Individuals who exceeded these criteria were not scanned because of the manufacturer's scanning area and set limitations on height and weight. Individuals who met these criteria but who did not fit within the DEXA scanning area were positioned so that a complete left-sided, half-body DEXA scan was performed. DEXA scans were analyzed using the computer software program Hologic QDR for Windows XP Software Version 12.1. The output for each analysis included the following information for whole body, left hip, and lumbar spine: BMD, BMC, bone area, bone mass, and hip and spine BMD t-scores and z-scores.

DIETARY INTAKE ANALYSIS

To estimate the participants' dietary intake at each time point, two 24-hour dietary recalls were administered at the participants' convenience. In brief telephone interviews, participants reported their food and beverage intake during the previous 24 hours. The unannounced telephone interviews were conducted twice at baseline and six-months by KPCHR trained staff: one to assess food and beverage intake during a weekday and one to assess intake during a weekend day. The Food Processor SQL (ESHA Research, Salem, OR) was used by KPCHR staff to analyze dietary composition. The values from the two interviews were averaged to approximate a participant's usual intake at each time point.

STUDY APPROVAL

Written informed consent was obtained and Notice of Privacy Health Insurance Portability and Accountability Act (HIPAA) forms were signed by each participant (referenced in Appendices A & B) at the baseline visit. The study's protocol was reviewed and approved by the Institutional Review Boards at OHSU and KPCHR and the State of Oregon Radiation Safety Committee.

STATISTICAL ANALYSIS

DATA TREATMENT

SPSS Version 15 (SPSS, Inc., Chicago, IL) was used to analyze the data. The data was first analyzed by looking at the data's distribution, outliers, and missing values. If the outcome variables were visibly skewed, they were log-transformed to approximate a normal distribution—only urinary potassium excretion met this criteria. Quantitative values of interest (i.e. protein and calcium intake) were organized into categorical values to examine potential relationships between categories of values and the dependent variables. Descriptive statistics and frequencies (for categorical variables) including mean, median, standard deviation, minimum, and maximum values were obtained for all variables, first by all participants together and then separated and analyzed according to diet groups.

Independent continuous variables that were analyzed included: age (years), height (cm), weight (kg), Body Mass Index (kg/m^2), urinary sodium excretion referenced to urinary creatinine excretion (mmol/mmol creatinine), energy intake (kcal/d), protein intake (g/d, % of total energy intake, g/kg), fat intake (g/d, % of total energy intake), carbohydrate intake (g/d, % of total energy intake), calcium intake (mg/d and mg/1000 kcal), sodium intake (mg/d and mg/1000 kcal), phosphorus intake (mg/d and mg/1000 kcal), and potassium intake (mg/d and mg/1000 kcal). Independent categorical variables that were analyzed included the following: gender (male and female), diet intervention (high-CHO or low-CHO), race (Caucasian and non-Caucasian) and relative tertiles of

protein intake in grams (g) of protein per kilogram (kg) of body weight: low (≤0.7 g/kg), medium (>0.7 and ≤1.5 g/kg), and high (>1.5 g/kg).

Independent sample t-tests were used to test for differences in baseline demographic and dietary data (e.g. weight, BMI, age, gender, race, energy intake, etc) between the diet groups. Significance was considered at $p<0.05$. The statistical analysis at baseline included all participants, independent of their returning status at six-months. However, the six-month data only included the participants that returned for six-month measurements. The changes and significances of the changes from baseline to six-months within groups only represented the individuals who returned for six-month measurements and completed the specific measurement (i.e. dietary data will have different sample sizes than urine data). Therefore, there will be different sample sizes among the different six-month measurement variables. In the statistical analysis and comparison of changes from baseline to six-months between groups, only the individuals who returned for all six-month measurements were analyzed; accordingly, the sample sizes for the Repeated Measures analysis are the same for all variables (41 for high-CHO and 51 for low-CHO).

CALCULATIONS

The following variables were computed to look for differences between time points and to examine the interrelationships between dietary variables and the effects on outcome variables. Differences between baseline and six-months for all variables were calculated by subtracting the baseline value from the six-month value. BMI was calculated with weight in kg, divided by height squared in meters. Percentage of weight

loss was calculated by the total amount of weight lost divided by the baseline weight, multiplied by 100. The protein to carbohydrate intake ratio was calculated by dividing the grams of protein by grams of carbohydrate. Nutrient density of all micronutrients (mg/1000 kcal) was calculated by dividing the average daily intake of the micronutrient by the average energy intake divided by 1000. The calcium to phosphorus intake ratio was calculated by dividing calcium intake by phosphorus intake (mg). The bone resorption to formation ratio was calculated by dividing urinary NTx concentration (nM BCE/mM creatinine) by serum BSAP concentration (U/L).

Repeated Measures

To test for differences from baseline values, six-month data was analyzed using General Linear Model (GLM) Repeated Measures with the repeated measure (time) having two values (baseline and six-month) and between-subject factor—dietary intervention (low-CHO and high-CHO). Other between-subject factors analyzed to elucidate differences between variables and time include gender and age. Outcome variables included urinary calcium excretion, bone turnover markers, bone mineral content and density (whole body, spine, and hip), and bone mineral density hip and spine t- and z-scores. Dietary data was also analyzed using Repeated Measures to look at changes in macronutrient and micronutrient intake from baseline within groups, and to also compare the differences between diet groups. Significance of changes and differences was considered at $p<0.05$.

REGRESSION ANALYSIS

Although regression analysis does not fulfill the primary aims of this study, regression analysis was performed to investigate the predictive value of the independent variables and their significance for the changes in outcome variables. The outcome variables were analyzed separately as changes from baseline (six-month values minus baseline values). Correlation between outcome variables were checked using Pearson's correlation coefficients and were considered to be significant if $p<0.05$. If the outcome variables were significantly correlated, Multivariate Analysis of Variance (MANOVA) was used to analyze the data set. Otherwise, the changes in outcome variables were analyzed using Analysis of Variance (ANOVA).

Forward and backwards stepwise progression methods were used to build multivariate and univariate regression models. All independent variables were entered into the regression equation simultaneously and significance was considered to be at $p<0.3$. Non-significant variables were removed from the equations in a stepwise manner. Independent variables were kept in the final models if $p<0.1$. For all independent variables, interactions and effect modifiers were assessed, and were considered significant if $p<0.2$. For simplicity, interactions were kept in the final model only if $p<0.05$. Confounding was assessed by looking at changes in significance and parameter estimates between models, and the more robust variable was kept in the model (the β value that changes the least when together, as well as remains significant in the final model). Correlations between independent variables were also assessed using Pearson's correlation coefficients, with the same criteria as above.

The first series of GLM regression models was designed to focus only on the diet group as the main "dietary" independent variable to emphasize the diets as "patterns" rather than composite parts of a whole. Other independent variables included in these analyses included change in urinary mineral excretion, age, gender, ethnicity, and weight. After significance of the diet group and other independent variables on the outcome variable of interest was assessed, the other dietary factors (macronutrient intake, micronutrient intake, and changes in intake) and bone-related parameters were included in the model and analyzed for significance.

POST-HOC ANALYSIS

For categorical variables with more than two groups or levels (protein intake levels and change in protein intake levels), post-hoc pairwise comparisons were performed using Tukey's studentized range and Fisher's Least Significant Differences (LSD) after Analysis of Variance (ANOVA) analysis to test for significant differences in outcome variables between levels.

RESULTS

DESCRIPTIVE STATISTICS

One hundred and fifteen participants were randomized to either the low-CHO diet (n=58) or the high-CHO diet (n=57). Figure 1 illustrates the number of participants who completed each measurement. Of the 115 participants that had baseline measurements, three participants (two from high-CHO group, one from low-CHO group) were excluded from analysis due to abnormal findings in the DEXA scans and one participant exceeding the manufacturer's set limits on size and weight. After the six-month intervention in the high-CHO group, eight participants were lost to follow-up, three participants missed 24-hour dietary recalls, and three participants missed 24-hour urine collection; therefore, 41 participants completed all six-month measurements of the original 57 high-CHO participants. After the six-month intervention in the low-CHO group, three participants were lost to follow-up, two participants missed 24-hour dietary recalls, and one participant missed BSAP analysis; accordingly, 51 participants completed all six-month measurements of the original 58 low-CHO participants. The total retention rate for the high-CHO group was 72% and the low-CHO group rate was 88%. After performing statistical tests examining two proportions, the retention rates were found to be significantly different ($p<0.01$).

Participant baseline anthropometric and demographic characteristics are presented in Table 3. There were no significant differences between groups in baseline values in most variables, except for urinary NTx and whole body BMD. Participant baseline and six-month diet composition, urinary mineral excretion and bone turnover

markers, and bone quality values are presented Tables 4 - 6. The tables present the means (or percent of the group's population for categorical variables) and standard deviations of the analyzed variables by diet group and by time point (baseline and six-months). The values were analyzed by independent sample t-tests to test for significant differences at baseline and six-months between groups. The values were also analyzed by paired t-tests to test for significant differences between baseline and six-month time points within groups.

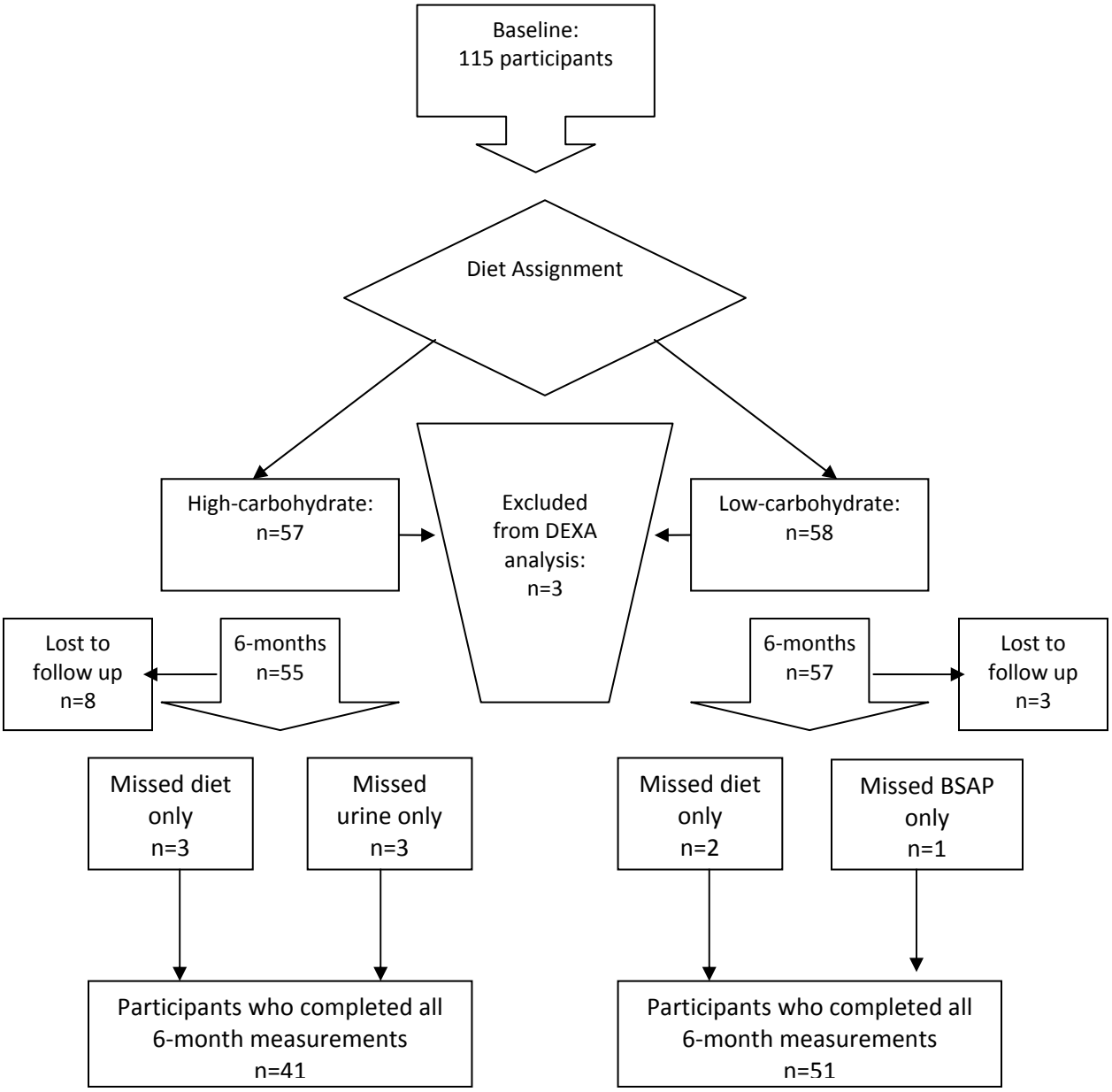

Figure 1: *Flow chart for sample size of participants and measurements collected*

Baseline characteristics are presented in Table 3. There were no significant differences in these variables between the two diet groups.

***Table 3**: Baseline characteristics by diet group*

	High-CHO *n=57*	**Low-CHO** *n=58*
Gender[1]	47 (82.5%)	43 (74.1%)
Race[2]	41 (82.5%)	48 (82.8%)
Age[3,4]	50 ± 10.8	49 ± 12.1
	[26 – 79]	[23 – 77]
Baseline Wt (kg) [3,4]	103 ± 18.3	103 ± 17.2
	[73 – 168]	[72 – 153]
BMI (kg/m^2) [3,4]	37 ± 5.7	36 ± 5.4
	[28 – 50]	[29 – 49]

[1]Number of females (% of group)
[2]Number of Caucasians (% of group)
[3]Mean ± SD
[4][Range]

As illustrated in Table 4, both groups demonstrated significant changes in dietary composition over six months. The high-CHO group (n=50) significantly decreased their mean energy intake, fat intake, and carbohydrate intake, which was consistent with the DASH dietary plan and goals. The high-CHO group also significantly increased their mean fiber intake, potassium intake, calcium nutrient density, potassium nutrient density, phosphorus nutrient density, and sodium nutrient density. The low-CHO group (n=55) significantly decreased their mean energy intake, carbohydrate intake, fiber intake, calcium intake, and sodium intake, consistent with a low-CHO dietary pattern. The low-CHO group significantly increased their mean fat intake, protein intake, phosphorus intake, calcium nutrient density, phosphorus nutrient density, and potassium nutrient density. There were also significant differences in dietary intake between groups at six-months. The following mean intake of macro- and micronutrients were significantly different between groups at six-months: carbohydrate, fat, protein, fiber, and calcium.

After examining the baseline values of all participants and comparing those values to those who returned for six-month measurements, no significant differences in dietary variables were found (data not shown). The significant differences within groups were also the same as those noted above.

***Table 4**: Baseline and six-month macronutrient and micronutrient dietary composition by diet group*

	High-CHO		Low-CHO	
	Baseline n=57	*6-months n=50*	*Baseline n=58*	*6-months n=55*
Energy (kcal/d)	1878 ± 539	1595 ± 484[b]	2010 ± 707	1575 ± 478[b]
Carbohydrates (g/d)	223 ± 67	201 ± 57[b]	242 ± 84	97 ± 43[bd]
% of energy	48% ± 8.3	51% ± 8.7[b]	49% ± 8.7	26% ± 11[bd]
Fat (g/d)	77 ± 33	56 ± 28[b]	81 ± 40	91 ± 40[d]
% of energy	36% ± 7.5	31% ± 7.8[b]	36% ± 7.2	51% ± 10[bd]
Protein (g/d)	76 ± 21	74 ± 20	77 ± 28	89 ± 32[ad]
% of energy	17% ± 4.6	19% ± 3.8	16% ± 3.9	23% ± 6.3[bd]
g/kg body wt	0.75 ± 0.23	0.8 ± 0.26	0.76 ± 0.29	1.02 ± 0.41[bd]
Protein intake[†]				
Low: ≤0.7 g/kg	23 (40%)	16 (36%)	27 (46%)	11 (21%)
Medium: 0.71 - 1.5 g/kg	34 (60%)	28 (62%)	30 (52%)	37 (70%)
High: >1.5 g/kg	0	1 (2%)	1 (2%)	5 (9%)
Protein:carbohydrate ratio	0.37 ± 0.15	0.38 ± 0.11	0.34 ± 0.12	1.12 ± 0.62[bd]
Fiber (g/d)	16.4 ± 6.4	21 ± 8[b]	16.8 ± 8.3	12 ± 5.4[bd]
Calcium (mg/d)	717 ± 286	793 ± 522	644 ± 247	613 ± 267[c]
(mg/1000 kcal)	392 ± 154	512 ± 276[b]	334 ± 124	401 ± 164[ac]
Phosphorus (mg/d)	730 ± 241	798 ± 324	732 ± 328	889 ± 344[b]
(mg/1000 kcal)	400 ± 109	508 ± 180[b]	375 ± 152	571 ± 165[b]
Calcium:phosphorus ratio	1.03 ± 0.43	1.13 ± 0.78	1 ± 0.5	0.74 ± 0.34[bd]
Potassium (mg/d)	1665 ± 549	2052 ± 812[b]	1809 ± 1044	1858 ± 818
(mg/1000 kcal)	941 ± 358	1327 ± 508[b]	924 ± 410	1188 ± 404[b]
Sodium (mg/d)	2891 ± 1146	2756 ± 1206	3059 ± 1082	2474 ± 1085[b]
(mg/1000 kcal)	1548 ± 467	1774 ± 763[a]	1568 ± 443	1567 ± 441

Mean ± SD

[†]Number of subjects in each level (% of group)

[a]Significantly different from baseline (p<0.05)

[b]Significantly different from baseline (p<0.01)

[c]Significantly different from high-CHO at 6-months (p<0.05)

[d]Significantly different from high-CHO at 6-months (p<0.01)

As shown in Table 5, urinary mineral excretion changed and varied between groups. In the high-CHO group, the only significant changes in mineral excretion were an increase in mean potassium excretion ($p<0.05$), signifying an increased intake of fruits and vegetables, and a decrease in mean sodium excretion rates ($p<0.05$). There were many more changes in urinary mineral excretion in the low-CHO group: an increase in UUN ($p<0.01$), increase in calcium ($p<0.01$), increase in phosphorus ($p<0.01$), and a decrease in sodium ($p<0.01$). The urinary mineral excretion values at six-months were also significantly different between groups. The following levels of excretion of minerals in urine were different at six-months between groups: calcium (in mg/24hr, mmol/mmol creatinine, and mmol/kg/d), UUN, potassium (mmol/mmol creatinine), and phosphorus (mmol/kg/d).

Table 5 also shows the values of bone turnover markers. The only significant difference in concentrations of the bone turnover markers was between groups at baseline in urine NTX excretion—NTx was initially lower in the low-CHO group compared to the high-CHO group ($p<0.05$). However, there was no difference between groups in NTx at six-months. There were no significant changes in the bone turnover markers in either group from baseline to six-months.

After examining the values of all participants versus those that returned for six-month measurements, no significant differences in absolute values were found between the values shown above and the values for the non-returnees (data not shown). Additionally, in the analysis that only included returnees, the low-CHO group experienced a decrease in BSAP and an increase in NTX; however, the difference between groups was not significant when the low-CHO group was compared to the

changes in the high-CHO group (which did not experience any significant changes in BSAP or NTx). Accordingly, it cannot be concluded that the low-CHO diet generated increased bone resorption or decreased bone formation compared to the high-CHO diet.

***Table 5**: Baseline and six-month urine mineral excretion and bone turnover markers*

	High-CHO		**Low-CHO**	
	Baseline n=57	*6-months n=46*	*Baseline n=58*	*6-months n=55*
Urinary creatinine excretion rate (mmol/24hr)	14 ± 5	13 ± 5	14 ± 5	14 ± 4
Urine urea nitrogen excretion rate (g/24hr)	11 ± 4	11 ± 3	11 ± 4	13 ± 5[be]
Urinary mineral excretion rate (mg/24hr)				
Calcium	176 ± 92	175 ± 85	194 ± 101	238 ± 145[ae]
Phosphorus	958 ± 360	892 ± 292	1042 ± 498	1066 ± 412[d]
Potassium*	64 ± 21	73 ± 24[a]	72 ± 27	66 ± 27
Sodium*	186 ± 69	160 ± 67[a]	192 ± 81	158 ± 63[a]
Urinary mineral excretion (mmol/mmol creatinine)				
Calcium	0.34 ± 0.25	0.37 ± 0.21	0.35 ± 0.19	0.45 ± 0.19[be]
Phosphorus	4.8 ± 1.5	6 ± 2.7[be]	5.2 ± 2	4.9 ± 1.7[b]
Potassium	1.5 ± 0.32	1.7 ± 0.41[a]	1.6 ± 0.35	1.5 ± 0.36[e]
Sodium	14 ± 5	13 ± 5	13 ± 4	12 ± 4[b]
Urinary mineral excretion (mmol/kg/d)				
Calcium	0.04 ± 0.03	0.05 ± 0.03	0.05 ± 0.03	0.07 ± 0.04[be]
Phosphorus	0.3 ± 0.11	0.31 ± 0.11	0.33 ± 0.12	0.39 ± 0.15[be]
Potassium	0.64 ± 0.24	0.79 ± 0.33[b]	0.71 ± 0.29	0.75 ± 0.33
Sodium	1.8 ± 0.68	1.8 ± 0.74	1.9 ± 0.71	1.8 ± 0.67
Serum BSAP (U/L)	36 ± 11	34 ± 11	34 ± 9	32 ± 9
Urine NTx (nM BCE/mM creatinine)	37 ± 23	42 ± 43	30 ± 15[c]	34 ± 12
NTx/BSAP ratio	1.2 ± 1.12	1.52 ± 2.44	0.91 ± 0.46	1.15 ± 0.48

Mean ± SD
*mmol/24hr
[a]Significantly different from baseline (p<0.05)
[b]Significantly different from baseline (p<0.01)
[c]Significantly different from high-CHO at baseline (p<0.05)
[d]Significantly different from high-CHO at 6-months (p<0.05)
[e]Significantly different from high-CHO at 6-months (p<0.01)

Table 6 illustrates the baseline and six-month values of weight and bone quality measurements. It can be seen that both groups experienced a significant reduction in weight and BMI over six months. The only other change in the high-CHO group was a significant increase in spine BMC ($p<0.05$) from baseline. The low-CHO group also experienced a significant increase in spine BMC ($p<0.01$), as well as whole body BMC ($p<0.05$). There were no other significant changes in either group from baseline to six-months. The whole body BMD was significantly higher in the low-CHO group than the high-CHO group at baseline and six-months ($p<0.05$), but did not change over time. At six-months, the low-CHO group had a lower BMI, reflective of a greater weight loss, and a higher whole body BMC than the high-CHO group. However, the low-CHO group tended to have a higher whole body BMC at baseline ($p=0.089$), which is most likely the cause of the higher whole body BMC at six-months.

After examining the values for all participants and comparing those to the values of the participants who completed all six-month measurements, no significant differences were found in any values (data not shown). All significant changes within groups and differences of bone quality at six-months were at similar levels of significance as those reported

Table 6: Baseline and six-month weight, body mass index, and bone quality values by diet group

	High-CHO		Low-CHO	
	Baseline n=57	*6-months n=47*	*Baseline n=58*	*6-months n=54*
Weight (kg)	103 ± 18	94 ± 16[b]	103 ± 17	90 ± 17[b]
Body Mass Index (kg/m²)	37 ± 6	34 ± 5[b]	36 ± 5	31 ± 5[bd]
Bone Mineral Content (g)				
Whole Body	2361 ± 323	2338 ± 301	2475 ± 371	2492 ± 395[ad]
Spine	62 ± 13.5	65 ± 11.8[a]	66 ± 12.9	67 ± 13.4[b]
Hip	35 ± 7	34 ± 6	35 ± 8	36 ± 8
Bone Mineral Density (g/cm^2)				
Whole Body	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.11[c]	1.2 ± 0.11[e]
Spine	1.1 ± 0.14	1.1 ± 0.13	1.1 ± 0.14	1.2 ± 0.15
t-score	0.13 ± 1.3	0.23 ± 1.3	0.17 ± 1.3	0.2 ± 1.3
z-score	0.92 ± 1.3	1 ± 1.3	0.8 ± 1.3	0.84 ± 1.4
Hip	1.0 ± 0.13	0.99 ± 0.13	1.0 ± 0.1	1.0 ± 0.16
t-score	0.33 ± 1.1	0.28 ± 1.1	0.32 ± 1.1	0.26 ± 1.2
z-score	0.83 ± 0.96	0.83 ± 0.99	0.75 ± 1.0	0.77 ± 1.1

Mean ± SD
[a]Significantly different from baseline (p<0.05)
[b]Significantly different from baseline (p<0.01)
[c]Significantly different from high-CHO at baseline (p<0.05)
[d]Significantly different from high-CHO at 6-months (p<0.05)
[e]Significantly different from high-CHO at 6-months (p<0.01)

GLM Repeated Measures

Mean changes by diet group in dietary intake, urine mineral excretion, bone turnover markers and bone quality values from baseline to six-months are presented in Tables 7 through 9. Values are presented as average changes and standard error of the mean for changes in variables. The mean changes shown in the tables represent only the participants who returned for their six-month visits, meaning the sample sizes for the high-CHO and low-CHO diet groups are the same across all variables. Significantly different changes from baseline between groups are also identified in these tables. Differences in dietary variables between groups were expected due to the intensive behavioral intervention designed to change diet and diet-related behaviors and to facilitate weight loss; furthermore, differences were expected due to the considerably different macro- and micronutrient composition of the diets.

In the earlier discussion, Table 4 illustrated the values of dietary variables at baseline and six-months, whereas Table 7 shows the mean change of these variables from baseline to six-months and the differences in the changes between groups. The following variables had significantly different changes between groups: carbohydrate, protein, fat, fiber, and phosphorus nutrient density. Both groups demonstrated a reduction in energy intake, but the change was not different between groups. This is interesting, because energy intake should be theoretically lower in the low-CHO group when the greater change in weight in the low-CHO group is considered. As expected, the low-CHO group displayed a greater reduction in carbohydrate intake and greater increases in protein and fat intake. Another interesting fact to note is that although there were changes in dietary variables within diet groups in Table 4, the only significant different changes between groups were in macronutrients, fiber, ratios between nutrients, and phosphorus nutrient density.

***Table 7**: Mean changes in dietary intake from baseline to six months, by diet group*

	Δ 6-month-Baseline	
	High-CHO n=41	*Low-CHO n=51*
Energy (kcal/d)	-343 ± 87	-447 ± 107
Carbohydrates (g/d)	-30 ± 11	-145 ± 13[b]
Protein (g/d)	-2.5 ± 3.8	11 ± 5.4[a]
Protein:carbohydrate ratio	0.03 ± 0.02	0.78 ± 0.08[b]
Fat (g/d)	-24 ± 5	9.1 ± 6.7[b]
Fiber (g/d)	4 ± 1.4	-4.7 ± 1.2[b]
Calcium (mg/d)	57 ± 87	-44 ± 43
(mg/1000 kcal)	122 ± 41	63 ± 25
Calcium:phosphorus ratio	0.08 ± 0.13	-0.28 ± 0.09[a]
Phosphorus (mg/d)	60 ± 54	150 ± 56
(mg/1000 kcal)	119 ± 26	196 ± 23[a]
Potassium (mg/d)	366 ± 115	35 ± 165
(mg/1000 kcal)	405 ± 72	265 ± 70
Sodium (mg/d)	-196 ± 202	-575 ± 204
(mg/1000 kcal)	253 ± 96	18 ± 79

Mean ± SEM
[a]Significantly different from High-CHO group ($p<0.05$)
[b]Significantly different from High-CHO group ($p<0.01$)

As previously described, Table 5 showed the baseline and six-month urinary and bone turnover values, and Table 8 shows the mean changes in these variables from baseline to six-months, as well as the significant differences in these changes between groups. The change in UUN was significantly greater in the low-CHO group compared to the high-CHO group, and urinary potassium and calcium excretion were significantly different between groups, whether indexed to time, creatinine, or body weight. Also, it is interesting to note that the mean changes in bone turnover markers were not significantly different between groups, showing that neither group promoted bone resorption or inhibited bone formation more than the other group.

***Table 8**: Mean changes in urine mineral excretion and bone turnover markers from baseline to six months, by diet group*

	Δ 6-months-Baseline	
	High-CHO	*Low-CHO*
	n=41	*n=51*
Urinary creatinine excretion rate (mmol/24hr)	-0.24 ± 0.5	-0.61 ± 0.48
Urine urea nitrogen excretion rate (g/24hr)	0.11 ± 0.57	2.3 ± 0.71[a]
Urinary mineral excretion rate (mg/24hr)		
Calcium	-3.5 ± 11	46 ± 17[b]
Phosphorus	-1.6 ± 1.7	0.98 ± 2.3
Potassium*	8.6 ± 3.6	-4.7 ± 3.4[b]
Sodium*	-23 ± 10.4	-28 ± 12.4
Urinary mineral excretion (mmol/mmol creatinine)		
Calcium	0.014 ± 0.23	0.099 ± 0.03[a]
Phosphorus	-.016 ± 0.097	0.18 ± 0.1
Potassium	1.1 ± 0.4	-0.26 ± 0.24[b]
Sodium	-1.3 ± 0.67	-1.4 ± 0.78
Urinary mineral excretion (mmol/kg/d)		
Calcium	0.004 ± 0.003	0.021 ± 0.005[b]
Phosphorus	0.01 ± 0.02	0.06 ± 0.02
Potassium	0.15 ± 0.04	0.05 ± 0.04[b]
Sodium	-0.09 ± 0.11	-0.03 ± 0.13
Serum BSAP (U/L)	-1.27 ± 0.88	-1.7 ± 0.64
Urinary NTx (nM BCE/mM creatinine)**	7.03 ± 4.1	5.28 ± 2.0
NTx/BSAP ratio***	0.35 ± 0.21	0.24 ± 0.07

Mean ± SEM
*mmol/24hr
**n=49 (high-CHO), n=49 (low-CHO)
***n=46 (high-CHO), n=54 (low-CHO)
[a]Significantly different from High-CHO group ($p<0.05$)
[b]Significantly different from High-CHO group ($p<0.01$)

As previously shown, Table 6 illustrated the baseline and six-month values of weight and bone quality measures, and Table 9 demonstrates the changes in these variables from baseline, as well as the significant differences in changes between groups. The only significant different changes between groups were in weight (in kg and percent of weight lost from baseline) and BMI, meaning that the low-CHO group lost a significantly greater amount of weight from baseline than the high-CHO group. The mean changes in the bone quality variables were not significantly different from each other. It is also important to note that although the low-CHO group experienced an increase in whole body BMC shown in Table 6, this table shows that the mean change was not different than the high-CHO group. This means that the effects of the dietary patterns on whole body BMC were not significantly different from each other. Also, both groups experienced an increase in spine BMC, and this table demonstrated that the mean changes were not significantly different. Therefore, it can be concluded from this analysis that although changes in bone quality markers were seen within groups, the changes between groups were not different from each other; additionally, there were no differential effects of the two diet groups on markers of bone quality.

Table 9: *Mean changes in weight and bone quality values from baseline to six months, by diet group*

	Δ 6-months-Baseline	
	High-CHO n=41	*Low-CHO n=51*
Weight (kg)	-8 ± 1	-13 ± 0.9[b]
Weight (% change from baseline)	-7.7 ± 0.8	-12.9 ± 0.8[b]
Body Mass Index (kg/m^2)	-3 ± 0.3	-4.7 ± 0.3[b]
Bone Mineral Content (g)		
Whole Body	-0.16 ± 5.8	16.7 ± 7.9
Spine	0.002 ± 0.005	1.5 ± 0.46
Hip	-0.002 ± 0.005	0.15 ± 0.55
Bone Mineral Density (g/cm^2)		
Whole Body	0.003 ± 0.003	0.01 ± 0.004
Spine	0.002 ± 0.005	-0.001 ± 0.005
t-score	0.04 ± 0.04	0.01 ± 0.06
z-score	0.03 ± 0.05	-0.01 ± 0.07
Hip	-0.002 ± 0.005	-0.009 ± 0.008
t-score	-0.04 ± 0.05	-0.07 ± 0.08
z-score	-0.04 ± 0.07	-0.01 ± 0.08

Mean ± SEM
[a]Significantly different from High-CHO group ($p<0.05$)
[b]Significantly different from High-CHO group ($p<0.01$)

CORRELATIONS

Correlations between changes in outcome variables were examined. The significant ($p<0.05$) correlations are presented in Table 7. Only a few outcome variables were highly correlated, hence multivariate regression analysis was only performed on the highly and expected correlated variables: the two measures of urinary calcium excretion (mmol/mmol creatinine and mmol/kg/d), BMC and BMD values for whole body, spine, and hip, and t- and z-scores for spine and hip. An interesting correlation observed is the significant positive correlation between change in urinary calcium excretion and the bone resorption to formation ratio ($p=0.021$), meaning that an increase in NTx in relation to BSAP was correlated with an increase in calcium excretion. This suggests a relationship between the ratio of bone resorption and formation and the amount of calcium excreted in the urine, but correlational analysis does not prove causation between the two variables. There is also a significant positive correlation between change in NTx and change in BMC, which is paradoxical since NTx theoretically would have a negative relationship with BMC. This demonstrates why correlational analysis does not explain much of the variation seen in the outcome variables.

***Table 10**: Significant correlations between changes in outcome variables using Pearson's coefficients*

Variable 1	***Variable 2***	**Statistics** *Pearson's coefficient*	*p-value*
Change in urinary calcium excretion (mmol/mmol creatinine)	Change in urinary calcium excretion (mmol/kg/d)	0.877	<0.001
Change in urinary calcium excretion (mmol/mmol creatinine)	Change in NTx/BSAP Ratio	0.231	0.021
Change in urinary N-telopeptide (nM BCE/mmol creatinine)	Change in whole body BMC	0.216	0.033
Change in urinary N-telopeptide (nM BCE/mmol creatinine)	Change in spine BMC	0.223	0.028
Change in whole body BMC (g)	Change in whole body BMD	0.339	0.001
Change in spine BMC (g)	Change in spine BMD	0.342	<0.001
Change in spine BMC (g)	Change in spine t-score	0.336	0.001
Change in spine BMC (g)	Change in spine z-score	0.271	0.007
Change in hip BMC (g)	Change in spine BMD	0.201	0.044
Change in hip BMC (g)	Change in hip BMD	0.599	<0.001
Change in hip BMC (g)	Change in hip t-score	0.524	<0.001
Change in hip BMC (g)	Change in hip z-score	0.542	<0.001
Change in spine BMD (g/cm^2)	Change in spine t-score	0.839	<0.001
Change in spine BMD (g/cm^2)	Change in spine z-score	0.602	<0.001
Change in hip BMD (g/cm^2)	Change in hip t-score	0.855	<0.001
Change in hip BMD (g/cm^2)	Change in hip z-score	0.835	<0.001

MULTIVARIATE LINEAR REGRESSION

MANOVA was used for the correlated outcome variables—urinary calcium excretion (mmol/mmol creatinine and mmol/kg/d), all bone mineral content and bone mineral density values (whole body, hip, and spine), and t- and z-scores (hip and spine). Since insignificant independent variables cannot be removed separately from each outcome variable's equation, ANOVA analysis for the correlated outcome variables was used to fully explore the linear relationships between the independent and dependent variables. The multivariate regression models are presented in Tables 11 through 15. Independent variables were kept in the equations if $p<0.1$ in at least one of the equations.

For the categorical variables in the linear regression models, each variable has its own reference group, meaning that the variable in the equation is the other variable. This means that the categorical variable in the equation has a greater or lesser effect on the outcome variable compared to the reference variable, depending on the slope. The reference variable for diet group is the high-carbohydrate diet group, leaving the low-carbohydrate diet group in the equation. The reference variable for gender is female, leaving males in the equation. The reference variable for race is non-Caucasian, leaving Caucasian in the equation. Any other reference variables for categorical variables are labeled in the specific equation. Summaries for MANOVA analysis follow each set of linear regression models for the correlated outcome variables.

As illustrated in Table 11, predicting urinary calcium excretion with MANOVA results in differences in the significance of the independent variables in the two models for the changes in outcome variables in the different measures (mmol/mmol creatinine and mmol/kg/d); however, significant predictors of urinary calcium excretion (percent of weight change and change in urinary sodium excretion) remained significant in both models for the outcome variables. Both models explain a significant amount of the variation in urinary calcium excretion as measured by the F statistic and r^2.

***Table 11**: Multivariate regression models for changes in urinary calcium excretion*

CHANGE IN URINARY CALCIUM EXCRETION (MMOL/MMOL CREATININE)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.011	0.002
Change in urinary sodium (mmol/mmol creatinine)	0.015	<0.001
Change in creatinine excretion rate (mmol/24hr)	0.002	0.696
Change in hip BMC	0.008	0.207
Change in hip BMD	-0.5	0.26
Diet group[1]	0.054	0.196
Gender[2]	0.102	0.145
Group x Gender[3]	-0.183	0.043
F (overall model significance)= 4.6 (p<0.001) r^2= 0.295		
CHANGE IN URINARY CALCIUM EXCRETION (MMOL/KG/D)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.002	<0.001
Change in urinary sodium (mmol/mmol creatinine)	0.002	0.001
Change in creatinine excretion rate (mmol/24hr)	0.003	<0.001
Change in hip BMC	0.001	0.146
Change in hip BMD	-0.091	0.196
Diet group[1]	-0.01	0.129
Gender[2]	0.017	0.117
Group x Gender[3]	-0.03	0.035
F (overall model significance)= 6.9 (p<0.001) r^2= 0.384 [1]Low-CHO group [2]Males [3]Males in low-CHO group		

As illustrated in Tables 12-15, the multivariate regression models for predicting changes in bone mineral density, bone mineral content, and t- and z-scores are not all significant, as measured by the F statistic. The predicting variables do not demonstrate the same relationships in magnitude, as measured by slope, or in the direction of the association, as measured by the positive or negative slope, with the outcome variables, nor do they have the same significance in all of the equations. This demonstrates the need for univariate regression analysis to fully explore the relationships between independent variables and changes in outcome variables.

***Table 12**: Multivariate regression models for changes in bone mineral content*

Change in Whole Body Bone Mineral Content (g)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.86	0.361
Change in protein to carbohydrate ratio	28.15	0.004
Change in calcium to phosphorus ratio	9.19	0.263
Change in potassium intake	0.12	0.015
Change in serum BSAP	-1.32	0.205
Change in urinary NTx	1.65	0.025
Change in NTX to BSAP ratio	-28.87	0.064
F (overall model significance)= 3.47 (p=0.003)		
r^2= 0.229		
Change in Spine Bone Mineral Content (g)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.97	0.409
Change in protein to carbohydrate ratio	-0.397	0.741
Change in calcium to phosphorus ratio	0.168	0.87
Change in potassium intake	0	0.594
Change in serum BSAP	0.101	0.439
Change in urinary NTx	-0.009	0.919
Change in NTX to BSAP ratio	1.387	0.476
F (overall model significance)= 0.875 (p=0.53)		
r^2= 0.07		
Change in Hip Bone Mineral Content (g)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	0.038	0.581
Change in protein to carbohydrate ratio	0.93	0.184
Change in calcium to phosphorus ratio	1.67	0.006
Change in potassium intake	-0.000087	0.813
Change in serum BSAP	0.061	0.419
Change in urinary NTx	0.065	0.224
Change in NTX to BSAP ratio	-1.151	0.308
F (overall model significance)= 1.79 (p= 0.1)		
r^2= 0.133		

***Table 13**: Multivariate regression models for changes in bone mineral density*

Change in Whole Body Bone Mineral Density (g/cm^2)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.002	<0.001
Change in protein to carbohydrate ratio	-0.006	0.177
Change in calcium to phosphorus ratio	0	0.969
Change in potassium intake	-0.0000012	0.604
Change in serum BSAP	0	0.709
Change in urinary NTx	0.001	0.093
Change in NTX to BSAP ratio	-0.11	0.1
F (overall model significance)= 4.2 (p= 0.001)		
r^2= 0.264		
Change in Spine Bone Mineral Density (g/cm^2)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	0	0.728
Change in protein to carbohydrate ratio	0.006	0.36
Change in calcium to phosphorus ratio	-0.008	0.167
Change in potassium intake	0.00000028	0.938
Change in serum BSAP	-0.00008	0.913
Change in urinary NTx	0	0.645
Change in NTX to BSAP ratio	0.000096	0.993
F (overall model significance)= 1.019 (p<0.001)		
r^2= 0.08		
Change in Hip Bone Mineral Density (g/cm^2)		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.001	0.566
Change in protein to carbohydrate ratio	0.003	0.724
Change in calcium to phosphorus ratio	0.018	0.36
Change in potassium intake	-0.0000012	0.808
Change in serum BSAP	0.001	0.289
Change in urinary NTx	0	0.559
Change in NTx to BSAP ratio	-0.009	0.565
F (overall model significance)= 1.09 (p=0.38)		
r^2= 0.085		

Table 14: *Multivariate regression models for changes in hip bone mineral density t-scores and z-scores*

CHANGE IN HIP T-SCORE		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.01	0.328
Change in protein to carbohydrate ratio	0.013	0.897
Change in calcium to phosphorus ratio	0.203	0.019
Change in potassium intake	-0.000024	0.648
Change in serum BSAP	0.005	0.641
Change in urinary NTx	0.008	0.264
Change in NTX to BSAP ratio	-0.177	0.272
F (overall model significance)= 1.4 (p=0.217) r^2= 0.107		
CHANGE IN HIP Z-SCORE		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.008	0.421
Change in protein to carbohydrate ratio	0.017	0.871
Change in calcium to phosphorus ratio	0.207	0.022
Change in potassium intake	-0.000042	0.441
Change in serum BSAP	0.002	0.846
Change in urinary NTx	0.011	0.171
Change in NTX to BSAP ratio	-0.222	0.187
F (overall model significance)= 1.48 (p=0.187) r^2= 0.112		

Table 15: *Final multivariate regression models for change in spine bone mineral density t-scores and z-scores*

CHANGE IN SPINE T-SCORE		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.008	0.297
Change in protein to carbohydrate ratio	0.046	0.53
Change in calcium to phosphorus ratio	-0.076	0.227
Change in potassium intake	0.000019	0.623
Change in serum BSAP	-0.002	0.802
Change in urinary NTx	0	0.958
Change in NTX to BSAP ratio	0.031	0.793
F (overall model significance)= 0.951 (p=0.472)		
r^2= 0.075		
CHANGE IN SPINE Z-SCORE		
Independent Variables	**Slope**	**p-value**
Change in weight (%)	-0.003	0.719
Change in protein to carbohydrate ratio	0.072	0.392
Change in calcium to phosphorus ratio	-0.51	0.479
Change in potassium intake	-0.000024	0.591
Change in serum BSAP	-0.004	0.634
Change in urinary NTx	0.006	0.361
Change in NTX to BSAP ratio	-0.064	0.638
F (overall model significance)= 0.789 (p=0.598)		
r^2= 0.063		

UNIVARIATE LINEAR REGRESSION

Linear relationships were assessed with GLM Univariate Analysis. The main focus was to look at changes in the outcome variables, so the changes in independent variables from baseline were entered into the regression equations rather than absolute values. ANOVA was performed first on the non-correlated outcome variables (BSAP, NTx, and NTx:BSAP ratio); additionally, after MANOVA was performed on the correlated outcome variables, ANOVA was then used to discover significant independent variables not found in MANOVA. Univariate regression models for changes in all outcome variables are presented in Tables 16 - 29. Summaries for each regression model briefly explain the relationships observed in the regression model.

As illustrated in Table 16, percent of weight change has a negative effect on urinary calcium excretion, meaning that participants who lost more weight had increased urinary calcium excretion (a negative percent of weight change from baseline). This is related to diet group as the low-carbohydrate diet group demonstrated a higher percentage of weight loss. Diet group was not included in the final model as weight change and diet group are significantly correlated (Pearson's correlation coefficient 0.429, $p<0.001$). Percent of weight change confounded the relationship between diet group and urinary calcium excretion, meaning that the slope of diet group changed significantly and was not a significant predictor when weight change was included in the model. Percent of weight change was the more robust variable, so it was kept in the model. Change in urinary sodium excretion also significantly predicted change in urinary calcium excretion. An increase in urinary sodium resulted in an increased urinary calcium excretion. The final model predicting change in urinary calcium excretion significantly predicted 22% of the outcome variable's variation ($p<0.001$).

Table 16: *Final univariate regression model for change in urinary calcium excretion*

CHANGE IN URINARY CALCIUM EXCRETION (MMOL/MMOL CREATININE)		
Independent variables	***Slope***	***p-value***
Change in weight (%)	-0.009	0.001
Change in urine sodium excretion	0.014	<0.001
F (overall model significance)= 13.8 (p <0.001) r^2=0.221		

As illustrated in Table 17, the same independent variables were significant in predicting change (as well as the same relationships) for urinary calcium excretion in mmol/kg/d compared to urinary calcium in mmol/mmol creatinine in Table 16, except for the addition of change in creatinine excretion rate in Table 17. This demonstrates the importance of controlling for creatinine excretion in urinary mineral excretion. The model also significantly predicted 30% of the variation in the outcome variable ($p<0.001$).

Table 17: *Final univariate regression model for change in urinary calcium excretion*

CHANGE IN URINARY CALCIUM EXCRETION (MG/KG/D)		
Independent variables	***Slope***	***p-value***
Change in weight (%)	0.002	0.002
Change in urine sodium excretion	-0.002	<0.001
Change in creatinine excretion rate	0.003	<0.001
F (overall model significance)= 13.8 (p<0.001) r^2= 0.301		

As shown in Table 18, it is interesting to note that the change in the resorption marker, NTx, is related to dietary micronutrient intake more than urinary calcium excretion in regression analysis. Changes in calcium and potassium intake have a positive relationship with NTx excretion, meaning that increased Ca and K both predict an increase in bone resorption. Changes in phosphorous, sodium, and calcium to phosphorus ratio intake have a negative relationship with NTx excretion, meaning that increased intake of these micronutrients is associated with decreased bone resorption. The predictive value of the micronutrients of the change in NTx is interesting because they are opposite than expected, especially calcium. Also, diet group was not a significant predictor in this model, showing that the mean change in NTx cannot be predicted by diet group. The model significantly predicted 33% of variation in the outcome variable ($p<0.001$).

Table 18: *Final univariate regression model for change in urinary N-telopeptide excretion*

CHANGE IN URINARY N-TELOPEPTIDE EXCRETION (NM BCE/MM CREATININE)		
Independent variables	***Slope***	***p-value***
Change in phosphorus intake	-0.031	0.002
Change in calcium intake	0.047	<0.001
Change in calcium to phosphorus ratio	-20.83	<0.001
Change in sodium intake	-0.005	0.002
Change in potassium intake	0.005	0.39
F (overall model significance)= 8.74 ($p<0.001$) r^2= 0.327		

In Table 19, it is shown that age, percent of weight change, and change in protein and fiber intake had positive relationships with the bone formation marker, BSAP. This means that an increase in these variables was associated with an increase in bone formation. Changes in carbohydrate and phosphorus intake, as well as the calcium to phosphorus intake ratio had a negative relationship with bone formation. The regression model significantly predicted about 20% of the outcome variable's variation.

***Table 19**: Final univariate regression model for change in serum bone-specific alkaline phosphatase*

CHANGE IN SERUM BONE-SPECIFIC ALKALINE PHOSPHATASE (U/L)		
Independent variables	***Slope***	***p-value***
Age	0.093	0.071
Change in weight (%)	0.173	0.062
Change in carbohydrate intake	-0.015	0.063
Change in protein intake	0.058	0.029
Change in fiber intake	0.171	0.001
Change in phosphorus intake	-0.007	0.007
Change in calcium to phosphorus intake ratio	-2.16	0.005
F (overall model significance)= 3.14 (p= 0.005) r^2= 0.198		

As illustrated in Table 20, changes in calcium intake and whole body BMC showed positive relationships with change in the resorption to formation ratio, meaning that increases in these variables was associated with increases in the NTx/BSAP ratio. Changes in protein intake and BMI was associated with a lower resorption to formation ratio. This model significantly predicted about 42% of the variation in the outcome variable ($p<0.001$).

Table 20: *Final univariate regression model for change in N-telopeptide to bone-specific alkaline phosphatase ratio*

CHANGE IN RESORPTION TO FORMATION RATIO (NTX/BSAP)		
Independent variables	***Slope***	***p-value***
Change in body mass index	-0.66	0.1
Change in protein intake	-0.007	0.012
Change in calcium intake	0.001	<0.001
Change in whole body BMC	0.003	0.055
F (overall model significance)= 115.48 ($p<0.001$) r^2= 0.419		

Table 21 demonstrates that changes in potassium intake and the protein to carbohydrate intake ratio had positive relationships with change in whole body BMC, meaning that increases in these dietary variables were associated with increases in bone mass. Gender also had a positive effect on bone mass, meaning that males were more likely to have greater increases in bone mineral content compared to females. Age had an expected negative relationship with change in whole body BMC. The regression model significantly predicted about 22% of the outcome variable's variation ($p<0.001$).

***Table 21**: Final univariate regression model for change in whole body bone mineral content*

CHANGE IN WHOLE BODY BMC		
Independent variables	***Slope***	***p-value***
Age	-1.416	0.004
Gender[1]	25.88	0.056
Change in protein to carbohydrate intake ratio	20.87	0.016
Change in potassium intake	0.009	0.053
F (overall model significance)= 6.268 ($p<0.001$)		
r^2= 0.216		
[1]Males		

In Table 22, the only significant predictor for change in spine BMC was change in NTx concentration, meaning that an increase in NTx predicted a loss of spine BMC. The model significantly predicted 5% of the outcome variable's variation (p=0.028).

Table 22: *Final univariate regression model for change in spine bone mineral content*

CHANGE IN SPINE BMC		
Independent variables	***Slope***	***p-value***
Change in urinary NTx	0.059	0.028
F (overall model significance)= 5.01 (p=0.028) r^2= 0.05		

In Table 23, the only "significant" (p<0.1) predictor for change in hip bone mineral content was the change in calcium to phosphorus intake ratio, meaning that an increase in calcium intake compared to phosphorus intake was associated with an increase in hip bone mineral content. The regression model does not significantly predict the variation in the outcome variable (p=0.078), and it only predicted about 3% of the variation in hip BMC.

Table 23: *Final univariate regression model for change in hip bone mineral content*

CHANGE IN HIP BMC		
Independent variables	***Slope***	***p-value***
Change in calcium to phosphorus ratio	0.759	0.078
F (overall model significance)= 3.17 (p=0.078) r^2= 0.033		

As illustrated in Table 24, changes in the bone resorption to formation ratio and percent change of weight both had negative relationships with change in whole body BMD, meaning that increases in these variables were related to decreases in bone density. Changes in NTx and BMI both had positive relationships with the outcome variable, meaning that they were associated with an increase in whole body BMD. Something noteworthy of this regression model is that the bone turnover markers significantly predicted change in BMD. The regression model significantly predicted about 27% of the outcome variable's variation ($p<0.001$).

Table 24: *Final univariate regression model for change in whole body bone mineral density*

CHANGE IN WHOLE BODY BMD		
Independent variables	***Slope***	***p-value***
Change in weight (%)	-0.004	0.001
Change in body mass index	0.007	0.042
Change in urinary N-telopeptide	0.001	0.044
Change in NTx/BSAP ratio	-0.012	0.043
F (overall model significance)= 8.2 ($p<0.001$) r^2= 0.265		

Table 25 demonstrates that the only significant predictor of change in spine bone mineral density was log-transformed change in urinary potassium excretion, meaning that an increase in urinary potassium excretion was associated with an increase in spine BMD. The model significantly predicted only about 5% of the variation in spine BMD (p=0.032). Note: There were no significant predictors of change for hip bone mineral density.

Table 25*: Final univariate regression model for change in spine bone mineral density*

CHANGE IN SPINE BMD		
Independent variable	***Slope***	***p-value***
Log-transformed change in urinary potassium excretion	0.018	0.032
F (overall model significance)= 4.728 (p= 0.032) r^2= 0.047		

Age, race, and the interaction between race and gender had negative relationships with change in hip t-score, meaning that being older or being Caucasian had a negative effect on hip t-scores in Table 26. Gender and change in calcium to phosphorus ratio had a positive relationship with change in hip t-score, meaning that being male and increasing calcium intake in relation to phosphorus intake was associated with an increase in hip t-score. The interaction means that Caucasian males had less of an increase in hip t-score than non-Caucasian males. However, this analysis should be taken with caution, given that there are only two non-Caucasian males, so the interaction and relationship to change in hip z-score is of suspect due to the low sample size in that sub-group. The regression model significantly predicted about 25% of the variation in the outcome variable ($p<0.001$).

Table 26: *Final univariate regression model for change in hip bone mineral density t-score*

CHANGE IN HIP T-SCORE		
Independent variables	***Slope***	***p-value***
Age	-0.016	<0.001
Gender[1]	1.24	0.009
Race[2]	-0.099	0.416
Gender x Race[3]	-0.86	0.072
Change in calcium to phosphorus ratio	0.223	0.001
F (overall model significance)= 5.79 (p<0.001) r^2= 0.248 [1]Males [2]Caucasians [3]Caucasian males		

As shown in Table 27, race was the only "significant" predictor of change in spine t-score, meaning that being male was associated with a positive change in spine t-score as compared to being female. The regression model did not significantly predict the change in the outcome variable (p=0.085), and it only explained 3% of the variation in spine t-score.

Table 27: *Final univariate regression model for change in spine bone mineral density t-score*

CHANGE IN SPINE T-SCORE		
Independent variable	***Slope***	***p-value***
Race[1]	0.164	0.085
F (overall model significance)= 3.03 (p= 0.085) r^2= 0.03 [1]Caucasians		

Table 28 illustrates that age had a significant negative effect on change in hip z-score, meaning that older participants had greater decreases in hip z-score compared to younger participants. Gender and change in calcium to phosphorus intake ratio both had positive effects on change in hip z-score, meaning that being male was associated with a positive change in z-score compared to being female and that increasing calcium intake in relation to phosphorus intake had a positive impact on hip z-score. The regression model significantly predicted about 21% of the variation in the outcome variable ($p<0.001$).

Table 28: *Final univariate regression model for change in hip bone mineral density z-score*

CHANGE IN HIP Z-SCORE		
Independent variables	***Slope***	***p-value***
Age	-0.017	0.001
Gender[1]	0.447	0.001
Change in calcium to phosphorus ratio	0.234	0.001
F (overall model significance)= 7.99 ($p<0.001$) r^2= 0.212 [1]Males		

As shown in Table 29, gender and change in protein intake had a negative relationship with change in spine z-score, meaning that being male and increasing protein intake were associated with a decrease in spine z-score. Race, the interaction between gender and race, change in sodium intake, and change in the protein to carbohydrate ratio had positive relationships with change in spine z-score. This means that Caucasians, Caucasian males, an increase in protein intake in relation to carbohydrate intake, and an increase in sodium intake (although the effect is very small) was associated with an increase in spine z-score. The interaction means that Caucasian males had a positive effect on hip z-score as compared to non-Caucasian females. Something to note is that there are only two non-Caucasian males, so the interaction and relationship to change in hip z-score is of suspect due to the low sample size in that sub-group. The regression model significantly predicted about 36% of the variation in the outcome variable ($p<0.001$).

Table 29: *Final univariate regression model for change in spine bone mineral density z-score*

CHANGE IN SPINE Z-SCORE		
Independent variables	***Slope***	***p-value***
Gender[1]	-2.023	<0.001
Race[2]	0.088	0.344
Gender x Race[3]	2.047	<0.001
Change in protein intake	-0.006	<0.001
Change in protein to carbohydrate ratio	0.242	0.001
Change in sodium intake	0.000085	0.011
F (overall model significance)= 8.13 ($p<0.001$) r^2= 0.359 [1]Males [2]Caucasians [3]Caucasian males		

POST-HOC ANALYSIS

Quantitative variables of interest (i.e. protein intake groups and change in protein intake groups) were organized into categorical variables—reference groups of intake to represent low, medium, and high intakes, and then changes in protein intake groups (i.e. from low-to-high protein intake groups or from medium-to-low protein intake groups) from baseline to six-months. The variables were then analyzed by ANOVA to compare means of outcome variables among the tertiles. Post-hoc analysis was completed using Tukey's studentized method and the Bonferroni-Sidak method. No significant differences in means of changes in outcome variables between groups were found.

DISCUSSION

SUMMARY

This prospective, parallel-cohort study with two dietary behavioral interventions compared the changes in bone-related parameters to estimate the effects of high-and low-carbohydrate diet patterns on skeletal health. The bone-related parameters measured were urinary calcium excretion, bone formation as measured by bone-specific alkaline phosphatase, bone resorption as measured by urinary N-telopeptide, and bone quality as measured by whole body, spine and hip bone mineral content, bone mineral density, and hip and spine bone mineral density t- and z-scores. Study participants were the 115 participants enrolled in the Insight Weight Loss Study that had hip and spine DEXA scans.

The primary aim of this study was to examine changes in urinary calcium excretion after six months of the high- or low-carbohydrate dietary interventions. The secondary aim of this study was to determine if changes in bone resorption and formation markers would be different between diet interventions. The tertiary aim of this study was to measure differences in the changes in bone quality (whole body, hip, and lumbar spine BMC, BMD, t-scores, and z-scores) between diet groups.

The primary hypothesis was accepted; increased urinary calcium excretion (in mg/24hr, mmol/mmol creatinine and mmol/kg/d) was observed in the low-CHO diet compared to the high-CHO diet. Urinary calcium excretion was significantly higher at six-months in the low-CHO group compared to baseline; also, change in urinary calcium excretion was significantly greater in the low-CHO group than the high-CHO group,

which did not demonstrate a significant change. From the three different mechanisms that can result in increased urinary calcium excretion, excessive bone resorption may not be the source since the change in urinary NTx concentration was not significantly different between diet groups nor was it significantly different from baseline in either group. Change in urinary calcium excretion also failed to significantly predict any changes in bone quality markers (BMC, BMD, t-scores, and z-scores) in linear regression models, meaning that the changes in bone quality could not be determined by the changes in urinary calcium excretion.

The mechanism by which a low-carbohydrate diet increases urinary calcium excretion is not clear in this study. Increased intestinal absorption of calcium may contribute, but this can only be theorized since fractional calcium absorption was not measured. A study that corresponds with this observation is that increased fat intake may increase intestinal calcium absorption by lengthening the amount of time food is in contact with the absorptive enzymes in the stomach and intestinal tract (76). This may be possible since the low-CHO diet group consumed a significantly higher amount of fat compared to the high-CHO group. An increased filtered load of calcium may be the cause of the increased urinary calcium excretion, but this may only be theorized at this point since serum calcium concentration and creatinine clearance, a surrogate marker of GFR, were not available for analysis. Vitamin D and PTH were also not measured, which limits the conclusion of this study to only theorize the cause of increased urinary calcium excretion. Other studies have shown that increased protein intake can increase urinary calcium excretion, either by increasing the renal acid load (48) or by consuming an abundance of certain types of amino acids—specifically sulfur-containing amino acids

(40) or aromatic amino acids (60). Since protein intake was significantly higher at six-months in the low-CHO diet group compared to the high-CHO diet group, it can be theorized that the increased protein content of the diet may have contributed to the increased urinary calcium excretion, as many other studies have found (52-57). However, protein intake failed to predict changes in urinary calcium excretion in the linear regression equations. Additionally, the increased protein intake in the low-CHO could also have contributed to an increase of intestinal calcium absorption (55), which can only be theorized at this point.

In the linear regression models predicting change in urinary calcium excretion, the only significant predictors were change in urinary sodium excretion and percent of weight change from baseline. Diet group also significantly predicted urinary calcium excretion, but weight change was significantly correlated to diet group—Pearson's correlation coefficient 0.435 and $p<0.001$—thereby confounding the relationship with the outcome variable. Weight change was the more robust variable, thus the reason why it was kept in the model and not diet group. Diet group was not significant in the final model with weight change in the model. Urinary sodium excretion is inherently related to urinary calcium excretion due to the physiological links between sodium and calcium movement in the kidney. Renal reabsorption of sodium and calcium parallel each other in the ascending limb of the Loop of Henle (26). Also, calcium's paracellular pathway in the kidney is dependent on sodium reabsorption. Although the movement of sodium and calcium is linked in the kidney, urinary sodium excretion did not change within the low-CHO group between baseline and six-months, but the changes were not significantly different between groups. So, although change in urinary sodium excretion

significantly predicted change in urinary calcium excretion, it still does not explain the difference in urinary calcium excretion between groups. Calcium's reabsorption in the kidney may have been inhibited in the low-CHO group due to the high protein content of the diet, because a higher urinary acid load from increased protein intake inhibits reabsorption in the luminal tubule (58). Another interesting finding was that a change in weight significantly predicted a change in urinary calcium excretion, meaning that weight loss was associated with increased calcium excretion. This could be due to the hypothetically increased bone resorption and bone loss that coincides with weight loss (77,78); however, bone resorption markers did not increase in the low-CHO group.

The secondary hypothesis was not accepted; the low-CHO diet did not result in higher urinary concentrations of NTx or serum lower concentrations of BSAP at six-months. The six-month values of the markers were not significantly different between groups, nor were the changes from baseline were not significantly different between groups This provides a foundation for the theory that a low-CHO diet pattern may increase urinary calcium excretion without increasing bone resorption or suppressing bone formation.

In linear regression analysis, many significant predictors were observed for the bone resorption and formation markers. Unlike urinary calcium excretion, intake of certain dietary variables significantly predicted changes in bone formation and resorption. Increased intake of the following variables was favorable to bone formation or decreased bone resorption: protein, fiber, phosphorus, sodium, and an increase in calcium intake in relation to phosphorus intake. Increased sodium and phosphorus intake had very small effects (β= 0.001 and -0.032, respectively). On the contrary,

increased intake of the following variables increased bone resorption or increased bone formation: carbohydrate, phosphorus, calcium, potassium, and increased calcium intake in relation to phosphorus intake. It is noted that increased phosphorus intake, as well as an increase in the calcium to phosphorus intake ratio, significantly predicted both increased formation and resorption, demonstrating the complex relationships between calcium and phosphorus intake and bone turnover. Non-dietary predictors of bone turnover include age, which increased BSAP, and weight change; weight loss decreased BSAP and a decrease in BMI led to increase in NTx/BSAP ratio.

The tertiary hypothesis was not accepted; the low-CHO diet did not impact bone quality, as measured by BMC and BMD. Six-month bone quality values were not significantly different from baseline within groups, and the changes in the values were not significantly different between groups. This is consistent with the results from the bone resorption and formation analyses—the low-CHO diet did not cause increased rates of bone loss or changes in bone quality. Also, significant changes in spine BMC were noted in both groups, and whole body BMC increased in the low-CHO group. The mean changes in these variables did not differ significantly between groups, which shows that neither diet was more beneficial or detrimental to bone quality after the six-month intervention.

Given that the changes were not significantly different between groups, there must be a reason aside from the diet intervention for the small increases in bone mineral content. It can be postulated that a loss of weight can result in more accurate DEXA scans and clearer images of bone mass, especially in the spine scan, due to a decrease in girth and fat mass. Furthermore, weight change from baseline was

significantly negatively correlated with change in whole body BMC ($p<0.01$) and BMD ($p<0.001$), meaning that a lower weight at six-months was correlated with a positive change in whole body BMD and BMC. This adds credence to the theory that a loss in weight may result in clearer DEXA images, although it cannot be proved with this evidence alone. Additionally, physical activity was an important part of the behavioral intervention, as all participants were counseled to exercise at a moderate intensity for 30 minutes most days of the week. Physical activity, especially load-bearing exercises, can mitigate bone loss caused by weight loss (78,79); accordingly, physical activity may have been a silent contributing factor to the increase in bone mineral content or the lack of bone loss. Physical activity data was collected for the Insight study, but was not available for analysis at the time of this study.

In linear regression analysis of changes in bone quality markers, only a few significant predictors of changes in the bone quality parameters were found. The dietary variables that had a positive impact on bone quality include the following: increased potassium and sodium intake, increased calcium intake in relation to phosphorus intake, and increased protein intake in relation to carbohydrate intake. Increased protein intake also significantly predicted a negative change in spine z-score. Other predicting variables include weight loss, NTX, urine potassium excretion, age, gender, and race. NTX demonstrated its predictive powers of changes in spine BMC and whole body BMD, meaning that an increase in the resorption marker predicted a decrease in bone quality.

STRENGTHS OF STUDY

The main strength of this study is the initial sample size of 115 participants recruited from the general population, although the six-month sample sizes were lower from baseline and between groups. The behavioral interventionists of the study and data collectors were blinded to the outcomes and the data collectors were blinded to the diet assignment. The interventionists at KPCHR that conducted the weekly group sessions were separated from the staff at OCTRI that collected and recorded the data. The number of outcomes used to assess bone health is also a strength because it allows for a full exploration of the impact on bone health. This study also had significantly different diet patterns between groups with differing macronutrient composition and micronutrient values, which allows a full analysis of the impact of changing dietary intake. Also, after doing back calculations to determine the power of the study to detect differences between groups, this study had an 81% power to detect significant differences in urinary calcium excretion between groups based on the effect size observed. Another significant strength is the largely homogenous population of the participants, who were mostly Caucasian females between the ages of 40-60 years old.

LIMITATIONS OF STUDY

There are many limitations to this study and the generalization of its results. The original study was powered to detect significant differences between diet groups in the main outcome variable of weight loss. Power of this sub-analysis to detect differences in the outcome variables was not computed before the initiation of the study, as the original study was designed and powered to detect differences in weight loss. After

using calculations to approximate the power this study had to detect differences in changes of bone-related outcome variables, it was observed that there was not adequate power (6-7%) to detect differences in changes in the two bone turnover markers between the two diet groups. The power was also inadequate (4-40%) to detect differences in changes in bone quality measures between groups. The highest degree of power was observed in the BMC analysis (40%), as compared to low power in BMD analysis (4-8%). Statistical analysis was limited by the type of methods used to compare the changes in the outcome variables. The absolute differences in the outcome variables were compared between groups rather than controlling for baseline values in the changes. This could have overestimated the significance of the changes within groups and differences between groups. A different method of statistical analysis that could have been used to better assess change from baseline is analysis of covariance (ANCOVA), also termed the conditional change model. This model takes into account the baseline values and different variances of the quantitative variables.

This sub-analysis of bone-related data was limited by the initial protocol's selection of bone turnover markers and the subsequent timing of sample analysis. Although samples were collected at baseline and six-months for PTH, vitamin D, and osteocalcin, these biomarkers were not measured at the time of this sub-analysis. Concentrations of PTH and vitamin D would have added a significant amount of understanding to changes in calcium homeostasis before and after the dietary interventions. Also, the N-telopeptide assay had a high interassay coefficient of variation (CV), 6-15%, compared to the bone-specific alkaline phosphatase assay interassay CV of 3-7%. This higher CV means that the NTx assay itself had relatively high variability and

inherent low reproducibility. This higher variation between sample duplicates can contribute to the inability to detect changes in bone resorption as measured by NTx.

This study relies on participants' self-reported dietary intake by 24-hour dietary recalls to estimate dietary intake of macronutrient and micronutrient intake. There may have been error resulting from participants under-reporting or over-reporting intake of certain foods or beverages. The inaccurate representation of dietary intake is consistent across different populations, with men overestimating energy and fat intake and women under-reporting overall dietary intake; also, under-reporting of dietary intake is especially evident in overweight people (90). This is evidenced by the great difference between estimated energy needs for weight maintenance in similar populations at ~3100 kcal/d, as shown by Dr. Diane Stadler and other researchers in unpublished studies, and the baseline mean energy intake reported by participants as 1878 ± 539 kcal/d in the high-CHO diet group vs 2010 ± 707 kcal/d in the low-CHO diet group. This disparity in reported energy intake when compared to energy needs to maintain weight in an overweight and obese population demonstrates the possible reporting error by the participants.

The accuracy of the 24-hour recalls also relies on the staff member that interviewed the participant. The recalls were gathered by different people with different methods, although all interviewers used the same multi-pass technique. Errors in diet recalls can also be attributed to differences in interpretation of the foods consumed or the amounts eaten from the interviewer and the person who entered the dietary data into the nutrient database. Also, another limitation to the use of the 24-hour recalls is the nutrient database itself, as all foods and different variations are not available in the

database, necessitating the use of substitutes that are available in the database. The inter-individual variation in dietary variables is also a limitation to diet analysis. For example, the range of energy intake was quite large (800-5000 kcal/d). In addition to all of the diet-related limitations, changing more than one dietary variable at a time prevents us from making conclusions regarding the effects of changing specific dietary constituents on the outcome variables.

One major limitation to the study is the fact that the changes in the outcome variables were only examined for a six-month period. Bone is a slow-moving system and bone quality should ideally be monitored long-term. Another limitation to the bone quality data is that physical activity assessment, while collected for the larger study, was not available for analysis at the time of this study. Physical activity can attenuate bone loss caused by weight loss (27), meaning that our participants may have lost bone mass, but it is not known how physical activity has affected changes in bone in this population.

Another limitation to this study is that information on women's menopausal status was not gathered, which can significantly alter any bone-related data and changes therein. The physiology of perimenopausal women can differ significantly from pre- and postmenopausal women, so that data would have been useful for stratification of subjects and post-hoc analysis. Also, participants' vitamin intake or changes in medication use were not recorded, which are items that may have affected dietary mineral composition, urinary mineral excretion, and bone-related changes. The generalization of this study's results is limited to the overweight and obese population and to Caucasian women in general. Despite concentrated efforts to include more men

and minorities, this study's population is mostly female and Caucasian, so extrapolation to all males and non-Caucasians should be done with caution.

FUTURE DIRECTIONS

Further research in this area should be continued to discover the dietary causes of increased urinary calcium excretion from the low-carbohydrate diet composition, or whether the increase was due to weight loss. It would be interesting to study weight loss and its effects on urinary calcium excretion and whether or not urinary calcium excretion is an estimate of bone resorption in weight loss. It is difficult to separate the diet's micronutrient composition from the diet pattern, so future research on low-carbohydrate diets could be conducted on the usual micronutrient intake of these diets, as well as the effects on mineral intake. Supplementation of calcium and other minerals that may be low in low-carbohydrate diets could possibly change the diet's effects on urinary mineral excretion, so supplementation may be an interesting area of study for people following low-carbohydrate diets. Also, it would be beneficial to know whether or not the increased urinary calcium excretion is a transient or a long-term effect after following a low-carbohydrate diet. If future studies on this topic were to include women, it would be interesting to know if the dietary effects on urinary calcium excretion differed among women's different states of menopause (pre-, peri-, and postmenopausal).

Since weight loss was the main objective of this study, it is difficult to pinpoint the exact causes (i.e. dietary intake, weight loss, or clearer DEXA images) for the changes seen in bone mineral content in both groups. Another interesting question this

study brings up is whether or not the changes in BMC as measured by DEXA scans, specifically in the spine, are related to a decrease in trunk area and/or waist circumference. Also, more research should be conducted in the area of bone mass, weight loss, and DEXA scans to discover how much weight loss is needed to see a change in bone content or density as a result in improved signal transmission. Also, if a person were to gain or regain weight, would the DEXA scan record a gain in bone mass to support the extra weight, or would the extra fat mass obscure the true bone content and mass in the scans? It is obvious that this study brings up a lot of questions that can be answered in future studies and research.

CONCLUSIONS

This study provides evidence to the theory that a low-carbohydrate dietary pattern increases urinary calcium excretion. Although the change in urinary calcium excretion was higher in the low-carbohydrate group, the evidence in this study suggests that this increase in urinary calcium excretion was not derived from increased bone resorption. The exact cause of urinary calcium excretion associated with the low-carbohydrate diet cannot be determined from the data and results presented here, but it is hypothesized that the low-carbohydrate, high-protein, and high-fat content of the diet had a synergistic effect on the increased urinary calcium excretion.

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APPENDIX

A: OHSU CONSENT FORM

	Oregon Health & Science University **Consent Form** **eIRB#: 777** **Protocol Approval Date: 01/13/2006**

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form-Cohorts 3 and 4

TITLE: *Metabolic Consequences of Low and High Carbohydrate Diets: The Insight Weight Loss Study*

PRINCIPAL INVESTIGATOR:	Diane Stadler, PhD, RD	(503) 494-0168
CO-INVESTIGATORS:	Njeri Karanja, PhD	(503) 335-2417
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	Angela Horgan, PhD, RD	(503) 494-6231

SPONSOR: National Center for Complementary and Alternative Medicine, National Institutes of Health

PURPOSE:

You have been invited to be in this study at OHSU because you are enrolled in the Insight Weight Loss Study conducted by the Kaiser Permanente Center for Health

Research. The purpose of the Insight Weight Loss Study is to compare the effects of the ATKINS diet (low-carbohydrate) and the DASH diet (high-carbohydrate) on weight loss, maintenance of weight loss, and overall health.

Up to 300 participants will be enrolled into the Insight Weight Loss Study and randomly assigned (assigned by chance) to follow either the Atkins-style diet or the DASH-style diet. Each participant will be enrolled in the study for 30 months and will attend three visits at the OHSU General Clinical Research Center (GCRC) to complete study related measurements. All participants, regardless of the group they are in, will complete a standard set of study measurements before they start the dietary intervention and again 6-months and 30-months after they start the dietary intervention. Each set of standard measurements takes about two hours to complete. In addition to completing the three standard measurement sets, 36 participants will be randomly selected to participate in the Resting Energy Expenditure Subsample. A different set of 48 participants will be randomly selected to participate in the Meal Tolerance Subsample.

PROCEDURES:

During each of the three visits to the General Clinical Research Center at OHSU you will be asked to complete the:

Standard Measurement Set:

- Arrive in the morning before you have eaten breakfast or any other food or beverage, except water, and before you have participated in any significant physical activity or exercise.
- Return your completed Quality of Life questionnaire distributed by the KPCHR study staff or, if necessary, complete the questionnaire at OHSU. This form may take up to 30 minutes to complete. This questionnaire contains about 130 questions and asks about nutrition-related issues and dieting and emotional well-being, vitality, general health status, sleep quality, daily function and work activity.
- Return all of the urine you collected during the past 24 hours into the container(s) provided to you by Kaiser Permanente Center for Health Research staff. If urine was spilled or if you forgot to collect some of your urine, you may be asked to repeat the collection and return it to the GCRC.
- Provide a urine sample for a pregnancy test if you are female. The results of the urine pregnancy test will remain private. We will inform you of the results and, if positive, refer you to your regular doctor or health care provider for on going care.
- Have your weight height, and waist circumference measured. This process will take about 10 minutes.
- Have your body composition (the amount of lean and fat tissue in your body) and bone density measured with whole body, left hip, and lumbar spine scans using a DEXA machine. This process will take about 30 minutes. You will be asked

to remove any clothing or jewelry that contains metal (for example, metal snaps, clasps, buckles, rings, ear-rings, etc). This procedure can only be performed if you weight less than 340 pounds and are less than 6'2" tall.

- Have your body composition measured by bioelectrical impedance. This process involves passing a very small, unperceivable electrical current between sets of electrodes attached to removable adhesive pads placed temporarily near your ankle and near your wrist. This is a painless, risk-free process that takes less than one minute to complete.
- Have your blood pressure measured twice after resting for 5 minutes. This step takes about 8 minutes.
- Provide a fasting blood sample of about four tablespoons from an arm vein. This step takes about 5 minutes to complete
- For those completing the standard measurement set, only, a breakfast meal will be provided after all measurements are done. You should plan to spend about 2 hours in the GCRC at each visit to complete the "Standard Measurement Set".
- At the 6- and 30 month visits you will be fitted with an activity monitor that is to be worn at your waist, attached to the waistband of your clothing. You will be asked to wear the activity monitor for 7 days and then to return the monitor to the Kaiser Center for Health Research in a self-addressed, stamped envelope.
- Within 4 weeks of your 6- and 30 month visits you will be contacted by telephone on two separate days to be interviewed about the foods and drinks that you've consumed during the past 24 hours. Each phone interview will take about 15 minutes to complete.

Resting Energy Expenditure Measurement:

If you are assigned to the resting energy expenditure subsample, you will complete the standard measurement set plus you will have your energy expenditure (calorie use) measured while you rest. This process takes about 1 hour to complete. A lightweight, clear, Plexiglas canopy will be placed over your head and chest so that samples of the air that you breathe out can be collected and analyzed. A trained research assistant will perform these procedures in a private room and care will be taken to maximize your comfort and feelings of relaxation. A breakfast meal will be provided after all measurements have been completed. You should plan to spend about three hours in the GCRC at each visit to complete all study related measurements.

Meal Tolerance Subsample:

If you are assigned to the meal tolerance subsample, you will be admitted to the inpatient unit of the GCRC the morning of or the evening before this set of measurements. If you are scheduled for these measurements on a weekday, you will complete the standard measurement set in addition to the meal tolerance procedure. If you are scheduled for the meal tolerance procedure on a weekend day, you will complete the blood sampling procedures only and the standard measurement set will be scheduled within 2 weeks on a weekday.

During the meal tolerance procedure, you will have a blood-sampling catheter inserted into one of your arm veins between 8:00-8:30 am and this catheter will remain inserted in your arm for up to nine hours. Blood samples of about 1 ½-2 tablespoons each will be collected before you eat a GCRC prepared breakfast meal and again ½, 1, 1 ½, 2, 2 ½, 3, 3 ½, 4, 6, and 8 hours after eating the breakfast meal (about 16 ½ tablespoons of blood in total). If the blood-sampling catheter stops working during the process a second catheter may need to be placed in your other arm. After the last blood sample has been taken, the blood drawing catheter will be removed from your arm. You will be encouraged to drink water during the blood collection procedure but you will be asked not to eat any food until after the last blood sample is collected. A lunch/dinner meal will be provided to you at the end of the procedure.

In between the three regularly scheduled OHSU visits, you may be asked to return to the GCRC for additional safety monitoring at the discretion of the study investigators. These visit(s) may include:

- Drawing additional blood samples of about one tablespoon total.
- Having a physical examination performed by one of the study physicians or their designee.
- Participating in an interview to review your medical history with one of the study physicians or their designee.

If you have any questions about the measurements taken at OHSU for the Insight Weight Loss Study, now or in the future, contact Diane Stadler at (503) 494-0168.

RISKS AND DISCOMFORTS:

Blood sampling will be performed by a registered nurse or a trained phlebotomist for those providing a fasting blood sample, only. A registered nurse will insert and draw blood from the blood sampling catheter inserted into an arm vein for those in the Meal Tolerance Subsample. You may feel some pain when your blood is drawn or when the blood sampling catheter is inserted. There is a small chance the needle will cause bleeding, a bruise, or an infection. There is also a small chance that part way through the meal tolerance test, the blood sampling catheter will stop working and that a new blood sampling catheter will need to be inserted into a vein in your other arm.

As a result of the DEXA scans (whole body, left hip, lumbar spine) performed in this study you will be exposed to some radiation (x-rays). The DEXA scans provide about the same exposure to x-rays as a cross-country airplane flight. While no amount of radiation has been proven to be safe, there is no direct evidence that small doses of radiation, similar to those used in the DEXA scans, cause harmful effects in the persons who are exposed.

There is no risk involved with having body composition analyzed by bioelectrical impedance.

There are no risks associated with having resting energy expenditure measured using a canopy air-collection system. However, some people report feeling claustrophobic or "closed-in."

The Quality of Life Questionnaire includes questions about the hassles associated with following a specific diet, nutritional health perceptions, and nutrition and social function. Some of these questions may seem very personal or embarrassing. They may upset you. You may refuse to answer any of the questions that you do not wish to answer. If the questions make you very upset, we will help you to find a counselor.

BENEFITS:

You may or may not personally benefit from being in this study. However, by serving as a subject, you may help us learn how to benefit patients in the future. Laboratory tests will be performed at no cost. You will be informed of any clinically significant abnormalities and these abnormal laboratory results will be provided to your physician upon your request.

ALTERNATIVES:

You may choose not to participate in any or all of the measurements taken in the General Clinical Research Center at OHSU. If you choose not to participate, you may be asked to withdraw from the Insight Weight Loss Study conducted by the Kaiser Permanente Center for Health Research.

CONFIDENTIALITY:

We will not use your name or your identity for publication or publicity purposes.

To have blood samples taken and analyzed at OHSU as part of this study you must have an OHSU medical record number. If you did not already have an OHSU medical record number, one was assigned to you as part of the GCRC scheduling process. To ensure that the medical record number assigned to you is unique, that this number has not been, or will not be, assigned to anyone else, you were asked to provide two forms of personal information such as your social security number and your mother's maiden name. Your OHSU medical record number will be provided to authorized data management staff at the Kaiser Permanente Center for Health Research. The data management staff will use your OHSU medical record number to identify your study-related blood sample results, only. All other OHSU measurement results will be transferred to the data management staff at the Kaiser Permanente Center for Health Research but this information will be labeled with the unique study code assigned by the Center for Health Research, only.

Some of the blood samples collected at OHSU will be sent to the University of Colorado Health Sciences Center, the University of California-Los Angeles, Pacific Biometrics, Inc or LipoScience, Inc for analysis. Some urine samples collected at OHSU will be sent to the University of Iowa Hospital and Clinics for analysis. Blood and urine samples sent to the University of Colorado Health Sciences Center or the University of Iowa Hospital and Clinics will be labeled with the unique study code assigned by the Center for Health Research, only.

Research records may be reviewed and/or copied by the sponsor of the study, the OHSU Institutional Review Board, the Office for Human Research Protections, the OHSU General Clinical Research Center, the National Center for Research Resources, the OHSU Laboratory and its contracted subsidiaries as required by law.

COSTS:

There are no costs associated with having measurements taken at OHSU for the Insight Weight Loss Study. All costs associated with collecting and analyzing the blood and urine samples and performing the body composition, resting energy expenditure, and meal tolerance measurements will be paid for by the study. You are not offered payment for being in this study.

LIABILITY:

If you believe you have been injured or harmed while participating in this part of the research study and require immediate treatment, contact Diane Stadler, PhD at (503) 494-0168.

It is not the policy of the U.S. Department of Health and Human Services, or any federal agency funding the research project in which you are participating, to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

The Oregon Health & Science University is subject to the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you suffer any injury and damage from this research project through the fault of the University, its officers or employees, you have the right to bring legal action against the University to recover the damage done to you subject to the limitations and conditions of the Oregon Tort Claims Act. You have not waived your legal rights by signing this form. For clarification on this subject, or if you have further questions, please call the OHSU Research Integrity Office at (503) 494-7887.

PARTICIPATION:

If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887. You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any

time. If you refuse to join or withdraw early from this part of the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

If you are a student or employee at OHSU, your participation in this research project is completely voluntary. You are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course. If you choose to withdraw from the study during your OHSU visit, we will request that you attend a final study interview at the Kaiser Permanente Center for Health Research.

Dr. Stadler or Dr. Gerhard may withdraw you from the measurements done at OHSU at any time if they believe it is in your best interest or if you are unable to follow instructions or complete the procedures. We will inform you of any new findings that may affect your willingness to continue or to withdraw from this part of the Insight Weight Loss Study.

We will give you a copy of this signed consent form.

SIGNATURES:

Your signature below indicates that you have read this entire form and agree to participate in this study.

Subject's Printed Name Subject's signature Date

Investigator's signature Date

Oregon Health & Science University

HIPAA RESEARCH AUTHORIZATION

AUTHORIZATION FOR THE CREATION, USE, AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR INSTITUTIONAL REVIEW BOARD APPROVED RESEARCH

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Title of Study:	Metabolic Consequences of Low and High Carbohydrate Diets (aka, Insight Weight Loss Study)
Name of Investigator:	Diane Stadler, PhD, RD
Phone Number:	503-494-0168
Sponsor:	National Institutes of Health (NIH): National Center for Complementary and Alternative Medicine (NCCAM)
IRB Number:	e777
Protocol Approval Date:	01-13-2006
Consent Form Approval Date:	01-25-2006

This authorization is voluntary, and you may refuse to sign this authorization. **If you refuse to sign this authorization, your health care and relationship with OHSU will not be affected. However, you will not be able to enter this research study.**

1. This form authorizes Oregon Health & Science University (OHSU) to use and disclose (release) certain protected health information about _____ (name of subject)

   that we will collect and create in this research study. The description of the information to be used or disclosed and the purposes of the requested use or disclosure are indicated in item number 8 of the authorization form.

2. The persons who are authorized to use and disclose your protected health information are:
   - ☒ All investigators listed on page one of the Research Consent Form
   - ☒ Others at OHSU who are participating in the conduct of this research protocol
   - ☒ The OHSU Institutional Review Board
   - ☒ Others: OHSU General Clinical Research Center and OHSU laboratories and its contracted subsidiaries

3. The persons who are authorized to receive this information are:
   - ☒ The sponsor of this study: NIH; National Center for Complementary and Alternative Medicine
   - ☒ Federal or other governmental agencies as required for their research oversight and public health reporting in connection with this research study:
     ☒ OHRP ☐ FDA ☒ Other: National Center for Research Resources
   - ☒ Others: Kaiser Permanente Northwest Center for Health Research
     University of Colorado Health Sciences Center
     University of Iowa Hospital and Clinics
     University of California, Los Angeles
     Liposcience, Inc. Raleigh, NC
     Pacific Biometrics, Inc Seattle, WA

4. We may continue to use and disclose protected health information that we collect from you in this study until:

☐ HIPAA Research Authorization expiration date _____

-OR-

☐ The study is completed _____

☐ Indefinitely

☒ Other: Five years after the IRB has accepted thefinal report as required by NIH

5. While this study is still in progress, you may not be given access to medical information about you that is related to the study. After the study is completed and the results have been analyzed, you will be permitted access to any medical information collected about you in the study.

6. You have the right to revoke this authorization and can withdraw your permission for us to use your information for this research by sending a written request to the Principal Investigator listed on page one of the research consent form. If you do send a letter to the Principal Investigator, the use and disclosure of your protected health information will stop as of the date he/she receives your request. However, the Principal Investigator is allowed to use and disclose information collected before the date of the letter or collected in good faith before your letter arrives. If you withdraw any tissue or blood samples that were collected from you, they either will be destroyed or stored without any information that identifies you. Revoking this authorization will not affect your health care or your relationship with OHSU.

7. The information about you that is used or disclosed in this study may be re-disclosed and no longer protected under federal law. However, Oregon law restricts re-disclosure of HIV/AIDS information; mental health information; genetic information; and drug/alcohol diagnosis, treatment, or referral information.

8. Description of the information to be used or disclosed and the purposes of the requested use or disclosure:

HEALTH INFORMATION **(Check as applicable)**	PURPOSE(S) *(Enter corresponding letter(s) from Purpose Categories)*
☐ ***Your complete existing health record *****	_____
☐ ***Limited information from your existing health record** (specify):***	
_____	_____

***** If we are requesting existing health records that are located outside of OHSU, you will need to complete an additional authorization to release these records to OHSU.***

THE FOLLOWING CHECKED ITEM(S) WILL BE GENERATED/COLLECTED DURING THE COURSE OF THIS STUDY:

☐ ***History and physical examinations***	_____
Reports: ☒ ***Laboratory*** ☐ ***Operative*** ☐ ***Discharge*** ☐ ***Progress***	***a, f***
☐ ***Photographs, videotapes, or digital or other images***	_____
☒ ***Diagnostic images/X-ray/MRI/CT***	***a***
☐ ***Bioelectric Output (e.g., EEG, EKG)***	_____
☒ ***Questionnaires, interview results, focus group survey, psychology survey, behavioral performance tests (e.g., memory & attention)***	***a, f***
☒ ***Tissue and/or blood specimens***	***a, f***
☒ ***Other: urine samples***	***a, f***
OHSU medical record number	***f***

PURPOSE CATEGORIES

a. To learn more about the condition/disease being studied
b. To facilitate treatment, payment, and operations related to the study
c. To comply with federal or other governmental agency regulations
d. For teaching purposes
e. To place in a repository or information/tissue "bank."
f. Other To analyze research results

9. If the information to be used or disclosed contains any of the types of records or information listed just below, additional laws relating to use and disclosure of the information may apply. You understand and agree that this information will be used and disclosed only if you **place your INITIALS** in the applicable space next to the type of information.

N/A Acquired immunodeficiency syndrome (AIDS) or human immunodeficiency virus (HIV) infection information
N/A Drug/alcohol diagnosis, treatment, or referral information
N/A Mental or behavioral health or psychiatric care
N/A Genetic testing information

You will receive a copy of this authorization form after you sign it.

Printed name of Research Subject

Signature of Subject | Date

-OR-

Printed name of Subject's Legally Authorized Representative

Signature of Subject's Legally Authorized Representative | Date

Description of Relationship to Subject: _____