Change in Vitamin D Concentration in Obese Individuals After a Six-month Weight Loss Intervention of High- versus Low-Carbohydrate Diets

by

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS AND ACRONYMS

$1,25(OH)_2D$	1,25-dihydroxy-vitamin D
25(OH)D	25-hydroxy-vitamin D
BECC	Body and Energy Composition Core
BMD	Bone Mineral Density
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
KPCHR	Kaiser Permanente Center for Health Research
NHANES	National Health and Nutrition Examination Survey
OCTRI	Oregon Clinical & Translational Research Institute
OHSU	Oregon Health & Science University
PTH	Parathyroid Hormone
UVB	Ultraviolet B or medium wave length radiation

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Abstract

Background: Vitamin D deficiency is more prevalent in overweight and obese individuals than in normal weight individuals. Reasons for this difference are not well understood but are attributed to reduced sun exposure and therefore reduced endogenous synthesis of vitamin D, lower intake of dietary vitamin D, and greater sequestration of vitamin D in adipose tissue in the those who are overweight or obese compared to their normal weight peers.

Objectives: The primary aim of this study was to determine how serum 25-hydroxy-vitamin D [25(OH)D] concentration, a marker of vitamin D status, changes with weight loss among individuals who are overweight and obese. A secondary aim was to determine whether demographic and lifestyle factors influence the change in circulating 25(OH)D concentrations with weight loss.

Method: A randomized controlled trial was used to assess the impact of a six-month weight loss intervention on change in mean serum 25(OH)D concentration in individuals adhering to either a high- (n=60) or low-carbohydrate (n= 59) diet. Weight, body composition, energy and nutrient intake, and fasting serum 25(OH)D and parathyroid hormone (PTH) concentrations were measured before and after the intervention.

Results: In this study, 92% of participants had serum 25(OH)D concentrations <30 ng/ml and were considered vitamin D insufficient. Furthermore, 40% of participants had serum 25(OH)D concentrations <20 ng/ml and were considered vitamin D deficient. These frequencies of vitamin D insufficiency and deficiency were seen before and after

weight loss and regardless of dietary assignment. Mean weight loss was 12 ± 6 kg and 6 ± 6 kg in the low-carbohydrate and high-carbohydrate diet groups, respectively. Fat mass decreased 6 ± 5 kg and 5.5 ± 5 kg in the low-carbohydrate and high-carbohydrate diet groups, respectively. Mean serum 25(OH)D concentration was 22 ± 7 at baseline and the mean change in 25(OH)D concentration was -2 ± 6 ng/ml after weight loss. The mean change in serum 25(OH)D concentration was not different between diet groups nor was it influenced by weight loss. Significant correlations were observed between serum 25(OH)D concentration and weight (r = -0.2), BMI (r = -0.2) and PTH concentration (r = -0.2) before and after weight loss. However, no significant correlations were observed between the change in serum 25(OH)D concentration and weight loss, fat mass, or BMI.

Conclusions: The prevalence of vitamin D deficiency and insufficiency was high among the overweight and obese individuals participating in this study. Mean serum 25(OH)D concentrations did not improve despite significant weight loss and loss of fat-mass. These results suggest that vitamin D supplementation may be necessary to achieve and maintain optimal vitamin D concentrations in overweight and obese individuals, especially those living in the Pacific Northwest. Additional research is needed to confirm these results and to better understand the physiological mechanisms that contribute the high risk of vitamin D deficiency and insufficiency among those who are overweight and obese.

Chapter 1

Introduction and Significance

Obesity and vitamin D deficiency are increasingly common in the US today. In its obesity surveillance by state for 1988, the Centers for Disease Control and Prevention (CDC) reported an overall prevalence of obesity of 10%-14%. In 2008, the prevalence of obesity was between 15-29%, with some states having prevalence rates >30% (1). In 2007-2008, the overall occurrence of obesity in the US was 34% (1-3). Studies also suggest that approximately 36% of healthy young adults, 18-29 years of age, and 41% of older adults 49-83 years of age are vitamin D deficient (4, 5). Prevalence studies also reveal that vitamin D insufficiency and deficiency are more common in overweight and obese individuals than normal weight individuals (6-16). Recent studies report that serum vitamin D concentration, the primary marker of vitamin D status, is inversely related to degree of obesity and body mass index (BMI) (4, 7, 10, 17, 18).

The relationship between obesity and vitamin D insufficiency/deficiency is not well understood. One explanation is that vitamin D is sequestered in adipose tissue to a greater extent in obese individuals than normal weight individuals. Another explanation is that vitamin D, synthesized endogenously in the skin, may not be as efficiently transported into the circulation of obese individuals as normal weight individuals. Yet other explanations are that obese individuals may not synthesize as much vitamin D endogenously due to lower sun exposure or they may not consume as much vitamin D from the diet or dietary supplements as their normal weight peers.

One question that has not yet been answered is whether weight loss increases serum vitamin D concentrations. Although a growing body of research confirms an association between obesity and low serum vitamin D concentrations, there is no rigorous research on the effect of weight loss and adipose tissue mobilization on circulating concentrations of the vitamin. The study reported here was undertaken to determine the effect of weight loss, generated through high- and low-carbohydrate diets, on fasting serum vitamin D concentrations in a well-defined and controlled population. This randomized trial was funded by grants from the National Center for Complimentary and Alternative Medicine and the National Center for Research Resources, components of the National Institute of Health.

Specific Aims and Hypotheses

The aim of this research was to evaluate vitamin D status as measured by serum total 25(OH)D concentration and how vitamin D status is affected by weight loss in healthy, overweight and obese adults. This work was part of a larger randomized controlled clinical trial to determine the effects of high and low carbohydrate diets on body weight and markers of disease risk. The specific aims and hypotheses of the present research are:

Aim 1: To measure serum total 25(OH)D concentration, by liquid chromatography tandem mass spectrometry (LC-MS/MS), and total body fat mass, by DEXA, before and after a six-month low- or high-carbohydrate dietary weight loss intervention.

Hypothesis: Serum total 25(OH)D concentration will be higher after weight loss and a reduction in body fat mass than before.

Aim 2: To determine if change in serum total 25(OH)D concentration is predicted by age, race, gender, season, smoking history, medication, dietary supplement use, and serum intact parathyroid hormone (PTH) concentration.

Hypotheses: Serum total 25(OH)D concentration will be predicted by season, sex, age, PTH concentration and use of dietary supplements and not predicted by diet or smoking history.

Chapter 2

Background

Vitamin D is commonly referred to as the "sunshine vitamin" and is well recognized for its role in regulating circulating concentrations of calcium and phosphate. Vitamin D also modulates neuromuscular function, reduces inflammation and influences the action of many genes (19, 20). There are two major physiological forms of vitamin D, ergocalciferol (Figure 1), also known as vitamin D_2, derived primarily from plant-based foods and dietary supplements, and cholecalciferol (Figure 2), also known as vitamin D_3, derived primarily from sun exposure, animal-based foods, or dietary supplements. After absorption or synthesis, both forms of vitamin D are converted to calcidiol, 25(OH) D, in the liver (Figure 3). Circulating 25(OH)D, the primary form of vitamin D in the blood, is then converted to calcitriol, 1,25(OH)2D, the biologically active form of vitamin D, in the kidney (Figure 4). The half-life of serum 25(OH)D is two to three weeks (21, 22), where as the half life of serum 1,25(OH)2D is 4-6 hours (21, 23). For this reason, measurement of serum 25(OH)D concentration is used to assess vitamin D status.

Low serum 25(OH)D concentrations can result from inadequate sunlight exposure, impaired endogenous synthesis in the skin, poor diet, insufficient intestinal absorption, or liver disease that prevents hydroxylation at carbon 25 (4, 9, 19). Although there is no consensus for accepted criterion to define vitamin D deficiency or insufficiency, serum 25(OH)D concentrations less than 20 ng/ml are generally considered to be deficient, concentrations between 20 to 30 ng/ml are considered to be

insufficient, concentrations between 30-50 ng/ml are considered to be adequate and concentrations greater that 50 ng/ml are considered to be necessary for optimal health. The classical disease of vitamin D deficiency is rickets in children and osteomalacia or osteoporosis in adults. Both conditions can cause bone and muscle pain and lead to a higher risk of fracture with increased age (20)(24). In addition to compromised skeletal health, vitamin D deficiency and insufficiency are linked to other chronic diseases including cardiovascular disease, diabetes, hypertension, cancer (19, 20), autoimmune disease, susceptibility to infection, (23) and obesity (9). Although a number of studies have shown an inverse relationship between circulating concentrations of vitamin D and obesity (9-11, 14, 15, 17, 25, 26), no studies have reported on the effect of weight loss on vitamin D concentrations in healthy overweight and obese individuals. Understanding how weight loss impacts vitamin D concentrations may lead to more effective, targeted interventions to prevent and treat vitamin D deficiency and insufficiency within this group who is also at increased risk for developing obesity-related co-morbidities.

Synthesis and Metabolism of Vitamin D

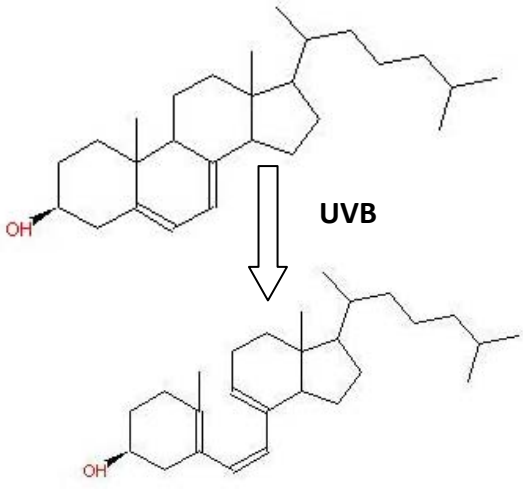


Figure 1. Structures of 7-Dehydrocholesterol and Cholecalciferol

Vitamin D, derived endogenously from 7-dehydrocholesterol, is a steroid that contains four rings; one of which is broken between carbon 9 and 10, yielding a 'seco-steroid' (27). In humans, synthesis of vitamin D occurs in the epidermis. Upon exposure to sunlight, specifically UVB radiation, 7-dehydrocholesterol (pre-vitamin D_3) is isomerized to cholecalciferol or vitamin D_3 in the stratum spinosum of the epidermis (4, 27-29). The stratum spinosum is located at the top of the basal layer of the epidermis near the dermal later which is highly vascularized (29). Vitamin D_3 is transported in the blood bound to a vitamin D binding protein (28, 29).

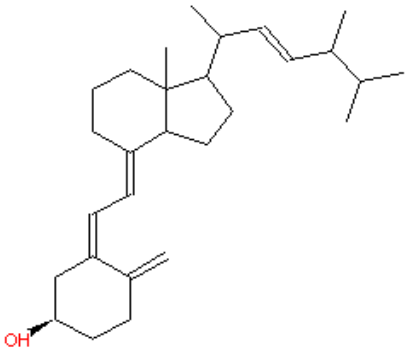


Figure 2. Structure of Vitamin D_3

Plants contain the steroid ergocalciferol which is also known as pre-vitamin D_2. This form of vitamin D can be extracted from plants and is used in dietary supplements. The structures of vitamin D_2 and vitamin D_3 differ only in their side chains; vitamin D_2 contains a double bond between carbons 22 and 23 and a methyl

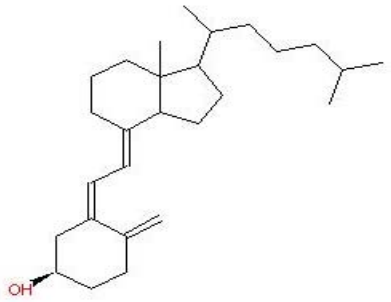


Figure 3. Structure of Vitamin D_2

group at carbon 24 (30). This structural difference causes vitamin D_2 to be less bioavailable than vitamin D_3 (30).

Hydroxylation of Vitamin D

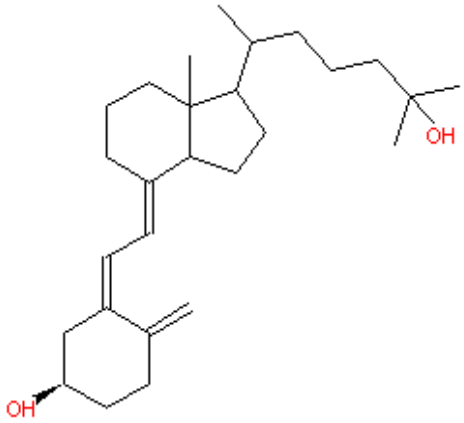


Figure 4. Structure of 25(OH)D_3

Once vitamin D is synthesized or ingested in the body, it goes through a series of hydroxylation steps to become active. Vitamin D, bound to vitamin D-binding protein, is transported to the liver (21, 27, 28). In the liver, vitamin D is hydroxylated by 25-hydroxylase to form 25-hydroxyvitamin D [25(OH)D]. 25(OH)D, bound to vitamin D binding protein, enters the portal blood stream (21, 22) and is transported to the kidney where it is hydroxylated to form 1,25$(OH)_2$D via 1α-hydroxylase. 1,25$(OH)_2$ vitamin D is the active metabolite which has a half life of only 4-6 hours. Hydroxylation of 25(OH)D and 1,25$(OH)_2$D also occurs at the 24 carbon by 24-hydroxylase, yielding, 24,25$(OH)_2$D and 1,24, 25$(OH)_3$D (21, 31). These metabolites are formed to prevent 1,25$(OH)_2$D toxicity (21).

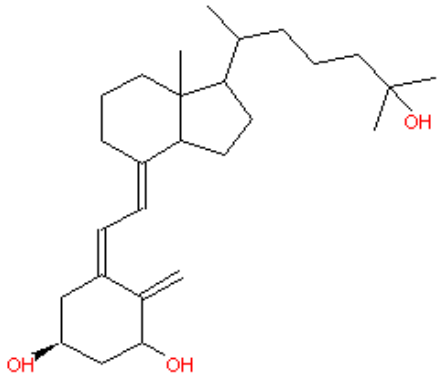


Figure 5. Structure 1,25$(OH)_2D_3$

Biomarkers of Vitamin D

The biomarkers most commonly used to assess vitamin D status are serum concentrations of 25(OH)D and parathyroid hormone (PTH), and the presence or absence of clinical signs of deficiency such as

rickets in children or osteomalacia in adults (21). Serum 25(OH)D is the major circulating form of vitamin D (9, 21, 27, 30), and has a half life of 2-3 weeks which reflects vitamin D status over a longer period of time than $1,25(OH)_2D$ (21, 31). Serum PTH concentration is a biomarker of vitamin D status because elevated PTH concentrations are associated with low serum 25(OH)D concentrations, low serum calcium concentrations, and a higher risk of osteoporosis (21). The normal range of serum PTH concentration is between 10-69 pg/ml. When serum concentrations of vitamin D or calcium are low or phosphorus concentrations are high, PTH is released from the parathyroid gland. Higher circulating concentrations of PTH stimulate hydroxylation of 25(OH)D to $1,25(OH)_2D$ in the kidney. Higher circulating concentrations of $1,25(OH)_2D$ increase calcium absorption in the intestine, calcium reabsorption in the kidney, and calcium resorption from bone to normalize blood calcium concentrations. Elevated blood PTH concentration due to low serum 25(OH)D concentration is known as secondary hyperparathyroidism (32).

Dietary and Supplemental Sources of Vitamin D

Foods high in preformed vitamin D_3 include foods of animal origin, such as liver, beef, egg yolk, and some saltwater oily fish. In the US, certain foods, milk and margarine in particular, and some cereals as well as orange juice, are fortified with vitamin D. Milk, fortified with vitamin D, contains 100 IU of vitamin D per 8 ounces. The amount of vitamin D in fortified cereals and orange juice products varies depending on the manufacturer (33). Over-the-counter vitamin D supplements are available independently or in combination with calcium or as part of a multivitamin. In the US,

over-the-counter vitamin D supplements contain vitamin D_2 or vitamin D_3 in concentrations of 200-5,000 IU per capsule. Prescription-strength vitamin D supplements may contain up to 50,000 IU of vitamin D_3 or D_2. In the US, only the vitamin D_2 form is available at prescription-strength.

Recommended Intake of Vitamin D

In 1997, the Food and Nutrition Board of the Institute of Medicine established a Dietary Reference Intake for vitamin D, including an adequate intake of and a tolerable upper limit for vitamin D. The adequate intake of vitamin D was established based on values known to affect bone health and calcium absorption (33). From birth to fifty years of age, the adequate intake of vitamin D is 200 IU (5 μg/d) per day, from 50-70 years of age the adequate intake of vitamin D is 400 IU per day (10 μg/d), and for those 71 years of age the adequate intake of vitamin D is 600 IU per day (15 μg/d) (34). In 2008, the American Academy of Pediatrics updated their policy guidelines to increase vitamin D recommendations for infants and children to 400 IU per day (35). In 2009, the Institute of Medicine's Food and Nutrition Board delegated a committee to revise the Dietary Reference Intakes of vitamin D and calcium for the adult population (36). The committee will review all of the currently available research and release their updated report by the end of summer 2010 (36). The tolerable upper limit for vitamin D is currently set at 2000 IU per day for children and adults and 1000 IU per day for infants under one year of age. The tolerable upper limit was established to prevent vitamin D-induced hypercalcemia, excess bone loss, and hyperphosphatemia (33).

In a recent risk assessment that evaluated current vitamin D intake recommendations, Hathcock, et al. concluded that the current tolerable upper limit of 2000 IU's is lower than research indicates as safe. Twenty-one studies, published between 1981-2006, investigated supplementation with doses of vitamin D that were above the current upper limit (2000 IU per day). Of the studies evaluated, fifteen involved healthy individuals who consumed vitamin D supplements of 800 IU per day to 100,000 IU every four months (37-40). No significant changes in serum calcium concentration, urinary calcium excretion, or toxic levels of serum 25(OH)D concentrations were reported (37, 38, 40, 41). This summary of the current literature points toward the safety of a higher recommendation for dietary vitamin D in the healthy adult population.

Ongoing Considerations of Vitamin D Recommendations

A topic of ongoing debate is the circulating concentration of 25(OH)D that yields the maximum benefit to the general public. In 2007, the 13th Workshop Consensus for Vitamin D Nutritional Guidelines was convened among 334 scientists, researchers, and nutrition experts representing 23 countries (42). Conclusions were that vitamin D deficiency and insufficiency were widespread among populations in many countries, recommendations for vitamin D intake needed to be increased, adequacy of vitamin D should be defined by a serum value that meets or exceeds 20 ng/ml, and vitamin D_3 fortification in more of the food supply is needed to achieve these goals (42). Those

attending the workshop presented evidence to support an increase in the tolerable upper limit of vitamin D to 10,000 IU per day.

Impact of Sun Exposure, Season and Latitude on Circulating Vitamin D Concentration

Under some circumstances, healthy people can meet their vitamin D requirements through sun exposure. UVB radiation at wavelengths of 290-315 nm must penetrate the skin to convert 7-dehydrocholesterol to vitamin D_3 (21, 33). The amount of UVB radiation exposure the skin receives depends on the season, latitude, time of day, duration of exposure, cloud cover, use of sunscreen or protective clothing, the amount of melanin in the skin, age, air quality, and altitude (21, 33). Year-round UVB exposure that produces vitamin D_3 in the skin is obtained in areas below the 34° N parallel, which in the United States, runs from Los Angeles, California to Columbia, South Carolina (33). UVB exposure obtained above the 42° N parallel is not sufficient to produce vitamin D from November through February. In the United States, the 42° N parallel runs from the northern border of California to Boston, Massachusetts (33). The farther above the 42° N parallel, the longer the time that UVB exposure is insufficient to generate vitamin D_3 (33).

Vitamin D Status of Individuals Living Portland, Oregon

Portland, Oregon is above the 45° N parallel and, as a result, people living in or around Portland are at risk for vitamin D deficiency due to insufficient sun exposure during the winter months. To achieve and maintain the recommended circulating vitamin D concentration from UVB exposure, an individual needs to receive 15-30

minutes of sun exposure to the legs, arms, face, or torso (without sunscreen) between 10am and 3pm, two times per week (33). In a study of 276 men, 65 years of age or older, living in Portland, the average total 25(OH)D concentration was 24.3 ng/ml (43). Almost 24% of those studied were vitamin D deficient (<20 ng/ml) in the summer and 47% were deficient in the winter (43). The authors concluded that a higher dietary intake of vitamin D through food or supplements is required, especially during the winter months when sun exposure is limited, to ensure adequate vitamin D status year-round. A study examining vitamin D status among internal medicine residents at Oregon Health & Science University in Portland, Oregon reported that 51% of these healthcare professionals had serum 25(OH)D concentrations <20 ng/ml (44). When analyzed by season, the average 25(OH)D concentration was slightly higher in the fall, 25 ± 8 ng/ml, than in the spring, 20 ± 8 ng/ml, ($p<0.001$) (44). These two studies reveal that vitamin D deficiency and insufficiency are common among young and old adults living in Portland, regardless of the time of year.

Relationship Between Vitamin D and Obesity

Vitamin D deficiency and insufficiency are common in the general healthy adult population (4, 9, 44-47), and even more common among the overweight and obese (9-15, 17, 25). A number of studies have shown an inverse relationship between circulating concentrations of vitamin D and level of obesity (9-11, 14, 15, 17, 25, 26). Goldner, et al. reported mean serum 25(OH)D concentrations of 19 ± 10 ng/ml and 36 ± 14 ng/ml ($p<0.0001$) in 41 pre-bariatric surgery patients with BMIs between 41-88 kg/m^2

compared to 41 non-obese controls with BMIs between 20-29 kg/m^2 (25). Of the pre-bariatric surgery patients, 61% were vitamin D deficient (<20 ng/ml), 29% were vitamin D insufficient (20-30 ng/ml) and only 10% were vitamin D sufficient (>30 ng/ml). Of the control group, 12% were vitamin D deficient, 32% were vitamin D insufficient, and 56% were vitamin D sufficient.

Parikh and colleagues studied the relationship between obesity and vitamin D status in obese (BMI>30 kg/m2) and non-obese (<30 kg/m2) Caucasian and African American participants (14). Mean serum 25(OH)D concentration was lower in the obese participants (23.5 ± 12 ng/ml) than the non-obese participants (31 ± 14 ng/ml) ($p<0.001$). In addition, negative correlations were observed between serum 25(OH)D concentrations and BMI and fat mass ($r=-0.4$; $p<0.0001$). There was no significant difference in mean serum 25(OH)D concentrations between the African American and Caucasian participants (14).

Kremer and associates observed an inverse relationship between serum 25(OH)D concentration and body fat mass in young Hispanic and Caucasian females (n=90), 16-22 years of age, living in California, (11). The girls were categorized as normal weight (BMI ≤ 25 kg/m^2) or overweight (BMI > 25 kg/m^2) and vitamin D insufficiency was defined as a serum 25(OH)D concentrations ≤ 29 ng/ml. The mean serum 25(OH)D concentration of the Hispanic girls was 27 ± 12 ng/ml which was significantly lower than mean serum 25(OH)D concentration of the Caucasian girls (35 ± 12 ng/ml; $p=0.002$). The average 25(OH)D concentration of girls categorized as normal weight or overweight was 34.3 ±

14 ng/ml and 24.6 ± 9.5 ng/ml ($p<0.001$), respectively. Regardless of weight status, Hispanic girls had lower serum 25(OH)D concentrations than Caucasian girls ($p=0.002$). Although vitamin D insufficiency was more common in Hispanics, after adjusting for BMI the significance of the difference diminished ($p=0.09$). These results suggest that despite living in Southern California, where UVB exposure is thought to be sufficient for adequate vitamin D_3 synthesis, the need for vitamin D supplementation may be higher than previously recognized to maintain optimal serum vitamin D concentrations. This may be especially pertinent to those who are overweight or who purposefully restrict sun exposure (11).

Effect of Obesity on Endogenous Synthesis of Vitamin D and Response to Therapeutic Vitamin D supplementation

To determine if obesity affects endogenous vitamin D synthesis, Wortsman and collaborators exposed White obese (BMI > 30 kg/m^2) and non-obese (BMI ≤ 25) control subjects to whole body UVB exposure (48). The average increase in serum vitamin D concentration, 24-hours after UVB exposure, was 57% lower in the obese participants (6.7 ± 1 ng/ml) than in the control participants (15.3 ± 2 ng/ml). BMI and serum 25(OH)D concentrations after UVB exposure were inversely correlated ($p=0.003$). The results of this study suggest that endogenous synthesis of vitamin D is lower and/or the transport of vitamin D from the skin to the blood is less efficient, in obese compared to non-obese individuals.

To better understand the reason for this difference in response to UVB exposure, the same authors measured the conversion of 7-dehydrocholesterol to vitamin D_3 in skin samples obtained from two obese and two non-obese subjects. Samples of epidermal and dermal tissue were obtained during surgery and 7-dehydrocholesterol and vitamin D_3 concentrations were measured by high-performance liquid chromatography. The percentage of 7-dehydrocholesterol that was converted to vitamin D_3 was not significantly different in the younger obese (9.4%) and non-obese (9.6%) subjects or the older obese (7.6%) and non-obese (7.3%) subjects. These results, although interpreted with caution because of the limited sample size, suggest that differences in the ability to synthesize vitamin D_3 do not explain the differences in circulating concentrations of vitamin D in obese and non-obese individuals.

These same authors then assessed the response of obese (n=19) and non-obese (n=19) individuals to a therapeutic oral dose of vitamin D (50,000 IU vitamin D_2) (63). Blood samples were taken before and 6, 10, and 24 hours after an oral vitamin D_2 loading dose was administered. The average peak vitamin D_2 concentration occurred approximately ten hours after the oral loading dose and was not significantly different between the obese, 72 ng/ml, and non-obese, 92 ng/ml, groups (p=0.06). Like the response to UVB exposure, BMI was inversely correlated with serum 25(OH)D concentration after supplemental D_2 treatment (p=0.007). These results suggest that intestinal absorption and transport of vitamin D into the circulation is similar among obese and non-obese individuals. When taken together, the combination of these

results does not explain why circulating concentrations of vitamin D are lower in obese than non-obese individuals.

Difference in Serum and Adipose Tissue Vitamin D Concentrations in Obese Individuals

Blum and associates measured vitamin D_3 concentrations in serum and subcutaneous adipose tissue samples from 17 obese individuals with a mean BMI of 51 ± 6.4 kg/m^2 (7). The average vitamin D_3 concentration in adipose tissue was 103 nmol/kg (~42 ng/ml) where as the average vitamin D_3 concentration in serum was 7.8 ± 4 nmol/l (~3.1 ng/ml). This large discrepancy in tissue concentration may be due, at least in part, to vitamin D_3 being stored in subcutaneous adipose tissue instead of entering the circulation, which may contribute to the low serum 25(OH)D concentration seen in the obese (7).

Effect of Body Composition on Serum Vitamin D Concentrations

Since vitamin D is a fat soluble vitamin that is present in adipose tissue, varying degree of adiposity and total fat mass may affect circulating vitamin D concentrations. To determine the effect of body weight and body composition on serum vitamin D concentration, Bolland and colleagues examined the effect of season on serum 25(OH)D concentration in post-menopausal women (n=1606) and older men (n=378) (49). Subjects were divided into quartiles based on body weight and fat mass and sine curves were generated to predict serum 25(OH)D concentration (49). With each additional kilogram of fat mass or body weight, similar changes were seen in peak, trough, and amplitude of vitamin D concentration between seasons. Those in the highest quartile of

fat mass had the smallest change in serum 25(OH)D concentration between seasons. The authors hypothesized that this inverse relationship was the result of vitamin D being sequestered in adipose tissue of overweight individuals leading to lower circulating concentrations of vitamin D. Alternative explanations were that, obese individuals were less likely to be outdoors, or when they were, more likely to wear protective clothing or to use sunscreen, that limited their sun exposure and thus their endogenous production of vitamin D.

Effect of Weight Loss on Serum and Adipose Tissue Concentrations of Vitamin D

Holick and associates measured vitamin D concentrations in serum and adipose tissue samples obtained from six bariatric surgery patients, three and six months after surgery. No significant change in serum vitamin D concentration was detected (9). In a different weight loss study (15), sixty overweight women 20-35 years of age, were assigned to one of two diets. One diet emphasized increasing consumption of vegetables to a minimum of three servings per day, the other diet emphasized increasing consumption of enriched cereals and cereal bars to a minimum of three times per day (50). Women in each diet group were separated into two subgroups: those with baseline serum 25(OH)D concentrations < 20 ng/ml and those with baseline serum 25(OH)D concentrations ≥ 20 ng/ml. After the two week dietary intervention, serum vitamin D concentrations were an average of 2 ng/ml higher in women with baseline concentrations < 20 ng/ml and 3 ng/ml higher in women with baseline concentrations ≥ 20 ng/ml group. Although average serum 25(OH)D concentrations increased in both

groups, those who consumed the high-cereal diet had a higher overall increase in serum 25(OH)D concentration (3 and 5 ng/ml, in the < 20 and ≥ 20 ng/ml subgroups, respectively) than those who consumed the high-vegetable diet (1 and 2 ng/ml, in the < 20 and ≥ 20 ng/ml subgroups, respectively). Those in the high-cereal group consumed more vitamin D (7 ± 2 μg/d) than those in the high-vegetable group (3 ± 2 μg/d) ($p<0.05$) (50).

The women with 25(OH)D concentrations ≥ 20 ng/ml at baseline demonstrated greater weight loss (1.7 ± 1.8 kg) than those with 25(OH)D concentrations < 20 ng/ml at baseline (0.5 ± 0.8 kg) ($p<0.001$). The participants in the high-cereal group, with serum 25(OH)D concentrations ≥ 20 ng/ml, lost an average of 2.7 ± 1.8 kg. Those in the high-vegetable group, with serum 25(OH)D concentration ≥ 20 ng/ml, lost an average of 0.7 ± 1.2 kg ($p<0.001$). It is important to recognize that this study is limited by the short duration (two weeks) of the intervention; since the half life of 25(OH)D in serum is approximately two weeks, which may not have allowed sufficient time for the diet or the weight loss to significantly influence serum 25(OH)D concentration (50).

In summary, it is well established that overweight and obese individuals have lower circulating concentrations of 25(OH)D and are at higher risk for vitamin D insufficiency and deficiency than normal weight individuals. What is not well established is whether weight loss and, as a result, mobilization of adipose tissue, a storage site for vitamin D, increases circulating concentrations of vitamin D among individuals who are

overweight or obese. Information derived from this analysis will help to answer this question.

Chapter 3

Methods

General Design

The work described in this thesis is a substudy of the Insight Weight Loss Study, a randomized controlled trial that evaluated the short- and long-term metabolic consequences of low-carbohydrate versus high-carbohydrate diets for weight loss. Study subjects were healthy adult men and non-pregnant, non-lactating women, over 21 years of age, who were overweight or obese (BMI 27-50 kg/m^2), and who wanted to lose weight. Individuals with controlled hypertension, mild hyperlipidemia (but not on lipid-lowering medications), and pre-diabetes were considered for enrollment if they met other eligibility criteria as shown in Table 1. Participants were assigned to the high- or low-carbohydrate intervention using a balanced allocation scheme that considered sex, age, BMI, total cholesterol, total triglycerides, and presence of metabolic syndrome. The Institutional Review Boards at Oregon Health & Science University (OHSU) and Kaiser Permanente Northwest, and the State of Oregon Radiation Safety Committee approved this study. Written informed consent was obtained and Notice of Privacy Health Insurance Portability and Accountability Act forms were signed by each participant at the baseline visit.

Recruitment and Screening

Several recruitment methods were used, including e-mail announcements, mailings, notices in various media, and referrals from Kaiser Permanente and community providers. Kaiser Permanente was the main source of recruitment, providing electronic, administrative, and medical databases to prescreen participants. The primary purpose of screening was to identify potential volunteers with debilitating health conditions who did not meet the inclusion criteria, and to give potential participants a better sense of the time commitment to the project.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Permission of primary care physician	Contraindication to weight loss
Age 21 years or older	Type 1 or 2 diabetes
BMI of 27-50 kg/m^2	Cardiovascular event in the past year
Normal blood pressure or well-controlled (<4 medications) stage I hypertension	Recent use of prescription weight loss medications in the past 3 months
Fasting glucose < 126 mg/dl	Current use of medications for treatment of psychosis or manic-depressive illness, diabetes mellitus, or lipid lowering
Fasting LDL cholesterol <160 mg/dl	History of gastric bypass surgery or liposuction
Fasting total triglycerides <300 mg/dl	Evidence of active cancer diagnosis (except for non-melanoma skin cancer) or treatment in past 2 years, defined as any diagnosis or any treatment within the past 2 years
Normal liver and kidney function	Excessive alcohol consumption or binge drinking
Willing to modify diet and other health behaviors	Weight loss of > 20 lbs in the past 3 months
Ability to give consent	Pregnant, breast-feeding, or planning to become pregnant

General Study Design

The Insight Study was a randomized controlled clinical trial whose goal was to address the health claims of popular low-carbohydrate weight loss diets compared to the high-carbohydrate Dietary Approaches to Stop Hypertension (DASH) diet (51). Phase I was an intensive behavioral weight loss intervention that lasted for six-months; Phase

II, a maintenance phase, lasted until the end of the study at 30 months. A total of 240 participants were enrolled into the study over three years and four cohorts were established based on the date of enrollment. Outcome measures for the Insight Study were collected at 0, 6, and 30 months, however, only data collected at 0 and 6 months were used for this substudy analysis. In addition, to control for season of enrollment, only participants enrolled in cohorts two and four, who completed both baseline and six-month appointments, were used for this substudy analysis.

Cohorts two and four were chosen because their baseline appointments and their six month appointments occurred in the summer or early fall and in the winter or early spring, respectively. Cohort two's baseline measurements took place between July and September of 2005 and six-month measurements took place between February and April of 2006. Cohort four's baseline measurements took place between September and November of 2006 and six-month measurements took place between April and May of 2007. The month of each appointment is important because in Portland endogenous vitamin D production occurs between March and October, provided adequate exposure to sunlight without the use of sun protection, such as sunscreen or excessive clothing.

During the first six months of the intervention, participants met in small groups with behavior change counselors for 90 minutes each week to discuss adherence to the high- or low-carbohydrate diets. If a participant was unwilling or unable to attend group sessions, individual counseling was provided by phone or in person. The goal of the counseling sessions was to increase adherence to the diet while giving participants the

tools to be successful at losing weight. Principles of social cognitive theory (52) motivational interviewing (53) and the transtheorectical model, also known as the stages-of-change model (54) were used to guide the counseling sessions. Participants were encouraged to identify barriers to losing weight and ways to overcome those barriers while finding the internal motivation necessary for behavior change to occur. The small group meetings were participant-focused and designed to support and encourage participants as they initiated behavior change for weight loss. During the first meeting, participants identified individual needs and established personal goals. A "Food and Fitness Guide" and a "Personal Lifestyle Planner" were provided and participants were asked to keep a three-day food record to assess their diet. Participants were also given a personal resource package that included a diet-specific cooking and meal pattern guide and other items such as measuring cups, water bottles, vegetable peelers, tote-sports bags, magnets, and logs and checklists to facilitate adherence to the diet and to help monitor progress.

A physical activity component was included in each group counseling session and was the same for both intervention arms. The goal was for participants to perform moderate-intensity aerobic activity for at least 30 minutes per day, four to five days per week. This recommendation is consistent with the Surgeon General's Report on Physical Activity and Health where moderate-intensity, aerobic, physical activity is defined as activity that increases the heart rate to 50 to 70% of maximal heart rate (55). The physical activity intervention focused on home-based programs that the participant

enjoyed and that addressed barriers to exercising and solutions around those barriers, such as scheduling a time for daily exercise and identifying an exercise partner.

Group Session Structure

All sessions were held at KPCHR in Portland, Oregon. Each group session followed the same basic structure. Participants checked-in, were weighed, and submitted self-monitoring records. Then, a discussion was held on one of six main curriculum components. The first session addressed fundamental topics such as meal patterns, the challenges of eating out, and identifying alternative types of moderate physical activity. The behavioral change component addressed potential barriers related to self-monitoring, progress reviews, identifying positive social support, group sharing, and making appropriate goals and action plans. The facilitators encouraged active participant involvement through taste-testing foods, watching cooking demonstrations, and practicing new ways to exercises.

Low-Carbohydrate Diet Plan

The low-carbohydrate diet plan was modeled after the Atkins diet (56) and progressed through four phases: weight loss induction, ongoing weight loss, pre-maintenance, and maintenance. Participants were encouraged to follow the weight loss induction phase for eight weeks. Participants were given 14 days of structured meal plans and encouraged to limit their carbohydrate intake to 20 grams per day. Foods consumed during this phase included meats, fish, poultry, eggs, cheese, nuts, butter, oil, and whipping cream. After the eight-week induction phase, participants were advised to

start the ongoing weight loss phase, during which, participants continued to restrict their carbohydrate intake but expand their food choices. Carbohydrate-gram counting guides were provided and participants were taught how to add carbohydrate into their diet slowly, incorporating an additional five grams of carbohydrate per week. Participants remained in the ongoing weight loss phase until they lost at least 10% of their baseline body weight. During the weight loss pre-maintenance phase, participants gradually increased their carbohydrate intake until they stopped losing weight and then lowered it slightly to continue very gradual weight loss. The maintenance phase, was achieved when participants reached their goal weight and were able to balance their carbohydrate intake with weight maintenance, which typically occurred after the first six months. If their goal weight was not achieved, participants were encouraged to return to the induction phase so weight loss would continue past six-months.

High-Carbohydrate Diet Plan

The high-carbohydrate diet was modeled after the Dietary Approaches to Stop Hypertension (DASH) diet (51) and emphasized fruit and vegetable intake along with consumption of low-fat dairy and meat products, and complex-carbohydrate grains. The high-carbohydrate diet was high in potassium, phosphorus, calcium, and magnesium, and low in saturated fat, sodium, and cholesterol. There were five main dietary goals: to reduce fat intake to no more than 25% of total energy, to consume 8-12 servings of fruits and vegetables daily, to eat 2-3 servings of low-fat dairy products daily, to reduce consumption of sweets and sweetened beverages, and finally, to reduce portion sizes to

reduce energy intake. At the initial counseling session, participants were encouraged to limit fat intake to 25% of their estimated energy needs and were given a specific amount of fat, in grams, to consume per day. Participants were instructed to spend the first eight weeks following a structured eating plan with energy-controlled meals, to initiate weight loss. A shopping guide was provided with shopping lists, tips, and guides to typical grocery stores to facilitate implementation of the diet. Subsequent sessions focused on adding fruits, vegetables, and low-fat dairy products into the diet, rather than removing foods from the diet; portion control and limited consumption of fats and sweets; planning small frequent meals, with salads; and unlimited consumption of vegetables.

Outcome Measurement Visits

Baseline measurements were scheduled up to two months before the intervention started, and follow-up measurements were scheduled five to seven months after the dietary intervention started. All measurements were performed at the Oregon Clinical & Translational Research Institute (OCTRI) at OHSU in Portland, Oregon. Participants arrived for their appointments between 0700 and 0830 after a 12-hour overnight fast. Urine, collected for the previous 24 hours, and fasting blood samples were obtained 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). Participants then changed from street clothing into a hospital gown and removed all metal-containing objects. Height, weight, and

body composition were measured by trained and licensed technicians in the OCTRI Body Energy and Composition Core (BECC) facility. After all samples were obtained and measurements were completed, participants changed back into their street clothes and were provided a complimentary continental breakfast.

Anthropometric Measurements

Weight was measured with a Scale-Tronix 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 by more than 0.5 kg, a third weight measurement was taken and the two closest measurements were averaged. Height, without shoes, was measured using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Ltd, Crymych, UK), and was recorded to the nearest 0.1 cm. Height was measured at the baseline visit only and carried forward to six-months. Body mass index (BMI) was calculated as the participant's weight in kilograms divided by his or her height in meters-squared.

Blood Sample Collection and Analysis

OCTRI nursing staff collected fasting blood samples from a peripheral arm vein by venipuncture using sterile technique. Blood samples were collected into 10 mL red-top tubes and green-top tubes that contained heparin to obtain serum and plasma, respectively. The green-top tube was sent immediately to the OHSU Clinical Chemistry Laboratory for analysis of a Complete Metabolic Screen, including plasma calcium concentration. The red-top tube was sent to the OCTRI Core Laboratory and allowed to

sit for 20 minutes to ensure complete clotting of red blood cells. The tube was spun in a centrifuge at 2800 rpm for 12 minutes to separate serum from red blood cells. The serum was harvested and transferred to polypropylene storage tubes and stored at -80° C for analysis of 25(OH)D and PTH concentrations at the end of the study.

Vitamin D concentration was measured as 25(OH)D_3 and 25(OH)D_2 using a high pressure liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method (57) in the OHSU Bioanalytical Shared Resource/Pharmacokinetics Core Laboratory. Serum samples (200 µl) were treated with 0.7 ml of acetonitrile:methanol (95:5) that contained 10 ng/ml of the internal standard, ([$^2H_6$]-25-OH-D_3). The sample was mixed, centrifuged at 14,000 x g for 10 minutes and 500 µl of the supernatant was transferred to an auto-sampler vial. Fifty µl of the treated sample was analyzed using a Shimadzu Prominence HPLC interfaced to an Applied Biosystems 4000 Q-TRAP hybrid triple quadrupole/linear ion-trap mass spectrometer with an atmospheric pressure ionization source (APCI) operating in the positive mode. The HPLC separation was conducted using a Thermo Betabasic-C18 (100 x 2.1mm, 5µ) column with 0.1% formic acid (solvent A) and methanol with 0.1% formic acid and 5 mM ammonium acetate. The flow rate of 0.4 ml/minute with 60% solvent B was increased to 98% solvent B over 2 minutes, maintained for 3 minutes, and then returned to starting conditions for 3 minutes. The 25(OH)D_3 and 25(OH)D_2 and internal standard, ([$^2H_6$]-25-OH-D_3, were watched in the eluant with multiple reaction monitoring. Source parameters were optimized for the dehydration of each analyte and consisted of curtain gas; ion spray voltage, 5000 V; nebulizer current; temperature, 450 °C; and ion source gas 1. Optimal multiple reaction

monitoring parameters were obtained for each analyte and consisted of the following parent/product ion pairs: 25(OH)D_3 m/z = 383.3 → 257.2; 25(OH)D_2 m/z = 395.4 → 209.3; and [$^2H_6$]-25-OH-D_3 m/z = 389.4 → 263.3. The dwell time was 100 msec with unit resolution. Standards ranging in concentration from 1 ng/ml to 200 ng/ml were prepared in phosphate buffered saline containing 4% bovine serum albumin and treated exactly as the serum samples. The concentration of stock solutions of 25(OH)D_3 and 25(OH)D_2 were determined by the absorbance at 265 nm using a Uvikon UV/VISABLE dual wavelength spectrophometer and a molar extinction coefficient of 18,200. Instrument control and data analysis were performed with Analyst 1.5 software (Applied Biosystems Carlsbad, CA). Peak area ratios of analyte to internal standard were obtained and the resulting linear regression equation was used to calculate serum 25(OH)D_2 and 25(OH)D_3 concentrations. The coefficients of variation and/or percent difference were less than 10% for control concentrations above 5 ng/ml and less than 20% for control concentrations of 1 ng/ml. The laboratory used this assay in conjunction with the National Institute of Standards and Technology (NIST)/National Institutes of Health (NIH) Vitamin D Metabolites Quality Assurance Program. Precision of the sample concentrations was within 8% of the NIST values and the calculated Z-score, a measure of the difference between control concentrations measured at OHSU and control concentrations measured by others, was -0.1 indicating that our results were within the established consensus values.

Intact PTH was measured in singlet, by an automated chemiluminescent assay, on the Immulite platform (Siemens Healthcare Diagnostics, Deerfield, IL) at the OCTRI

Core Laboratory. The analytical sensitivity of the assay was 4.0 pg/ml and the lowest reportable value was 5.0 pg/ml (coefficient of variation was 6% to 12% depending on the concentration of the control sample). Plasma calcium was measured in singlet by an ion selective electrode method (Beckman Coulter, Fullerton, CA) by staff of the OHSU Clinical Chemistry Laboratory.

Urine Collection and Analysis

Participants were given two non-reactive and light-protective plastic jugs to store their collected urine. Participants were instructed to collect urine for 24 hours starting 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 and 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. A minimum volume of the 24-hour urine collection was considered to be 800 ml. If the 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 a graduated cylinder and recorded by the OCTRI nursing staff. Aliquots derived from the total volume were transferred to the OCTRI Core Lab for storage at -80° C and batched analysis at the end to the study. Urine samples were analyzed for creatinine concentration and urinary mineral concentration, including calcium and phosphorus, at the Kaiser Permanente Northwest Regional Laboratory (Portland, OR).

Urinary calcium was measured spectrophotometrically, and urinary phosphorus and creatinine concentrations were measured by colorimetric assay (58).

Body Composition Analysis

Body composition was measured by dual energy X-ray absorptiometry (DEXA) whole body scan. 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. DEXA scans were only performed on individuals who weighed < 340 pounds and who were < 74 inches tall. Individuals who were over the weight or height limit were not scanned because they exceeded the limits set by the manufacturer. Individuals who met the criteria but did not properly fit within the DEXA scanning area were positioned so that a complete left-sided, half-body DEXA scan was performed. When a half body scan was performed, values were multiplied by two to estimate total body composition parameters. DEXA scans were analyzed using the Hologic QDR for Windows XP Software Version 12.1. The output for each analysis included whole body bone mineral density, bone area, bone mass, fat mass, lean mass, and fat-free mass.

Dietary Intake Analysis

Energy and nutrient intake was estimated by two unannounced 24-hour dietary recalls. 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 twice at six months by trained study staff. At each time

point, one of the interviews was performed to assess food and beverage intake during a weekday, and the other was performed to assess food and beverage intake during a weekend day. The Food Processor SQL (ESHA Research, Salem, OR) was used by KPCHR Insight study staff to analyze dietary composition. The values from the two interviews were averaged to approximate each participant's usual intake at the baseline and six-month time points.

Statistical Analysis

Subject characteristics of age, race, sex, BMI, and vitamin/mineral supplement use were characterized by means and standard deviations for continuous measures or proportions for categorical outcomes. Associations between total 25(OH)D concentration and measures of body composition including BMI, lean mass, and fat mass were tested using multiple linear regression. Binary indicators of dietary intervention and cohort membership were included, along with interactions between these binary indicators and BMI, lean mass and fat mass. Three separate models were built for serum total 25(OH)D concentration at baseline, six months, and for the difference in serum total 25(OH)D concentration between these times. The impact of season on vitamin D concentration was addressed by testing significance of the cohort indicator (cohort 2 or 4), or any interaction involving this indicator, and the other predictors of interest. The effect of diet was assessed in a similar manner, with attention given to the binary indicator for diet (low-carbohydrate versus high-carbohydrate) together with interactions involving other predictors and diet. The models were

adjusted for the demographic variables, age, sex, and race. The statistical analyses at baseline included all participants that returned for six-month measurements so the sample size was the same at both time points.

Independent continuous variables that were analyzed included: age, weight, body mass index, serum 25(OH)D_{2+3}, and PTH concentrations, plasma calcium concentration, urinary calcium and phosphorus excretion in mg/d and referenced to urinary creatinine excretion, lean mass and fat mass as determined by DEXA; and energy carbohydrate, protein, fat, vitamin D, and calcium intakes. Independent categorical variables that were analyzed included: gender (male and female), diet group (low-carbohydrate or high-carbohydrate), cohort (2 or 4), race (White or non-White), supplement use (vitamin D, calcium, or none), multivitamin use (yes or no), medication interaction (yes or no) and smoking status (yes/previously or no). Independent sample t-tests were used to test for differences in demographic and dietary data (e.g., BMI, age, energy intake, etc) within and between diet groups. No adjustments for multiple comparisons were made. Difference were considered significant at $p<0.05$.

Calculations

STATA, Version 11.0 (StataCorp, College Station, TX), was used to analyze the data. The data were first analyzed by looking at the distribution, outliers, and missing values. Participants with missing values were excluded from the analysis as indicated in Figure 1. None of the outcome variables were visibly skewed therefore no transformation of the data was necessary. Descriptive statistics and frequencies (for

categorical variables) including mean, standard deviation, minimum, and maximum values were obtained for all variables, for the entire sample together, and by diet group.

The following variables were computed to determine differences between time points and to examine the interrelationships between dietary variables and effects on outcome variables. The change in values from baseline to six-months between groups was calculated by subtracting the baseline value from the six-month value. Total serum 25(OH)D concentration was calculated as the sum of $25(OH)D_3$ plus $25(OH)D_2$ concentrations. Urinary calcium and phosphorus concentrations were converted from mg/dl to mg/day to mmol/l and then indexed to creatinine and reported as mmol calcium or phosphorus/mmol creatinine. Macronutrient intake (g/d) was converted to kcal/d by multiplying each macronutrient (protein, carbohydrate, and fat) by its respective energy density value (4, 4 and 9 kcal/g, respectively) and then divided by total energy intake (kcal/d) and multiplied by 100 to calculate percent of total energy intake. Nutrient density of vitamin D and calcium (mg/1000 kcal) was calculated by dividing the daily intake of each nutrient by the daily energy intake in kcal divided by 1000.

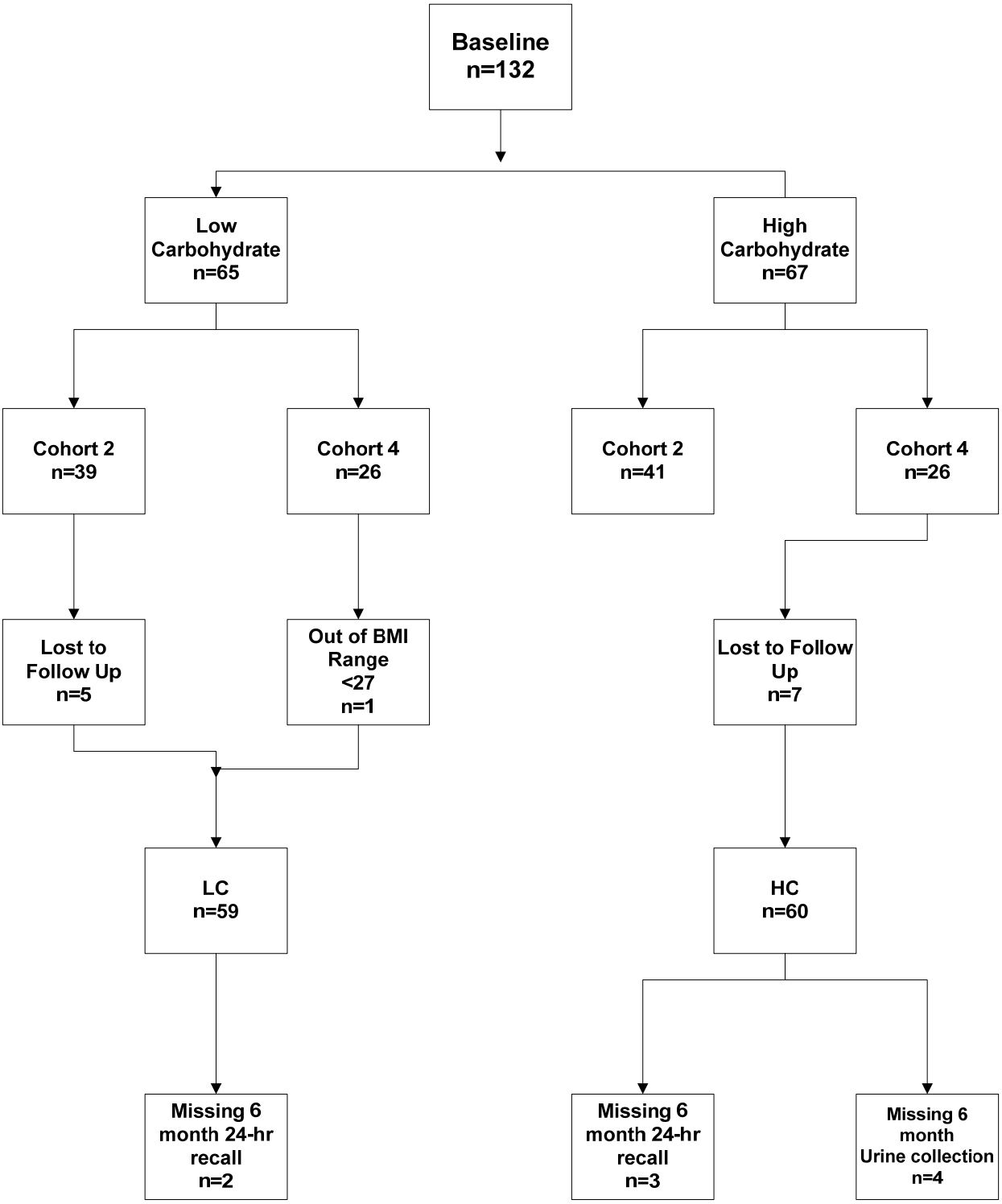
Chapter 4

Results

Descriptive Statistics

Figure 6 shows the distribution of participants to dietary arms and cohorts of the Insight Weight Loss Study. One-hundred thirty-two participants were randomized to either the low- or high-carbohydrate dietary intervention. Sixty-seven were assigned to the high-carbohydrate group, and 65 were assigned to the low-carbohydrate group. Of those in the high-carbohydrate group, 41 were in cohort two and 26 were in cohort four; and in the low-carbohydrate group, 39 were in cohort two and 26 were in cohort four. Among those in the high-carbohydrate group, seven participants were lost to follow up, in the low-carbohydrate group, five were lost to follow up and one participant had a BMI of less than 27 kg/m^2 and was excluded from analysis. Statistical analysis was performed on data derived from a total of 119 participants, 60 in the high-carbohydrate group and 59 in the low-carbohydrate group. Four participants in the high-carbohydrate group had missing urine samples at the six-month follow-up visit; two declined to complete the urine collection and two had low urine volume (< 800 ml) and were asked to repeat the urine collection but declined. These four participants were excluded from the statistical analysis involving urine data. Five participants, three in the high-carbohydrate group and two in the low-carbohydrate group, did not complete a six-month dietary intake recall and therefore were excluded from the dietary intake analysis.

Figure 6. Distribution of Participants to Dietary Arms and Cohorts of the Insight Weight Loss Study



Baseline Characteristics

Participant characteristics at baseline are presented in Table 2. All baseline characteristics were similar between dietary intervention groups. The average age was 51 and 53 years among those in the low-carbohydrate and high-carbohydrate groups, respectively. The average weight was 100 and 102 kg and the average BMI was 34 and 36 kg/m^2 in the low-carbohydrate and high-carbohydrate groups, respectively. The majority of participants were female, as well as Caucasian. None of the participants were taking medications that would significantly affect vitamin D metabolism, such as anti-convulsants, bile acid sequestrants, and/or corticosteroids. Potential medication interactions noted included oral contraceptives and anti-hypertensive drugs such that 24% and 32% had potential drug-nutrient interactions in the low-carbohydrate and high-carbohydrate groups, respectively. Of those who took dietary supplements, 20% and 23% took a vitamin D supplement and 19% and 22% took a calcium supplement in the low-carbohydrate and high-carbohydrate groups, respectively. In addition, 73% and 63% reported taking a multivitamin supplement in the low-carbohydrate and high-carbohydrate groups, respectively. At baseline, 40% and 37% of participants reported either currently smoking or smoking in the past in the low-carbohydrate and high-carbohydrate groups, respectively.

Table 2. Baseline Characteristics of Participants by Dietary Intervention

Characteristic	Dietary Intervention	
	Low-Carbohydrate Diet (n=59)	High-Carbohydrate Diet (n=60)
Age (yr)[1]	51 ± 11	53 ± 11
Weight (kg)[1]	100 ± 16	102 ±18
BMI (kg/m^2)[1]	34 ± 5	36 ± 6
Female / Male	73% / 24%	75% / 25%
White / Non-white	95% / 5%	82% / 18%
Multivitamin	73% Yes / 27% No	63% Yes / 37% No
Vitamin D / Calcium supplement /none	20% / 19% / 61%	23% / 22% / 55%
Potential Medication Interaction	24% Yes / 76% No	32% Yes / 68% No
Smoking History	40%	37%
[1]Mean ± SD		

Dietary Intake Before and After the Six-Month Dietary Weight Loss Intervention

Dietary intake reported by participants in the two diet groups is presented in Table 3. Total energy intake at baseline was 1940 ± 685 kcal/d for the low-carbohydrate group and 2047 ± 625 kcal/d for the high-carbohydrate group and these values were not significantly different. Both groups demonstrated a significant reduction in average energy intake at six-months to 1556 ± 493 kcal/d in the low-carbohydrate group and 1757 ± 692 kcal/d in the high-carbohydrate group; differences were not significant between groups. Dietary macronutrient composition was similar between groups at baseline and when expressed as a percent of total energy intake, was 48 ± 9% carbohydrate, 16 ± 4% protein, and 36 ± 8% fat. At six-months, the low-carbohydrate group's average dietary macronutrient composition was 24 ± 10% carbohydrate, 23 ± 5% protein, and 53 ± 5% fat; whereas the high-carbohydrate group's average dietary macronutrient composition was 51 ± 11% carbohydrate, 18 ± 5% protein, and 31 ± 11% fat. The macronutrient content of the diet was significantly different between groups at six-months for each component.

Vitamin D intake by both groups at each time point was significantly less, on average, than the recommended intake of 5-10 μg/d (200-400 IU/d). The average vitamin D intake of the low-carbohydrate group was 1.75 ± 2.4 μg/d (75 ± 65 IU/d) at baseline and 1.3 ± 3.5 μg/d (62 ± 90 IU/d) at six months. The average vitamin D intake of the high-carbohydrate group was 2.2 ± 2.4 μg/d (86 ± 129 IU/d) at baseline and 2.3 ±2.6 μg/d (86 ± 153 IU/d) at six months. Vitamin D intake was not significantly different

between diet groups at baseline (p=0.3) or six-months (p=0.065), nor was the change in vitamin D intake significantly different between groups (p=0.78). Only two people at both baseline and six-months consumed more than 10 μg/d of vitamin D; the highest reported intake of vitamin D was 25 μg/d by one participant in the high-carbohydrate group at six-months.

Average calcium and phosphorus intakes were also lower in both groups than recommended. The average calcium intake at both time points among both diet groups ranged from 643-878 mg/d and was low compared to the Recommended Dietary Allowance of 1200 mg/d. Mean calcium intake was not significantly different between diet groups at baseline (p=0.3) but intake was significantly higher in the high-carbohydrate group than the low-carbohydrate group at six-months (p=0.004). Phosphorus intake was significantly different between groups at baseline (p=0.018) but not at six-months (p=0.6).

Table 3. Dietary Intake Before and After the Six-Month Dietary Weight Loss Intervention

Dietary Intervention	Low-Carbohydrate Diet			High-Carbohydrate Diet		
Time-point	Baseline (n=59)	6 Months (n=57)	Change	Baseline (n=60)	6 Months (n=57)	Change
Energy Intake (kcal/d)	1940 ± 685	1556 ± 493[b]	-381 ± 720	2047 ± 625	1757 ± 692[b]	-322 ± 932
Carbohydrate (g/d)	234 ± 106	89.5 ± 40[b]	-143 ± 99[c]	246 ± 100	220 ± 91[a]	-29 ± 132
Protein (g/d)	76.5 ± 38	91 ± 36[b]	14 ± 43[c]	81 ± 28	77 ± 27[a]	-5 ± 37
Fat (g/d)	78 ± 29	92 ± 38[b]	14 ± 43[c]	80 ± 30	63 ± 44[a,b]	-19 ± 55
Vitamin D (µg/d)	1.75 ± 2.4	1.3 ± 3.5	-0.4 ± 4	2.2 ± 2.4	2.3 ± 2.6	0.2 ± 3
Vitamin D µg/1000 kcal	0.87 ± 0.98	0.86 ± 2.4	0.03 ± 3	1.2 ± 1.3	1.4 ± 1.5	0.3 ± 2
Calcium (mg/d)	722 ± 352	643 ± 295	-88 ± 428	794 ± 412	878 ± 528[a]	67 ± 604
Calcium mg/1000 kcal	389 ± 161	429 ± 193	35 ± 202	394 ± 182	525 ± 277[a,b]	128 ± 293
Phosphorus (mg/d)	696 ± 364	887 ± 404[b]	186 ± 524[c]	846 ± 319[a]	855 ± 363	-0.3 ± 498
Phosphorus mg/1000 kcal	365 ± 144	500 ± 260[b]	132 ± 277	429 ± 149[a]	458 ± 277	33 ± 296

Mean ± SD
[a]Significantly different from low-carbohydrate group ($p<0.05$)
[b]Significantly different from baseline ($p<0.01$)
[c]Significantly different change from high-carbohydrate ($p<0.05$)

Body Weight and Body Composition Before and After the Six-Month Dietary Weight Loss Intervention

As illustrated in Table 4, participants in both diet groups lost weight and demonstrated changes in body composition after the six-month dietary weight loss intervention. Participants in the low-carbohydrate group lost an average of 12 ± 6 kg and those in the high-carbohydrate group lost an average of 6 ± 6 kg. The difference in weight loss between the groups was significantly different ($p<0.0001$). Baseline BMI was 35 ± 5 kg/m^2 in the low-carbohydrate group and 37 ± 6 kg/m^2 in the high-carbohydrate group. The average decrease in BMI was -3 ±2 kg/m^2 in both groups after the six-month intervention. Fat mass decreased by 6 ± 5 kg and body fat percentage decreased by 3 ± 3% in both groups. All body composition parameters were significantly different ($p<0.00001$) between time points within diet groups, indicative of weight loss, however, change over time was not different between groups for any of these parameters.

Table 4. Body Weight and Body Composition Before and After the Six-Month Dietary Weight Loss Intervention*

Body Composition Parameter	Low-Carbohydrate Diet (n=59)			High-Carbohydrate Diet (n=60)		
Time point	Baseline	6 months	Change	Baseline	6 months	Change
Weight (kg)	100 ± 16	89 ± 16[a]	-12 ± 6[a]	102 ± 18	96 ± 17	-6 ± 6
Lean Mass (kg)	59 ± 11	57 ± 11	-3 ± 2	60 ± 8	57 ± 8	-3 ± 2
Fat Mass (kg)	39 ± 9	32.5 ± 10[a]	-6 ± 5	44 ±12	38 ± 12	-6 ± 5
% Body Fat	41 ± 7	38 ± 8	-3 ± 3	40 ± 6	37 ± 7	-3 ± 3
BMI (kg/m^2)	35 ± 5	32 ± 5	-3 ± 2	37 ± 6	34 ± 6	-3 ± 2

*Mean ± SD
[a]Significantly different from high-carbohydrate group ($p<0.05$)

Serum 25(OH)D Concentration Before and After the Six-Month Dietary Weight Loss Intervention

The baseline average serum total 25(OH)D concentration [25(OH)D_3+25(OH)D_2] for all participants combined was 22 ± 7 ng/ml. At baseline among those in the high-carbohydrate group, the average serum total 25(OH)D concentration was 22 ± 7 ng/ml and among those in the low-carbohydrate group the average serum total 25(OH)D concentration was 22 ± 8 ng/ml as shown in Table 5. At six-months, the average serum total 25(OH)D concentration was 20 ± 8 ng/ml and 20 ± 7 ng/ml for the low-and high-carbohydrate groups, respectively. There was no significant difference in serum total vitamin D concentration between the groups at baseline ($p=0.8$) or six months ($p=0.9$). The predominant component of serum total 25(OH)D was 25(OH)D_3, comprising about

90% of the total 25(OH)D concentration. The $25(OH)D_2$ concentration was very low in almost all participants. Forty-two participants at baseline and six months had $25(OH)D_2$ concentrations less than 1 ng/ml; and approximately 65% of those studied did not have any measurable $25(OH)D_2$ in their blood. The change from baseline in serum total 25(OH)D concentration was -2.5 ± 6 ng/ml for both diet groups, which was not significantly different between groups (p=0.8). When weight loss was taken into consideration, the average change in total serum total 25(OH)D concentration was not different. Those who lost 5 kg or less had an average change in serum total 25(OH)D concentration of -3 ± 6 ng/ml; those who lost more than 5 kg had an average change in serum total 25(OH)D concentration of -2 ± 6 ng/ml.

Table 5. Serum 25(OH)D Concentration Before and After the Six-Month Dietary Weight Loss Intervention*

Serum Vitamin D Concentration	Low-Carbohydrate Diet (n=59)			High-Carbohydrate Diet (n=60)		
	Baseline	6 Months	Change	Baseline	6 Months	Change
$25(OH)D_3$ (ng/ml)	20.5 ± 8.2	18.0 ± 8.0	-2.5 ± 6.3	20.4 ± 6.7	18.0 ± 7.4	-2.5 ± 6.0
$25(OH)D_2$ (ng/ml)	1.2 ± 2.2	1.5 ± 2.7	-0.31 ± 1.9	1.7 ± 3.0	1.8 ± 3.0	-0.1 ± 2.0
$25(OH)D_{2+3}$ (ng/ml)	21.7 ± 8.0	19.5 ± 7.6	-2.2 ± 6.2	22.1 ± 6.5	19.7 ± 7.1	-2.4 ± 5.5
*Mean ± SD						

Table 6 illustrates the stratification of participants based on vitamin D status at baseline and six-months. When the entire sample (n=119) was considered, the majority,

92% of participants, were either vitamin D deficient or insufficient at baseline and six-months. No participants had vitamin D concentrations in the optimal range of 51-149 ng/ml at either time point. Classification of vitamin D status was similar between groups at both time points. A higher percentage of participants were considered severely deficient at six-months than at baseline which may be due, at least in part, to the collection of six month samples during the winter and spring seasons when endogenous synthesis of vitamin D would have been very low.

Table 6. Classification of Vitamin D Status Before and After the Six-Month Dietary Weight Loss Intervention

Serum 25(OH)D Concentration & Classification	Low-Carbohydrate Diet (n=59)		High-Carbohydrate Diet (n=60)		Total Sample (n=119)	
	Baseline	6 Month	Baseline	6 Month	Baseline	6 Month
Severe Deficiency < 10 ng/ml	5%	10%	3%	8.5%	4%	9%
Deficient < 20 ng/ml	37%	45%	29%	36%	33%	40%
Insufficient 20-29 ng/ml	43%	33%	65%	52.5%	55%	43%
Sufficient 30-50 ng/ml	15%	12%	3%	3%	8%	8%
Optimal 51-149 ng/ml	0	0	0	0	0	0
Toxic >150 ng/ml	0	0	0	0	0	0

Serum PTH and Calcium Concentration Before and After the Six-Month Dietary Weight Loss Intervention

Table 7 illustrates serum PTH and calcium concentrations before and after the six-month dietary intervention. Among all participants combined (n=119), the average serum PTH concentration at baseline was 55 ± 22 pg/ml with a range of 10.7 to 136 pg/ml. The average serum PTH concentration at six-months was 53 pg/ml with a range of 14 to 144 pg/ml. The normal range for serum PTH concentration by this method of analysis is 10-69 pg/ml (59). Values greater than 69 pg/ml suggest hyperparathyroidism. Based on this definition, 22% and 17% of participants at baseline and six-months, respectively, had elevated serum PTH concentrations. There were no significant differences in mean PTH concentrations within or between groups except that the change in PTH was -5 ± 16 pg/ml in the low-carbohydrate group and 0.8 ± 14 pg/ml in the high carbohydrate group ($p<0.05$).

The average plasma calcium concentration for all participants was within the normal range of 8.8-10.3 mg/dl at both time points, 9.4 ± 0.3 at baseline and 9.2 ± 0.3 at six-months. Ten participants had serum calcium concentrations outside of the normal range, nine were below normal (8.3-8.7 mg/dl) and one was above normal (10.8 mg/dl). However, none of these values were judged to be indicative of hypo- or hypercalcemia (60, 61). There were no differences in plasma calcium concentrations within or between groups.

Urinary Calcium and Phosphorus Excretion Before and After the Six-Month Dietary Weight Loss Intervention

Table 7 also illustrates urinary excretion of calcium and phosphorus before and after the six-month dietary intervention. Baseline urinary calcium excretion in the low-carbohydrate group was 197 ± 106 mg/day and in the high-carbohydrate group was 180 ± 87 mg/day. At six-months, urinary calcium excretion was 226 ± 140 mg/day in the low-carbohydrate group and 180 ± 92 mg/d in the high-carbohydrate group. The average change in urinary calcium excretion was 29 ± 119 mg/day in the low-carbohydrate group and 0.8 ± 91 mg/day in the high-carbohydrate group, which were not significantly different (p=0.2). The average urinary calcium excretion indexed to creatinine excretion was similar at baseline and six-months for both diet groups. The average change in urinary calcium excretion when indexed to creatinine excretion was also similar between groups (p=0.2). Urinary phosphorus excretion was 1050 ± 551 mg/day in the low-carbohydrate group and 947 ± 338 mg/day in the high-carbohydrate group at baseline. At six-months, the average urinary phosphorus excretion was 1125 ± 443 mg/day in the low carbohydrate group and 940 ± 354 mg/day in the high-carbohydrate group. Among those in the low-carbohydrate group, urinary phosphorus excretion indexed to creatinie excretion was 2.5 ± 0.55 and 2.8 ± 0.80 mmol phosphorus/mmol creatinine at baseline and six-months, respectively. Urinary phosphorus excretion indexed to urinary creatinine excretion was 2.3 ± 0.63 and 2.4 ± 0.64 mmol phosphours/mmol creatinine at baseline and six-months respectively, among those in the high-carbohydrate group. The change in urinary phosphorus excretion indexed to

creatinine excretion was 0.33 ± 0.84 mmol phosphorus/mmol creatinine in the low-carbohydrate group and 0.12 ± 0.70 mmol phosphorus/mmol creatinine in the high-carbohydrate group which were not significantly different between groups (p=0.14).

Table 7. Serum PTH and Calcium Concentration and Urinary Calcium and Phosphorus Excretion Before and After the Six-Month Dietary Weight Loss Intervention*

Diet Group	Low-Carbohydrate Diet (n=59)			High-Carbohydrate Diet (n=60)		
Time point	Baseline	6 Months	Change	Baseline	6 Months	Change
PTH (pg/ml)	57 ± 26	52 ± 21	-5 ± 16	53 ± 18	53 ± 22	0.8 ± 14[a]
Plasma calcium (mg/dl)	9.4 ± 0.3	9.2 ± 0.3	-0.2 ± 0.4	9.4 ± 0.3	9.2 ± 0.3	-0.2 ± 0.4
Urinary Calcium (mg/day)	197 ± 106	226 ± 140	29 ± 119	180 ± 87	180 ± 92	0.8 ± 91
Urinary Calcium/ Creatinine Ratio (mmol/mmol)	0.37 ± 0.20	0.44 ± 0.23	0.07 ± 0.18	0.35 ± 0.17	0.38 ± 0.20	0.03 ± 0.16
Urinary Phosphorus (mg/day)	1050 ± 551	1125 ± 443	57 ± 537	947 ± 338	940 ± 354	-16 ± 354
Urinary Phosphorus/ Creatinine Ratio (mmol/mmol)	2.5 ± 0.55	2.8 ± 0.80	0.33 ± 0.84	2.3 ± 0.63	2.4 ± 0.64	0.12 ± 0.70

*Mean ± SD

[a]Significantly different from low-carbohydrate group ($p<0.05$)

Correlational Analyses

As shown in Table 8, correlations between outcome variables were examined and significance was established at $p<0.05$. There was a significant negative correlation between serum total 25(OH)D concentration and PTH at baseline (r= -0.22 p=0.018), this relationship was also seen at six-months (-0.20 p=0.027). There was a significant negative correlation between serum total 25(OH)D concentration and BMI at baseline (r= -0.18 p= 0.049) and at six-months (r= -0.23 p=0.01). Weight was also negatively correlated with serum total 25(OH)D concentration at baseline (r= -0.23 p=0.01) and at six-months (r= -0.21 p=0.02). There were no significant correlations using the change in values from baseline to six-months among any of the body composition or biochemical parameters. Even though significant relationships existed between serum total 25(OH)D concentration and weight and BMI, there was no significant relationship between serum total 25(OH)D concentration and fat mass or lean mass as measured by DEXA. Likewise, no significant relationship was seen between serum total 25(OH)D concentration and plasma calcium concentration. When comparing urinary mineral excretion in mg/day or when indexed to creatinine excretion, no significant relationships were observed with serum total 25(OH)D concentration.

Table 8. Significant Correlations Between Serum Vitamin D, PTH, BMI and Weight at Baseline and Six-Months

		Baseline		6 Months	
		Coefficient	**P-value**	**Coefficient**	**P-value**
Serum 25(OH)D (ng/ml)	PTH (pg/ml)	-0.22	0.018	-0.202	0.027
	BMI (kg/m^2)	-0.18	0.049	-0.23	0.01
	Weight (kg)	-0.19	0.037	-0.21	0.02

Regression Analysis

To determine the correlation between serum total 25(OH)D concentration and body fat mass at baseline and six-months, regression models were built that incorporated the predictive variables, diet, cohort, age, race, gender, smoking status, and medication and supplement use. Partial correlations were generated and compared to raw, unadjusted correlation coefficients. Table 9 displays both the raw correlation coefficients and the partial correlation coefficients adjusted for the predictive variables noted above. There were no significant relationships among the raw or partial correlations at either time-point.

Table 9. Correlation Coefficients Between Serum Total 25(OH)D and Body Fat Mass at Baseline and Six-Months

Time Point	Raw Correlation Coefficients	Partial Correlation Coefficients
Baseline	-0.14 (p=0.12)	-0.12 (p=0.19)
6 months	-0.12 (p=0.21)	-0.074 (p=0.43)
Change	0.0045 (p=0.96)	-0.0895 (p=0.36)

Table 10 shows that after adjusting for cohort, no predictive relationships were established between the change in serum total 25(OH)D concentration and the following variables: change in body weight, change in PTH concentration, age, gender, diet group, race, smoking status, medication use, or multivitamin or supplement use (p=0.25). Change in PTH concentration was not a significant predictor of the change in serum total 25(OH)D concentration, whereas baseline PTH concentration was predictive of the change in serum total 25(OH)D concentration (p=0.04) when baseline serum total 25(OH)D concentration was in the model as well (p<0.0001). The prediction equation estimates show that when baseline serum total 25(OH)D concentration is 22 ng/ml and PTH concentration is 53 pg/ml, the mean change in serum total 25(OH)D concentration would be -2.2 ng/ml (p<0.0001). The baseline values chosen to create this predictive equation were based on the mean values of all participants (n=119).

Table 10 also displays the influence of the predictor variables on the change in serum total 25(OH)D concentration based on a reference group of White, females, in cohort two, in the low-carbohydrate group, and not taking any dietary supplements. In

this model, baseline serum total 25(OH)D concentration, baseline PTH concentration, and cohort were significant predictors of the change in serum total 25(OH)D concentration ($p=0.0001$). Other variables were not significant predictors of the change in serum total 25(OH)D concentration. Neither calcium nor phosphorus excretion indexed to creatinine excretion significantly predicted the change in serum total 25(OH)D concentration ($p=0.56$) and therefore these variables were not included in the regression model.

Table 10. Association of Predictor Variables on the Change in Serum Total 25(OH)D Concentration

Predictor Variable	Effect (ng/ml)	95% Confidence Interval	Two-sided p value
Male	-0.82	-3.4, 1.8	0.53
Female	Reference group		
White	Reference group		
Non-white	-0.02	-3.2, 3.2	0.99
Vitamin D supplement use	1.0	-1.7, 3.8	0.5
Calcium supplement use	0.9	-1.6, 3.5	0.5
Multivitamin	0.38	-1.9, 2.7	0.7
No supplementation	Reference group		
Age (by decade)	0.88	-0.7, 1.8	0.07
Baseline PTH (pg/ml)	-0.06	-0.11, -0.01	0.03
Change in PTH (pg/ml)	-0.02	-0.08, 0.05	0.66
Baseline 25(OH)D_{2+3} (ng/ml)	-0.33	-0.5, -0.2	<0.0001
Weight Loss (kg)	-0.02	-0.19, 0.14	0.80
Cohort 2	Reference group		
Cohort 4	+2.4	0.40, 4.5	0.02
Low Carbohydrate	Reference group		
High Carbohydrate	-0.33	-2.4, 1.8	0.76
Reference Group: female, white, cohort 2, low carbohydrate, and no supplementation	-3.9	-6.0, -1.7	<0.0001

Dietary intervention was not a significant predictor of the change in serum total 25(OH)D concentration but cohort was a significant predictor. Table 11 describes the effect of diet and cohort on the change in serum total 25(OH)D concentration and the 95% confidence intervals after adjusting for baseline serum total 25(OH)D

concentration. Among those in the high-carbohydrate group who were in cohort two, the predicted change in total serum 25(OH)D concentration was -3.4 ng/ml whereas for those in cohort four, the predicted change in serum total 25(OH)D concentration was -0.87 ng/ml. Among those in the low-carbohydrate group, who were in cohort two, the predicted change in serum total 25(OH)D concentration was -3.3 ng/ml whereas for those in cohort four, the predicted change was -0.74 ng/ml. The difference in the predicted change in serum total 25(OH)D concentration between diet groups was -0.12 ng/ml and was not significantly different (p=0.9). The difference in the predicted change in serum total 25(OH)D concentration between cohorts was 2.51 ng/ml which was significantly different (p=0.01).

Table 11. Effect of Diet and Cohort on the Predicted Change in Serum Total 25(OH)D Concentration

	High-Carbohydrate Diet	Low-Carbohydrate Diet
Cohort 2	-3.4 [-5.0, -1.8]	-3.3 [-4.8, -1.7]
Cohort 4	-0.87 [-2.7, 0.93]	-0.74 [-2.6, 1.1]
[95% confidence interval] Diet effect (p=0.9) Cohort Effect (p=0.01)		

Chapter 6

Discussion

Summary and Conclusions

This substudy investigated whether weight loss and loss of fat mass improved serum total 25(OH)D concentrations and whether the type of weight loss diet followed influenced this result. We also determined if serum total 25(OH)D concentration was related to weight and total body fat mass and if the change in serum total 25(OH)D concentration was related to the change in weight and total body fat mass. In addition, we assessed whether the change in serum total 25(OH)D concentration was predicted by age, race, gender, season of sample collection, smoking history, medication and dietary supplement use, and change in serum PTH concentration.

The primary hypotheses, that serum total 25(OH)D concentration would be higher after weight loss and loss of fat mass than before and that serum total 25(OH)D concentration would be inversely related to weight and body fat mass, were rejected, at least in part. Serum total 25(OH)D concentration was not higher after weight loss and loss of fat mass than before nor was it significantly correlated with fat mass. Instead, the average serum total 25(OH) D concentration was 2 ± 0.5 ng/ml lower after weight loss in both dietary intervention groups and serum 25(OH)D concentration was significantly negatively correlated with weight, and BMI.

The reduction from baseline in average serum total 25(OH)D concentration may be due, at least in part, to differences in seasons and sun exposure when the baseline and six-month blood samples were obtained. Baseline blood samples were obtained in

the summer and fall months during the highest sun exposure seasons and six-month samples were obtained in the winter and spring months during the lowest sun exposure seasons. The difference in season of sample collection would likely impact endogenous vitamin D synthesis and thus circulating serum total 25(OH)D concentrations. Other researchers have reported a similar affect of season of sample collection on circulating concentrations of 25(OH)D. Tangpricha and associates reported an average difference of 5 ng/ml in 25(OH)D concentrations between those studied in the summer versus those studied in the winter who lived in Boston, Massachusetts (5). Hyppönen and collegues reported an average difference of 7.6 ng/ml in serum 25(OH)D concentrations between those studied in the summer or fall versus those studied in the winter or spring living in the United Kingdom.

The hypotheses that serum total 25(OH)D concentration would be inversely related to weight, fat mass, BMI, and PTH concentration was accepted in part. We observed significant negative correlations between serum 25(OH)D concentration and weight ($r = -0.2$), BMI ($r = -0.2$) and PTH concentration ($r = -0.2$) but no significant correlation with fat mass before and after weight loss. Correlations between the change in serum 25(OH)D concentration and the amount of weight lost, change in fat mass, or change in BMI were not significant. Unlike our results, Arunabh and colleagues reported no significant correlations between serum 25(OH)D concentration and weight or BMI and a significant negative correlation with body fat percentage (62). Tangpricha, et al. reported a significant negative correlation between serum 25(OH)D and PTH concentration (5), which was similar our observations. Snijder and associates reported

that serum 25(OH)D concentration decreased with increased total body fat percentage, BMI, and waist circumference (16).

The secondary hypotheses that serum total 25(OH)D concentration would be predicted by season, gender, age, PTH concentration, and use of dietary supplements, and not predicted by diet or smoking was accepted in part. A predictive relationship was established with season, baseline PTH, and baseline serum 25(OH)D concentration, but not fat mass, weight, change in PTH concentration, age, gender, diet, medication and supplement use, or smoking history. O'Sullivan also reported non-significant predictive relationships between serum 25(OH)D concentration and age, gender, and smoking history (63).

Our study demonstrated a high prevalence of vitamin D deficiency and insufficiency among overweight and obese individuals living in the Portland, Oregon, metropolitan area. Of those studied, the mean baseline serum 25(OH)D concentration was 22 ± 7 ng/ml. Ninety-two percent of participants had serum 25(OH)D concentrations <30 ng/ml and were considered vitamin D insufficient. In addition, 40% of participants had serum 25(OH)D concentrations <20 ng/ml and were considered vitamin D deficient. Other researchers have reported similar results (Tables 12 and 13). Parikh, et al. (14) and Arunabh and associates (62) reported mean serum 25(OH)D concentrations of 23.5 ± 12 ng/ml and 21.6 ± 14 ng/ml, respectively, which are comparable to our results. Goldner and collaborators studied obese and non-obese individuals living in Omaha, Nebraska and observed a significant difference in serum

25(OH)D concentration between the groups. The average serum 25(OH)D concentration among the obese participants was 19 ± 10 ng/ml which was significantly lower than the average serum 25(OH)D concentration among the non-obese individuals of 36 ± 14 ng/ml (25). Of those studied, 90% of the obese subjects were vitamin D insufficient compared to 32% of the non-obese controls. O'Sullivan and colleagues (63) reported that 90% of the healthy Irish adults that they studied were vitamin D insufficient with serum 25(OH)D concentrations < 30 ng/ml, and that the percentage of individuals who were vitamin D insufficient was higher in the winter (97%) than in the summer (85%). Likewise, Hyppönen, et al. reported that 87% of the 7437 British adults who they studied had serum 25(OH)D concentrations < 30 ng/ml and were vitamin D insufficient (64). Among the healthy individuals living in Boston and studied by Tangpricha and colleagues the prevalence of vitamin D deficiency was 30% in winter and 11% in the summer (5). In a study of overweight Spanish women (n=66), Rodriguez and associates (15) reported that 89% of women were vitamin D insufficient with serum 25(OH)D concentration < 36 ng/ml.

In summary, this research showed a high prevalence of vitamin D insufficiency and deficiency among overweight and obese individuals in the Portland Oregon metropolitan area. A negative correlation between serum 25(OH)D concentration, weight, BMI, and PTH concentration was demonstrated. As seen in previous research, a predictive relationship between the change in serum 25(OH)D concentration and season was displayed.

Table 12. Summary of Studies Reporting Serum Vitamin D Concentrations

	Study Design & Year	Baseline (ng/ml)	End of Study (ng/ml)
Miksa et al. (n=119)	Randomized Clinical Trial 2010	22 ± 7	20 ± 7
Parikh et al. (14) (n=154)	Cross Sectional 2004	23.5 ± 12	NA
Arunabh et al. (17) (n=410 females)	Cross sectional 2003	21.6 ± 14	NA
Tangpricha et al. (5) (n= 165 winter) (n=142 summer)	Cross Sectional 2002	Winter: 30 ± 10	Summer: 35 ± 10
Goldner et al. (25) (n=41)	Cross Sectional 2008	obese 19 ± 10 non-obese 36 ± 14	
Hyppönen et al. (64) (n=2850 winter/spring) (n=4587 sumer/fall)	1958 British birth cohort at 45 years old 2007	Winter/spring: 16.4 Summer/Fall: 24.0	

Table 13. Classification of Vitamin D Status in Published Studies of Populations Living Above the 40° N Latitude

Prevalence Studies	Location & Year	Severely Deficient < 10 ng/ml	Deficient < 20 ng/ml	Insufficient < 30 ng/ml	Sufficient ≥ 30 ng/ml
Miksa et al. n=119	Portland, OR (45°N) 2010	Baseline: 4% 6 Months: 9%	Baseline: 37% 6 Months: 50%	Baseline: 50% 6 Months: 33%	Baseline: 9% 6 Months: 8%
O'Sullivan et al. (63) Summer n=39 Winter n=31	Dublin, Ireland (53°N) 2008			85% summer 97% winter	15% summer 3% winter
Snijder et al. (16) n=453	Amsterdam (52° N) 2005	8% male 14% female	45% male 56% female		47% male 30% female
Hyppönen et al. (64) n=7437	Great Britain (54° N) 2007			87%	
Tangpricha et al. (5) Summer n=16 Winter n=49	Boston, MA (42°N) 2002		Summer: 11% Winter: 30%		
Goldner et al. (25) Obese n=41 Non-obese n=41	Omaha, NE (41.3°N) 2008		61% obese 12% non-obese	90% obese 32% non-obese	
Rodriguez et al. (15) n=66 females	Madrid, Spain (40° N) 2009			< 36 "low D" 89% Average BMI 29 ± 3	≥ 36 "high D" 11% Average BMI 26 ± 1

Strengths

The main question asked in this study, whether weight loss improves circulating vitamin D concentrations in healthy overweight and obese individuals, has not, to our knowledge, been addressed or answered by other investigators. Therefore, the results of this study are novel and have implications for future research.

This study was a collaboration between researchers at KPCHR and OHSU. A randomized controlled design was used, and the behavioral intervention was conducted at KPCHR and the data collection was conducted at OHSU. This design component separated intervention activities from data collection and analysis activities, which allowed for single-blinding of study personnel and minimized potential ascertainment bias. The six-month low- and high-carbohydrate dietary interventions generated significant weight loss and the sample size of 119 participants provided ample power to detect clinically-relevant and significant differences in 25(OH)D concentrations if they existed. Complete sets of baseline and six-month data were available for all primary outcome variables which reduced bias associated with loss to follow up or to the use of imputation to account for missing data. The methods used to measure serum total 25(OH)D concentrations and body composition are considered "gold standard" methods. Vitamin D was analyzed using liquid chromatography, tandem-mass spectrometry (LC/MS-MS), which measures 25(OH)D_2 and 25(OH)D_3 individually, quantitatively and at concentrations as low as 1 ng/ml. Body composition measures was measured by DEXA, which provides discrete information on lean mass and fat mass, and bone mass.

Limitations

Despite the strengths mentioned above, this study also had limitations which impact the generalization of results. Despite targeted efforts to recruit and enroll men and minorities, this study sample was 78% female and 89% Caucasian. Application of these results to men and individuals of other racial or ethnic groups should be done with caution. Although, participants' dietary supplement use was recorded, it was done in an elementary fashion without details about the type or amount of the supplement used. It was therefore not possible to quantify supplemental vitamin D intake or to determine if supplementation affected serum 25(OH)D concentration.

The effect of cohort on serum 25(OH)D concentrations seen in this analysis, most likely reflects the difference in season during which baseline and six-month measurements were taken. Since Portland, Oregon is above the 45° N latitude and because endogenous synthesis of vitamin D from sun exposure occurs between March to October, the timing of sample collection likely impacted serum 25(OH)D concentrations. Baseline appointments occurred during summer and fall when participants were able to synthesize vitamin D endogenously. Six-month appointments occurred during the winter and spring months when endogenous synthesis of vitamin D was minimal.

Another limitation of this study was that dietary data were collected by 24-hour recall. This method relies on a participants' ability to accurately report their food and beverage intake during the previous 24-hour period and is subject to recall bias. In addition to limitations imposed by recall bias, the interviews were conducted by

different people whose interviewing methods to gather dietary intake information may have differed slightly, leading to ascertainment bias. This, in turn, may have resulted in differences in recording and analyzing the types and amounts of foods consumed and thus to differences in accuracy of the amounts of energy and nutrients consumed.

Another potential limitation was the nutrient analysis program used to calculate energy and nutrient intakes. Nutrient analysis programs are limited by the completeness and currency of their databases. Specific foods and brands of foods may be missing from the database and require substitutions. Substitutions may not reflect accurate nutrient content of the actual food item consumed.

Future Directions

Additional research is needed to better define the effect of weight loss on serum 25(OH)D concentrations in overweight and obese adults. Studies are needed to confirm the result reported here, that weight loss does not increase serum 25(OH)D concentrations. Ideally, a randomized, controlled study that assesses the impact of weight loss and lack of weight loss on serum 25(OH)D concentrations in overweight and obese individuals during the winter months, when endogenous synthesis of vitamin D is minimal, should be performed to answer this question. Studies are also needed to explain why vitamin D deficiency is more prevalent among overweight and obese individuals than normal weight individuals so that targeted interventions can be designed to prevent this nutritional deficiency. Likewise, randomized-controlled-dosing trials are needed to determine effective treatment regimes for overweight and obese individuals who are vitamin D deficient or insufficient.

Conclusion

The prevalence of vitamin D deficiency and insufficiency is extremely high among overweight and obese individuals living in Portland, Oregon and may be attributed to very low dietary intake of vitamin D and limited sunlight exposure. Mean serum 25(OH)D concentrations did not improve despite significant weight loss and fat-mass loss. Routine supplementation with moderately high doses of vitamin D along with changes in lifestyle behaviors that increase exposure to sunlight to enhance endogenous synthesis of vitamin D are needed to improve the vitamin D status of this high-risk population.

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