

**THE EFFECT OF A 12-MONTH WEIGHT LOSS INTERVENTION ON VITAMIN D
STATUS IN SEVERELY OBESE CAUCASIANS AND AFRICAN AMERICAN ADULTS**

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Vitamin D deficiency is a potential risk factor for several chronic conditions, including cardiovascular disease, diabetes and immune disorders. Several studies have found an association between Vitamin D deficiency and obesity, yet it is unclear whether Vitamin D contributes to obesity, or obesity itself causes Vitamin D deficiency. The purpose of this study is to determine the relationship between obesity and Vitamin D status and to examine the effects of weight loss and physical activity on Vitamin D status in severely obese Caucasians and African Americans. 50 class II and class III obese women participated in a 12-month diet and exercise intervention. Vitamin D status was examined at baseline, 6 and 12 months to determine the relationship between Vitamin D and weight loss, fat loss, race and physical activity. At baseline, both the Class I and Class III obese subjects were considered Vitamin D insufficient (20-30 ng/mL). The Class II subjects were considered deficient (<20 ng/mL). The normal weight subjects were classified as sufficient (>30 ng/mL). Caucasians in the Class I and III obese groups had significantly higher Vitamin D than African Americans. Vitamin D increased significantly following 6 months of weight loss. There was no significant relationship between change in Vitamin D and change in body weight or body fat. Vitamin D decreased at 12 months despite a period of weight maintenance yet remained significantly greater than baseline. Meal replacement shakes containing Vitamin D were taken twice per day from baseline to 6 months and once per day from 6-12 months, contributing to the increase in Vitamin D at 6 months and 12 months.

Throughout the intervention, Vitamin D status was significantly greater in Caucasians than African Americans. There was no relationship between Vitamin D status and physical activity at 6 or 12 months. The results of this study confirm that Vitamin D deficiency or insufficiency is related to degree of adiposity and is more prevalent in African Americans than Caucasians. Moreover, a dietary weight loss intervention significantly increased Vitamin D in severely obese women, indicating that lower Vitamin D in obesity is amenable to weight loss intervention.

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PREFACE

I would first like to thank my research advisor on this project, Dr. Bret Goodpaster. Without Dr. Goodpaster's interest in pursuing this topic, this project would not have been possible. I would also like to thank the fellow members of Dr. Goodpaster's laboratory for their assistance in carrying out this project, particularly Dr. James DeLany. Dr. Delany devoted hours helping me with my statistics and interpretation of the results.

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1.0 INTRODUCTION

Obesity is one of the most significant issues in public health, affecting more than 150 million adults in the United States [1]. Although weight loss can have tremendous health benefits, weight loss typically results in decreases in bone mineral density and total lean body mass and may be influenced by both racial differences and the method of weight loss. It is thought that bone loss is primarily due to a reduced mechanical strain on the skeleton and can be mediated by a high Calcium intake. Vitamin D may also play a role [2]. Exercise has been suggested to increase Vitamin D levels, potentially through greater UV exposure or increases in Vitamin D receptor genes in skeletal muscle. It is unknown what happens to serum vitamin D levels and vitamin D intake during weight loss, or how Vitamin D status affects body composition changes, whether through diet alone or in combination with exercise. Given that there are Vitamin D receptors in both skeletal and adipose tissue, weight loss may potentially be influenced by Vitamin D intake and circulating levels in the body. Alternatively, weight loss may improve Vitamin D status, given its storage in adipose tissue.

1.1 RATIONALE

Vitamin D is necessary for the absorption of calcium by the small intestine and calcium storage in bone. Vitamin D deficiency has been studied as a risk factor for osteoporosis and low bone mineral density, yet the health benefits of Vitamin D may extend beyond bone health [3].

Vitamin D status is classified into three categories: Vitamin D sufficiency, insufficiency and deficiency [4, 5]. Vitamin D insufficiency and deficiency are receiving attention as potential risk

factors for several chronic conditions, including cardiovascular disease, diabetes, metabolic syndrome and obesity [6, 7]. Vitamin D status is most commonly determined through serum 25-hydroxyvitamin D (25OHD) and may be influenced by lifestyle factors, age, gender, adiposity, body weight and seasonal variations. Vitamin D has been reported to decline with age and be lower in women than men [8]. Cross sectional studies have correlated Vitamin D insufficiency and deficiency with increased incidence of cardiovascular disease, insulin resistance, high blood pressure and other non-skeletal outcomes [9]. Those at risk of vitamin D deficiency include the elderly, those living indoors or having a covered-up style of dress, pregnant women, darker-skinned individuals, and groups recognized as being at increased risk of diabetes [10].

Studies have suggested a relationship between Vitamin D and specific components of body composition, including fat and muscle mass. Vitamin D as well as several other vitamins and minerals have been shown to decrease dramatically following surgically-induced weight loss, yet it is unclear from these studies how much of the insufficiency is due to the rapid decrease in weight or the malabsorptive nature of the procedures [11]. Evidence for the argument of malabsorption of vitamin D exists in the numerous studies correlating obesity with Vitamin D deficiency. No studies have examined if Vitamin D status, without supplementation, naturally improves with a loss of body fat or weight.

Vitamin D deficiency is commonly found in obese, sedentary subjects, particularly those with darker skin pigmentation (e.g. African Americans). Low serum Vitamin D is attributed to diminished synthesis of vitamin D in the skin because of increased pigment. Additionally, African Americans typically have higher rates of obesity, total adiposity, cardiovascular disease and diabetes as compared to Caucasians. Given that African Americans traditionally exhibit less weight loss and greater retained adiposity following weight loss interventions, it stands to reason

that Vitamin D insufficiency or deficiency may be a contributing factor, and that certain ethnic populations may benefit from supplementation during weight loss [12].

At present, research into the relationship between Vitamin D insufficiency, body weight and adiposity has been primarily cross-sectional or observational in nature and indicates an inverse relationship between Vitamin D and health parameters including body weight, BMI, waist circumference, insulin resistance, bone health and cardiovascular disease [10, 13-17]. It is unclear whether adiposity and body weight are influenced by Vitamin D status, or poor Vitamin D status is an effect of excess body weight due to storage in adipose tissue and other hormonal factors involved in the pathogenesis of obesity.

1.2 SPECIFIC AIMS

- 1) To compare baseline serum Vitamin D levels between obese, severely obese, and normal weight individuals.
- 2) To compare serum Vitamin D levels between severely obese Caucasians and African Americans across a 12-month weight loss intervention.
- 3) To examine the effect of weight loss on serum Vitamin D levels in Class II and III I(severely) obese individuals.
- 4) To examine the effect of added physical activity to weight loss on serum Vitamin D levels.

1.3 HYPOTHESES

- 1) Baseline serum Vitamin D levels will be greater in lean and Class I obese controls versus Class II and III obese subjects and will be significantly correlated with body weight across all subjects.

- 2) Serum Vitamin D levels will be greater in Caucasians than African Americans at baseline, 6 and following 6 and 12 months of weight loss.
- 3) Vitamin D status at 6 and 12 months will be associated with change in adiposity and body mass.
- 4) Increased physical activity will be associated with greater improvements in Vitamin D status at 6 and 12 months.

1.4 SIGNIFICANCE

Obesity is a significant public health issue in the United States. Vitamin D insufficiency and deficiency are emerging as risk factors for several comorbidities commonly found in obesity. Given Vitamin D's association with obesity and its comorbidities, recommendations on how to optimize weight loss are warranted and may include achieving an optimal Vitamin D status. Currently, there are no studies that have observed Vitamin D status over an extended non-surgical weight loss intervention. This study examined Vitamin D status and body composition changes during weight loss with and without exercise and in Caucasians versus African Americans. The results of the study could contribute significantly to the knowledge base of Vitamin D insufficiency. First, it may provide evidence for a greater need for Vitamin D supplementation in obese and obese African American populations. Second, Vitamin D insufficiency may be identified at baseline as a potential predictor of less than optimal weight loss. Finally, Vitamin D status may emerge as one of the health parameters improved during weight loss, potentially even more so when combined with physical activity.

2.0 REVIEW OF LITERATURE

2.1 INTRODUCTION

Obesity rates are increasing worldwide, contributing to the burden of its associated chronic diseases. Dietary restriction and exercise interventions produce favorable changes in body composition, which in turn improves the status of the associated chronic diseases [18]. Vitamin D deficiency is increasing in prevalence and being tied to various chronic diseases beyond bone health in common with obesity, including diabetes mellitus, insulin resistance, cancers and cardiovascular disease [19, 20]. Given the numerous studies observing an inverse relationship between body fatness, muscular strength and Vitamin D, and the potential benefit of improving Vitamin D status on obesity-related disease, it is necessary to further clarify the relationship between changes in body fatness and Vitamin D status [21].

2.2 VITAMIN D DEFICIENCY

Vitamin D is a fat-soluble vitamin that does not occur naturally in most food sources and is frequently added as a dietary supplement. It was first identified by scientist Edward McCollum in 1922 while experimenting with treatments to cure rickets in dogs. It was not until 1923 that it was synthesized in the body's skin via exposure to ultraviolet light [22]. Vitamin D deficiency can result from impaired synthesis or absorption via limited exposure to sunlight, inadequate dietary intake, or problems associated with converting Vitamin D to its active form. Populations who are vulnerable to Vitamin D deficiency include those with limited sun exposure, those who do not frequently leave their home, dark-skinned individuals, elderly, those with fat absorption

issues, and those who are obese or have undergone gastric bypass surgery [23-25]. Several studies have found an association between Vitamin D deficiency and obesity, yet it is unclear whether Vitamin D contributes to obesity, or obesity itself causes Vitamin D deficiency [24, 26-33]. Research is currently investigating the various physiological mechanisms to explain these associations.

Vitamin D deficiency continues to be investigated as a contributor to Type II diabetes mellitus, a disease most frequently found in overweight and obese populations. Furthermore, low Vitamin D levels are significantly associated with an increased risk of obesity related diseases such as metabolic syndrome, yet research is lacking in any prospective studies examining the role of low Vitamin D in disease pathology. Positive associations of Vitamin D supplementation on insulin resistance have been found, yet the intakes associated with improvements are higher than the current dietary recommendations [34]. Intervention studies that have been conducted were either too short in duration, or manipulated dietary intake of Vitamin D, preventing a true analysis of the natural changes in Vitamin D intake and status with weight loss. There is a general lack of consistency in vitamin D intervention outcomes, potentially due to various levels of supplementation, different subject populations and seasonal variations in Vitamin D levels [35]. It is unclear what variations occur in Vitamin D levels during non-supplemented, non-surgical weight loss, how this may differ between African Americans and Caucasians, and what specific changes in body composition during weight loss may be related to Vitamin D.

2.3 SERUM VITAMIN D AND HEALTH - OVERVIEW

Vitamin D is produced by the cells of the skin following UV exposure and obtained from food intake and dietary supplements [36]. There are two major forms of Vitamin D, ergocalciferol, or D₂, and cholecalciferol, or D₃. Both are derived from supplements and food, but cholecalciferol alone is synthesized in the body following UV exposure. In order to achieve its active form in the body, Vitamin D must undergo two hydroxylation reactions. The first occurs in the liver to create the pre-hormone 25-hydroxyvitamin D [25(OH)D], or calcidiol. Calcidiol is also considered total Vitamin D, or the combined measurement of D₂ and D₃. The second reaction occurs at the level of the kidneys to form the physiologically active steroid hormone form of Vitamin D [1,25(OH)₂D], known as calcitriol. Any additional calcidiol not required by the kidneys to synthesize calcitriol is stored in tissues throughout the body or interact with Vitamin D receptors. Vitamin D receptors are present in various cells throughout the body, underscoring the importance of adequate Vitamin D status far beyond bone health. In a randomized control trial examining Vitamin D supplementation, Vitamin D intake of 1100 IU per day decreased the risk of non-skin cancers [19]. In a prospective cohort study on cardiovascular health and colorectal cancer prevention, optimal benefits were achieved when serum total vitamin D, 25(OH)D, fell between 25 and 37 ng/mL, with an intake of 700 to 1000 IU per day [37]. The current recommendations for supplementation do not exceed 600 IU per day regardless of age or gender, indicating that Vitamin D supplementation trials are warranted for a wide variety of health outcomes, including obesity.

2.3.1 Vitamin D Measurement

Vitamin D status is best measured by total serum 25-hydroxyvitamin D or calcidiol. It remains in blood circulation for approximately 15 days, making it a reliable measure of dietary intake and skin response to UV exposure [38]. Calcitriol is not a reliable measure of Vitamin D status given its short half-life and tight regulation by parathyroid hormone, calcium and phosphate [38]. In addition, calcitriol does not typically decrease in serum measurements until Vitamin D deficiency is severe, as the kidneys receive priority over the rest of the body tissues in receiving circulating calcidiol [39].

Clinically acceptable measures of Vitamin D status are continually being researched and refined, and are dependent on gender, age, and health status. Furthermore, because seasonal variations affect the diagnosis of Vitamin D deficiency, year round measurements are necessary yet are rarely conducted [40]. A major issue in the clinical and research monitoring of Vitamin D is defining clinical decision limits for the interpretation of 25(OH)D assays [41]. According to the Vitamin D council, an acceptable serum 25(OH)D measure for children and adults is approximately 50 ng/ml. The most recent fact sheet published by The Office of Dietary Supplements, regulated by the National Institute of Health, defined optimal Vitamin D status for bone health as ≥ 20 ng/mL. The data was derived from a report by the Institute of Medicine in 2010 [5] See Table 1.

Table 1. Serum 25(OH)D Concentrations and Health

ng/mL	Health Status
<12	Associated with Vitamin D deficiency, leading to rickets in infants in children and osteomalacia in adults
12-20	Generally considered inadequate for bone health in healthy individuals
>20	Generally considered adequate for bone and overall health in healthy individuals
>50	Emerging evidence links potential adverse effects to such high levels, particularly >60 ng/mL

A recent study by Gomez-Alonzo which reviewed results from Vitamin D supplementation trials proposed a functional classification of 25(OH)D to be >33ng/mL [42]. A long-term follow-up in Roux-en-Y gastric bypass patients considered post-operative optimal 25(OH)D to be >27 ng/mL; suboptimal as 17-27 ng/mL; deficient as <17 ng/mL [43] This discrepancy in recommendations underscores the need for more research in Vitamin D supplementation and longitudinal research interventions.

2.3.2 Adipose Tissue

People with a BMI of >30 commonly have low serum 25(OH)D, with levels continuing to decrease with an increase in body fat [24, 32, 44]. It is believed that due to Vitamin D's storage in adipose tissue, increased subcutaneous fat in obesity alters Vitamin D's release into circulation [24, 28]. Across several studies, an increasing amount of adipose tissue is associated with both decreased serum 25(OH)D and 1-25(OH)D, indicating that morbid obesity may be coupled with severe Vitamin D deficiency. A study by Moschonis examining post-menopausal women found that 25(OH)D was inversely related to total and regional fat mass and positively correlated with

total lean mass, independent of age, parathyroid hormone levels, physical activity and UV exposure [45]. An obese person may require a greater amount of UV exposure and dietary supplementation to combat the increased sequestering of 25(OH)D in the adipose tissue. However, given that adipose tissue is a storage site for Vitamin D, it may be difficult to assess if the diet is already sufficient in Vitamin D, or if any increases in supplementation will improve Vitamin D status without a loss of adipose tissue.

It has been suggested that low Vitamin D intake and exposure contributes to obesity itself [29], yet lean individuals have also been found to have low 25(OH)D levels, making the relationship between body fat and Vitamin D less straightforward [46]. It is possible that Vitamin D stored in fat cells has a direct influence on the fat cell's ability to store and release Calcium, which plays a role in the storage and release of fat from adipocytes. A study by Shi found that in mice, elevated 1-25(OH)D levels in adipose cells increased calcium in adipocytes, which causes increased formation of fat cells (lipogenesis) and decreased breakdown of fat cells (lipolysis) [47]. Calcium levels also have a direct effect on serum parathyroid hormone (PTH) which is increased in obese subjects and declines with weight loss [24, 48-50]. It has been suggested that PTH may promote accumulation of adipose tissue through increased calcium concentration in adipocytes [51]. Therefore, Vitamin D that is sequestered in adipose cells may promote obesity by elevating intracellular calcium and therefore increasing PTH, which may inhibit lipolysis and promote lipogenesis.

A higher Calcium intake is associated with lower body weight, yet no clinical weight loss studies yielded statistically significant results when coupled with Calcium supplementation. It is amounts of adipose tissue, or that the sequestering of Vitamin D in the adipose tissue further

contributes to obesity through alterations in calcium storage and release. No studies have examined the effect of weight loss on improving Vitamin D status.

2.3.3 Vitamin D and the Endocrine System

In addition to its relationship with body weight and body fat, Vitamin D is thought to have both direct and indirect effects on the endocrine system and is involved in the pathophysiology of Type II diabetes, hyperparathyroidism, and metabolic syndrome [16, 30, 52, 53] which is characterized by having three of the five following: increased waist circumference >102cm in men and >88cm in women; elevated serum triglycerides > 1.70 mmol/l; reduced serum high density lipoprotein (HDL) cholesterol <1.04 mmol/l in men and 1.30mmol/l in women; fasting plasma glucose (FPG) >6.1mmol/l; elevated systolic blood pressure > 130 mmHg and/or diastolic blood pressure >85 mmHg [54]. Studies examining the relationship between metabolic syndrome, also known as syndrome X and Vitamin D have been inconclusive, but suggest that Vitamin D deficiency may be an avoidable risk factor [10, 54]. Low serum 25(OH)D acts on both the Vitamin D receptor cells of the pancreas, causing beta-cell dysfunction, and the cells of adipose and lean tissue which are responsible for insulin action and glucose metabolism [30]. Several studies have indicated that baseline serum 25(OH)D is associated with markers of insulin resistance, higher serum fasting glucose, and increased risk of Type II diabetes[14, 16, 17, 20, 53, 55, 56]. In a study of Asians living in East London, where diabetes mellitus is four times more prevalent than the United States, 95% of those at high risk for insulin resistance were considered Vitamin D deficient [57]. Given the prevalence of insulin resistance, Type II diabetes and metabolic syndrome in obese populations, it may be possible that Vitamin D status is a mediator for this relationship.

Recent evidence has suggested that optimal Vitamin D status, which down-regulates PTH, may reduce risk for obesity, diabetes and insulin resistance [56]. The down-regulation of PTH alone is not expected to produce weight loss, but may potentiate the fat loss achievable with caloric restriction and exercise. Support for this indirect effect of Vitamin D come from observational studies following gastric bypass patients. Post-surgery, patients have exhibited linear increases in PTH with linear decreases in serum 25(OH)D, suggesting malabsorption of calcium and up-regulation of PTH [58, 59]. The severity of hyperparathyroidism is related to the extent of removed intestine, where the fat-soluble vitamins are absorbed [11, 51]. The decrease in 25(OH)D found with large weight loss in gastric bypass, however, cannot discount the possibility of 25(OH)D levels improving with non-surgical weight loss where malabsorptive changes have not occurred [60].

2.4 WEIGHT LOSS AND VITAMIN D STATUS

2.4.1 Vitamin D Deficiency in Obese Populations

Obesity is associated with low circulating levels of 25(OH)D, whether measured through BMI, adiposity, or waist-to-hip ratio. In one study in women, 51% of severely obese women had clinical Vitamin D deficiency as compared to only 22% of non-obese patients [32]. A study by Konraden found that with increasing BMI group, there were significant decreases in serum 25(OH)D; those with a BMI of >39.9 had 24% lower 25(OH)D than those with a BMI of <25 [61]. Finally, a 20-week study involving weight loss in obese women found that baseline serum 25(OH)D was inversely correlated with various measures of obesity including PTH, insulin levels and markers of insulin resistance [51]. Vitamin D deficiency in obesity may be due solely

to its deposition in the adipose tissue and has been suggested to inhibit the development of fat cells [24, 62]. However, these studies are all observational in nature and do not imply that fat mass affects serum 25(OH)D or vice versa [63]. It is also plausible that overweight and obese individuals consume less Vitamin D from dietary sources. In a study assessing Vitamin D intake in males and females, Vitamin D intake was an independent predictor of BMI [64]. The majority of foods rich in Vitamin D are also high in Calcium, which has been thoroughly examined in both cross-sectional and longitudinal weight loss studies. Research has suggested that weight loss, specifically body fatness, is enhanced with calcium supplementation when coupled with Vitamin D [65-67]. The mechanisms for this result are unclear and would be clarified by a weight loss study in which voluntary Calcium and Vitamin D intake were observed while measuring Vitamin D status.

2.4.2 Racial Differences

African Americans are at greater risk for several chronic diseases, including obesity, heart disease, and Type II diabetes than Caucasians [68]. Additionally, they have lower circulating levels of 25(OH)D and have greater rates of Vitamin D insufficiency [23, 69]. 25(OH)D is correlated with fat mass, fat distribution and anthropometric measures in adult African Americans [31]. The higher prevalence of Vitamin D insufficiency in African Americans versus whites is primarily due to the fact that their skin pigmentation causes reduced Vitamin D production in the skin [69]. However, several social and lifestyle factors that contribute to their obesity may increase the prevalence of Vitamin D insufficiency beyond their skin's ability to produce Vitamin D. As with Caucasians, the greater adipose tissue may sequester Vitamin D, and obese individuals are more likely to cover their skin and have limited sunlight exposure

[70]. Obese individuals, and more so, African Americans, have a lower dietary intake of both Vitamin D and Calcium as well. Although African Americans appear to be resistant to the effects of low Vitamin D on the skeleton, as indicated by their lower risk of fragility fractures and osteoporosis, it is unknown how their Vitamin D status affects the other chronic diseases which are associated with Vitamin D insufficiency, and how their Vitamin D status may influence attempts at non-surgical weight loss [71]. Considering that African Americans traditionally report less weight loss during behavioral interventions than whites, identifying a relationship between Vitamin D and weight loss may be particularly beneficial for populations at greater risk for obesity and its associated chronic diseases. The Vitamin D insufficiency found in African Americans may be one of the main factors contributing to the increased rates of diabetes and intramuscular fat which cannot be explained by total or central adiposity alone [68].

2.4.3 Physical activity and UV exposure

The addition of an exercise program to dietary restriction may mediate the relationship between Vitamin D status and obesity. Those who exercise both at weight maintenance and weight loss exhibit greater losses of body fat and a shift from fat to fat-free mass. Decreased 25(OH)D and increased parathyroid hormone are associated with increased fat mass and sarcopenia [31, 72]. Theoretically, if 25(OH)D is sequestered in the fat mass, loss of body fatness should result in elevated serum levels of 25(OH)D, as well as a preservation or gain of skeletal muscle. However, studies examining the relationship between exercise score and Vitamin D are inconclusive and do not support a direct relationship. It is more likely that physically active people have less body fatness, take in great amounts of dietary Vitamin D, and spend more time outside, thus increasing

their UV exposure. Even when exposed to equal amounts of ultraviolet light, obese subjects exhibit lower 25(OH)D levels, indicating that increasing exposure to the skin may not be enough to improve Vitamin D status in obese populations without weight loss [29]. There is potential for the increased lean body mass and decreased fat mass associated with physical activity to influence serum 25(OH)D. Furthermore, since muscle mass influences insulin sensitivity, increasing serum 25(OH)D levels through a loss of body fat and preservation of lean mass through diet and exercise may clarify the relationship between Vitamin D and insulin resistance [20].

Studies examining Vitamin D status and UV exposure have been conducted throughout the world, yielding results which conflict with the typical observations of an inverse relationship between obesity and Vitamin D status. A study in Saudi Arabian found that females with a lower BMI were deficient in Vitamin D, while males were deficient in Vitamin D and PTH simultaneously. Saudi Arabian women, regardless of BMI classification, experience very little sun exposure and diets low in Vitamin D, indicating the importance of both variables in Vitamin D status regardless of body fatness [25]. If weight loss can improve Vitamin D status through the release of Vitamin D from adipose tissue, exposure to UV rays, subsequent Vitamin D synthesis and dietary supplementation must be sufficient.

2.5 VITAMIN D SUPPLEMENTATION

A challenge to Vitamin D supplementation trials lies in that there is no widely-accepted definition for optimal Vitamin D levels [56]. Current Vitamin D recommendations are designed to prevent bone disease and have not been broadened to other diseases influenced by Vitamin D [19]. See Table 2.

Table 2. Recommended Dietary Allowances (RDAs) for Vitamin D [5]

Age	Male	Female
0-12 months	400 IU	400 IU
1-70 years	600 IU	600 IU
>70 years	800 IU	800 IU

The Office of Dietary supplements defines optimal Vitamin D intake for healthy adults to be 600 IU per day [73]. Clinical recommendations do not take into account body fatness and body size, potentially leaving obese patients with insufficient supplementation. In a meta-analysis examining optimal Vitamin D levels across various chronic diseases, a mean serum 25(OH)D level of approximately 30 to 44 ng/mL provided optimal benefits on health without risk of Vitamin D toxicity. Oral intake and supplementation to achieve these levels ranged from 1,800 to 4,000 IU vitamin D per day [37]. One study by Cannell et al suggested upwards of 5000 IU per day in Vitamin D deficient obese individuals [19]. Finally, a study by Hathcock found no toxicity indications when supplementation reached 10,000 IU per day. There is a clear need for refining optimal Vitamin D intake, particularly for at risk populations. In studies examining the effect of calcium and Vitamin D supplementation during weight loss, changes in 25(OH)D were negatively associated with baseline anthropometric measurements, and supplementation enhanced the beneficial effect of weight loss on lipid and lipoprotein profiles [27, 43]. In a study assessing the relative risk of developing Type II diabetes, a combined intake of >1200 IU of Calcium and >800 IU of Vitamin D resulted in a 33% reduced risk [74]. In one study examining supplementation on weight loss alone, supplementation itself did not significantly impact weight loss, but did increase 25(OH)D levels and decreased cardiovascular disease risk markers [75]. Research on Vitamin D supplementation in obese patients is warranted, and a greater

understanding of how weight loss influences Vitamin D status will assist in determining necessary supplementation in this population.

2.6 SUMMARY

Although low 25(OH)D levels are associated with several chronic diseases including obesity, it remains unclear whether this is a contributor or simply a consequence of poor health, creating a challenge for supplementation trials [7, 76]. If weight loss, specifically loss of body fat, results in elevated levels of 25(OH)D, this may provide evidence for much greater levels of supplementation in obese patients and identify a potential mediator of the chronic diseases associated with obesity. A recent study found that obese children and adolescents with low Vitamin D status are at an increased risk of developing impaired glucose metabolism, stressing the need for adequate Vitamin D interventions across all clinical populations [56]. A 20-week study in obese women found a non-significant increase in 25(OH)D levels with a 10% weight loss and a significant association with 25(OH)D and decreased insulin levels [20]. However, this study was limited by a small sample size and short duration. A long-term, multi-cohort study monitoring Vitamin D levels during weight loss with or without physical activity could further clarify the relationship between Vitamin D status and obesity and contribute to the scientific knowledge base on proper Vitamin D supplementation and the chronic diseases commonly associated with obesity.

3.0 METHODOLOGY

3.1 INTRODUCTION

Obesity is a significant public health issue which continues to increase in prevalence. Lifestyle interventions to decrease the severity of obesity and its associated chronic diseases are of increasing importance. Vitamin D deficiency is associated with obesity, yet the relationship has not been examined over a long-term weight loss intervention without direct manipulation of Vitamin D intake or surgically-induced weight loss. The purpose of this study was to examine the effects of weight loss and body composition changes on Vitamin D status in severely obese individuals following a 12-month lifestyle intervention.

3.2 SUBJECTS

One-hundred and thirty individuals participated in the RENEW study, a 12-month lifestyle intervention consisting of caloric restriction and progressive physical activity. The sub-study included 90 women. 50 were in the intervention group and consisted of Class II and III obese, half African American, half Caucasian. The intervention sample was restricted to 50 women to both obtain a full completer analysis as well as minimize cost of sample preparation. The remaining 40 women made up the control group and were classified as either normal weight (BMI <29 kg/m²) or Class I obese (BMI >29 and <35 kg/m²). The control group also consisted of 31 Caucasians and 9 African Americans. The selection of the control group was random, but restricted to females that completed the 12 month follow-up visit. See Figure 1.

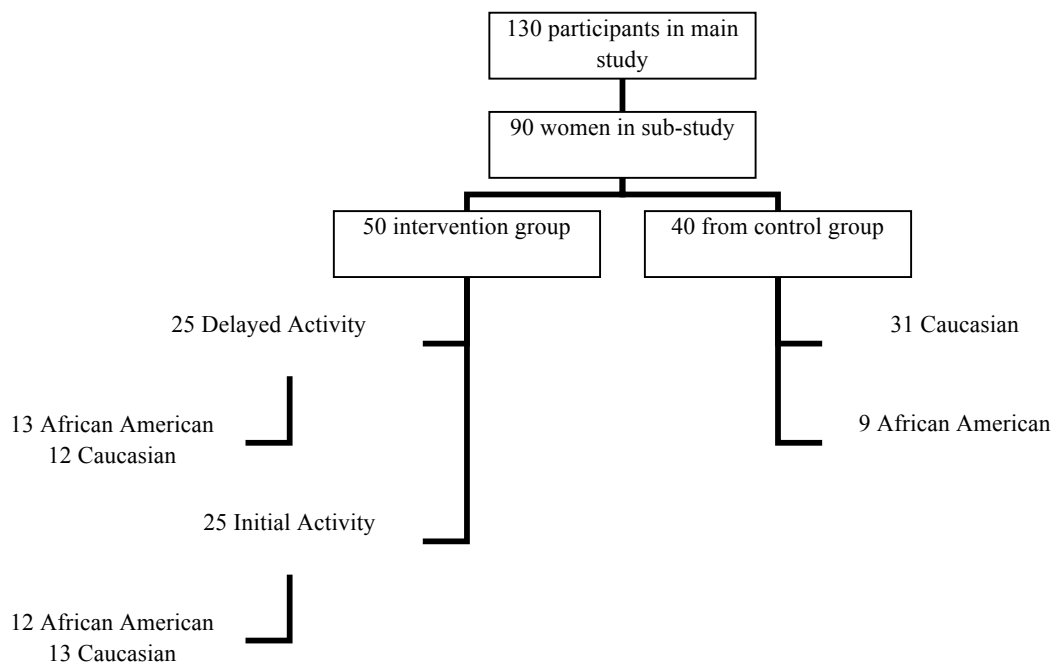


Figure 1. Study Participants

The purpose of this study was to examine the effects of an intensive lifestyle intervention on weight loss in severely obese adults, and whether the adoption of a physical activity program in addition to a dietary intervention would result in additional weight loss compared with dietary intervention alone. The study also sought to examine if the outcomes were different in African Americans and white individuals. This study provided optimal design for examining the effects of weight loss, fat loss and physical activity on serum Vitamin D levels and comparing these outcomes in whites versus African Americans. All Vitamin D analyses were conducted after the completion of the main study. Participants were eligible for the main study if they met the following criteria:

- 1) Between the ages of 30 and 55 years
- 2) Class II or class III obesity, defined as BMI between 35 and 39.9 or above 40, respectively
- 3) Able to walk without assistance

- 4) Commit to the schedule of intervention and assessment visits
- 5) Obtain medical clearance for intervention.

Participants were recruited via television, newspaper advertising, and mass mailings. All participants provided informed consent to participate in the study. The University of Pittsburgh Institutional Review Board approved the ongoing clinical trials in the main study and the sub-investigation presented here.

3.2.1 Exclusion Criteria

Candidates were excluded if they had any of the following:

- 1) History of cancer within the past 5 years
- 2) History or were currently being treated for coronary heart disease
- 3) Had enrolled in a weight loss program in the past year
- 4) History of bariatric surgery
- 5) Reported losing greater than 5% of their body weight in the previous 6 months
- 6) Uncontrolled hypertension, diabetes, or pregnancy during the previous 6 months.

3.2.2 Randomization

Participants consented to be randomized to one of two weight loss interventions groups, with blocking according to gender, level of obesity and race/ethnicity (African American versus White). Given that race and ethnicity have been shown to affect Vitamin D status, this randomization ensured equal racial/ethnic representation across study groups.

3.3 EXPERIMENTAL DESIGN

Participants were randomized into one of two weight loss interventions consisting of dietary modifications and physical activity. Interventions were conducted by Dr. Jakicic and the staff at the Physical Activity and Weight Management Research Center. One intervention group undertook diet and physical activity immediately, with the exercise prescription increasing in total minutes per week over the first 6 of 12 months. The second group began with dietary recommendations alone for the first 6 months, and incorporated physical activity recommendations at month 6.

3.3.1 Intervention components

The lifestyle intervention program consisted of a combination of group, individual and telephone contacts over the 12-month period. The first 6 months were more intensive, delivering 3 group meetings and 1 individual meeting per month. During months 7 through 12, participants received 2 group sessions and 2 telephone contacts per month. Diet was monitored weekly with the use of food intake journals, and participants were asked to record the type of food, calorie value, fat grams and time of day that the food was consumed. Vitamin supplementation was not recorded. All participants were prescribed a daily calorie intake based on initial body weight that would result in sustained weight loss of 8-10% at 12 months. Energy intake was prescribed at 1200 to 2100 calories per day, with a recommendation macronutrient composition of 20 to 30% fat, 50-55% carbohydrate and 20 to 25% protein. There were no specific foods recommended, however, to improve compliance to calorie recommendations, participants were given liquid and

prepackaged meal replacements at no charge. During the first 6 months, participants received 2 meal replacement shakes and 2 snack bars per day; in the final 6 months, they received one shake and one snack bar. Each shake contained 210 IUs of Vitamin D, or approximately 35% of the recommended daily value based on a 2000 calorie diet.

3.3.2 Physical Activity

As mentioned, a progressive physical activity program was prescribed to both groups of participants. The initial activity group was prescribed moderate-intensity physical activity akin to brisk walking, until a final goal of 60 minutes, 5 days per week at the end of month 6. This level was then maintained from months 7-12. Participants in the delayed physical activity group were provided with the same recommendations, yet were delayed until month 7. Participants were provided with a pedometer to monitor steps and were asked to record their physical activity in their food intake journal. To increase adherence to physical activity guidelines, participants were provided with exercise videos, pedometers and portable exercise equipment such as resistance bands and stability balls. No specific recommendations regarding strength training or flexibility training were provided.

3.4 ASSESSMENTS

3.4.1 Vitamin D status

Vitamin D status was assessed at baseline, 6 and 12 months in 50 class II and II obese women as well as 39 lean controls. The levels of the compounds ergocalciferol (Vitamin D₂), calciferol

(Vitamin D₃) and calcidiol (25(OH)D) were extracted using the Waters ACQUITY TQD system, an HPLC-MS assay, performed at Endocrinology and Metabolism Research Center of the University of Pittsburgh Medical Center. In a study comparing commercially available assays, the HPLC-MS method was found to be the most valid [77]. The Institute of Medicine's report defines deficiency as <12 ng/mL, insufficiency as 12-20 ng/mL, and sufficiency as >20 ng/mL [5]. For purposes of this investigation, Vitamin D status was classified according to the 20-week study by Tzotsas et al, examining the effect of weight loss on Vitamin D status. Vitamin D was classified according to the following measurements: deficient (<20ng/mL), insufficient (<30ng/mL and sufficient (>30ng/mL) [51].

3.4.2 Body Composition

Body parameters were measured at baseline, 6 and 12 months. Body weight, height and waist circumference were measured using standard protocols [78]. Body fat and fat free mass were measured by either dual-energy x-ray absorptiometry or by air displacement plethysmography depending on the size of the participant. Computed tomography scans were performed at baseline and 6 months to determine the quantity of adipose tissue in the abdomen and liver.

3.4.3 Dietary Intake

Dietary intake and nutrient composition was determined at baseline with the use of a food frequency questionnaire. Participants were asked to record food description as well as calories and fat per food item for each meal. The frequency per day of meals eaten and meal replacements used was input into a database to determine compliance with dietary recommendations and to monitor the use of the Slim-Fast products. Slim-fast meal replacement

shakes contain 210 IUs of Vitamin D, approximately 35% percent of the Recommended Daily value (RDV) of Vitamin D, 600 IUs, based on a 2000 calorie diet.

3.4.4 Physical Activity

Participants were given a multisensory physical activity monitor (Sensewear Pro3; Body Media, Pittsburgh, Pennsylvania) to wear between 7 and 11 consecutive days at baseline, 6 and 12 months to capture their physical activity at these time points. The activity monitor provides measures in total caloric burn (kcal), metabolic equivalents (METs) and general activity classifications such as low, moderate and vigorous activity (>6 METs). For purposes of this investigation, average METs (AVGMETS) per day as well as total steps per day will be examined.

3.5 STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS statistical software (version 19.0). Statistical significance for all analyses was set at $p < .05$. Data was first analyzed to provide descriptive characteristics of BMI, weight, fat mass, Vitamin D status and physical activity as reported in averages METs as well as steps per day for the study participants.

3.5.1 Baseline Comparisons

A one-way ANOVA was used to establish the baseline comparisons of Vitamin D status between the lean controls, class I obese controls and the intervention subjects. In addition, an independent

t-test was performed to compare the baseline Vitamin D status in African Americans versus Caucasians. Correlation analyses was used on the entire study group, controls and intervention subjects, to test the hypothesis that body weight would associated with baseline Vitamin D status. To examine the seasonal variations in Vitamin D levels, a one-way ANOVA was used to determine the differences in Vitamin D by season. Given that none of the intervention subjects were examined in October, November or December, the first 9 months of the year were used.

3.5.2 Intervention Analysis

Data at 6 and 12 months was analyzed using analysis of variance (ANOVA). Two-way repeated measures analysis of variance (ANOVA) was used to compare the 6 and 12 month differences in Vitamin D status between the initial and delayed physical activity groups. Correlational analysis was used to examine the relationship between body weight and adiposity and Vitamin D status at 6 months. Two-way repeated measures ANOVA was used to compare the 6 and 12 month differences in serum Vitamin D status between Caucasians and African Americans. Finally, correlation analysis was used to determine the relationship between physical activity and Vitamin D status.

3.5.3 Power Analysis

Power analyses were conducted to determine the necessary sample size to detect significant changes in Vitamin D status at 6 and 12 months. Based on results from a previous study which examined Vitamin D status in lean versus obese adults, a 20-30% difference should be expected [29]. At a significance level of .05 for a 2-tailed t-test and power set at .80, a sample size of 31 participants per experimental group would be required to detect this difference. Furthermore, the

literature indicates that African Americans exhibit approximately a 50% lower serum Vitamin D level than Caucasians, regardless of seasonal variation [79]. At a significance level of .05 for a 2-tailed t-test and power set at .80, a sample size of 7 per experimental group would be required. Therefore, the amount of participants in the intervention group (25 African American women, 25 Caucasian women) as well as the 41 lean controls is sufficient to detect any true differences in Vitamin D status.

4.0 RESULTS

4.1 BASELINE CHARACTERISTICS

Subjects in this investigation were 50 women (25 Caucasian, 25 African American) between 30 and 55 years of age, with an average BMI of 44 kg/m². The control group consisted of 40 women (31 Caucasian and 9 African American) with an average BMI of 27 kg/m². Using the total of 25(OH)D₂ and 25(OH)D₃, both the Class I and Class III obese subjects were considered Vitamin D insufficient (20-30 ng/mL). The Class II subjects were considered deficient (<20 ng/mL). The normal weight subjects were classified as sufficient (>30 ng/mL). Because it was below the detection limit (<4 ng/mL) in all but 3 intervention subjects and 3 control group subjects, Vitamin D₂ (ergocalciferol) was not included further analysis. 25(OH)D was used as the measurement of Vitamin D in the intervention analysis.

Table 3. Baseline Characteristics by Body Mass Index

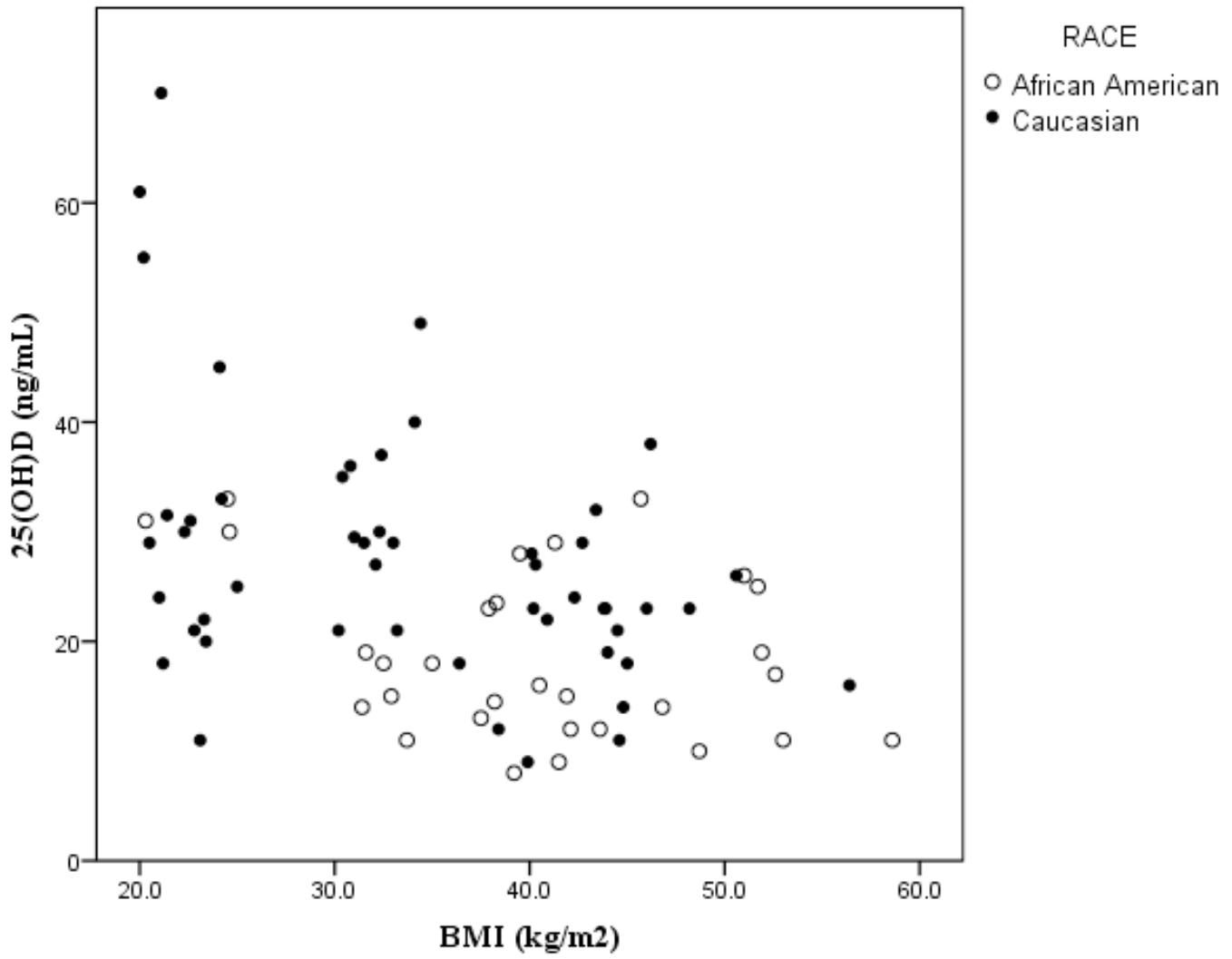
Outcome Variable	Normal Weight	Class I	Class II	Class III	<i>p</i> value
N=	22	18	12	38	
25(OH)D (total)	31.9± 14.8	27.2± 10.0	17.1±7.7	20.5±7.2	<.01
25(OH)D ₃	30.8±14.3	25.5 ±10.0	15.8±7.8	20.2±7.3	<.01
Weight (kg)	61.6± 6.9	85.5±6.4	99.9± 6.8	123.1±13.7	<.01
Fat mass (kg)	19.7± 3.9	38.7± 4.1	46.0± 4.8	63.6 ±10.4	<.01
Steps per day	9717.24± 3230.6	8869.0± 2150.6	9197.4±3375.9	6276.5± 2190.8	<.01
Average METs	1.58 ±.26	1.25± .13	1.29± .24	1.06± .17	<.01

*Group means ±SD; variables examined using one-way ANOVA

4.1.1 Vitamin D by BMI

One-way analysis of variance (ANOVA) revealed significant differences between the weight classes (normal weight, class I, class II and class III obese) in all examined variables (Table 3). Post-hoc examined revealed significant differences in 25(OH)D between the normal weight and Class II and Class III obese groups, but not between the normal weight and Class I obese, or Class II and class III obese (Table 3).

Pearson correlation was used on the entire group (normal weight and obese) to test the relationship between baseline BMI and Vitamin D (Figure 2). Baseline BMI was significantly correlated with 25(OH)D ($-0.45, p < .01$). The correlation was similar between 25(OH)D and fat mass ($-0.47, p < .01$). To further examine seasonal differences, a one-way analysis of variance was conducted on the intervention subjects to examine Vitamin D by season (Figure 3). Given that none of the intervention subjects were examined in October, November or December, the first 9 months of the year were used. There was no significant effect of season ($p = .46$). The control subjects were also examined. There were no significant differences in Vitamin D status by season in the control group ($p = .70$) Therefore, subsequent analysis did not include controlling for seasonal variation.



Significant correlation between 25(OH)D and BMI, $r=-.45, p<.01$)

Figure 2. Relationship Between Baseline 25(OH)D and BMI

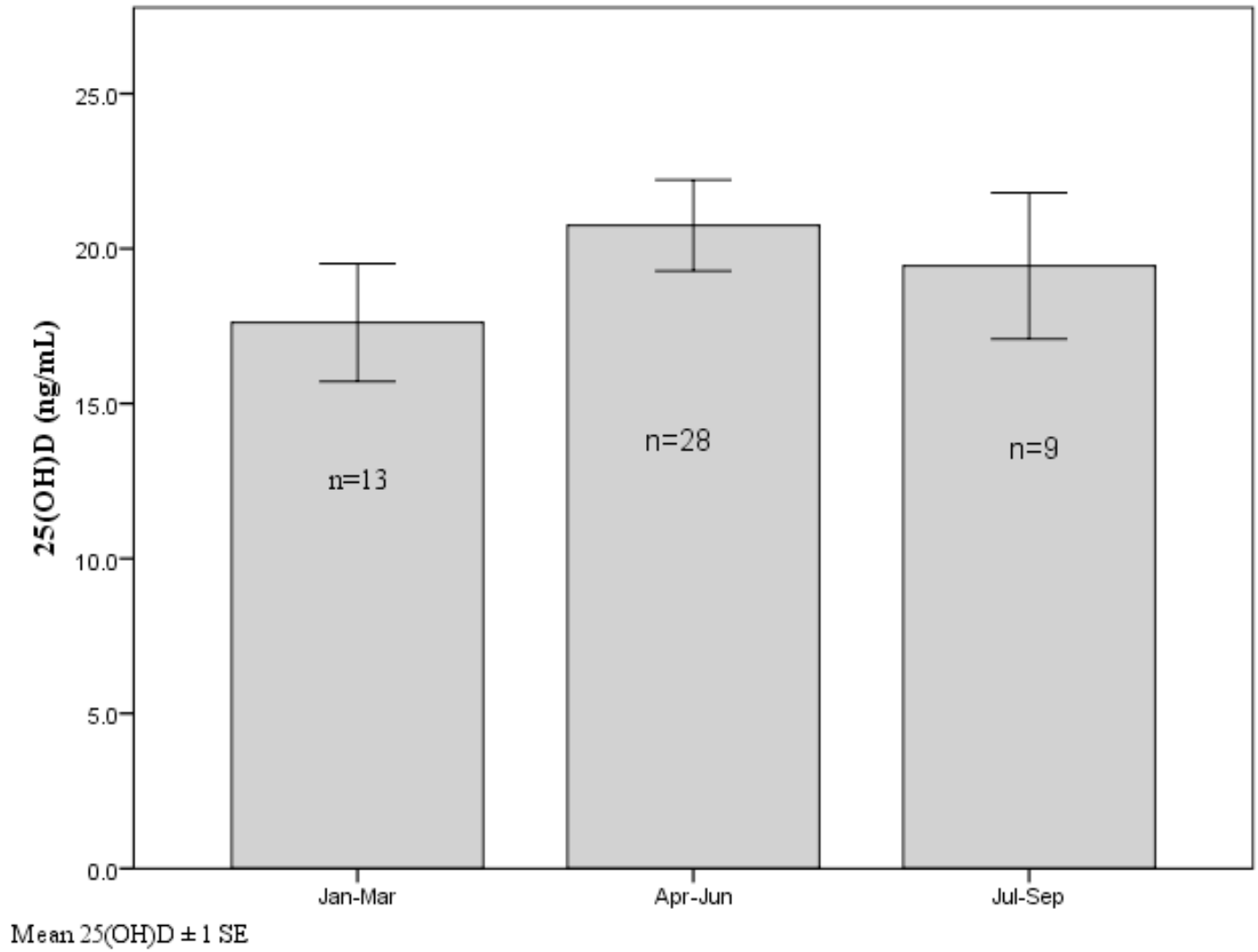
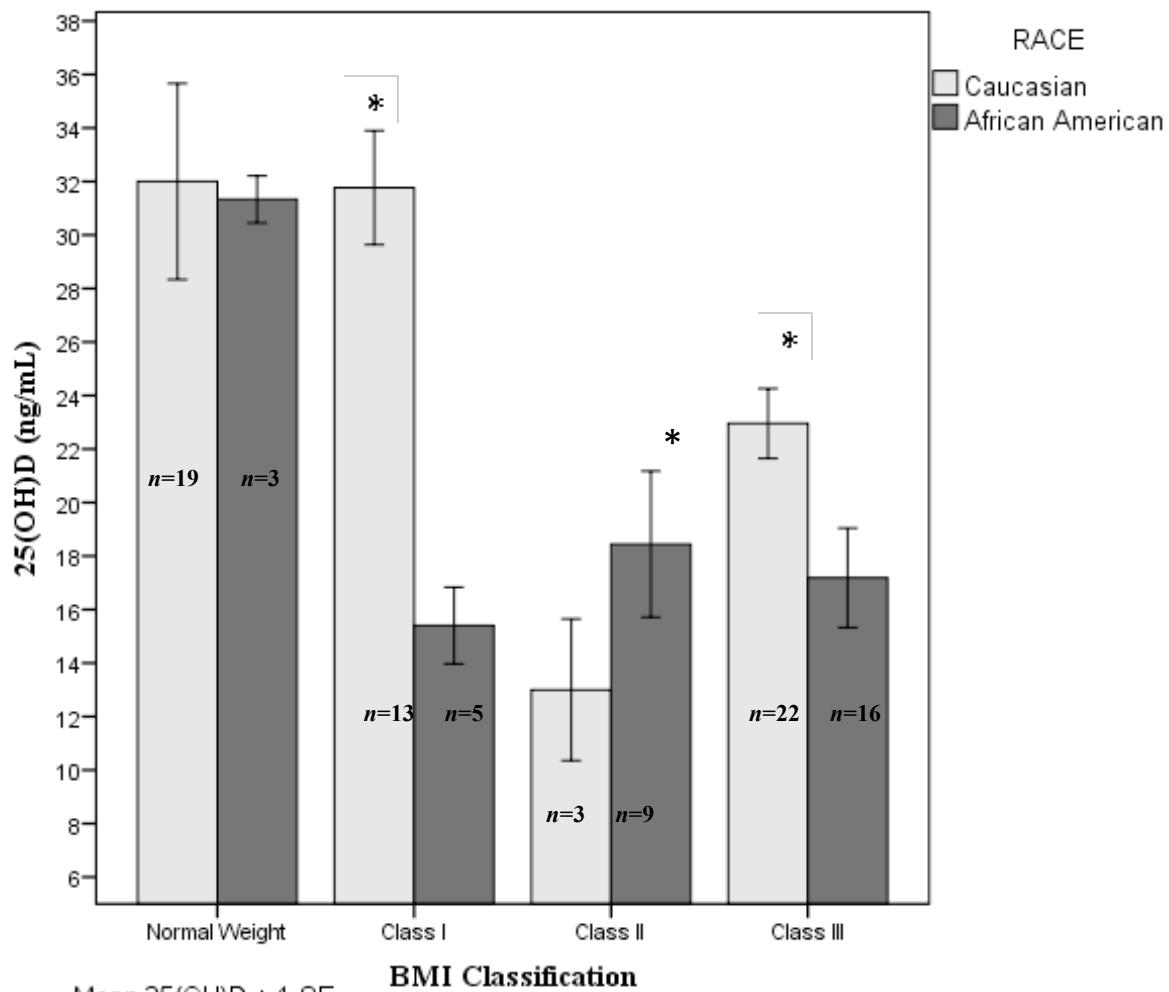


Figure 3. 25(OH)D by Time of Year

4.1.2 Racial Differences

At baseline, total 25(OH)D was significantly greater in Caucasians than African Americans (27.5 ± 11.8 versus 18.6 ± 7.9 , $p < .01$). Analysis of variance was conducted to compare baseline 25(OH)D in African Americans versus Caucasians by BMI classification. Caucasians in the Class I and III obese groups had significantly higher 25(OH)D than African Americans Figure 4). African Americans in the Class II obese group had significantly greater 25(OH)D than Caucasians. There was no significant difference between normal weight Caucasians and African Americans. Although racial breakdown by obesity level is presented in Figure 4, a few of the groups had a small number of subjects and it is not possible to make definitive conclusions on the differences in 25(OH)D by racial group.



Mean 25(OH)D \pm 1 SE
 *significant difference between racial groups determined by analysis of variance, $p < .05$.

Figure 4. Baseline Differences in 25(OH)D By Race and BMI

4.2 INTERVENTION

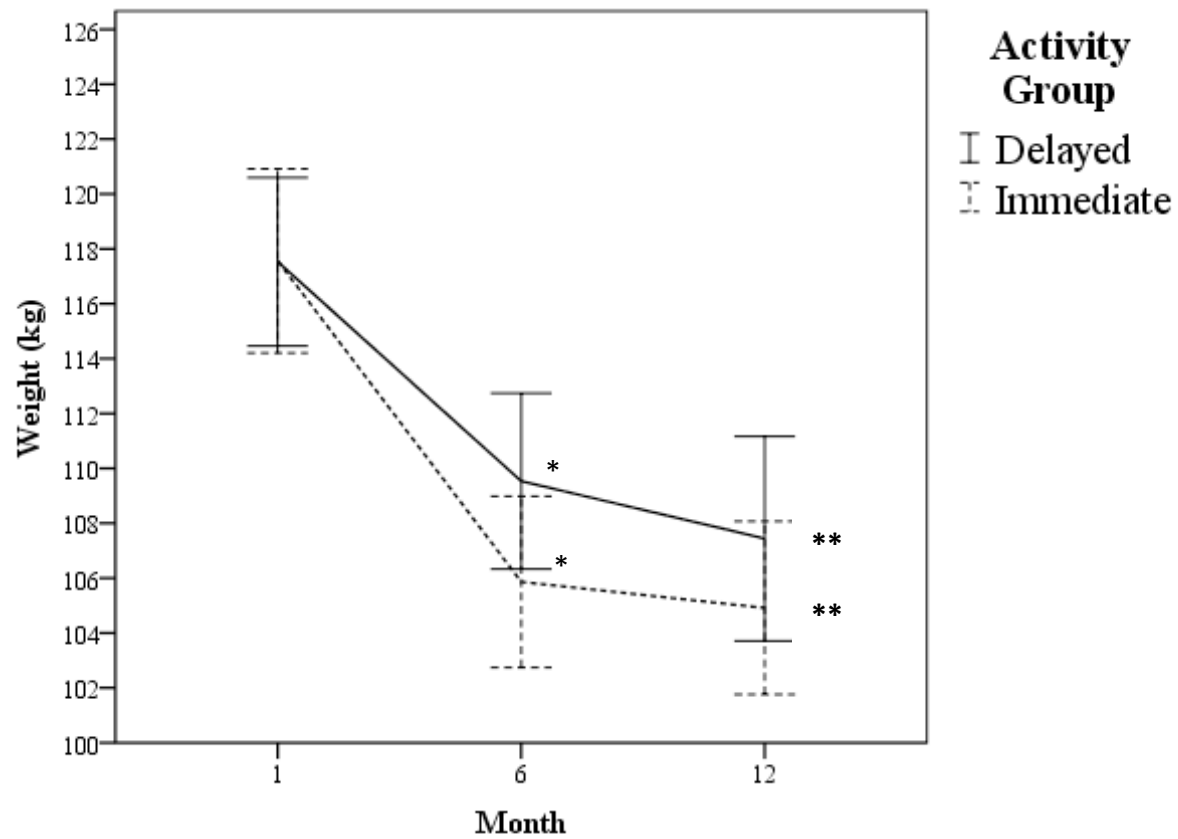
4.2.1 Changes in Vitamin D, Body Composition and Physical Activity

At month 6, both intervention groups lost a significant amount of body weight. The delayed physical activity group lost an average of 8.1 ± 6.3 kg, while the initial physical activity group lost significantly more, an average of 11.8 ± 7.2 kg. See Figure 5. Analysis of variance was performed to compare Vitamin D, body composition and physical activity at baseline, 6 and 12 months in

the initial versus delayed physical activity groups (Table 4). There was a significant main effect of time for 25(OH)D and all measures of body composition, but not for measurements of physical activity. However, there was a main effect of intervention group for average METs and steps per day. At both months 6 and 12, 25(OH)D was significantly greater than baseline in both intervention groups (Figure 6). However, 25(OH)D decreased significantly from month 6 to month 12 in both intervention groups ($p < .01$). Body fat also decreased significantly from baseline to 6 month and baseline to 12 months. There were no significant interaction effects between intervention group and time for any of the variables.

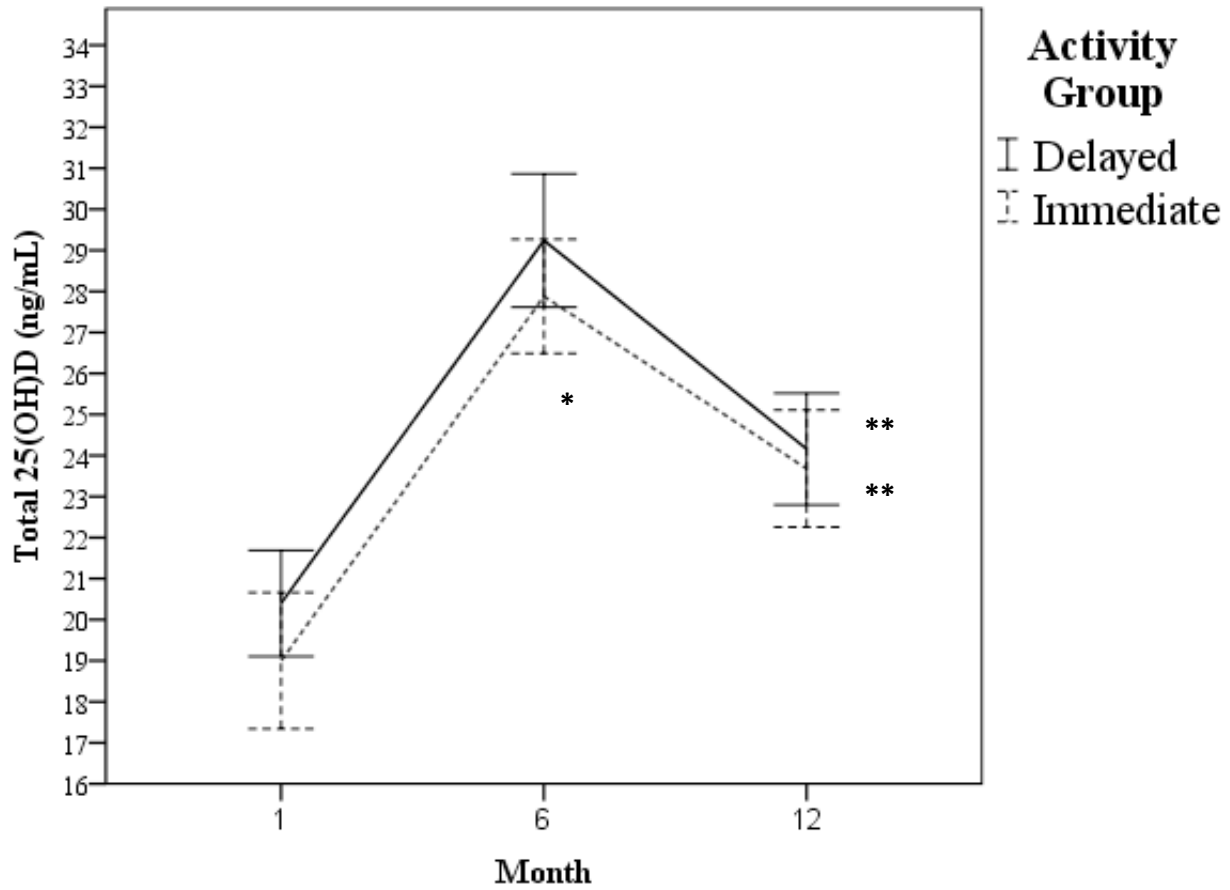
4.2.2 Relationship Between Change in Vitamin D, Body Weight and Adiposity

Pearson correlation was conducted for 6-month change in 25(OH)D and body weight, as well as 6-month change in 25(OH)D and fat mass. There was no significant correlation between change in 25(OH) D and body weight or body fat at 6 months. Follow-up independent t-tests were conducted to compare changes between intervention arms at baseline and 6 months. There were no significant differences between change in 25(OH)D, body weight or body fat between physical activity groups. Only change in body weight between physical activity groups approached significance ($p = .06$).



Mean weight by intervention group at baseline, 6 and 12 months.
 *significant decrease from baseline to month 6, $p < .01$
 **significant decrease from baseline to month 12, $p < .01$
 Error Bars: ± 1 SE

Figure 5. Change in Body Weight by Intervention Group



Mean total 25(OH)D by intervention group at baseline, 6 and 12 months.
 *significant increase from baseline to month 6, $p < .01$
 **significant increase from baseline to month 12, $p < .01$
 Error Bars: ± 1 SE

Figure 6. Absolute Change in 25(OH)D by Intervention Group

Table 4. Change in Body Composition and Physical Activity by Activity Group

Variable	Assessment period			Time		Group
	Baseline	6 months	12 months	Month 6 v baseline	Month 12 v baseline	
Body Fat (%)						
Delayed	50.1 ± 4.4	48.5 ± 4.7	48.2 ± 5.2	<i>p</i> <.01	<i>p</i> <.01	<i>p</i> =.327
Initial	50.1 ± 3.9	47.4 ± 4.0	47.1 ± 4.7			
Fat-free mass (kg)						
Delayed	56.7 ± 6.5	54.5 ± 6.9	54.5 ± 6.9	<i>p</i> <.05	<i>p</i> <.05	<i>p</i> =.684
Initial	57.1 ± 5.0	53.8 ± 5.1	53.6 ± 5.9			
Fat (kg)						
Delayed	59.4 ± 11.8	53.8 ± 12.0	55.1 ± 12.7	<i>p</i> <.01	<i>p</i> <.01	<i>p</i> =0.383
Initial	59.2 ± 12.5	50.6 ± 11.2	50.3 ± 11.6			
Steps/day						
Delayed	6580.1 ± 2261.6	6873.9 ± 2047.2	7296.8 ± 2467.5	<i>p</i> =0.169	<i>p</i> =0.072	<i>p</i> <.01
Initial	7399.3 ± 3224.6	8684.8 ± 3039.7	8828.4 ± 3391.1			
Average METs						
Delayed	1.08 ± .17	1.09 ± .16	1.14 ± .22	<i>p</i> =0.482	<i>p</i> =0.187	<i>p</i> <.05
Initial	1.16 ± .24	1.21 ± .25	1.22 ± .22			

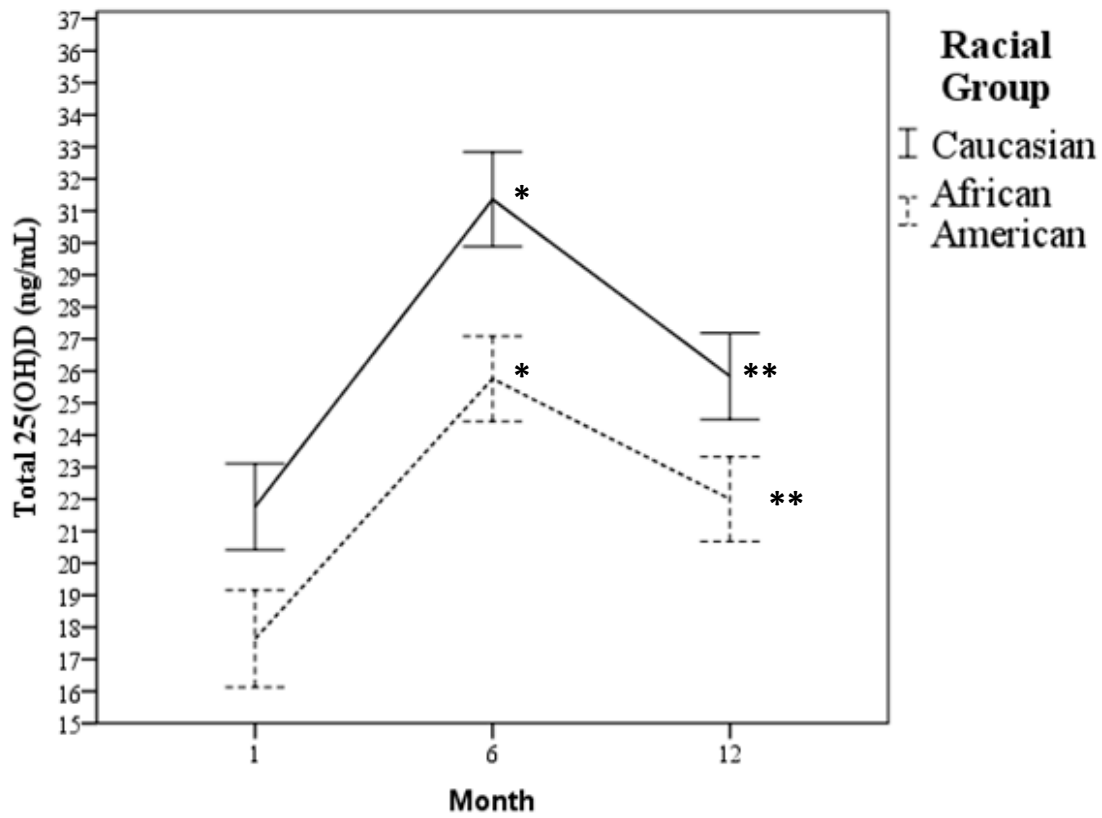
4.2.3 Change in Vitamin D by Race

Two-way analysis of variance was performed to compare 25(OH)D body composition and physical activity at baseline, 6 and 12 months (Table 5). There was a main effect of time, both from baseline to 6 months and baseline to 12 months, for 25(OH)D and all measurements of body composition. There was a main effect of racial group for body weight ($p<.05$), 25(OH)D ($p<.01$) and fat-free mass. At each time point, Caucasians had significantly greater Vitamin D status than African Americans (Figure 7). Both racial groups improved Vitamin D status at 6 months, but then experienced a decrease at 12 months (Figure 7). Both racial groups decreased in body weight significantly from baseline to month 6 and 12 (Figure 8). Caucasians experienced significantly greater weight loss than African Americans at 6 months (-12.7 ± 6.9 versus -7.0 ± 5.8 , $p<.01$). There were no significant interaction effects of racial group by time for any of the examined variables.

Table 5. Change in Body Composition and Physical Activity by Racial Group

Variable	Assessment period			Time		Race
	Baseline	6 months	12 months	Month 6 v baseline	Month 12 v baseline	
Body Fat (%)						
Caucasian	50.6 ± 3.1	48.2 ± 3.7	47 ± 4.9	<i>p</i> <.05	<i>p</i> <.01	<i>p</i> =.996
AA	49.6 ± 4.9	47.8 ± 5.2	48.4 ± 5.0			
Fat-free Mass (kg)						
Caucasian	55.9 ± 4.7	52.6 ± 5.0	52.2 ± 4.7	<i>p</i> <.05	<i>p</i> <.05	<i>p</i> <.01
AA	57.9 ± 6.7	56.0 ± 6.7	56.0 ± 7.2			
Fat (kg)						
Caucasian	59.2 ± 9.2	50.4 ± 9.01	48.0 ± 11.5	<i>p</i> <.01	<i>p</i> <.01	<i>p</i> =0.092
AA	59.4 ± 14.5	53.9 ± 13.72	54.4 ± 12.9			
Steps/day						
Caucasian	6987.8 ± 2221.4	7891.4 ± 3078.5	8717.5 ± 2886.6	<i>p</i> =0.18	<i>p</i> =0.079	<i>p</i> =0.311
AA	7027.3 ± 3382.3	7701.9 ± 2382.1	7403.1 ± 2805.2			
Average METs						
Caucasian	1.12 ± .19	1.17 ± .24	1.25 ± .23	<i>p</i> =0.485	<i>p</i> =0.189	<i>p</i> =0.099
AA	1.11 ± .24	1.14 ± .20	1.11 ± .18			

At 6 months, Caucasians in the intervention group moved from a classification of Vitamin D insufficiency to sufficiency, while African Americans moved from Vitamin D deficiency to insufficiency and remained as so at the 12 month visit despite a significant decrease from 6 months to 12 months (*p*<.01) (Figure 7).



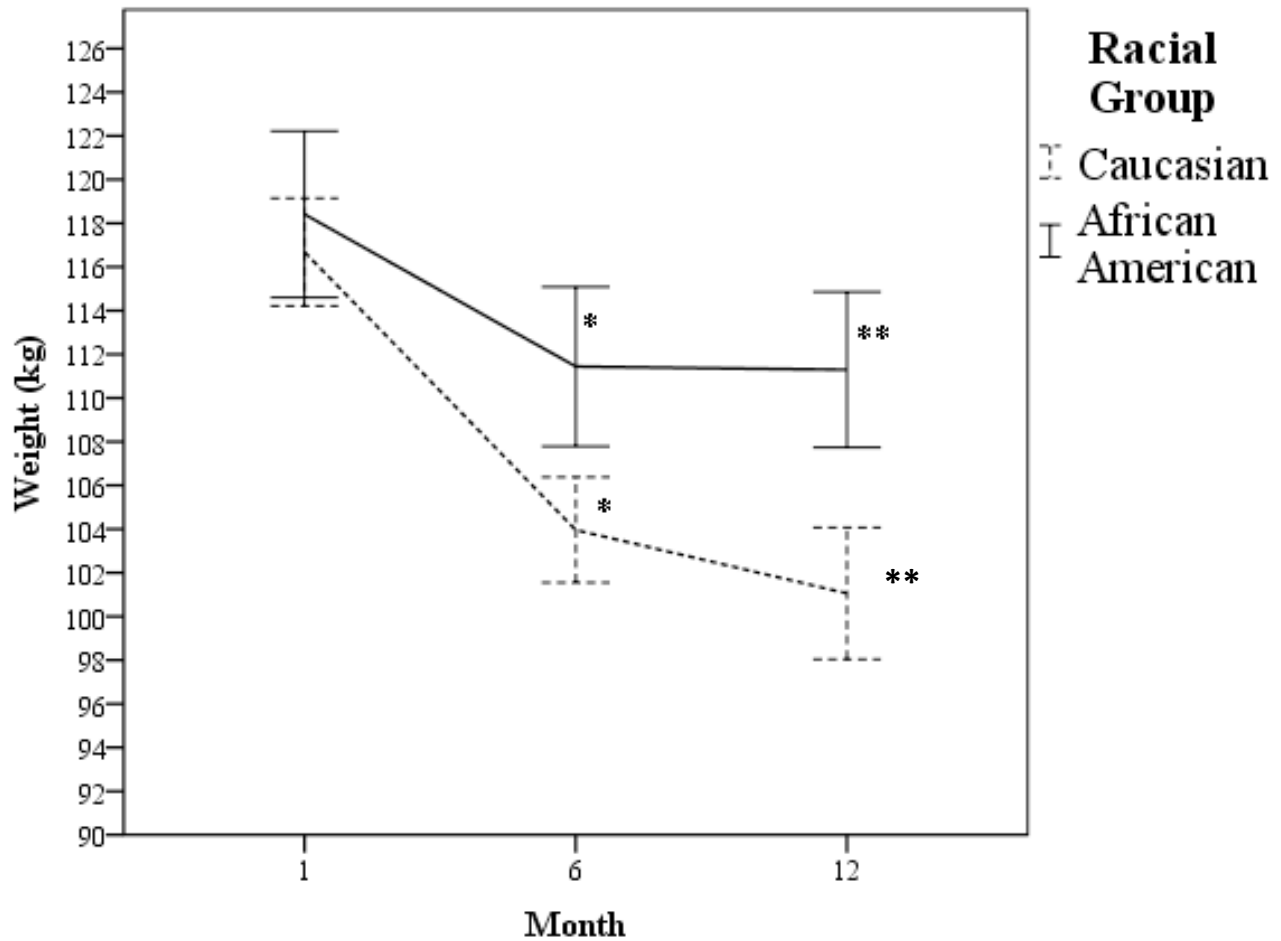
Mean total 25(OH)D by racial group at baseline, 6 and 12 months.

*significant increase from baseline to month 6, $p < .01$

**significant increase from baseline to month 12, $p < .01$

Error Bars: ± 1 SE

Figure 7. Change in 25(OH)D by Race



Mean weight by racial group at baseline, 6 and 12 months.
 *significant increase from baseline to month 6, $p < .01$
 **significant increase from baseline to month 12, $p < .01$
 Error Bars: ± 1 SE

Figure 8. Change in Body Weight by Race

4.2.4 Vitamin D and Physical Activity

At month 6 and 12, both average METs and steps per day were not significantly greater than baseline (Table 4). However, the initial physical activity group had significantly greater changes in physical activity than the delayed physical activity group. There were no significant differences between change in 25(OH)D or body composition between physical activity groups.

Pearson correlation revealed no significant relationship between change in 25(OH)D and steps per day ($r=.08$, $p=.59$) or change in 25(OH)D and average METs ($r=.21$, $p=.14$).

Table 6. Correlations between 6-Month Change in Body Weight, Vitamin D and Physical Activity

	Weight (kg)	25(OH)D ng/mL	Fat mass (kg)	Average METs	Steps/day
Weight (kg)	***	-.16	.93*	-.17	-.21
Sig.		.25	.00	.25	.14
25(OH)D ng/mL	-.16	***	-.20	.21	.08
Sig.	.25		.16	.14	.59
Fat mass (kg)	.93*	-.20	***	-.17	-.18
Sig.	.00	.16		.24	.20
Average METs	-.17	.21	-.17	***	.80*
Sig.	.25	.14	.24		.00
Steps/day	-.21	.08	-.18	.80*	***
Sig.	.14	.59	.19	.00	

*Pearson correlation , significant at $p<.01$

5.0 DISCUSSION

To our knowledge, this is the first long-term study to examine the relationship between Vitamin D, body composition and physical activity. Previous cross-sectional or short-term studies have observed an inverse relationship between BMI and Vitamin D, but without long-term monitoring of weight loss. Thus, it has been unclear whether poor Vitamin D status contributes to obesity or obesity causes Vitamin D deficiency due to sequestering of Vitamin D in fat tissue. At baseline, only the normal weight controls (BMI<29) were considered sufficient in Vitamin D. The Class I obese (BMI 30-35) were classified as insufficient, while class II and class III were deficient and slightly above deficient, respectively. Of particular note is that the obese Caucasians exhibited significantly greater Vitamin D levels than lean African Americans, which supports the possible need for greater supplementation in African Americans, especially those who are obese. This intervention revealed significant improvement in Vitamin D status in both Caucasians and African Americans who are more prone to Vitamin D insufficiency or deficiency due to decreased synthesis of Vitamin D at the level of the skin due to sun exposure.

Following 6 months of weight loss and usage of meal replacement shakes containing a total of 70% of the recommended dietary intake of Vitamin D, both Caucasian and African American intervention subjects reached a Vitamin D status of sufficiency or insufficiency, respectively yet remained in the insufficient range at 12 months following a period of weight maintenance between 6 and 12 months (Table 5). Both racial groups also lost a significant amount of body weight and body fat at 6 months.

Several previous studies have confirmed that Vitamin D deficiency or insufficiency is widespread among obese individuals [10, 13-17, 24, 32, 50, 51]. Vitamin D status had a significant negative correlation with body weight and body fat at baseline and was highest in normal weight and Class I participants (Table 3). These findings are in agreement with the Tzotzas study which determined a negative correlation between 25(OH)D and fat mass, body weight, waist circumference, and BMI [51] as well as the study by Villarasa, et al which found that 25(OH)D in severely obese women was inversely associated with body weight, body fat and waist-to-hip ratio [32].

Several studies have linked poor Vitamin D status to several chronic diseases commonly coupled with obesity, namely Type II diabetes. A study by Forouhi et al found that Vitamin D status was inversely associated with future glycemia and insulin resistance [16]. Due to the presence of Vitamin D receptors in the pancreas, Vitamin D deficiency is believed to cause beta-cell dysfunction [30]. It is possible that obesity contributes to the development of Type II diabetes by sequestering Vitamin D in adipose tissue and rendering it unavailable for endocrine function. Future long-term weight loss interventions examining the relationship between insulin resistance and Vitamin D status are warranted.

In order to examine Vitamin D status, it was necessary to determine differences in Vitamin D by season. Seasonal variations have been reported in previous studies and were suggested to be necessary in interpreting clinical tests for Vitamin D sufficiency [40]. There were no significant differences in intervention subjects by season, although Vitamin D status was greatest in April through July. The lack of statistical significance could possibly be related to the small sample size examined at baseline per season. Obese individuals may also have a diet poor in Vitamin D that likely remains constant throughout the year. Vitamin D₂,

ergocalciferol, was undetectable in all but 3 intervention and 3 control subjects. Therefore, the measurement of 25(OH) D was comprised nearly entirely of Vitamin D₃. This finding is not unexpected, as Vitamin D₂ is derived through supplementation alone, while Vitamin D₃ is derived via diet, supplementation and sun exposure. Obese individuals may spend less time outside or cover a greater area of their skin than normal weight individuals, thus minimizing the opportunity for sun exposure at all times of year.

The second aim of this investigation was to compare serum Vitamin D levels between Caucasians and African Americans across a 12-month weight loss intervention. It was hypothesized that serum Vitamin D levels will be greater in Caucasians than African Americans at baseline, 6 and 12 months of the intervention. At all 3 time points, the Caucasian participants in both the intervention and control groups exhibited significantly greater Vitamin D levels than African Americans (Figure 7). Similarly, body weight was significantly lower in Caucasians than African Americans at each time point (Figure 8). A study by Harris et al which examined seasonal changes in 25(OH)D in Caucasians versus African Americans found that African Americans exhibited lower 25(OH)D throughout the year, and had smaller increases from winter to summer months than Caucasians [79]. Lower 25(OH)D in African Americans is primarily due to the reduced production of Vitamin D in response to sun exposure. Another study by Harris et al suggested that in most latitudes in North America, even young, normal weight African Americans cannot achieve optimal Vitamin D status at any time of the year. Furthermore, African Americans also consume less Vitamin D in food and supplementation [80]. Continuous National Health and Nutrition Examination Survey (NHANES) studies attribute lower dietary intake of Vitamin D to both lactose intolerance and limited intake of milk, milk products and fortified grains [81]. These factors, coupled with

obesity, puts African Americans at a serious risk of Vitamin D deficiency and its associated comorbidities. Along with Type II diabetes, cardiovascular disease is increased in prevalence in the African American population, and Vitamin D deficiency may be a contributing factor. A study examining risk for myocardial infarction found a decreased risk with elevated Vitamin D₃ levels regardless of season, supporting that increased sun exposure is protective against cardiovascular disease [65]. A systematic review by Pittas et al reviewed Vitamin D and cardiometabolic outcomes. In 3 of the cohorts examined, low 25(OH)D was associated with hypertension. In 5 out of 7 cohorts examined in the study, low 25(OH)D was associated with cardiovascular disease. 4 clinical trials found no beneficial effect of supplementation on cardiovascular outcomes [7]. These findings highlight both the need to achieve optimal Vitamin D status, particularly in susceptible populations, as well as the need for clarification of how obesity and Vitamin D interact to contribute to the development of cardiovascular disease.

The next aim of this investigation and possibly the most valuable in clarifying the relationship between Vitamin D and obesity was to examine the effect of weight loss on serum Vitamin D levels in Class II and Class III obese individuals. To our knowledge, this was the longest non-surgical weight loss study in which change in Vitamin D status was examined. Previous weight loss studies involving Vitamin D have been short term, cross-sectional, or conducted in patients following weight loss surgery. Both the delayed and initial physical activity groups lost a significant amount of body fat and weight at 6 months. Furthermore, both groups significantly increased Vitamin D status at 6 months. Vitamin D decreased in both groups between month 6 and 12, despite no further significant weight loss. However, 12-month Vitamin D status remained significantly greater than baseline. If the loss of body fat does indeed increase serum 25(OH)D, one might have expected Vitamin D status to be maintained

from 6-12 months. However, participants decreased their intake of meal replacement shakes containing Vitamin D from 2 to 1 per day, possibly contributing to the decrease in serum 25(OH)D.

Of significance is that with weight loss and increased intake of Vitamin D via meal replacement shakes, African Americans moved from Vitamin D deficiency to insufficiency, and Caucasian participants moved from Vitamin D insufficiency to sufficiency. This effect has clinical significance when determining Vitamin D status. Weight loss may be a component of strategies to increase Vitamin D status in obese participants long-term, and supplementation may need to include adjustment for adiposity. Further studies examining supplementation in African Americans and Caucasians are necessary to examine the difference in Vitamin D requirements between racial groups.

Despite the fact that Vitamin D significantly increased simultaneously with a decrease in body weight and body fat, there was no statistical relationship between change in Vitamin D and change in body weight and body fat at 6 or 12 months. A similar result was found in the Tzotsas study, which demonstrated improved Vitamin D status with a significant weight loss at 20 weeks (approximately 10%), but no significant correlation between the two. There was, however, a significant correlation between change in Vitamin D status and improvement in insulin sensitivity. It is possible that the improvement in 25(OH)D is not wholly attributed to fat loss, but rather to the increased Vitamin D intake in the form of meal replacement shakes. Participants were given meal replacement drinks twice daily for the first 6 months of the study to increase adherence to calorie recommendations. Each drink contained 210 IUs or 35% of the Recommended Daily Allowance (RDA) for Vitamin D, providing 70% of the recommended dietary intake based on a 2000 calorie diet. A study by Zitterman et al which examined

Vitamin D supplementation with 83 micrograms, or approximately 3300 IUs per day, during a weight loss intervention found that 25(OH)D improved with weight loss in both the placebo and supplement group, but the supplement group experienced greater increases in 25(OH)D. Furthermore, both groups lost a similar amount of body weight, suggesting that Vitamin D supplementation may not enhance weight loss [75]. In this study, it is possible that the weight loss at 6 months allowed the Vitamin D intake via meal replacement shakes to increase serum Vitamin D more so than if weight loss had not occurred. A study by Wortsman, et al which examined the response to oral supplementation of 50000 IUs in obese and normal weight individuals found that BMI was inversely correlated with serum Vitamin D concentration following supplementation [24]. Therefore, the increase in 25(OH)D at month 6 is likely due to both increased intake of vitamin D and weight loss. Furthermore, the reduction to one meal replacement shake per day from months 6-12 is likely the reason for the decrease in 25(OH)D during weight maintenance. Research on optimal supplementation for obese populations has indicated that the intake from the meal replacement shakes in this investigation is less than optimal in obese patients [19, 34], but it appears that the combination of increased Vitamin D intake coupled with significant weight loss was sufficient in this severely obese population to improve Vitamin D status. A future study which examines different levels of supplementation, with and without weight loss, may shed light on the interplay between Vitamin D supplementation and long-term significant weight loss.

The final aim of the study was to examine the effect of weight loss plus exercise on serum Vitamin D levels. It was hypothesized that physical activity would be associated with greater improvements in Vitamin D status at 6 and 12 months. It was expected that physical activity would promote greater fat loss, thereby freeing more 25(OH)D from the fat tissue, as

well as promote greater synthesis of Vitamin D at the skin via greater time spent outside exercising. There was no significant correlation between change in 25(OH)D and change in average METs or steps per day at 6 or 12 months. Although the initial physical activity group exhibited significantly greater physical activity at 6 months than the delayed physical activity group, there was no significant difference in physical activity measures in either group from baseline to 6 months or from baseline to 12 months, nor was there a difference in Vitamin D status at 6 or 12 months between the intervention groups. Therefore, it is difficult to determine whether or not greater levels of physical activity would have improved 25(OH)D.

Previous studies examining UV exposure and Vitamin D in obese populations revealed that obese individuals exhibit lower 25(OH)D levels than normal weight individuals when exposed to equal amounts of UV light [29]. The physical activity prescription given to participants in the main study did not require participants to conduct their exercise outside, nor was it able to capture the environment in which exercise was done. Physical activity was prescribed in minutes per day at a moderate intensity, akin to brisk walking. Walking was the most commonly reported method of activity, much of which was done via use of in-home exercise videos provided through the study. The exercise prescription was not designed specifically to increase Vitamin D exposure. Exercise may not directly improve Vitamin D status, but may help to explain the relationship between Vitamin D and insulin sensitivity. To date, there have been no investigations to examine the effect of adding physical activity to weight loss on Vitamin D status. Future weight loss studies incorporating outdoor exercise are warranted to clarify the relationship between physical activity and Vitamin D status.

This investigation was not without limitations. First, data on Vitamin D intake throughout the investigation was not captured. It is therefore not possible to attribute the improvement in

Vitamin D status to weight loss alone. As mentioned previously, many of the participants utilized Slim-fast meal replacement drinks, which had a significant effect on serum Vitamin D status. It is also possible that participants increased their intake of low-fat dairy throughout the study. A second limitation was that physical activity was not supervised and did not include recommendations beyond minutes per day in activity of moderate intensity. There were also no specific recommendations to exercise indoors or outdoors, limiting the ability to connect physical activity with increased UV exposure. Another limitation was that only a subset of subjects were analyzed, potentially resulting in a reduced statistical power to detect significant associations with physical activity or changes in body composition. Lastly, the investigation included only women, which may limit the clinical applications of the study findings.

5.1 CONCLUSIONS

Obesity is a significant public health issue in the United States and increasingly, worldwide. Obesity and several chronic diseases are both associated with Vitamin D deficiency. The purpose of this study was two-fold. First, this study sought to determine the relationship between obesity and Vitamin D status in both Caucasians and African Americans. Second, this study sought to examine the effects of weight loss and body composition changes on Vitamin D status in severely obese individuals following a 12-month weight loss intervention, and to compare these findings between African Americans and Caucasians. This was the first study to examine Vitamin D status over an extended non-surgical weight loss intervention. The results of this study confirm that Vitamin D deficiency or insufficiency is common to obese individuals and is related to the level of adiposity. Vitamin D deficiency is more prevalent in African Americans

than Caucasians, but improves in both racial groups with weight loss intervention including additional Vitamin D intake. It is possible that obesity itself may cause Vitamin D deficiency, and this may help to explain the relationship between obesity and several chronic diseases that are associated with poor Vitamin D status. This investigation has revealed that severely obese individuals (BMI >35) are able to improve Vitamin D status through increased Vitamin D intake at the current recommended reference intakes and may be enhanced by weight loss. Therefore, clinicians should both consider the degree of adiposity when prescribing Vitamin D supplementation, and recommend strategies for weight loss in obese patients diagnosed with Vitamin D deficiency. Future interventions designed to examine the independent effects of Vitamin D supplementation and fat loss on Vitamin D status are warranted. Additionally, long-term studies which monitor Vitamin D intake and include exercise intervention designed to enhance UV exposure are needed to support the relationship between weight loss, physical activity and Vitamin D status.

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