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The Relationship Between Vitamin D Status and Body Mass Index in a Racially Diverse Urban Population of Male and Female Pre- and Early Adolescents

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ACCEPTANCE

This thesis, THE RELATIONSHIP BETWEEN VITAMIN D STATUS AND BODY MASS INDEX IN A RACIALLY DIVERSE URBAN POPULATION OF MALE AND FEMALE PRE- AND EARLY ADOLESCENTS, by Sarah M. Cork was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the College of Health and Human Sciences, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

THE RELATIONSHIP BETWEEN VITAMIN D STATUS AND BODY MASS INDEX IN A RACIALLY DIVERSE URBAN POPULATION OF MALE AND FEMALE PRE- AND EARLY ADOLESCENTS

by

Sarah M. Cork

Objectives: To assess the association between serum 25(OH)D and body mass index (BMI) in pre- and early-adolescents and to determine whether this association varies by demographic/clinical characteristics.

Methods: Vitamin D status was determined using serum 25(OH)D in healthy pre- and early adolescents in Pittsburgh, PA (deficiency=<12 ng/mL, insufficiency=12-<20 ng/mL, sufficiency=≥20 ng/mL). Adiposity was quantified using BMI percentile (normal=<85^{th}, overweight=≥85^{th}-95^{th}, obese=≥95^{th}). The relationship between serum 25(OH)D and adiposity was assessed in the total population and after stratification by gender, race, Fitzpatrick skin type, age, and Tanner stage.

Results: 294 children (mean age 10.2 ± 2.1 years; 60% African American; median serum 25(OH)D=27.0 ng/mL) were studied. Serum 25(OH)D was significantly lower in obese (n=72) vs. overweight (n=48) and normal weight (n=171) participants at 23.6, 29.5, and 28.2 ng/mL, respectively; p=0.015. This trend remained significant for early adolescents but did not differ after stratification by other demographic/clinical characteristics. A significant negative correlation was found between BMI and serum 25(OH)D (r = -0.315; p=0.000). Regression analysis predicted that 25% of the variance in serum 25(OH)D levels was attributed to BMI, gender, race, skin type, age, pubertal status, daily vitamin D

and calcium intake, sun exposure, and sunscreen use, with Tanner stage being the only significant independent predictor.

Conclusions: A significant inverse association between serum 25(OH)D and adiposity was observed in a population of pre- and early adolescents. This relationship was stronger in early adolescents. A meta-analysis to further explore this association in pediatric populations is warranted.

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ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D AKA calcitriol
25(OH)D	25-hydroxyvitamin D
AAP	American Academy of Pediatrics
AI	Adequate Intake
BF%	Body Fat Percentage
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CPGs	Clinical Practice Guidelines
D_2	Ergocalciferol
D_3	Cholecalciferol
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
IOM	Institute of Medicine
IRB	Institutional Review Board
IU	International Units
kg	kilogram
L	Liter
LC-MS/MS	Liquid chromatography tandem mass spectrophotometry assay
µg	microgram
m	meter

mL	milliliter
nmol	nanomole
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
OR	Odds Ratio
PA	Pennsylvania
PTH	Parathyroid Hormone
RDA	Recommended Dietary Allowance
SAT	Subcutaneous Adipose Tissue
SPSS	Statistical Package of Social Science software
TFM	Total Fat Mass
TSF	Triceps Skinfold
UPMC	University of Pittsburgh Medical Center
U.S.	United States of America
UVB	Ultra-violet B rays
VAT	Visceral Adipose Tissue
WC	Waist Circumference

CHAPTER I

THE RELATIONSHIP BETWEEN VITAMIN D STATUS AND BODY MASS INDEX IN A RACIALLY DIVERSE URBAN POPULATION OF MALE AND FEMALE PRE- AND EARLY ADOLESCENTS

INTRODUCTION

The primary role of vitamin D is in the development and maintenance of good bone health, through regulation of calcium and phosphorus homeostasis.[1] In the absence of sufficient vitamin D, production of parathyroid hormone (PTH) is upregulated as a compensatory measure, resulting in secondary hyperparathyroidism.[2] Hypovitaminosis D and hyperparathyroidism are risk factors for bone loss[3] and both conditions have independently been found to be associated with the development of metabolic syndrome and other chronic diseases.[2] In the United States (U.S.), the prevalence of vitamin D deficiency in healthy weight, overweight, obese, and severely obese children between 6 to 18 years of age has been found to be 21%, 29%, 34%, and 49%, respectively.[4] In children, vitamin D deficiency can lead to health conditions including rickets, osteomalacia, osteoporosis, acute respiratory infection, asthma, atopic dermatitis, and food allergies.[5] Youth at high risk of vitamin D deficiency include the obese, dark-skinned individuals, females, infants who are exclusively breastfed without vitamin D supplementation, and children with disorders causing fat malabsorption.[6]

Childhood obesity is a major public health concern. In the U.S., 17% of children and adolescents aged 2-19 years are obese.[7] Children who are obese are at an increased risk of developing health conditions including hypertension, cardiovascular disease, type

2 diabetes, respiratory issues, joint pain, fatty liver, gallstones, and gastroesophageal reflux disease.[8] The negative relationship between serum vitamin D and adiposity has been well established in adult populations.[9,10] Despite consistent findings in adults, research studies that have examined the relationship between vitamin D status and obesity in the pediatric population have shown variable results. The majority of studies in children and adolescents have found that measures of adiposity, specifically body mass index (BMI)[11-16] has been negatively associated with serum levels of the vitamin D metabolite 25-hydroxyvitamin D (25(OH)D). However, several studies have observed no association between vitamin D status and BMI,[17-19] or have observed a negative association in obese[16] participants only. The frequency of vitamin D deficiency in some of these populations was markedly low[17] or high.[18,19] A large study population with a normal serum vitamin D frequency distribution is essential to determine associations between body composition and vitamin D status.

Determining the relationship between vitamin D status and adiposity in children and adolescents is further complicated by demographic and anthropometric factors that may be independent risk factors for hypovitaminosis D. African American race[14,20,21] and dark skin tone[13,22] have consistently been found to be a significant predictors of low vitamin D status in children and adolescents. Studies examining the relationship between vitamin D status and other demographic characteristics, such as age and gender, have produced less consistent results. Several studies in children and adolescents have found female gender to be a positive risk factor for low levels of 25(OH)D[16,19-21,23-25] while others have found no association[12-15,26] with gender, or that the association is dependent

on race.[11] Similarly, age and pubertal development have been found to be both inversely associated[11,14,15,19,23,24] and unrelated[12,26] to vitamin D status.

The purpose of the proposed study is to examine the association between serum 25(OH)D levels and BMI in a population (n = 295) of racially diverse pre-and early-adolescents (ages 6 to 14 years) residing in Pittsburgh, Pennsylvania (PA). Participants were recruited from 2006 to 2008 for an observational vitamin D and sunlight exposure study and from 2008 to 2011 for a randomized controlled trial designed to determine the effect of vitamin D supplementation on serum parathyroid hormone and markers of bone turnover. This study will use Institute of Medicine (IOM) cutoffs to define vitamin D deficiency as serum 25(OH)D <12 ng/mL (30 nmol/L), insufficiency as 25(OH)D 12-20 ng/mL (30-50 nmol/L), and sufficiency as 25(OH)D ≥20 ng/mL (≥50 nmol/L).[1] This cutoff for deficiency is the lowest in use in research and clinical practice. Use of this definition increases the likelihood that those who are identified as vitamin D deficient are truly deficient. While previous studies have examined how gender, race, skin type, age, and pubertal status independently relate to vitamin D status, this study also aims to examine the modifying effects of these variables on the relationship between serum 25(OH)D and BMI. Studies in the current literature tend to examine either race or skin type, and age or pubertal status. This study is further differentiated by the examination of both race with skin type and age with pubertal status, allowing for comparisons to be made between similar cohorts. The association between serum 25(OH)D and BMI and the influence of gender, race, skin type, age, and pubertal status on this relationship is of particular interest due to conflicting evidence regarding the complex interactions between these variables in pediatric populations. Understanding of the association between

vitamin D status and body composition by demographic characteristics is necessary to guide the development of population-specific recommendations for vitamin D intake and the conduct of intervention studies including clinical trials of vitamin D supplementation to determine the dosages needed to prevent or correct deficiencies.

Specific Aim 1: To describe the association between serum 25(OH)D and BMI in pre- and early-adolescent children.

Hypothesis 1: An inverse relationship will be observed between serum 25(OH)D and BMI.

Null Hypothesis 1: No association between serum 25(OH)D and BMI will be observed.

Specific Aim 2: To determine whether the association between serum 25(OH)D levels and BMI is modified by gender, race, skin type, age, or pubertal status in pre- and early-adolescents.

Hypothesis 2A: A stronger inverse association between serum 25(OH)D and BMI will be observed in female participants than in male participants.

Null Hypothesis 2A: The strength of the inverse association between serum 25(OH)D and BMI will not differ by gender.

Hypothesis 2B: A stronger inverse association between serum 25(OH)D and BMI will be observed in African American participants than in Caucasian participants.

Null Hypothesis 2B: The strength of the inverse association between serum 25(OH)D and BMI will not differ by race.

Hypothesis 2C: A stronger inverse association between serum 25(OH)D and BMI will be observed in dark skin types (Fitzpatrick skin types IV and V) than in light skin types (types I-III).

Null Hypothesis 2C: The strength of the inverse association between serum 25(OH)D and BMI will not differ by skin type.

Hypothesis 2D: A stronger inverse association between serum 25(OH)D and BMI will be observed in early adolescents (ages 12 to 14) but not in preadolescents (ages 6 to 11).

Null Hypothesis 2D: The strength of the inverse association between serum 25(OH)D and BMI will not differ by age group.

Hypothesis 2E: A stronger inverse association between serum 25(OH)D and BMI will be observed in late pubescent participants (Tanner stages IV and V) than in mid-pubescent (stages II and II) or prepubescent participants (stage I).

Null Hypothesis 2E: The strength of the inverse association between serum 25(OH)D and BMI will not differ by pubertal status.

CHAPTER II

LITERATURE REVIEW

Vitamin D

Vitamin D Sources

Vitamin D, also known as calciferol, is fat-soluble vitamin that plays a critical role in the human body, particularly in bone health. The two major forms of vitamin D are vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol). Both vitamins D_2 and D_3 are synthesized in response to ultraviolet (UV) radiation of sterols.[3] Ergocalciferol is synthesized in molds, yeast, and vascular plants, and cholecalciferol is synthesized in the skin of mammals.[3,27] Vitamin D_3 produced by the skin accounts for >90% of vitamin D stores in the human body.[14,28] In the dermis, 7-dehydrocholesterol is converted to previtamin D_3 by UV-B rays from the sun.[27,28] In a process known as thermal isomerization, heat in the skin facilitates the transformation of previtamin D_3 into an inactive form of vitamin D_3, which attaches to vitamin D binding protein and enters systemic circulation.[1]

Vitamin D occurs infrequently in either form as a natural component of foods.[27] Dietary sources of naturally occurring vitamin D are limited, and include egg yolk, fatty fish, certain fish oils (such as cod liver oil), and liver and fat from aquatic mammals.[1,27] In the U.S., foods such as milk, infant formula, orange juice, margarine, and breakfast cereals are frequently fortified with vitamin D to increase dietary intake.[1,28] Both vitamins D_2 and D_3 are used for fortification and in supplements,[1] however added D_3 is

the primary source of vitamin D in the American diet.[27] Despite fortification, Americans on average do not consume enough dietary vitamin D to maintain desirable serum levels of 25(OH)D.[29] As a fat-soluble vitamin, vitamin D is absorbed with other dietary fats in the small intestine. Both forms of vitamin D from the diet are integrated into micelles that enter enterocytes via passive diffusion.[1] Within the enterocyte, vitamin D is repackaged into chylomicrons that are transported to circulation via the lymphatic system.[1]

Vitamin D Physiology

Vitamin D is considered a prohormone, and must be metabolized to its hormonal form in order to function.[1] After entering systemic circulation via the skin or the lymph, vitamin D is cleared within hours via uptake by the liver or peripheral tissues, particularly adipose and skeletal muscle.[1] In the liver, vitamin D_3 is hydroxylated to become 25-hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D.[27] In the kidneys, 25(OH)D is again hydroxylated to produce 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol).[27] Calcitriol is the biologically active hormonal form of vitamin D, and is responsible for both the calciotropic and noncalcitropic actions of vitamin D.[1] Both vitamins D_2 and D_3 exhibit identical responses in the body when activated.[1] Calcitriol production in the kidneys is closely regulated by mineral status, whereas 25(OH)D production in the liver is related to vitamin D availability from sun exposure and dietary intake.[3] As a result, serum concentration of 25(OH)D is most reflective of overall vitamin D stores, and is used in clinical practice and in research to determine vitamin D status.

Vitamin D Functions

The primary role of vitamin D is in the development and maintenance of good bone health, through regulation of calcium and phosphorus homeostasis.[1] Serum calcium levels are tightly controlled by the action of calciotropic hormones, including calcitriol, calcitonin, and PTH. When serum calcium levels are low, calcitriol and PTH work in unison to increase the levels of both calcium and phosphorus in the blood by increasing absorption in the intestine, promoting release from the bone, and enhancing reabsorption in the kidneys.[2] In the absence of sufficient vitamin D, production of PTH is upregulated as a compensatory measure, resulting in secondary hyperparathyroidism.[2] Both hypovitaminosis D and hyperparathyroidism are risk factors for osteomalacia, osteoporosis, and bone loss.[3] Further, both conditions have independently been found to be associated with the development of diabetes mellitus, heart disease, and metabolic syndrome.[2]

Vitamin D deficiency has also been linked to the pathogenesis of a vast range of acute and chronic diseases in adult and pediatric populations, including hypertension, cardiovascular disease, diabetes mellitus, Crohn's disease, rheumatoid arthritis, multiple sclerosis, schizophrenia, and several cancers.[1,5,27] This is due to the wide variety of roles vitamin D plays in many organ systems, including the cardiovascular, renal, endocrine, and immune systems.[1,5,27] Noncalcitropic functions of vitamin D include facilitating hematopoiesis, reducing inflammation in vascular cells, decreasing renin expression, increasing insulin secretion, and inducing immune cell differentiation.[30] The mechanisms behind many of these functions of vitamin D remain unclear, and are the subjects of ongoing research.[1,5,27]

Due to the multitude of physiological actions regulated or enhanced by vitamin D, vitamin D sufficiency is considered essential for overall health. Maintaining adequate vitamin D status is especially important in childhood and adolescence, as these are peak times for bone growth and development.[5] Children and adolescents are at a higher risk for vitamin D deficiency than adults,[31] the consequences of which are even greater during the first 18 years of life.[5] In children, deficiencies in vitamin D lead to potentially irreversible complications such as rickets, impaired bone mass acquisition, and decreased bone mineralization.[5] In addition to rickets, childhood-specific disorders that have been associated with vitamin D deficiency include acute respiratory infection, asthma, atopic dermatitis, and food allergies.[5]

Vitamin D Requirements, Status, and Intake in Children and Adolescents

Vitamin D Requirements in Children and Adolescents

The Dietary Reference Intake (DRI) for vitamin D was established using bone health as the primary indicator.[1] Dietary recommendations for vitamin D and calcium intake were first published in 1997.[1] From 1997-2010, the Adequate Intake (AI) for vitamin D was 400 international units (IU).[1] Daily intake of this amount was determined to be sufficient to maintain serum 25(OH)D levels of 12-20 ng/mL (30-50 nmol/L), and that concentrations of at least 20 ng/mL were necessary for good bone health.[1] A decade later, an international task force of researchers and nutrition experts evaluated a large body of contemporary literature, concluding that a minimum serum 25(OH)D concentration of 30 ng/mL (≥75 nmol/L) was necessary for maintenance of bone and general health in children and adults, and that current intake recommendations were too

low to achieve desirable levels.[29] Based on mounting evidence for greater vitamin D needs to support bone health, growth, and remodeling, the IOM committee to review dietary reference intakes for vitamin D and calcium increased the recommendations for vitamin D in 2010.[1] The Recommended Dietary Allowance (RDA) for vitamin D was increased from 400 IU (10 µg) to 600 IU (15 µg) per day for all children, adolescents, and adults ages 1-70 years.[1]

Despite this progress, a substantial amount of data suggests that the RDA still does not reflect research published within the last 10 years, and that average vitamin D needs are even higher than the 600 IU currently recommended by the IOM.[29] There is a consensus among experts that the RDA should be further increased to 800 IU (20 µg) per day to maintain vitamin D sufficiency.[29,31] For children and adults without regular sun exposure, expert recommendations are even higher, ranging from 800 IU (20 µg) to 2000 IU (50 µg) per day.[31] The Endocrine Society concluded that children determined to be "at risk" for deficiency, such as obese children, may require two to three times greater intake of vitamin D than healthy children to maintain adequate blood serum levels.[32] The Society's recommendations for children at risk, such as children who are obese, are 400-1,000 and 600-1,000 IU/day for children under 1 year of age and aged 1-18 years, respectively.[32] Recommendations for sufficient sun exposure depend on a multitude factors, including skin pigmentation, age, latitude, season, time of day, and sunscreen use.[1,31]

Vitamin D Intake in Children and Adolescents

Less than 10% of vitamin D in the body is derived from the diet.[28] Despite this minimal contribution, dietary intake of vitamin D rich foods, particularly dairy, has been shown to have significant impact on vitamin D status.[33,34] Analysis of NHANES 2005-06 data found vitamin D intakes in all age groups to be less than the Estimated Average Requirement (EAR) of 400 IU necessary to maintain serum 25(OH)D concentrations of at least 30 ng/mL.[1] Mean vitamin D intake from food and dietary supplements in children ages 1-3, 4-8, 9-13, and 14-18 years was found to be 350, 344, 304 and 238 IU/day, respectively.[1] This pattern suggests that vitamin D intake decreases in children and adolescents as they age. This decline is likely due to the concurrent reduction in milk consumption with age.[35]

Total milk consumption in children and adolescents has also declined, related in part to an increase in consumption of soft drinks and juice in these age groups[34] and in part to a decrease in milk consumption in the U.S. in general.[35] This is significant, as milk consumption is a strong determinant of both vitamin D intake[33] and serum 25(OH)D concentration.[36] Over the past four decades in the U.S., milk consumption has decreased as prevalence of hypovitaminosis D has increased.[3,35] Milk contributes over 50% of the daily intake of vitamin D for Americans of all races and age groups.[35] Low milk and yogurt consumption have both been found to be negatively related to serum 25(OH)D levels.[34]

Vitamin D Status in Children and Adolescents

Standard definitions of vitamin D deficiency, insufficiency, and sufficiency have yet to be established by consensus or central authority within the scientific community. Cutoff concentrations of serum 25(OH)D used to define deficiency in research and clinical practice vary drastically, ranging from <10 ng/mL (25 nmol/L)[15,16] to <50 ng/mL (125 nmol/L).[1] This fivefold difference reflects ongoing debate regarding vitamin D requirements. The IOM defines vitamin D deficiency as serum 25(OH)D <12 ng/mL (30 nmol/L), insufficiency as 25(OH)D 12-20 ng/mL (30-50 nmol/L), and sufficiency as 25(OH)D ≥20 ng/mL (≥50 nmol/L).[1] This cutoff is based on the minimum serum level necessary to prevent the development of rickets as determined by the IOM.[1] The National Institute of Health (NIH) uses these same values to define vitamin D status.[6]

In contrast, the cutoffs set by the American Academy of Pediatrics (AAP) are slightly higher, defining vitamin D deficiency as serum 25(OH)D ≤15 ng/mL (≤37.5 nmol/L), insufficiency as 15-20 ng/ml (37.5-50 nmol/L), and sufficiency as 20-100 ng/mL (50-250 nmol/L).[27] The Endocrine Society Clinical Practice Guidelines (CPGs) further raise the minimum standard for deficiency, defining vitamin D deficiency as 25(OH)D less than 20 ng/ml (50 nmol/L), insufficiency as 20-<30 ng/mL (50-<75 nmol/L), and sufficiency as ≥30 ng/mL (≥75 nmol/L).[32] This definition is based on the Endocrine Society's determination of a serum level of 20 ng/ml to be the minimum at which bone and general health can be maintained.[32] Finally, the Vitamin D Council defines deficient as ≤30 ng/mL (≤75 nmol/L), insufficient as 31-39 ng/mL (77.5-97.5 nmol/L), and 40-80 ng/mL (100-200 nmol/L) as sufficient.[19] This definition of deficiency is more than two times less specific than the IOM's definition.

Higher cutoffs for vitamin D deficiency complicate the identification of individuals with true deficiency and their shared attributes, making it challenging to establish definitive risk factors for low vitamin D status. Further, this lack of consensus convolutes comparisons of "vitamin D deficiency" between studies and populations. In absence of an agreed-upon definition, estimates of the prevalence of "vitamin D deficiency" should be carefully assessed. A Center for Disease Control and Prevention (CDC) analysis of NHANES data from 2003-06 determined prevalence of vitamin D deficiency in the U.S. population based on two cutoff points.[3] When vitamin D deficiency was defined by IOM guidelines (<12 ng/mL), 8.1% of the overall US population ages 1 year and older was found to be deficient.[3] The prevalence of deficiency in non-Hispanic blacks was 31.1%, compared with 11.3% in Mexican-Americans and 3.6% in non-Hispanic whites.[3] In females, the prevalence of deficiency was 9.9%, and in males it was 6.3%. The prevalence of vitamin D deficiency in pre-adolescents ages 6-11 was 1.8%, compared to 8.5% prevalence in adolescents ages 12-19 years.[3]

In the same report, when vitamin D deficiency was defined according to Endocrine Society CPGs (<20 ng/ml), overall and demographic-specific prevalence rates were found to be significantly higher. By this standard, 31.7% of the overall population was considered vitamin D deficient. The prevalence of deficiency in non-Hispanic blacks was now 70.6%, 44.2% in Mexican-Americans, and 21.7% in non-Hispanic whites.[3] The prevalence of deficiency rose to 34% in females and 29.4% in males. Preadolescents were found to have 15.9% prevalence of deficiency, compared to 32.7% prevalence in adolescents. Although the prevalence of vitamin D deficiency differed significantly depending on the definition used, demographic patterns in prevalence remained

consistent. Non-Hispanic blacks had significantly higher prevalence of deficiency compared to other races. More females than males were found to be vitamin D deficient, and more adolescents were deficient than pre-adolescents. Obese individuals of all ages have also been found to have a higher prevalence of hypovitaminosis D than normal weight individuals in populations around the world.[15,26] A study of the same 2003-06 NHANES data found in US youth aged 6-18 years, the prevalence of vitamin D deficiency in healthy-weight, overweight, obese, and severely obese children is 21%, 29%, 34%, and 49%, respectively.[4] Another retrospective chart review of patients in an urban children's hospital found the prevalence of hypovitaminosis D (<20 ng/ml) to be 100% in obese girls and 91% in obese boys.[26]

Relationship between Vitamin D and Adiposity

Low serum levels of 25(OH)D have consistently been found to be associated with higher adiposity in children and pre- and early-adolescents worldwide. Anthropometric measurements of body fatness that have been found to be negatively correlated with serum vitamin D levels include BMI,[11-16] body fat percentage (BF%),[13,37] total fat mass (TFM),[12,37] waist circumference (WC),[37,38] mid-upper arm circumference (MAC),[37] triceps skinfold,[37] and visceral (VAT) [11] and subcutaneous (SAT)[10-13] adipose tissue. In absence of body composition data, BMI is particularly useful as measure of adiposity, as it has been validated as a direct predictor of body fatness in children and adolescents.[39] Further, studies in adults have identified a direct causal link between higher BMI and lower levels of 25(OH)D.[10] A bidirectional Mendelian randomization analysis of multiple international adult cohorts determined that higher BMI directly leads to lower serum

25(OH)D.[10] The inverse relationship between BMI and serum 25(OH)D has been observed when BMI has been analyzed as both a continuous[11-15] and categorical[14-16] variable. Dairy intake has also been observed to be inversely associated with BMI.[40] In analysis of data from two cross-sectional surveys in Canada (n=8958), children who consumed greater than two glasses of milk a day were less likely to be obese than those who consumed less than one glass a day.[33]

A multitude of factors have been proposed to explain the significant association between low vitamin D status and adiposity. Physiological mechanisms related to the metabolism, production, and mobilization of vitamin D have been found to be altered in the obese. Reductions in dermal synthesis, dermal release into circulation, and intestinal absorption have been observed.[2,5,38] Lifestyle factors associated with obese populations, such as lower dairy consumption and sun underexposure, may further contribute the increased prevalence of hypovitaminosis D in these individuals.[2,5,15,26,38] However, the most strongly supported explanation is that increased body fat leads to increased sequestration of vitamin D in the adipose tissue, decreasing circulating levels of 25(OH)D and overall bioavailability.[1,2,10,12,24,41]

The uptake of vitamin D into adipose tissue is perpetuated in a positive feedback loop. Adipose tissue is the primary site of vitamin D storage, and does not release vitamin D to compensate in response to low serum calcium levels.[1] Decreased bioavailability of vitamin D triggers a hypothalamic response that leads to increased hunger and decreased energy expenditure.[41] Further, the resulting secondary hyperparathyroidism leads to an upregulation of lipogenesis.[41] As the amount of adipose tissue increases, uptake and clearance of vitamin D are further enhanced.[1] Conversely, serum 25(OH)D levels have

been found to increase with weight loss without increasing dietary intake or sun exposure, suggesting that vitamin D is released from adipose stores as adipose is lost.[1] Results from clinical trials in adults suggest that decreasing vitamin D storage sites by reducing fat mass is the only way to restore normal serum 25(OH)D levels in the overweight and obese.[2]

Relationship between Vitamin D and Demographic Variables

Relationship between Vitamin D, Race and Skin Type

The IOM designates both obesity and African American ancestry as significant confounders that negatively impact 25(OH)D concentration.[1] In both adult and pediatric African American populations, the prevalence of vitamin D deficiency has been found to be consistently higher than in other races.[3,12,27] Further, African American children and adolescents have been found to be at a greater risk for hypovitaminosis D[11,22] and have significantly lower serum levels of 25(OH)D.[11,27] African American race increases the risk of vitamin D deficiency primarily due to darker complexion related to increased amounts of the pigment melanin in the skin.[22] Melanin reduces vitamin D production by acting as a natural sunscreen, preventing UV-B rays from reaching the layers of the epidermis that contain the highest concentrations of 7-dehydrocholesterol, the precursor to endogenous D3.[28] Darker skinned individuals may require 5-10 times longer sun exposure to synthesize the same amount of vitamin D_3 that lighter skinned individuals produce in 10-15 minutes.[27]

Skin pigmentation is the most important determinant of vitamin D synthesis by the skin.[27] In turn, vitamin D produced in the skin is the major determinant of overall

vitamin D status in the body,[22] and darker skin significantly increases the risk of insufficiency.[13,28] Darker skin types have been found to be both negatively associated with[13] and predictive of[11] serum concentrations of 25(OH)D in pediatric populations. Children and adolescents with darker skin types have been found to have significantly lower median serum 25(OH)D levels than those with lighter skin types, regardless of race.[13] The NIH considers both dark skin tone and a BMI ≥30 kg/m^2 to be risk factors for vitamin D inadequacy.[6] African American individuals' risk for hypovitaminosis D is further increased by low intake of dairy.[35] Analysis of NHANES 2005-06 showed that non-Hispanic blacks consume significantly less milk and dairy products than Mexican Americans and non-Hispanic whites, likely due to increased prevalence of lactose intolerance in this population.[35]

Both fat mass and non-white ethnicity have been found to be significantly negatively correlated with serum 25(OH)D.[11,12,15] In a retrospective study by Alemzadeh et al. (2008), a diverse population of 127 obese (BMI >95th% for age) pre- and early-adolescents (6-17.9 years) in the Midwestern U.S., the total prevalence of hypovitaminosis D (defined as 25(OH)D <30 ng/ml) was found to be 74%.[12] Black and Hispanic participants were found to have significantly higher prevalence of vitamin D deficiency (87.2% and 76.9% respectively, $p<0.05$), defined as <20 ng/mL, than Caucasian participants (59.1%). Ethnicity was significantly correlated with vitamin D status, and fat mass was found to have the greatest effect on serum 25(OH)D levels. Fat mass also accounted for the greatest proportion of the variance in levels of serum 25(OH)D. Accordingly, the vitamin D deficient group had higher mean BMI ($p<0.001$) and FM ($p<0.001$) that the insufficient or sufficient group. Vitamin D status was

significantly impacted by adiposity, race, vitamin D intake, and season. All three groups were similar in age, proportion of females, and Tanner stage.

More recent studies of children and adolescents have produced similar findings. In 2013, a cross-sectional study by Vierucci et al. (2013) examined demographic, anthropometric, and lifestyle predictors of vitamin D deficiency, including BMI, ethnicity, gender, age, residence, season, sun exposure, and sunscreen use. The overall prevalence of hypovitaminosis D (defined as 25(OH)D <30 ng/mL) was 79.5% in this diverse group of 625 normal weight, overweight, and obese participants (2-21 years). BMI was found to significantly increase the risk of low serum concentrations of serum 25(OH)D in all participants, and a strong negative correlation was confirmed between the two variables. Overweight and obese children and adolescents had serum 25(OH)D levels significantly lower and an odds ratio (OR) of hypovitaminosis D five times greater than those with a normal BMI. Non-white race increased the risk for vitamin D deficiency by 11-fold. Only 2.7% non-white participants were found to be vitamin D sufficient (25(OH)D ≥30 ng/mL), compared to 21.6% of white participants ($p<0.0001$). Low sun exposure and regular sunscreen use also significantly increased the risk of hypovitaminosis D. Adolescents (11-21 years) were found to have lower mean 25(OH)D levels than children (2-10.9 years), but adolescent age was not associated with increased risk of hypovitaminosis D. Similarly, gender was not associated with vitamin D status.

Relationship between Vitamin D and Gender

Although NHANES data has shown greater prevalence of vitamin D deficiency in girls, conflicting study results continue to limit understanding of the relationship between

gender and vitamin D status. A cross-sectional study by Cizmecioglu et al. (2008) examined the prevalence of hypovitaminosis D (defined as 25(OH)D <20 ng/ml (50 nmol/l)) in a group of 301 obese and overweight Turkish adolescents (11-19 years).[16] The overall prevalence of hypovitaminosis D was found to be 65% in the total population ($p=0.001$). Vitamin D deficiency, defined as 25(OH)D >10 ng/ml, was two times more prevalent in females than in males ($p<0.001$). Females were also found to have a significantly lower mean serum 25(OH)D ($p=0.01$). When BMI was examined as a categorical variable, no relationship between obesity status (BMI <85th%, ≥85-<95th%, or ≥95th%) and vitamin D categories was found. When examined as a continuous variable, BMI was found to have a significant inverse association with 25(OH)D in overweight and obese participants with vitamin D levels <20 mg/ml (50 nmol/l) in both genders ($r = -0.186$, $p<0.01$)**.**

A retrospective, cross-sectional study by Aypak et al. (2014) examined the modifying effects of gender, puberty, and adiposity on vitamin D status.[19] The prevalence of hypovitaminosis D was found to be extremely high in this population of 168 obese and non-obese Turkish participants ages 4-16 years. Over 98% of all participants had a serum 25(OH)D concentration <20 ng/mL. This unusually large proportion of hypovitaminosis D may explain the lack of association detected between BMI and 25(OH)D levels, a finding which is inconsistent with the majority of the literature on the subject. Although BMI was not found to be a correlate when examined as either a continuous or categorical variable, female gender and pubertal status were both found to be significantly inversely associated with serum 25(OH)D and were determined to be independent predictors of

vitamin D status. A negative association between insulin resistance and serum 25(OH)D concentration was seen in pubertal obese subjects only.

Relationship between Vitamin D, Age, and Pubertal Status

The relationship between age, pubertal development, and vitamin D remains unclear despite findings of increased prevalence of hypovitaminosis D in adolescents compared to children and preadolescents.[3] A retrospective study of 102 Norwegian overweight and obese children and adolescents (8-19 years) by Lagunova et al. (2011) found BMI, body weight, and fat mass to be inversely associated with 25(OH)D concentrations.[14] The prevalence of vitamin D deficiency (defined as <20 ng/mL) in the entire population was 19%. Obesity and higher BMI were significantly more prevalent in Vitamin D deficient children. Teenagers 13-19 years old showed a significantly higher prevalence of vitamin D deficiency and insufficiency than in preadolescents aged 8-12 years. Children aged 11-12 years were found to have the highest concentrations of 25(OH)D.

A cohort study of a diverse group 237 normal weight and overweight children and adolescents (ages 8-18 years) in Pittsburgh, PA by Rajakumar et al. (2011) found VAT, race, female gender, pubertal status, and season to be independent predictors of vitamin D status.[13] Participants with vitamin D deficiency (defined as <20 ng/ml) were found to have the highest BMI, BMI percentile, BF%, VAT, SAT, and proportion of obesity. Mean serum 25(OH)D concentrations were significantly lower in obese, black, and pubertal (Tanner stages II-V) children compared to non-obese, white, and prepubertal (Tanner stage I) children, respectively. Further, the proportion of children classified as

vitamin D deficient and odds of vitamin D deficiency were significantly higher in obese, black, female, and pubertal participants.

Lifestyle characteristics that vary by age also play an important role. Children and preadolescents tend to spend more time playing outdoors than adolescents, increasing their exposure to UV-B radiation and further contributing to adequate vitamin D status.[14,28] Children and preadolescents also consume significantly more milk than early adolescents and adolescents, resulting in a greater intake of vitamin D and calcium.[35] Similarly, male children and adolescents have been found to consume significantly more milk than their female counterparts.[35]

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CHAPTER III

MANUSCRIPT IN STYLE OF JOURNAL

Introduction

The primary role of vitamin D is in the development and maintenance of good bone health, through regulation of calcium and phosphorus homeostasis.[1] In the absence of sufficient vitamin D, production of parathyroid hormone (PTH) is upregulated as a compensatory measure, resulting in secondary hyperparathyroidism.[2] Hypovitaminosis D and hyperparathyroidism are risk factors for bone loss[3] and both conditions have independently been found to be associated with the development of metabolic syndrome and other chronic diseases.[2] In the United States (U.S.), the prevalence of vitamin D deficiency in healthy weight, overweight, obese, and severely obese children between 6 to 18 years of age has been found to be 21%, 29%, 34%, and 49%, respectively.[4] In children, vitamin D deficiency can lead to health conditions including rickets, osteomalacia, osteoporosis, acute respiratory infection, asthma, atopic dermatitis, and food allergies.[5] Youth at high risk of vitamin D deficiency include the obese, dark-skinned individuals, females, infants who are exclusively breastfed without vitamin D supplementation, and children with disorders causing fat malabsorption.[6]

Childhood obesity is a major public health concern. In the U.S., 17% of children and adolescents aged 2-19 years are obese.[7] Children who are obese are at an increased risk of developing health conditions including hypertension, cardiovascular disease, type 2 diabetes, respiratory issues, joint pain, fatty liver, gallstones, and gastroesophageal

reflux disease.[8] The negative relationship between serum vitamin D and adiposity has been well established in adult populations.[9,10] Despite consistent findings in adults, research studies that have examined the relationship between vitamin D status and obesity in the pediatric population have shown variable results. The majority of studies in children and adolescents have found that measures of adiposity, specifically body mass index (BMI)[11-16] have been negatively associated with serum levels of the vitamin D metabolite 25-hydroxyvitamin D (25(OH)D). However, several studies have observed no association between vitamin D status and BMI,[17-19] or have observed a negative association in obese[16] participants only. The frequency of vitamin D deficiency in some of these populations was markedly low[17] or high.[18,19] A large study population with a normal serum vitamin D frequency distribution is essential to determine associations between body composition and vitamin D status.

Determining the relationship between vitamin D status and adiposity in children and adolescents is further complicated by demographic and anthropometric factors that may be independent risk factors for hypovitaminosis D. African American race[14,20,21] and dark skin tone[13,22] have consistently been found to be a significant predictors of low vitamin D status in children and adolescents. Studies examining the relationship between vitamin D status and other demographic characteristics, such as age and gender, have produced less consistent results. Several studies in children and adolescents have found female gender to be a positive risk factor for low levels of 25(OH)D[16,19-21,23-25] while others have found no association[12-15,26] with gender, or that the association is dependent on race.[11] Similarly, age and pubertal development have been found to be both inversely associated[11,14,15,19,23,24] and unrelated[12,26] to vitamin D status.

The purpose of the proposed study is to examine the association between serum 25(OH)D levels and BMI in a population (n = 295) of racially diverse pre-and early-adolescents (ages 6 to 14 years) residing in Pittsburgh, Pennsylvania (PA). Participants were recruited from 2006 to 2008 for an observational vitamin D and sunlight exposure study and from 2008 to 2011 for a randomized controlled trial designed to determine the effect of vitamin D supplementation on serum parathyroid hormone and markers of bone turnover. While previous studies have examined how gender, race, skin type, age, and pubertal status independently relate to vitamin D status, this study also aims to examine the modifying effects of these variables on the relationship between serum 25(OH)D and BMI. The association between serum 25(OH)D and BMI and the influence of gender, race, skin type, age, and pubertal status on this relationship is of particular interest due to conflicting evidence regarding the complex interactions between these variables in pediatric populations. Understanding of the association between vitamin D status and body composition by demographic characteristics is necessary to guide the development of population-specific recommendations for vitamin D intake and the conduct of intervention studies including clinical trials of vitamin D supplementation to determine the dosages needed to prevent or correct deficiencies.

We aim to describe the association between serum 25(OH)D and BMI in pre- and early-adolescent children and to determine whether the association between serum 25(OH)D levels and BMI is modified by gender, race, skin type, age, or pubertal status in pre- and early-adolescents. We hypothesize that an inverse relationship will be observed between serum 25(OH)D and BMI and that this association will be stronger in females, in

African Americans, in participants with dark skin types, in early adolescents and in late pubescence.

Materials and Methods

Participants

The study population consists of 295 healthy, racially-diverse pre-and early-adolescents (ages 6 to 14 years) residing in Pittsburgh, PA.[22] Participants were recruited from the Primary Care Center at Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center (UPMC).[22] Recruitment was conducted between 2006 and 2008 for an observational vitamin D and sunlight exposure study, and between 2008 and 2011 for a randomized controlled trial designed to determine the effect of vitamin D supplementation on serum PTH and markers of bone turnover.[22] Exclusion criteria included individuals with hepatic or renal disease, cancer, malabsorptive disorders, and disorders of vitamin D or calcium metabolism.[22] Participants taking anticonvulsants, systemic glucocorticoids, oral contraceptives, or depot medoroxyprogesterone were also excluded.[22]

Study Design

This cohort study is a secondary analysis using demographic, anthropometric, nutrition and serum vitamin D data collected as part of two studies conducted by Dr. Kumaravel Rajakumar at Children's Hospital of Pittsburgh of UPMC.[22] The first NIH-funded (K23 grant) observational study was conducted between 2006 and 2008. This study examined racial and seasonal variations in vitamin D status as well as sunlight

exposure and vitamin D intake in pre- and early-adolescents.[22] The second NIH-funded (R03 grant) clinical trial was conducted between 2008 and 2011. In this study, participants were randomly assigned to a vitamin D supplement (1000 IU D_3 or placebo to determine the impact of supplementation on vitamin D status.[43] Both protocols were approved by The University of Pittsburgh Institutional Review Board (IRB) and required signed parental consent. The present analysis was approved by the IRB at both the University of Pittsburgh and Georgia State University.

Data Variables

Serum 25(OH)D is considered the gold standard biomarker for assessing vitamin D status, and is the most accurate reflection of vitamin D stores in individuals.[11] Serum 25(OH)D was measured using liquid chromatography tandem mass spectrophotometry (LC-MS/MS) assay, which measures both serum 25(OH)D_2 and 25(OH)D_3.[22] Vitamin D status was defined according to IOM guidelines, which define deficiency as serum 25(OH)D <12 ng/mL, insufficiency as 25(OH)D 12-<20 ng/mL, and sufficiency as 25(OH)D ≥20 ng/mL.[1] BMI and BMI percentile were used to quantify adiposity, and were analyzed as both categorical and continuous variables. BMI categories were defined according to CDC reference values: normal = BMI <85th percentile, overweight = BMI ≥85th to <95th percentile, and obese = BMI ≥95th percentile.[44] Participant BMI was calculated from weight and height of participants measured at study entry and exit. Both gender (male or female) and race (African American or Caucasian) were analyzed as nominal variables. Racial categorization was parent-identified at study entry. Skin color data was also parent-reported, and was quantified using Fitzpatrick sun-reactive

skin typing.[45,46] This scale categorizes light-skinned individuals into three skin types: Type I, pale white skin (always burns, never tans), Type II, white skin (burns easily, tans minimally), and Type III, light brown skin (burns moderately, tans uniformly). Dark-skinned individuals are classified as either skin Type IV, moderate dark skin (burns minimally, always tans well) or Type V, dark brown skin (burns rarely to never, tans markedly). Fitzpatrick skin type was analyzed using the two categories of light-skinned (Types I, II, and III) and dark-skinned (types IV and V). When stratified by age, preadolescence was defined as 6-11 years and early adolescence as 12-14 years. Pubertal status was defined using the Tanner scale (Tanner stage I = prepubertal, Tanner stages II and III = mid-pubertal, Tanner stages IV and V = late pubertal).[47] Tanner staging for all participants was conducted during the initial enrollment physical examination by a study investigator. All participants were asked to complete a food frequency questionnaire (FFQ) at baseline and 6 months later. The FFQ, titled Eating Survey, K-95-1, (Harvard Medical School, © 1995 Brigham and Women's Hospital) was analyzed at Brigham and Women's Hospital. After processing and analysis, nutrient intake data comprised 17 nutrients including total calories, dietary calcium, and vitamin D.

Data Analysis

Frequency analysis was used to describe the demographic, anthropometric, and nutrition characteristics of the total study population and stratified by vitamin D status. A Kolmogorov-Smirnov test was used to analyze the distribution of all continuous variables. Differences in population characteristics by vitamin D status were analyzed

using a Kruskal Wallis H Test for continuous variables and Chi-squared test for categorical variables. Body mass index and BMI percentile were analyzed as both a continuous and categorical variable.[44] Differences in mean serum 25(OH)D levels by BMI category were analyzed using a Kruskal-Wallis H test for the entire cohort, and after subdivision by gender, race, Fitzpatrick skin type, age, and Tanner stage. Differences in BMI by vitamin D status category were also analyzed using a Kruskal-Wallis one-way analysis of variance for the entire cohort, and after subdivision by gender, race, Fitzpatrick skin type, age, and Tanner stage. Significances in the differences in the proportions of demographics within vitamin D status groups was assessed using a Chi-squared test.

Spearman's rank correlation coefficient was used to assess the relationship between serum 25(OH)D and BMI as continuous variables in the entire cohort and after subdivision by gender, race, skin type, age, and pubertal status. Multivariate regression analysis was used to assess the modifying effects of demographic, anthropometric, nutrition, and lifestyle variables on serum 25(OH)D. The first model used blockwise entry and assessed BMI alone, as BMI has been found to be a known predictor of serum 25(OH)D concentration. [11-16] The second model used forced entry and included BMI, gender, race, skin type, age, pubertal status, total caloric intake, dietary vitamin D and calcium intake, duration of sun exposure, sunscreen use, and use of multivitamins. All statistical analyses were carried out using IBM's Statistical Package of Social Sciences (SPSS) software program version 23.0. A p value <0.05 was considered significant.

Results

Descriptive Characteristics

A total of 294 healthy, 6- to 14-year old (mean age 10.2 $\pm$ 2.1 years) preadolescents [n=232 (78.9%)] and early adolescents [n=62 (21.1%)] from the urban Pittsburgh, PA area were studied (Table 1). One participant was removed from the population prior to any analysis due to a very high BMI (outlier) indicative of a calculation error. The majority of the population was male [n=158 (53.7%)], African American [177 (60.2%)], dark skin type [n=180 (61.2%)], preadolescent [n=232 (78.9%)], and pre-pubertal [n=161 (54.8%)]. The majority of the population was of normal weight [n=173 (58.8%)], whereas 16.3% (n=48) were overweight and 24.8% (n=73) were obese. The prevalence of vitamin D deficiency, insufficiency, and sufficiency was 8.2%, 21.9%, and 69.8%, respectively.

The demographic, anthropometric, nutrition intake, and lifestyle characteristics of the total population and after stratification by vitamin D status are described in Tables 2 and 3. Median serum 25(OH)D in the total population was 27.0 ng/mL, which the IOM classifies as vitamin D sufficient. The majority of the participants were found to be vitamin D sufficient [n=203 (69.0%)], whereas 21.8% (n=64) were insufficient, and 8.2% (n=24) were deficient. Participant age, weight, height, and BMI differed significantly by vitamin D status ($p = 0.000$). Those in the vitamin D deficient group were older, taller, heavier, and had a higher BMI than those in the insufficient and sufficient groups. The median BMI in the total population was 19.0 kg/m^2, or normal weight. The distribution for all continuous variables, with the exception of age, were found to be skewed.

The median daily vitamin D intake for the total population was 260.0 IU, or 43% of the RDA of 600 IU (Table 2). Only 5% (n=15) of participants in the total population met the RDA for vitamin D intake. Of these, 11 were vitamin D sufficient, and 0 were deficient. The median daily calcium intake for the total population (1182.5 mg) fell short of the RDA for children 9-13 years of age (1300 mg) but met the RDA for children for 4-8 years of age (1000 mg). Vitamin D and calcium intake differed by serum vitamin D status, with the vitamin D deficient group consuming significantly less ($p=0.005$ and $p=0.042$, respectively). Of the 80.6% of total participants that reported greater than 2 hours of daily sun exposure, half (49.4%) wore sunscreen outside. The vitamin D deficient group was less likely than the insufficient and sufficient groups to both spend time in the sun and wear sunscreen, but not at a significance level of $P<0.05$.

African American participants were significantly more likely to be exposed to greater than 2 hours of sun daily than Caucasian participants [n=150 (89.8%) vs. n=87 (76.3%), respectively; $p=0.002$] (Table 4). However, African American children were significantly less likely to wear sunscreen than Caucasian children [n=39 (22.7%) vs. n=78 (67.8%), respectively; $p=0.000$]. Total daily kilocalorie intake also differed by race, with African American children consuming significantly more energy than Caucasian children (2442.4 kcal vs. 2126.5 kcal, respectively; $p<0.002$). Intake of vitamin D and calcium did not differ significantly by race.

Females and early adolescents were found to consume fewer calories and less vitamin D and calcium than males and preadolescents, respectively, but difference was not statistically significant. No significant differences were observed by gender in any nutrition or lifestyle characteristic. Obese participants were found to consume

significantly less vitamin D than normal weight or overweight participants, without significant differences in intake of total calories or calcium (p=0.047). Although obese participants spent a similar amount of time in the sun as overweight and normal weight participants, they were much less likely to wear sunscreen [n=21 (29.2%), n=17 (36.2%), n=79 (47.0%), respectively, p=0.028].

Demographic Characteristics by Vitamin D Status and Body Mass Index

Proportions of demographic and anthropometric characteristics differed significantly by vitamin D status (Table 5). The vitamin D deficient group was comprised of a significantly higher proportion of females (66.7%), African Americans (100%), dark skin types (91.3%), early adolescents (54.2%), and late pubertal participants (54.2%) than the sufficient group. Caucasian and light-skinned participants were significantly more likely to be vitamin D sufficient than African American and dark-skinned participants. None of the Caucasian participants were found to be vitamin D deficient, and three quarters (75.4%) were sufficient. In contrast, 13.6% African Americans participants were vitamin D deficient (and comprised 100% of the deficient group) and 66.1% were vitamin D sufficient.

Older participants (early adolescents and late pubertals) were significantly more likely to be vitamin D deficient than younger participants. Only 4.8% of preadolescents were vitamin D deficient, compared to 21.0% of early adolescents. Similarly, almost half (43.3%) of late pubertal individuals were vitamin D deficient and only 16.7% were sufficient, whereas only 4.4% of prepubertal participants were deficient and 83.8% were sufficient. Only half (54.2%) of obese participants were vitamin D sufficient, in contrast

to 73.1% of normal weight participants and 81.3% of overweight participants. Further, 15.3% of obese participants were found to be vitamin D deficient, compared to only 6.4% of normal weight and 4.2% of overweight participants.

In the total population, serum 25(OH)D concentrations differed significantly by weight category (p=0.015), gender (p=0.013), age category (p=0.000), and Tanner stages (p=0.000), but not by race or skin type (Table 6). Normal weight, male, preadolescent, and prepubertal participants had significantly higher serum 25(OH)D levels than obese, female, early adolescent, and mid- and late- pubertal participants. Preadolescent and prepubertal participants were the only groups with a median 25(OH)D level >30 ng/mL (sufficient). In contrast, early adolescents and late pubertal participants were the only groups to have median serum D levels <20 ng/mL (insufficient). When stratified by weight category, serum vitamin D level differed significantly only in Caucasians and early adolescents, with the highest 25(OH)D level observed in normal weight participants, and the lowest level in obese participants (p=0.007) (Table 7). Serum 25(OH)D concentrations were also higher in normal weight participants than in obese participants in both males and females, African Americans, both skin types, both age categories, and in pre- and late pubertal participants, but these differences were not statistically significant. Those in the overweight category had the overall highest serum 25(OH)D concentrations, in the total population and after subdivision in all demographic groups with the exception of Caucasians and prepubertal individuals.

In the total population, BMI differed significantly by vitamin D status, gender, age category, and Tanner stages, but not by race or skin type. Body mass index was significantly higher in vitamin D deficient (p=0.000), female (p=0.000), early adolescent

(p=0.000), and late pubertal (p=0.000) participants (Table 8). The highest median BMI (22.4 kg/m^2) was observed in participants who were vitamin D deficient, and the lowest median BMI (18.1 kg/m^2) was observed in sufficient participants (p=0.000). This significant linear trend was also observed after subdivision by gender, race, skin type, and age category. No significant difference in BMI by serum vitamin D status was observed in any Tanner stage group.

Relationship between Serum 25(OH)D and Body Mass Index

In the total population, a significant negative correlation existed between BMI and serum 25(OH)D concentrations (p=0.000) (Table 9; Figure 1). However, the strength of the association was only fair (ρ=-0.315). A significant negative correlation of fair strength between these variables was also observed in both males and females, Caucasians, both skin type categories, and in all weight categories. The strongest inverse associations were found in Caucasian (ρ=-0.408, p=0.000), overweight (ρ=-0.407, p=0.003), and obese (ρ=-0.430, p=0.000) participants. A significant negative relationship between BMI and serum 25(OH)D was observed in African Americans, preadolescents, and prepubertal participants, but the strength was weak. No significant association was observed in early adolescents, mid-pubertal, or late pubertal participants. Body mass index and serum 25(OH)D were also not found to be associated when stratified by vitamin D status category.

A multiple linear regression analysis model found BMI to be a significant predictor of serum 25(OH)D (p=0.000) (Table 10). This model predicted 9.2% of the variance in serum 25(OH)D could be attributed to BMI alone (R^2=0.092, p=0.000).

Further, the model predicted a 0.835 ng/mL decrease in serum 25(OH)D for every 1 kg/m^2 increase in BMI (*B*=-0.835, p=0.000). A second multiple linear regression analysis model assessed the independent effects of BMI, gender, race, skin type, age, pubertal status, total daily calories, daily vitamin D intake, daily calcium intake, sun exposure, and sunscreen use on serum 25(OH)D levels. This model predicted that 25% of the variance in serum 25(OH)D levels could be attributed to these variables (R^2=0.250, p=0.000). Only Tanner stage was found to be a significant independent predictor of serum 25(OH)D concentrations (*B*=-3.323, p=0.002). When BMI was entered as the dependent variable in the same model (with serum 25(OH)D substituted as an independent variable), only gender (*B*=1.821, p=0.002) and age (*B*=0.441, p=0.033) were found to be significant predictors.

Discussion

We found a significant inverse relationship between serum 25(OH)D concentration and BMI in a population of Caucasian and African American pre- and early adolescents. BMI was a significant negative predictor of serum 25(OH)D levels, and obese participants had a significantly lower median serum D concentrations than overweight or normal weight participants. In turn, vitamin D deficient participants had a significantly higher median BMI than those in the insufficient or sufficient groups. Therefore, we reject the primary null hypothesis that there would be no association between serum vitamin D and BMI in our population.

The prevalence of vitamin D deficiency was higher in female, African American, dark-skinned (Fitzpatrick skin types IV-V), early adolescent (12-14 years), and late

pubertal (Tanner stages IV-V) participants than in their counterparts. Correlation statistics revealed that the negative association between BMI and serum 25(OH)D was statistically significant in male and female, Caucasian and African American, light and dark skin type, preadolescent and prepubertal participants. Therefore, we fail to reject the null hypotheses that the strength of the inverse association between serum 25(OH)D and BMI will not differ by gender, race, and skin type. We further reject the null hypothesis that the strength of the inverse association will not differ by age and Tanner stage. However, we anticipated that the association would be stronger in early adolescents and those in late puberty.

The inverse relationship between serum 25(OH)D and BMI has been reported frequently in studies of both children and adults. The association observed in our population is likely due to increased sequestration of serum 25(OH)D into the adipose that occurs in the presence of increased adipose tissue.[1,2,10,12,24,41] The uptake of vitamin D into adipose tissue is perpetuated in a positive feedback loop. Adipose tissue is the primary site of vitamin D storage, and does not release vitamin D to compensate in response to low serum calcium levels.[1] Decreased bioavailability of vitamin D triggers a hypothalamic response that leads to increased hunger and decreased energy expenditure.[41] Further, the resulting secondary hyperparathyroidism leads to an upregulation of lipogenesis.[41] As the amount of adipose tissue increases, uptake and clearance of vitamin D are further enhanced.[1]

Lifestyle factors associated with obese populations, such as lower dairy consumption and sun underexposure, have also been shown to contribute to the prevalence of hypovitaminosis D in these individuals.[2,5,15,26,38] In our population, obese

participants consumed the least amount of vitamin D, which may have contributed to diminished vitamin D status. However, the obese children were also significantly less likely to wear sunscreen, but spent equal amounts of time in the sun as normal weight and overweight children. This is notable, as obesity has also been associated with reductions in dermal vitamin D synthesis and release into circulation. [2,5,38] The interactions between mechanisms underlying the relationship between serum D status and adiposity remain unclear. In the absence of additional anthropometric and biochemical data, it is impossible to determine which contributing factor (adipose sequestration, inadequate vitamin D intake, dermal irregularities, etc.) was most responsible for the low serum levels observed in obese participants in our population.

An interesting finding that emerged from this study is that overweight participants had higher serum 25(OH)D concentrations than normal weight participants, both in the total population and when stratified by demographic group. The only exceptions were Caucasians and prepubertal individuals. In these groups, normal weight participants had the highest vitamin D levels and obese the lowest, as was anticipated. To our knowledge, the finding of overweight participants to have the high serum 25(OH)D concentrations has not been replicated in the literature, and cannot be easily explained based on the data from the current population. This finding is in contrast with the overall finding of a negative correlation between BMI and serum 25(OH)D, both in our population and in the majority of adult and pediatric populations studied. [11-16] Overweight participants in our population did not consume significantly more vitamin D or calcium or spend more time in the sun. The overweight group was significantly less likely to wear sunscreen than the normal weight group, which may have contributed to their vitamin D status.

The association between race, skin tone, and hypovitaminosis D was less straightforward in our population than in many previous studies, in which the relationship between dark skin and low serum 25(OH)D has been well established. African American children, adolescents, and adults have consistently been found to be at a greater risk for hypovitaminosis D[11,22] and have significantly lower serum levels of 25(OH)D.[11,27] An Italian cross-sectional study of 625 participants 2-21 years found non-white children to be at an eleven-fold greater risk for hypovitaminosis D, and to have serum D levels 53% lower than white children.[15] Based on findings such as these, we hypothesized we would see a similar trend in our population. Body mass index was found to be significantly higher in vitamin D deficient participants in both races and skin type groups. However, we found that the negative correlation between serum D and BMI in our population was stronger in Caucasians and light-skinned participants. Caucasians were the only racial or skin tone group in which serum 25(OH)D concentrations were significantly lower in obese individuals than in normal weight or overweight individuals.

Further, African Americans did not have significantly lower serum D levels than Caucasians. These findings are in contrast with previous studies,[15] including those of almost-identical populations.[11] A cohort study of a diverse group of 237 normal weight and overweight children and adolescents from Pittsburgh, PA by Rajakumar et. al (2011) found lower serum 25(OH)D levels in black participants.[11] However, this study also found that proportion of children classified as vitamin D deficient were significantly higher in obese, black, female, and pubertal participants, findings which were mirrored by our own. The vitamin D status groups in our population differed significantly by racial composition. A significantly larger proportion of African American and dark-skinned

participants were found to be vitamin D deficient or insufficient; African Americans comprised 100% of the deficient group when stratified by race. The composition was similar when stratified by Fitzpatrick skin type (dark skin types comprised >90% of the deficient group). These findings are consistent with previous reports that the prevalence of vitamin D deficiency in both adult and pediatric African American populations is significantly higher than in other races.[3,12,27]

The findings of the current study are consistent with previous studies that have shown darker skin to significantly increase the risk of hypovitaminosis D,[11,13,22] and support IOM and NIH determinations of African American race and dark skin as risk factors for hypovitaminosis D.[1,6] Skin pigmentation is the most important determinant of vitamin D synthesis by the skin.[27] In turn, vitamin D produced in the skin (>90%) is the major determinant of overall vitamin D status in the body,[22] and darker skin significantly increases the risk of insufficiency.[13,15,28] However, sun exposure habits are also among the strongest predictors of serum vitamin D levels.[15] African American children in our study were more likely to spend more than 2 hours in the sun and less likely to wear sunscreen than Caucasian children. These lifestyle factors increase the opportunity for exposure to UV-B rays and dermal synthesis, contributing to vitamin D status.[15] Differences in these lifestyle factors between races may help explain our finding that African American and dark skin types did not have significantly lower median serum levels of 25(OH)D than Caucasian and light skin types.

As predicted, the inverse relationship between serum vitamin D levels and BMI was stronger in females. Females comprised less than half of the total population but two-thirds of the vitamin D deficient group. In our population, females were observed to have

significantly lower median serum 25(OH)D levels and higher BMIs. These findings are consistent with previous findings that female gender is a positive risk factor for hypovitaminosis D.[16,19-21,23-25] Specifically, with a 2008 Turkish cross-sectional study by Aypak (2014) that also found females to have a have significantly lower mean serum 25(OH)D than males. This study also found vitamin D deficiency to be two times as prevalent in females, consistent with the finding in our population that 5.1% of males and 11.9% of females were deficient. Although NHANES data has shown female children and adolescents consume less dairy than males, no significant differences existed in intake of total calories, vitamin D, or calcium between genders.[35]

Age and pubertal status were the strongest determinants of both vitamin D status and BMI. Early adolescent, and late pubertal participants had significantly lower levels of serum vitamin D and higher prevalence's of deficiency than preadolescent and prepubertal participants. These results are similar to previously reported inverse associations between age, pubertal development, and serum 25(OH)D levels.[11,14,15,19,23,24] A study of 102 Norwegian children and adolescents found that teenagers 13-19 years old had a significantly higher prevalence of vitamin D deficiency and insufficiency than in preadolescents aged 8-12 years**.** Similarly, the aforementioned study by Rajakumar et. al (2011) found mean serum 25(OH)D concentrations were significantly lower and odds of deficiency were higher in pubertal (Tanner stages II-V) than in prepubertal (Tanner stage 1) participants. In our study, Tanner stage was the only variable found to be independently predictive of serum 25(OH)D concentrations, and early adolescents were one of only two subgroups in which a significant difference in serum 25(OH)D levels existed across weight categories.

The positive association between female gender, adolescence, and pubertal status with hypovitaminosis D is likely related to the positive association of these characteristics with increased adiposity.[42] Gender is a strong predictor of overall adiposity in children and adolescents.[42] Cross-sectional and longitudinal studies have found that by age 5, girls have greater TFM and BF% than boys, and that this gender difference continues to increase until the age of 18.[42] Changes in body composition associated with the onset of puberty have been well established.[36,42] Post-infancy, BMI and TFM are typically stable until 6-8 years of age, when a second "adiposity rebound" occurs.[42] This rebound refers to the increase in BMI, TFM, VAT, and SAT that occurs in late childhood and early adolescence.[42] This rise continues into mid- to late adolescence, and stabilizes around the ages of 16 in girls and 18 in boys.[42] Pubertal development and sexual maturation positively correlate with accumulation of total body fat, particularly in girls.[42]

There are several limitations to this study. The determination of a cause-and-effect relationship is not possible due to the cross-sectional nature of the study. Our population consisted of a much greater proportion of African Americans to Caucasians and preadolescents to early adolescents, which makes identifying trends more challenging. Future studies in populations with more even distributions of these characteristics may yield more generalizable results. Further, although BMI has been validated as a direct predictor of body fatness in children and adolescents, a measure such as BF% or TFM would have been superior, as this would have allowed for a direct analysis of the relationship between fat mass and vitamin D status. A strength of this study is the use of the gold-standard biomarker for vitamin D (serum 25(OH)D), ensuring vitamin D status was recorded as accurately as possible, and that vitamin D status was

defined according to IOM guidelines, which specifies the lowest cutoff point for vitamin D deficiency (serum 25(OH)D <12 ng/mL),. This definition of deficiency is more specific than the Endocrine Society CPG used in the used in the majority of research studies (25(OH)D <20 ng/mL) and is more useful for screening for true negatives.

Clarifying the risk factors of vitamin D deficiency have important implications for clinical practice. Vitamin D deficiency has serious health implications in children, and can lead to chronic conditions including rickets, type 1 diabetes, hypertension, chronic kidney disease, and pediatric cancers. Understanding patterns in vitamin D deficiency prevalence by demographic characteristic allows health care professionals to more easily screen for patients at highest risk for deficiency. Vitamin D deficiency should be considered and screened for in obese individuals, females, dark-skinned individuals, and adolescents. Lifestyle patterns that contribute to vitamin D deficiency, such as vitamin D and calcium intake, sun exposure, and sunscreen use, and physical symptoms of vitamin D deficiency, such as skeletal malformations and muscle aches, should be assessed in these patients. If concern for deficiency emerges from this screening, serum 25(OH)D labs should be ordered. Establishing defined correlates of low serum vitamin D is important for providers for identifying patients at risk and expediting the process of treating the deficiency. Further, the Endocrine Society has determined that children "at risk for deficiency", including obese, female, dark-skinned, and older children, may require 2-3 times greater dosage of vitamin D to maintain adequate serum D levels.[32] Understanding which demographic factors increase vitamin D needs is essential for healthcare providers to identify when supplementation dosages need to be increased.

We conclude that serum 25(OH)D and BMI are inversely correlated in a population of Caucasian and African American pre- and early adolescents. This association was strongest in obese, female, Caucasian, light skin type, preadolescent, and prepubertal participants. Obesity, female gender, African American race, dark skin tone, preadolescence, prepubescence, and lack of multivitamin use were significant negative predictors of vitamin D status. Age and Tanner stage were the strongest demographic predictors of serum 25(OH)D. The results of this study support previous findings that obesity, female gender, African American race, dark skin tone, and pubescence are independent risk factors for hypovitaminosis D. However, the lack of uniformity in definitions of vitamin D deficiency used in research continues to complicate the comparison of prevalence rates across populations and determination of demographic and nutrition characteristics that contribute to deficiency. This also makes it difficult to determine if certain characteristics contribute to vitamin D deficiency in some types of populations but not others. A comprehensive meta-analysis, using a singular definition of vitamin D deficiency to analyze the vast amounts of relevant literature, is warranted to get a true picture of vitamin D status in populations across the world and to identify common risk factors.

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APPENDIX

Table 1 – Demographic and Clinical Characteristics of the Total Population

	n (%)
Total Population	294 (100)
Gender	
Male	158 (53.7)
Female	136 (46.3)
Race	
Caucasian	117 (39.8)
African American	177 (60.2)
Skin Type	
Light (Skin types I-III)	111 (37.8)
Dark (Skin types IV-V)	180 (61.2)
Age Category	
Preadolescent (6-11 years)	232 (78.9)
Early adolescent (12-14 years)	62 (21.1)
Tanner Stage	
Prepubertal (Stage I)	161 (54.8)
Mid-pubertal (Stages II-III)	103 (35.0)
Late pubertal (Stages IV-V)	30 (10.2)
Weight Category	
Normal (BMI <85%)	173 (58.8)
Overweight (BMI ≥85-<95%)	48 (16.3)
Obese (BMI ≥95%)	73 (24.8)
Vitamin D Status*	
Deficient (<12 ng/mL)	24 (8.2)
Insufficient (12-<20 ng/mL)	64 (21.8)
Sufficient (≥20 ng/mL)	203 (69.0)

*n=291

BMI – body mass index, kg – kilogram, m – meter, ng – nanogram, mL – milliliter

Table 2 – Demographic, Anthropometric and Nutrition Characteristics of Total Population and by Serum Vitamin D Status (Continuous Variables)

	Total Population N=294	Deficient n=24	Insufficient n=64	Sufficient n=203	Significance
Age (years)*	10.2 ± 2.1	12.0 ± 2.1	11.4 ± 2.0	9.7 ± 1.9	**0.000**
Weight (kg)**	37.6 (30.2, 49.3)	58.6 (40.6, 75.2)	48.1 (37.7, 61.9)	34.5 (28.4, 43.0)	**0.000**
Height (cm)**	140.1 (130.6, 151.3)	158.1 (143.0, 165.0)	149.9 (138.7, 159.3)	136.6 (129.3, 145.3)	**0.000**
BMI (kg/m^2)**	19.0 (16.2, 22.5)	22.4 (18.0, 30.3)	20.7 (18.1, 24.9)	18.1 (16.4, 20.8)	**0.000**
Serum 25(OH)D (ng/mL),***	27.0 (18.0, 38.0)	9.0 (6.7, 10.9)	16.1 (14.7, 17.9)	33.0 (25.2, 42.0)	**0.000**
Total daily calories (kcal)**	2242.4 (1727.2, 2938.6)	2427.0 (1385.5, 3001.7)	2151.7 (1662.6, 2792.7)	2300.0 (1805.0, 2957.5)	0.239
Daily vitamin D intake (IU)**	260.0 (154.7, 407.2)	183.1 (98.7, 243.1)	224.0 (146.4, 384.5)	309.4 (168.7, 415.6)	**0.005**
Daily calcium intake (mg)**	1182.5 (771.4, 1624.8)	1078.3 (599.2, 1294.7)	1068.6 (750.6, 1464.9)	1249.5 (817.0, 1669.6)	**0.042**

*Data are expressed as mean ± standard deviation. Independent samples Kruskal-Wallis test; significance level P = 0.05.

**Data are expressed as median (25%, 75%). Independent samples Kruskal-Wallis test; significance level P = 0.05.

***N=291

Deficient = <12 ng/mL, Insufficient = 12-<20 ng/mL, Sufficient = ≥20 ng/mL.

kg - kilogram, cm - centimeter, BMI - Body Mass Index, m – meter, ng = nanogram, mL = milliliter, kcal - kilocalories, IU - International Units, mg – milligrams.

Table 3 – Nutrition and Lifestyle Characteristics of the Total Population and by Serum Vitamin D Status (Categorical Variables)*

	Total Population N=294	Deficient n=24	Insufficient n=64	Sufficient n=203	Significance
Takes multivitamin**	37 (12.6)	0 (0)	3 (4.9)	33 (16.4)	**0.010**
Takes vitamin D supplement**	1 (0.3)	0 (0)	0 (0)	1 (0.5)	0.813
Takes calcium supplement**	4 (1.4)	0 (0)	1 (1.6)	3 (1.5)	0.834
≥2 hours daily sun exposure**	237 (80.6)	18 (81.8)	50 (83.3)	167 (85.2)	0.879
Wears sunscreen outside**	117 (39.8)	5 (22.7)	28 (45.9)	81 (40.3)	0.164

*Data are expressed as *n* (% within vitamin D category).

**Chi-Square test; significance level P=0.05.

Deficient = <12 ng/mL, Insufficient = 12-<20 ng/mL, Sufficient = ≥20 ng/mL.

Table 4 – Nutrition and Lifestyle Characteristics of Population by Select Demographic and Anthropometric Characteristics

	Continuous Variables*			Categorical Variables**		
	Total daily calories (kcal)	**Daily vitamin D intake (IU)**	**Daily calcium intake (mg)**	**Takes MVI**	**≥2 hours daily sun exposure**	**Wears sunscreen**
Gender						
Male	2250.0 (1753.2, 2930.0)	303.4 (166.5, 428.4)	1225.0 (757.3, 1622.2)	23 (14.7)	127 (84.7)	65 (41.9)
Female	2210.1 (1725.1, 2957.5)	243.6 (146.4, 383.0)	1144.4 (817.0, 1631.4)	14 (10.6)	110 (84.0)	52 (39.4)
Significance	0.792	0.073	0.635	0.296	0.873	0.662
Race						
Caucasian	2126.5 (1707.1, 2668.2)	292.8 (163.7, 422.7)	1156.1 (780.9, 1575.6)	16 (14.0)	87 (76.3)	78 (67.8)
African American	2442.4 (1769.8, 3200.3)	254.2 (151.3, 396.9)	1199.1 (750.8, 1661.3)	21 (12.1)	150 (89.8)	39 (22.7)
Significance	**0.002**	0.224	0.819	0.626	**0.002**	**0.000**
Age						
Preadolescent (6-11 years)	2268.7 (1769.6, 2979.8)	278.2 (164.4, 404.73)	1224.8 (770.9, 1660.5)	35 (15.4)	188 (85.5)	94 (41.6)
Early adolescent (12-14 years)	2151.7 (1550.1, 2880.5)	221.3 (141.6, 429.3)	1089.1 (787.6, 1545.7)	2 (3.3)	49 (80.3)	23 (37.7)
Significance	0.117	0.512	0.117	**0.021**	0.330	0.583
Weight Category						
Normal (BMI <85%)	2181.0 (1781.1, 2922.8)	299.5 (188.7, 469.3)	1186.6 (784.0, 1582.9)	29 (17.1)	139 (84.8)	79 (47.0)
Overweight (BMI ≥85-<95%)	2613.7 (1744.6, 3737.1)	289.1 (188.7, 469.3)	1308.4 (871.2, 1819.8)	5 (10.9)	38 (80.9)	17 (36.2)
Obese (BMI ≥95%)	2210.1 (1697.1, 2736.6)	193.1 (129.1, 383.2)	1115.0 (645.7, 1586.4)	3 (4.2)	60 (85.7)	21 (29.2)
Significance	0.124	**0.047**	0.120	**0.013**	0.758	**0.028**

*Data are expressed as median (25%, 75%). Independent samples Kruskal-Wallis test; significance level P = 0.05.

**Data are expressed as n (% within demographic category). Chi-Square test; significance level P=0.05.

kcal - kilocalories, IU - International Units, mg – milligram

Table 5 – Proportions of Demographic and Anthropometric Characteristics by Serum Vitamin D Status

	Deficient n=24	Insufficient n=64	Sufficient n=203	Significance
Gender				**0.003**
Male	8	26	122	
% within gender	5.1%	16.7%	78.2%	
% within D category	33.3%	40.6%	60.1%	
Female	16	38	81	
% within gender	11.9%	28.1%	60.0%	
% within D category	66.7%	59.4%	39.9%	
Race				**0.000**
Caucasian	0	28	86	
% within race	0%	24.6%	75.4%	
% within D category	0%	43.8%	42.4%	
African American	24	36	117	
% within race	13.6%	20.3%	66.1%	
% within D category	100%	56.3%	57.6%	
Skin Type				**0.008**
Light (Skin types I-III)	2	22	84	
% within skin type	1.9%	20.4%	77.8%	
% within D category	8.7%	35.5%	41.4%	
Dark (Skin types IV-V)	21	40	119	
% within skin type	11.7%	22.2%	66.1%	
% within D category	91.3%	64.5%	58.6%	
Age				**0.000**
Preadolescent (6-11 years)	11	38	180	
% within age category	4.8%	16.6%	78.6%	
% within D category	45.8%	59.4%	88.7%	
Early adolescent (12-14 years)	13	26	23	
% within age category	21.0%	41.9%	37.1%	
% within D category	54.2%	40.6%	11.3%	
Tanner Stage				**0.000**
Prepubertal (Stage I)	7	19	134	
% within Tanner stage	4.4%	11.9%	83.8%	
% within D category	29.2%	29.7%	66.0%	
Mid-pubertal (Stages II-III)	4	33	64	
% within Tanner stage	4.0%	32.7%	63.4%	
% within D category	16.7%	51.6%	31.5%	
Late pubertal (Stages IV-V)	13	12	5	
% within Tanner stage	43.3%	40.0%	16.7%	
% within D category	54.2%	18.8%	2.5%	
Weight Category				**0.009**
Normal (BMI <85%)	11	35	125	
% within weight category	6.4%	20.5%	73.1%	
% within D category	45.8%	54.7%	61.6%	
Overweight (BMI ≥85-<95%)	2	7	39	
% within weight category	4.2%	14.6%	81.3%	
% within D category	8.3%	10.9%	19.2%	
Obese (BMI ≥95%)	11	22	39	
% within weight category	15.3%	30.6%	54.2%	
% within D category	45.8%	34.4%	19.2%	

Chi-Square test; significance level P=0.05

Deficient - <12 ng/mL, Insufficient - 12-<20 ng/mL, Sufficient - ≥20 ng/mL.

Table 6 –Body Mass Index (kg/m^2) and Serum 25(OH)D Levels (ng/mL) Level by Demographic, Anthropometric, and Clinical Characteristics*

	BMI (kg/m^2)	Significance	25(OH)D (ng/mL)	Significance
Gender				
Male	18.1 (16.5, 20.6)	**0.000**	29.0 (21.0, 39.0)	**0.013**
Female	19.8 (17.7, 24.1)		24.0 (15.9, 36.2)	
Race				
Caucasian	18.4 (16.4, 21.1)	0.080	26.7 (19.9, 35.4)	0.387
African American	19.1 (17.0, 23.1)		27.5 (16.1, 39.0)	
Skin Type				
Light (Skin types I-III)	18.2 (16.4, 21.6)	0.046	27.1 (21.1, 37.3)	0.293
Dark (Skin types IV-V)	19.2 (17.0, 22.9)		27.7 (16.7, 39.0)	
Age Category				
Preadolescent (6-11 years)	18.2 (16.4, 21.7)	**0.000**	30.0 (21.3, 40.0)	**0.000**
Early adolescent (12-14 years)	20.4 (19.1, 23.9)		16.9 (12.6, 22.8)	
Tanner Stage				
Prepubertal (Stage I)	17.4 (16.2, 20.5)	**0.000**	31.8 (23.5, 40.1)	**0.000**
Mid-pubertal (Stages II-III)	20.0 (17.9, 23.3)		23.6 (16.2, 36.5)	
Late pubertal (Stages IV-V)	23.0 (19.9, 29.7)		14.6 (9.6, 18.4)	
Weight Category				
Normal (BMI <85%)	17.1 (16.0, 18.4)	**0.000**	28.2 (19.0, 38.0)	**0.015**
Overweight (BMI ≥85-<95%)	20.3 (19.2, 23.0)		29.5 (22.3, 41.0)	
Obese (BMI ≥95%)	25.5 (23.2, 30.8)		23.5 (15.9, 33.8)	
Vitamin D Status**				
Deficient (<12 ng/mL)	22.4 (18.0, 30.3)	**0.000**	9.0 (6.7, 10.9)	**0.000**
Insufficient (12-<20 ng/mL)	20.7 (18.1, 24.9)		16.1 (14.7, 17.9)	
Sufficient (≥20 ng/mL)	18.1 (16.4, 20.8)		33.0 (25.2, 42.0)	

*Data are expressed as median (25%, 75%). Independent samples Kruskal-Wallis test; significance level P = 0.05.
**n=291
BMI – body mass index, kg – kilogram, m – meter, ng – nanogram, mL - milliliter

Table 7 – Serum 25(OH)D level (ng/mL) by Demographic and Weight Category*

	Normal n=173	Overweight n=48	Obese n=73	Significance
Gender				
Male	29.7 (21.0, 40.0)	30.0 (23.2, 41.0)	24.8 (18.2, 34.9)	0.311
Female	26.2 (16.1, 36.1)	29.0 (19.9, 41.0)	18.8 (15.0, 34.0)	0.107
Race				
Caucasian	27.6 (21.5, 39.0)	25.7 (23.4, 48.0)	21.3 (16.2, 26.7)	**0.027**
African American	29.0 (15.8, 38.3)	32.0 (21.2, 41.0)	23.5 (15.3, 39.0)	0.220
Skin Type				
Light (Skin types I-III)	27.5 (21.7, 39.0)	28.4 (23.2, 51.0)	24.0 (15.3, 27.0)	0.075
Dark (Skin types IV-V)	29.5 (16.0, 38.7)	31.0 (21.7, 40.5)	23.5 (16.0, 38.0)	0.189
Age				
Preadolescent (6-11 years)	30.9 (21.6, 39.3)	32.0 (23.7, 42.0)	25.1 (16.9, 38.3)	0.061
Early adolescent (12-14 years)	18.4 (13.4, 27.2)	18.5 (16.6, 23.9)	13.1 (7.8, 17.5)	**0.016**
Tanner Stage				
Prepubertal (Stage I)	32.5 (23.7, 41.0)	32.0 (24.1, 40.5)	27.9 (18.8, 39.8)	0.289
Mid-pubertal (Stages II-III)	22.2 (15.5, 36.3)	29.0 (16.6, 42.0)	23.1 (16.2, 29.6)	0.255
Late pubertal (Stages IV-V)	15.1 (10.2, 22.4)	18.0 (7.3, 22.5)	11.4 (7.3, 16.9)	0.297

*Data are expressed as median (25%, 75%). Independent samples Kruskal-Wallis test; significance level P = 0.05. Normal - BMI <85%, Overweight - BMI ≥85-<95%, Obese - BMI ≥95%.

Table 8 – Body Mass Index (kg/m^2) by Demographic and Vitamin D Status*

	Deficient n=24	Insufficient n=64	Sufficient n=203	Significance
Gender				
Male	22.4 (18.4, 29.9)	19.5 (16.6, 21.9)	17.8 (16.4, 20.0)	**0.008**
Female	23.5 (17.9, 31.1)	22.3 (18.8, 26.7)	19.2 (16.7, 22.5)	**0.000**
Race				
Caucasian	-	20.5 (18.2, 24.5)	18.0 (15.8, 20.4)	**0.000**
African American	22.4 (18.0, 30.3)	20.8 (18.0, 26.5)	18.4 (16.6, 21.1)	**0.000**
Skin Type				
Light (Skin types I-III)	35.6 (24.9, -)	20.5 (16.7, 24.4)	18.0 (15.9, 20.4)	**0.003**
Dark (Skin types IV-V)	22.0 (17.8, 28.0)	21.1 (18.7, 26.8)	18.6 (16.6, 21.1)	**0.000**
Age				
Preadolescent (6-11 yrs)	18.4 (17.3, 28.3)	21.0 (17.5, 25.1)	17.9 (16.2, 20.6)	**0.003**
Early adolescent (12-14 yrs)	24.9 (21.1, 32.6)	20.5 (19.0, 24.5)	20.0 (18.1, 22.1)	**0.024**
Tanner Stage				
Prepubertal (Stage I)	17.8 (16.5, 31.0)	18.2 (16.4, 21.9)	17.2 (15.9, 19.5)	0.232
Mid-pubertal (Stages II-III)	20.2 (17.5, 40.3)	20.6 (18.7, 24.4)	19.4 (17.8, 22.5)	0.140
Late pubertal (Stages IV-V)	24.9 (21.2, 30.2)	23.0 (20.1, 34.6)	19.8 (18.7, 22.8)	0.191

*Data are expressed as median (25%, 75%). Independent samples Kruskal-Wallis test; significance level P = 0.05. Deficient - <12 ng/mL, Insufficient - 12-<20 ng/mL, Sufficient - ≥20 ng/mL.

Table 9 – Correlation Statistics between Body Mass Index (kg/m^2) and Serum 25(OH)D (ng/mL) Levels by Demographic and Anthropometric Characteristics and Vitamin D Status

	Spearman's ρ	Significance
Total Population	-0.315*	**0.000**
Gender		
Male	-0.255*	**0.001**
Female	-0.320*	**0.000**
Race		
Caucasian	-0.408*	**0.000**
African American	-0.246*	**0.001**
Skin Type		
Light (Skin types I-III)	-0.340*	**0.000**
Dark (Skin types IV-V)	-0.267*	**0.000**
Age Category		
Preadolescent (6-11 years)	-0.216*	**0.001**
Early adolescent (12-14 years)	-0.232	0.070
Tanner Stage		
Prepubertal (Stage I)	-0.182**	**0.021**
Mid-pubertal (Stages II-III)	-0.110	0.275
Late pubertal (Stages IV-V)	-0.150	0.430
Weight Category		
Normal (BMI <85%)	-0.391*	**0.000**
Overweight (BMI ≥85-<95%)	-0.407*	**0.003**
Obese (BMI ≥95%)	-0.430*	**0.000**
Vitamin D Status		
Deficient (<12 ng/mL)	0.071	0.741
Insufficient (12-<20 ng/mL)	0.135	0.286
Sufficient (≥20 ng/mL)	-0.128	0.069

*Significance level P = 0.05 (2-tailed)

**Significance level P = 0.01 (2-tailed)

BMI – Body mass index, kg – kilogram, m – meter, ng – nanogram, mL - milliliter

Table 10 – Summary of Regression Analysis for Variables Predicting Serum 25(OH)D Concentration

		Unstandardized Coefficients		Standardized Coefficients	
	Variable	**Beta**	**Standard Error**	**Beta**	**Significance**
Model 1	BMI	-0.835	0.155	-0.303	**0.000**
	R^2		0.092		
	F for change in R^2		29.117		**0.000**
Model 2	BMI	-0.272	0.170	-0.098	0.110
	Gender	-0.909	1.664	-0.032	0.585
	Race	0.480	2.405	0.017	0.842
	Skin type	-0.904	1.003	-0.072	0.368
	Age	-1.075	0.569	-0.162	0.060
	Tanner stage	-3.323	1.083	-0.265	**0.002**
	Total calories	-0.001	0.001	-0.074	0.471
	Vitamin D intake	0.003	0.009	0.033	0.755
	Calcium intake	0.001	0.003	0.059	0.690
	Multivitamin use	5.058	2.590	0.121	0.052
	Sun exposure	-0.161	2.186	-0.004	0.941
	Sunscreen use	-1.833	1.758	-0.064	0.298
	R^2		0.250		
	F for change in R^2		7.280		**0.000**

Figure 1 – Correlation of Serum 25(OH)D Levels (ng/mL) and Body Mass Index (kg/m^2) in Total Population