Vitamin D Moderators and Supplementation Outcomes

By

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Dedication

To my husband who without his unwavering support and devotion, I could not have achieved my dreams. You are a perfect husband and friend. To my mother who can remember the first time I said I wanted to be a nurse even when I cannot. I will never be able to live up to my reflection that I see in her eyes. And to my sons, thank you for always making me laugh.

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Thank you all for going on this journey with me, I can't wait to see where our next journey will take us! So…

"be your name Buxbaum or Bixby or Bray

Or Mordecai Ali Van Allen O'Shea,

You're off to Great Places!

Today is your day!

Your mountain is waiting.

So…*get on your way!*"

Dr. Seuss. (1990). *Oh, the places you'll go!* New York, NY: Random House

Abstract

Robyn L. Havens
Vitamin D Moderators and Supplementation Outcomes
(Under the direction of Dr. Elizabeth NeSmith)

Vitamin D insufficiency is a global health concern affecting approximately 1 billion people, including about one third of the American population. Vitamin D insufficiency promotes the development of chronic diseases. The people most at risk for developing chronic diseases from vitamin D insufficiency are those individuals in the vulnerable populations who experience poor health outcomes. Currently, researchers and clinicians disagree as to the recommended daily allowance and therapeutic range supporting sufficient serum vitamin D concentrations. To provide data to resolve this disagreement, the objectives of this secondary analysis were to determine if age, sex, and body mass index were moderators of serum vitamin D concentration and if varying dosages of vitamin D supplementation affected serum interleukin-6 concentrations.

The data records of 60 healthy male and female African American participants were examined who were aged 13-45 years, categorized as overweight or obese, and exhibited a baseline serum vitamin D concentration ≤ 50 nmol/L. The participants were randomized into four treatment groups for the original study: 1) a control group that received a placebo; (2) a group that received monthly supervised doses of 18,000 IU (equivalent to 600 IU/day); (3) a group that received monthly supervised doses of 60,000 IU (equivalent to 2,000 IU/day); and (4) a group that received monthly supervised doses

of 120,000 IU (equivalent to 4,000 IU/day). After 16 weeks of vitamin D supplementation, the only statistically significant interaction found was with sex as a moderating variable despite the small sample size of men. No other significant interactions were found, including no interaction with vitamin D supplementation and interleukin-6. Despite lacking statistical significance, the data results suggested that the 2,000 or 4,000 IU/day dosages of vitamin D supplementation was needed for the overweight/obese African American participants to achieve a sufficient serum vitamin D concentration > 50 nmol/L as recommended in the 2011 Institute of Medicine report. These results also suggest that the overweight/obese, African American adolescents and adults needed much more vitamin D supplementation than the 600 IU/day recommended by National Institute of Health researchers. Lastly, the findings suggest that the national clinical guidelines published by the Endocrine Society may warrant revision to at least 2,000 IU/day to be effective for individuals in vulnerable populations. Future research is needed to further elucidate the role vitamin D plays in maintaining overall good health and the benefits of vitamin D supplementation.

Keywords: vitamin D, vitamin D insufficiency, vitamin D supplementation, age, sex, body mass index, interleukin-6, vulnerable populations conceptual model

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Chapter 1: Introduction

This chapter introduces a doctoral research project regarding vitamin D, the influence of demographic characteristics on its serum concentration, and the effect of vitamin D supplementation on the level of a serum inflammatory biomarker. After providing a brief background concerning vitamin D insufficiency, this chapter describes the problem underlying vitamin D supplementation treatment, the research question, purpose statement, specific aims, and hypotheses of this study. This chapter concludes with the long-term goals and significance of this research.

Background

Vitamin D insufficiency, defined as a serum vitamin D concentration < 50 nmol/L, affects approximately 1 billion people worldwide in a pandemic proportion (Cashman et al., 2016; Wilson, Tripkovic, Hart, & Lanham-New, 2017). Furthermore, one third of the American population may also be deficient in vitamin D (Looker et al., 2011). Vitamin D insufficiency causes rickets and osteomalacia as well as promotes the development of autoimmune diseases such as multiple sclerosis and diabetes, colon and breast cancer (Lips, 2006; Samefors, Scragg, Lanne, Nyström, & Ostgren, 2017), dementia (Annweiler, 2016), and cardiovascular disease (Alkhatatbeh, Abdul-Razzak, Khasawneh, & Saadeh, 2017; Dong, Pollock, et al., 2010; Mousa, Naderpoor, de Courten, Scragg, & de Courten, 2017; Perez-Hernandez et al., 2016). People at risk for vitamin D insufficiency include premature infants, the elderly, individuals with pigmented skin, low sunshine exposure, malabsorption conditions, and those who are obese or had bariatric surgery (Holick, 2004). Individuals also at risk for vitamin D insufficiency are those taking medications that enhance the catabolism of 25-hydroxyvitamin D (25(OH)D; calcifediol) and

1,25-dihydroxyvitamin D (1,25$(OH)_2$D; calcitriol) (Holick et al., 2011). Individuals with chronic granuloma-forming disorders, lymphomas or primary hyperparathyroidism are also at risk for vitamin D insufficiency (Lips, 2006). Wallace, Reider, and Fulgoni (2013) included people with low-incomes as an at-risk population since they may not be able to afford the foods rich in vitamin D or vitamin D supplements. While the use of sunscreen and clothing protects people from acquiring skin cancer due to prolonged sun exposure, doing so hampers the absorption of ultraviolet (UV) light by the skin, promoting vitamin D insufficiency (Holick et al., 2011).

Problem Statement

In vitro, in vivo, and clinical trial research findings over the past 20 years indicate that vitamin D may influence more physiological functions than calcium and phosphorus metabolism for bone health (Baeke, van Etten, Gysemans, Overbergh, & Mathieu, 2008). Vitamin D may also have an important role in disease prevention and treatment. For example, vitamin D may act to suppress pro-inflammatory cytokines and increase the anti-inflammatory ones (Deluca & Cantorna, 2001; Schleithoff et al., 2006). Recent research also suggests that vitamin D supplementation may be a treatment for autoimmune diseases characterized by inflammation (Grossman & Porth, 2014). In these cases, serum levels of the inflammatory biomarkers such as interleukin-6 (IL-6) may indicate the treatment effectiveness of vitamin D supplementation. However, few studies were found that measured serum levels of inflammatory biomarkers in association with serum vitamin D concentration.

Thus far, most vitamin D insufficiency-related research has been conducted on healthy normal-weight individuals. Research is needed to identify optimal dosages of vitamin D supplementation for various subpopulations such as individuals with varying body mass indexes (BMIs) as well as those persons in the diverse demographic groups of age, sex, race, and geographical regions of residency. This information will assist

researchers in determining the role of vitamin D in disease prevention and treatment, so clinicians have evidence-based guidelines for achieving and maintaining optimal serum vitamin D concentrations in their clients.

Research Question and Purpose Statement

The relationships between serum vitamin D concentration, vitamin D treatment supplementation, demographic characteristics, and inflammatory biomarkers are unknown. Thus, the research question for this study was: How do the demographic characteristics of a vulnerable population affect the serum vitamin D concentration and IL-6 level when the subjects were given various dosages of vitamin D supplementation? To answer this question, this study examined these relationships via a secondary analysis of a large data set acquired from a previous study.

The original study [*Vitamin D Supplementation in Obese African American Adults and Youth* (D-SUNNY; Dong, 2012)] used a double-blind, randomized, placebo-controlled experimental design with four specific aims (Bhagatwala et al., 2015):

1. compare the effect of vitamin D supplementation on serum vitamin D concentration, parathyroid hormone (PTH) serum concentration, and serum and urine calcium concentration;
2. compare the effect of vitamin D supplementation on vascular measures;
3. compare the effect of vitamin D supplementation on telomerase activity, expression, and length; and
4. examine the effect of vitamin D supplementation on arterial physiology and renal function in healthy young overweight and obese African Americans with vitamin D insufficiency residing in the southeastern region of the United States.

To achieve these specific aims, the participants in the D-SUNNY study were randomized into four treatment groups; each group was given a different dosage of vitamin D supplementation over 16 weeks:

1. control group who received a placebo;
2. group who received supervised monthly doses of 18,000 international units (IU) (equivalent to 600 IU/day);
3. group who received supervised monthly doses of 60,000 IU (equivalent to 2,000 IU/day); and
4. group who received supervised monthly doses of 120,000 IU (equivalent to 4,000 IU/day).

The present secondary analysis research project differed from the original study as it focused solely on the effect vitamin D supplementation had on the serum vitamin D concentration and IL-6 level to highlight the vitamin D issues associated with a vulnerable population. This secondary analysis built on the original study by exploring the relationships between vitamin D status, sex, age, and BMI as well as the influence of vitamin D supplementation on the immune system via IL-6. To explore these relationships, the data records from the original D-SUNNY were re-examined for 60 of the original participants who were healthy male and female individuals, aged 13-46 years with a BMI in the overweight and obese categories, and who had a baseline serum vitamin D concentration ≤ 50 nmol/L. All subjects were African American who lived in the same area of the southeastern region of the United States. Therefore, race and geographical location (i.e., sunshine exposure) were controlled variables to minimize any confounding influences they may exert on serum vitamin D concentration.

Specific Aims

Aim 1: Determine whether age, sex, and BMI affected serum vitamin D concentration at differing dosages of supplementation in a sample of African American individuals living in the southeastern region of the United States.

- Hypothesis 1a: Individuals aged 13 to 21 years will require higher dosages of vitamin D supplementation than individuals aged 22 to 45 years to reach a sufficient serum vitamin D concentration ≥ 50 nmol/L.
- Hypothesis 1b: Women will require higher dosages of vitamin D supplementation than men to reach a sufficient serum vitamin D concentration ≥ 50 nmol/L.
- Hypothesis 1c: Obese individuals will require higher dosages of vitamin D supplementation than overweight individuals to reach a sufficient serum vitamin D concentration ≥ 50 nmol/L.

Aim 2: Determine whether vitamin D supplementation affected serum levels of the IL-6 inflammatory biomarker in a sample of African American individuals living in the southeastern region of the United States.

- Hypothesis 1: After 16 weeks of vitamin D supplementation treatment, serum levels of the IL-6 inflammatory biomarker will decrease compared to the baseline values.

Significance

The importance of vitamin D for establishing healthy bones has been established since the early 1920's (McCollum, Simmonds, Becker, Shipley, 1922). However, the use of vitamin D supplementation to treat disease conditions other than vitamin D insufficiency is controversial, especially with treating diseases associated with the inflammatory process and inhibition of IL-6 inflammatory biomarker. Questions arise regarding the appropriate dosages needed to obtain/maintain health, particularly since researchers and clinicians disagree as to the safe, upper tolerable limits of vitamin D. For example, the 2011 Institute of Medicine report titled *Dietary Reference Intakes for Calcium and Vitamin D* (IOM, 2011), which is the most recent report on this topic,

identified the minimum serum vitamin D concentration as 50 nmol/L and the risk of vitamin D toxicity at serum concentrations above 124 nmol/L. However, some researchers disagree with this parameter. They believe that a minimum serum vitamin D concentration of 75 nmol/L is needed for overall health as well as muscle strength and bone mineral density (Holick et al., 2011, 2012), and vitamin D toxicity does not occur until concentrations of at least 375 nmol/L (Kennel, Drake, & Hurley, 2010).

Results from this secondary analysis research project will provide important clinical knowledge that significantly impacts the scientific community. For example, the data from this secondary analysis will inform researchers as to which investigations are needed to determine optimal vitamin D dosages for members of vulnerable populations. These studies are needed to guide clinicians when prescribing vitamin D supplements to their clients who are overweight or obese, male or female, and of varying ages and races. As front-line health care providers, nurses must possess the knowledge to assess, diagnose, and treat those who may be experiencing vitamin D insufficiency.

Summary

Vitamin D insufficiency is a global health concern. While vitamin D is needed for calcium and phosphorus metabolism and bone health, the role of vitamin D in preventing disease remains elusive. The purpose of this secondary analysis research project was to examine vitamin D supplementation dosages and their relationship to age, sex, BMI, and IL-6 within vulnerable populations. The results should better guide clinicians when prescribing vitamin D to clients who are obese or overweight, male or female, and in varying age groups.

Chapter 2: Review of the Literature

This chapter presents a review of the literature in two parts. Part 1 discusses vitamin D and its relationships with age, sex, BMI, and the IL-6 inflammatory biomarker. Part 2 discusses the Vulnerable Populations Conceptual Model (VPCM) developed by Flaskerud and Winslow (1998) that was used as the theoretical framework for this research project. The VPCM model defines vulnerable populations as those individuals or groups of people who have an increased risk for adverse health outcomes. Thus, this model provided an appropriate theoretical context for this secondary analysis since participants from vulnerable populations (women, African Americans, and children) were used in the original D-SUNNY study (Dong, 2012).

Part 1: Vitamin D

Literature Search Strategy

A literature search was conducted to determine the current state of the science for vitamin D supplementation in overweight and/or obese African Americans with vitamin D insufficiency. The Medline (1946-2017), CINAHL (1947-2017), PubMed (1936-2017), and Web of Science (1950-2017) databases were searched with these key words: vitamin D, vitamin D sufficiency, vitamin D insufficiency, vitamin D inadequacy, vitamin D dosage, vitamin D history, vitamin D supplementation, vitamin D and immune system, and interleukin-6. The inclusion and exclusion criteria for this search were:

- Inclusion criteria: data-based research papers published in peer-reviewed journals written in the English language, all ages, children, adolescents, adults, all races, men and women, non-obese, obese and overweight, animal studies, in vivo and in vitro studies, and clinical trials.

- Exclusion criteria: research studies that contained outdated information or involved pregnant women. Pregnant women were not included in the original D-SUNNY study nor are a population included in the aims of this secondary analysis project.

Search Results

More than 25,142 articles were found with this search strategy with an estimated 25% duplication of articles among the four databases. Titles and abstracts were reviewed to determine the most relevant research publications. In addition, 100 more articles were found after reviewing the reference lists of selected articles for pertinent publications. A total of 562 published research articles were reviewed for this literature search; most of the research had been conducted in the United States. The following discussion details the current state of the science concerning vitamin D in support of this research project's specific aims.

Vitamin D

Vitamin D is a steroid vitamin associated with a group of fat-soluble prohormones that enhances the absorption and metabolism of calcium and phosphorous. Food is one source of vitamin D, particularly salmon, mackerel, herring and eggs (Harel, Flanagan, Forcier, Harel, 2011). Cod liver oil and sundried mushrooms also naturally contain ~ 400-500 international units (IU) of vitamin D per serving (Holick, 2005). In the United States, foods such as milk, yogurt, cheese, orange juice, cereal, and bread have been fortified with vitamin D since the 1930s (Holick, 2005). For example, milk and orange juice typically contain 115-172 IU of vitamin D per serving, while the amount of vitamin D in cereal, bread, yogurt, and cheese ranges from 7 to 342 IU per serving depending on the brand (Gebhardt & Thomas, 2002). Vitamin D supplements in the form of vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol) are not only used to fortify food, but are also available as supplements in a variety of dosages.

The major source of vitamin D is sunlight, which when absorbed through the skin, provides ~ 50-90% of this valuable vitamin to the body (Lips, 2010). Unobstructed UV light is the only source of vitamin D, and without sun exposure, people are not able to obtain adequate concentrations of vitamin D from food (Holick et al., 2011, 2012). Research has shown that a healthy Caucasian male obtained 10,000-20,000 IU of vitamin D_2 when exposed to sunlight or a lamp emitting ultraviolet-B (UV-B) radiation for 5-15 minutes between the hours of 10 am and 3 pm on the arms and legs (Holick et al., 2011). This sunlight exposure is just long enough to cause a slight pinkness to the skin. While the body does store vitamin D in the tissue during the summer months, research involving healthy young Caucasian men from Nebraska demonstrated that only 80% of the vitamin D needed during the winter months came from this cutaneous accumulation (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). Thus, obtaining an adequate amount of sunlight exposure is important throughout the year.

The gold standard for determining an individual's vitamin D status is by obtaining a blood sample and measuring the amount of circulating vitamin D_3 (Holick, 2009). The 2011 IOM report established four vitamin D status levels for all ages: 1) deficiency < 30 nmol/L; 2) inadequacy 30-49 nmol/L; 3) sufficient 50-125 nmol/L; and 4) possibly harmful > 125 nmol/L (IOM, 2011; Looker et al., 2011). While members of the Endocrine Society agreed with these basic definitions, they maintained that a serum vitamin D concentration of ≥ 75 nmol/L was necessary for good health (Holick et al., 2011, 2012). Although the primary source of vitamin D is exposure to UV-B radiation from sunlight, prolonged sunlight exposure may damage the skin causing skin cancer or the eyes causing cataracts (American Cancer Society, 2017). Consequently, the American Cancer Society (2017) has warned the public to decrease their long-term sun exposure, wear protective clothing, and frequently apply sunscreen lotion. The public, heeding this advice, is now at risk for vitamin D insufficiency because sunscreen lotion also prevents

vitamin D absorption through the skin. Other controllable factors affecting serum vitamin D concentration are obesity and region of residence. Factors that cannot be controlled are skin pigmentation, age, and sex (Dong, Pollock, et al., 2010; Holick et al., 2011).

History. The history of vitamin D parallels that of rickets. When families migrated into the cities of northern Europe during the 1600s, the children began experiencing the crippling bone disease known as rickets. Affecting nearly 80% of the children living in cities of Europe and North America by the late 1800s, cod liver oil was prescribed to reduce the bone growth retardation and deformities caused by rickets (Holick, 2005). By 1900, scientists postulated that rickets may be due to a lack of an essential dietary factor – a notion that led to several landmark studies (Wolf, 2004). For example, Mellanby (1919) found that when puppies were given a bread and low-fat milk diet for 3-4 months, they exhibited the same signs and symptoms of rickets as children. After adding various types of food one at a time, Mellanby found that rickets was preventable in the puppies when buttermilk and/or cod liver oil was added to their diet. Hess and Unger (1921) discovered that rickets was a seasonal disease due to the seasonal differences in sunlight. Chick, Dalyell, Hume, Mackay, and Henderson-Smith (1922) found that rickets could be prevented and cured in children by giving them whole milk, cod liver oil, and sunshine. Various studies also demonstrated that not only did natural sunlight prevent and cure rickets, but artificial light also had the same effect when children were exposed to a quartz-mercury lamp for at least two months (Wolf, 2004). Lastly, McCollum et al. (1922) used cod liver oil in their experiments to isolate the essential dietary factor preventing rickets. These researchers found that the fat-soluble factor A (aka vitamin A) was really composed of two factors: vitamin A and a different one that prevented rickets. The water-soluble factor vitamin B had already been discovered, and since McCollum and his colleagues (1922) had found that vitamin C prevented and cured scurvy, the fat-soluble factor that prevented and cured rickets was named vitamin D.

By the 1950s, scientists made three important discoveries concerning vitamin D (Wolf, 2004). First, when food such as whole milk was irradiated, the food became antirachitic, which markedly reduced the incidence of rickets in children. Second, calciferol (vitamin D_2) was identified and that this substance was composed of ergosterol and cholecalciferol (vitamin D_3). Third, the process of the skin's ability to absorb sunlight was elucidated including how ergosterol changed into calciferol through the photochemical and thermal reactions (Wolf, 2004). Eventually, Holick et al. (1980) identified the specific molecular events that turned sunlight on the skin into the cholecalciferol (a metabolite of vitamin D_3) for utilization throughout the body.

A major study concerning the health of the American population is the National Health and Nutrition Examination Survey (NHANES). This survey has been conducted since the 1960s by the National Center for Health Statistics (CDC, 2017c). NHANES assesses the health and nutritional status of adults and children throughout the United States using interviews, physical examinations, and blood samples to provide vital health statistics. Researchers have used the NHANES data to determine disease prevalence, risk factors, and nutritional status as well as correlations with health promotion and disease prevention. The 2001-2006 NHANES data revealed that children older than 1 year of age were 67% sufficient in vitamin D, 24% inadequate, 8% insufficient, and 1% possibly had a harmful serum vitamin D concentration level (Looker et al., 2011). Researchers have also found a link between vitamin D and autoimmune diseases such as diabetes, multiple sclerosis, and Crohn's disease (Cantorna, Hayes, & Deluca, 1996; Cantorna, Munsick, Bemiss, Mahon, 2000; Mathieu, Waer, Laureys, Rutgeerts, & Bouillon, 1994; Samefors et al., 2017), dementia (Annweiler, 2016), and cardiovascular disease (Alkhatatbeh et al., 2017; Dong, Pollock, et al., 2010; Mousa et al., 2017; Perez-Hernandez et al., 2016).

Metabolism. Vitamin D exists in two forms: vitamin D_3 that is synthesized in the skin from UV sunlight, and Vitamin D_2 that is absorbed from the intestinal tract after eating fortified food and/or irradiated plants (Lips, 2006). As shown in Figure 1, UV light absorbed through the skin changes 7-hydrocholesterol to cholecalciferol (vitamin D_3) and/or food absorbed in the intestinal track changes ergocalciferol (vitamin D_2) to cholecalciferol. Once cholecalciferol is made, "it immediately begins to thermally equilibrate to vitamin D by a temperature-dependent process. This thermal equilibration takes approximately 10 hours to reach completion at body temperature" (Avioli & Krane, 1998, p. 128). Vitamin D is then hydroxylated twice: the first time in the liver where it is converted to calcifediol. Calcifediol then travels via its vitamin D binding protein through the circulation to the kidneys where it is hydroxylated a second time to calcitriol, the biologically active form of vitamin D (Holick, 2007).

The hydroxylation process of calcifediol and calcitriol in the liver and kidneys, respectively, enables these metabolites to enter the cell and bind to a calcium binding protein. Calcitriol production is promoted or diminished by calcium, phosphorus, and fibroblast growth factor in the blood (Holick, 2007). When increased calcium is needed during pregnancy, lactation or a child's growth spurt, sex steroids, prolactin, growth hormone, and insulin-like growth factor 1 trigger the kidneys to produce calcitriol (Holick, 2005). Calcitriol sustains calcium homeostasis by enhancing calcium absorption in the intestine and obtaining calcium from the skeletal bone (Holick, 2009). Calcitriol also decreases the synthesis and secretion of PTH, which increases the serum calcium concentration. Once calcium/phosphorus homeostasis is reached, calcitriol triggers its own elimination via the expression of 25-hydroxyvitamin d-24-hydroxylase that in turn catabolizes calcitriol into biologically inactive, water-soluble calcitroic acid (Holick, 2007).

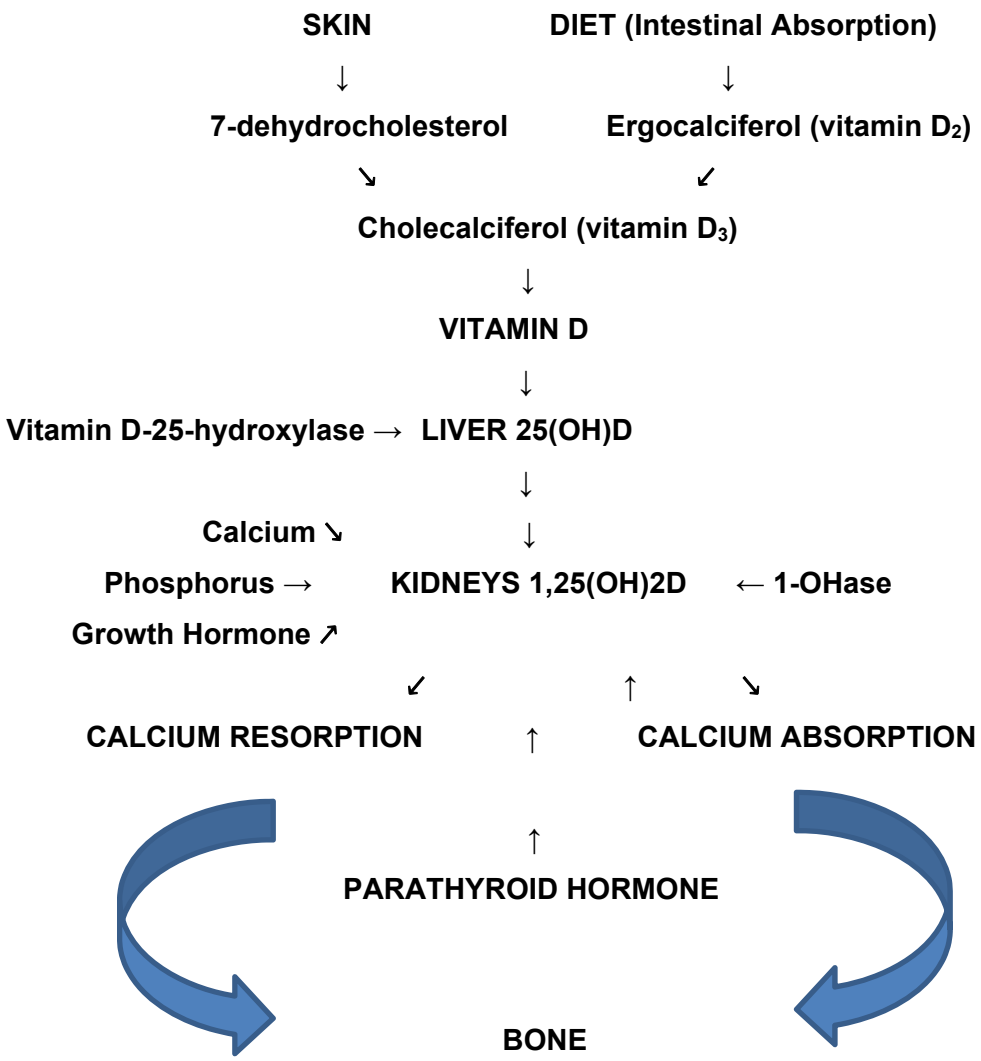

Figure 1. Vitamin D metabolism. Original artwork by R. Havens that was inspired by Holick, 2005, 2007; Lips, 2006.

For calcium absorption to occur, calcitriol must be present and vitamin D receptors must be intact (Lips, 2006). Inadequate/deficient vitamin D concentrations in the blood promotes inadequate calcium absorption; the resulting hypocalcemia triggers an increased secretion of PTH (Compher, Badellino, & Boullata, 2008). Increased PTH results in bone resorption and decrease in bone mineral density, osteoporosis, and fractures (Holick et al., 2011; Lips, 2006). Vitamin D receptors are found in all body

tissues since calcitriol regulates cell growth, activated T and B lymphocytes, and macrophages as well as activates insulin secretion and impedes renin production (Canela, Nemere, & Norman, 1988; Holick, 2005).

Serum concentrations and daily allowances. Presently, a serum vitamin D concentration of 40 nmol/L is considered sufficient to cover the requirements of approximately half the American population, and 50 nmol/L is sufficient to cover the requirements for 97.5% of the American population (IOM, 2011). Despite the disagreement as to what is the best level, most experts agree that a serum vitamin D concentration range of 50-100 nmol/L for adults is optimal for muscle strength, bone mineral density, and overall good health (Aloia, Talwar, Pollack, & Yeh, 2005; Holick et al., 2011, 2012; IOM, 2011; Looker et al., 2011).

The body uses ~ 3,000-5,000 IU of vitamin D per day (Heaney et al., 2003; Holick, 2005). The current recommended daily oral intake allowance of vitamin D is 400 IU/day for 0-12 months of age, 600 IU/day for 1-70 years of age, and 800 IU/day for people over 70 years of age (National Institutes of Health [NIH], 2017; IOM, 2011; Holick et al., 2011). These recommended dosages should maintain a sufficient serum vitamin D concentration in healthy individuals (Ross et al., 2011). Additionally, a few experts have suggested that supplemental vitamin D be administered in combination with calcium due to the notion that calcium maintains higher vitamin D concentrations (Heaney, 2008). However, McCullough et al. (2009) found that calcium did not impact serum vitamin D concentration when they compared participants receiving both calcium and vitamin D supplements versus those participants only receiving vitamin D supplement.

The NIH and IOM recommended daily allowances of vitamin D may not be high enough to raise a person's low serum vitamin D concentration to the sufficient status level (Arunabh, Pollack, Yeh, & Aloia, 2003; Heaney & Holick, 2011; Harel et al., 2011; Rajakumar, Fernstrom, Holick, Janosky, & Greenspan, 2008; Wortsman Matsuoka,

Chen, Lu, & Holick, 2000). Thus, members of the Endocrine Society recommended these oral dosages for individuals exhibiting a deficient or inadequate vitamin D status: at least 1,000 IU/day for children aged 0-18 years and 1,500-2,000 IU/day for individuals aged 19-70+ years (Holick et al., 2011, 2012). Despite the recommendations of the NIH, IOM, and Endocrine Society organizations, differences of opinion exist concerning the need for vitamin D supplementation. For example, the IOM (2011) report asserts that while vitamin D is necessary for skeletal health, there was not enough research evidence to support the theory that vitamin D influenced diseases such as cancer, cardiovascular disease, diabetes, and various autoimmune diseases. This report also determined that there was not a widespread health problem concerning vitamin D and did not see a need for routine screening (IOM, 2011; Rosen et al., 2012).

The Endocrine Society members disagreed with the IOM report, arguing that there was no research evidence supporting the supposition that a serum vitamin D concentration > 50 nmol/L only benefited bone health (Holick et al., 2012). Furthermore, the clinicians and researchers of the Endocrine Society asserted that vitamin D insufficiency was a widespread American problem, a person could not obtain all their daily requirements of vitamin D from sunshine and food, and cancer, cardiovascular disease, diabetes, and autoimmune diseases were all affected by an insufficient serum vitamin D concentration (Holick et al., 2012; NIH, 2017). Lastly, members of the Endocrine Society supported the establishment of 75 nmol/L as the sufficient level of serum vitamin D concentration (Vieth, 2011).

Toxicity. A century ago, vitamins were a food supplement and not considered to be toxic even in large doses. However, several deaths occurred in England and the United States in 1935 due to high doses of vitamin D given to treat arthritis (Leake, 1936). Consequently, researchers reconsidered the possible toxic effects of vitamin D as a condition caused by *toxisterol* (a byproduct of over-irradiated ergosterol) and

physicians became conservative when prescribing vitamin D (and all other vitamins) to their patients.

The IOM (2011) report identified the harmful vitamin D status as a serum concentration > 124 nmol/L. However, a few scientists disagreed with this parameter and suggested that vitamin D toxicity does not occur until a serum concentration of > 375 nmol/L (Holick, 2005; Kennel et al., 2010) and possibly as high as > 750 nmol/L (Jones, 2008). The 2011 Endocrine Society Clinical Practice Guideline stated that no scientific evidence has been found demonstrating negative effects with high serum vitamin D concentrations in adults and children except for individuals with chronic granuloma-forming disorders or lymphomas (Holick et al., 2011, 2012). Furthermore, Araki et al. (2011) and Koul et al. (2011) estimated the toxic dosage of vitamin D to be > 100,000 IU per day for at least a month.

Vitamin D toxicity is rare. For example, Araki et al. (2011) found only 76 documented cases of vitamin D toxicity in their review of literature; some of these cases were due to manufacturing and/or mislabeling errors of vitamin D dietary supplements, while others were due to the over-fortification of milk and nut oil with vitamin D. Lowe, Cusano, Binkley, Blaner, and Bilezikian (2011) also examined nine cases of vitamin D toxicity due to a mislabeled vitamin D supplement. However, Koul et al. (2011) described 10 cases of toxicity due to a physician over-prescribing of vitamin D supplements in the Kashmir valley of India, an area with a high prevalence of harmful vitamin D status. Lastly, Vieth, Pinto, Reen, and Wong (2002) described a case whereby family members may have been purposefully poisoned with vitamin D supplements. In this incident, two previously-healthy male family members presented with renal failure and the family's white table sugar contained extremely high concentrations of vitamin D.

Vitamin D intoxication (i.e., overdose, toxicity) cannot occur from UV irradiation of the skin; the amount of 7-dehydrocholesterol absorbed by the skin is limited and

additional UV irradiation converts to inactive products (Plum & DeLuca, 2010). While the underlying cause of intoxication has evolved from the early toxisterol theory, several theories currently exist as to how specific metabolic processes produce elevated levels of vitamin D metabolites (Jones, 2008; Koul et al., 2011; Lowe et al., 2011; Masterjohn, 2006; Vieth, 2004). The three dominant theories are (1) high plasma calcitriol increases cellular calcitriol; (2) high plasma calcitriol increases serum concentrations over the vitamin D binding protein binding capacity, causing free calcifediol to enter the cell affecting gene expression; and (3) high concentrations of vitamin D metabolites exceed the vitamin D binding protein binding capacity, causing release of free calcitriol that enters target cells (Jones, 2008). These theories involve a high serum concentration of a vitamin D metabolite contacting the vitamin D receptor in the nucleus of the target cell, which results in an exaggerated gene expression (Koul et al., 2011).

Another theory suggested by Masterjohn (2006) postulates that the metabolic process causing toxicity is a depletion of vitamin K. This author explained that vitamin D requires vitamin K in the protein expression of osteocalcin and matrix Gla (MGP). Osteocalcin's function concerns bone mineralization; MGP protects soft tissues from calcification. These proteins cannot be activated without vitamin K. Therefore, toxic vitamin D concentrations increase the expression of osteocalcin and MGP, which in turn uses more vitamin K for activation. However, the available reserves eventually become depleted due to a limited amount of vitamin K (Masterjohn, 2006). Regardless of the theories as to why vitamin D toxicity occurs, the result is hypercalcemia (Holick, 2005; Jones, 2008; Koul et al., 2011; Lowe et al., 2011; Masterjohn, 2006; Vieth, 2004).

Besides playing a pivotal role in vitamin D metabolism, calcium is a mineral needed for healthy bones, muscle contractibility, heart function, and blood clotting (Burchum & Rosenthal, 2016). Hypercalcemia is defined as a serum calcium concentration > 10.2 mg/dl (Dudenkov et al., 2015), which results from elevated

intestinal calcium absorption and bone resorption (Koul et al., 2011; Vieth, 2007). Patients experiencing vitamin D toxicity will present with early signs and symptoms of hypercalcemia such as weakness, fatigue, nausea, vomiting, anorexia, abdominal cramping, and constipation (Araki et al., 2011; Burchum & Rosenthal, 2016; Jones, 2008; Kennel et al., 2010; Lowe et al., 2011; Masterjohn, 2006; Veith, 1990, 2004, 2007). With time, calcium deposits in the soft tissue may occur resulting in damage to the heart, vessels, lungs, and kidneys and bone resorption causes osteoporosis. Persisting vitamin D toxicity in children may suppress growth for 6 months or longer. As the vitamin D toxicity progresses, the kidneys are affected resulting in polyuria, nocturia, and proteinuria and eventually neurologic symptoms occur such as seizures, confusion, and ataxia. Eventually, cardiac symptoms occur such as dysrhythmia and coma (Burchum & Rosenthal, 2016).

Young children are at higher risk for vitamin D intoxication due to accidental ingestion and extreme dosing by parents (Burchum & Rosenthal, 2016; Vieth, 1990). Elderly persons are also at risk for vitamin D intoxication, especially those individuals with decreased liver or kidney function, diminished estrogen levels, granuloma-forming diseases and malignancies, and diseases that over-produce calcitriol. Lastly, people who purposefully ingest excessive amounts of calcium are at risk for vitamin D intoxication (Plum & DeLuca, 2010; Vieth, 1990).

Vitamin D supplement sales doubled between 2009-10, making it the most popular supplement in the United States (Araki et al., 2011). Additionally, Araki et al. (2011) reported that over 50% of Americans use a dietary supplement; 60-70% of these individuals fail to report their supplement usage to their healthcare provider when asked what medications they are taking. Therefore, the potential for hypercalcemia is high and all etiologies should be considered even with the presence of an underlying pathology (Lowe et al., 2011). Since vitamin D toxicity may last weeks to months as calcifediol has

a half-life of 2-3 weeks, aggressive hydration, bisphosphonates, pamidronate, steroids, glucocorticoids, and diuretics are necessary treatments to prevent permanent damage (Araki et al., 2011; Burchum & Rosenthal, 2016). Presently, only vitamin D intoxication studies with animals (in vivo or vitro) have been performed due to ethical considerations of inducing intoxication in humans (Jones, 2008). Additional animal model studies are needed to elucidate the safe upper tolerable limits of vitamin D and determine the precise mechanisms of toxicity (Masterjohn, 2006; Vieth, 2004).

Characteristic factors acting as moderators may affect an individual's vitamin D status due to changing the strength and/or direction of the relationship between an independent and dependent variable (Mackinnon & Luecken, 2008). The moderators most frequently affecting an individual's vitamin D status are obesity, race, age, sex, and region of residence (Alemzadeh, Kichler, Babar, & Calhoun, 2008; Aloia et al., 2005; Compher et al., 2008; Dawson-Hughes, 2004; Dong, Pollock, et al., 2010; Goldner et al., 2008; Harel et al., 2011; Holick et al., 2011; Konradsen, Ag, Lindberg, Hexeberg, Jorde, 2008; Kumar, Muntner, Kaskel, Hailpern, & Melamed, 2009; Lips, 2010). The following sections describe each of these moderators and their relationship to vitamin D.

Vitamin D Moderators

Obesity. Obesity has become a global health concern. The World Health Organization (2017) reported that ~ 2.8 million people a year were dying from obesity-related diseases. Between 1980 and 2000, obesity rates in the United States doubled among adults and children, tripling among adolescents (CDC, 2017a). Consequently, many scientists such as Olshansky et al. (2005) have suggested that the high rate of obesity would soon decrease life expectancy among Americans. The diseases know to be obesity-related include coronary heart disease, stroke, hypertension, Type 2 diabetes, endometrial, breast, and colon cancers, as well as liver and gallbladder disease, sleep apnea, osteoarthritis, infertility, and mental health conditions (Bray, 2004;

CDC, 2017a). Furthermore, the CDC (2017a) estimates that obesity-related morbidity translates into medical costs in the United States of an estimated $147 billion total, which is $1,429 higher per person than the medical costs of a normal weight individual. Lastly, obesity has been linked to decreased employee work productivity and chronic absenteeism (CDC, 2017a).

The causes of obesity are complex; controllable elements are diet and physical activity while uncontrollable elements are developmental stage, age, and genes (Kopelman, 2000). Although 33.8% of the American population is reportedly obese, an estimated 44.1% of them are African Americans entailing 37.3% of the men and 49.6% of the women (Flegal, Carroll, Odgen, Curtin, 2010). Also, recent research findings suggest that low serum vitamin D concentrations promote increase adiposity and weight gain (McCarty & Thomas, 2003; Wood, 2008) due to percentage of body fat content being inversely related to serum vitamin D concentration (Alemzadeh et al., 2008; Arunabh et al., 2003; Compher et al., 2008; Dong, Pollock, et al., 2010; Goldner et al., 2008; Konradsen et al., 2008; Wortsman et al., 2000).

Measurement. Singla, Bardoloi, and Parkash (2010) define obesity as "…when energy intake, principally stored as triglycerides, exceeds energy expenditure" (p. 76). The current gold standard of measurement for obesity, BMI is calculated as weight (kilograms) divided by height (meters) squared; overweight adults are defined as having a BMI of 25.0-29.9 kg/m^2 and obese adults ≥ 30 kg/m^2 (Flegal et al., 2010). BMI is age- and sex-specific for children and adolescents, commonly expressed as a percentile: < 5^{th} percentile is considered underweight, 5^{th} percentile to < 85^{th} percentile is considered a healthy weight, ≥ 85^{th} percentile to < 95^{th} percentile is considered overweight, and ≥ 95^{th} percentile is considered obese (CDC, 2017a). In a cross-sectional study of comparing BMI in adolescents, Dong, Pollock, et al. (2010) found that visceral and subcutaneous abdominal adipose tissue (i.e., fat) were consistently inversely related to serum vitamin

D concentration – a relationship independent of fat site or distribution. Thus, these researchers concluded that BMI alone was an appropriate parameter to use when investigating the relationship between adiposity and vitamin D. Cheng et al. (2010) also found that low serum vitamin D concentrations were inversely correlated with adipose tissue in lean individuals with low to normal BMIs, especially visceral adiposity. However, these researchers suggested that visceral adiposity was more indicative of serum vitamin D concentration than BMI. Recently, Drincic, Armas, Van Diest, and Heaney (2012) suggested that body weight rather than BMI should be used to evaluate vitamin D status because BMI does not reflect a person's size variation in fat tissue.

Effects of obesity on metabolism. Adipose tissue (fat) was originally thought to be an energy storage organ. Adipose tissue is now considered an endocrine organ manufacturing adipokines that modulate metabolic processes in the body (Singla et al., 2010). The greater the amount of adipose tissue, the greater concentration of adipokines, which then increases the effect adipokines have on macronutrient metabolism. For example, adipokines affect energy and vascular homeostasis, glucose and lipid metabolism, immune response, and reproduction; these processes result in the complications of obesity such as diabetes and infertility (Singla et al., 2010).

***Vitamin D and obesity*.** Several theories currently exist explaining why percentage of body fat content is inversely related to the serum vitamin D concentration. The most popular theory proposes that sequestered vitamin D in adipose tissue reduces the amount of circulating calcifediol; vitamin D is fat-soluble and therefore easily incorporated into body fat (Lips, 2006). Other theories include 1) an increase in vitamin D catabolism is related to the 24-hydroxylase enzyme found in adipose tissue, 2) the liver is slower in synthesizing calcifediol in obese individuals, 3) obese individuals are less likely to participate in outdoor activities, thus reducing the opportunities for sunlight exposure and vitamin D absorption through the skin, 4) an inability of the skin to change

7-dehydrocholesterol to vitamin D_3, and 5) a negative feedback of high calcitriol and PTH on the hepatic synthesis of vitamin D (Earthman, Beckman, Masodkar, & Sibley, 2012; Wortsman et al., 2000). To further illuminate this issue, Drincic et al. (2012) investigated the effect of obesity on vitamin D in normal weight and obese women. Their findings led these researchers to suggest that the inverse relationship between obesity and vitamin D was due to volumetric dilution, whereby oral or cutaneous vitamin D was diluted in the large fat mass of the obese person.

Several studies have shown that people with higher BMIs require a higher intake of vitamin D supplementation than their lean counterparts to attain a sufficient serum vitamin D concentration (Arunabh et al., 2003; Dong, Stallmann-Jorgensen, et al., 2010; Drincic et al., 2012; Harel et al., 2011; Rajakumar et al., 2008; Wortsman et al., 2000). For example, Harel et al. (2011) used dosages as high as 50,000 IU/week (7,143 IU/day) and still had 72% of obese Caucasian and African American adolescents fail to reach sufficient serum vitamin D concentrations after repeated treatment. Dong, Pollock, et al. (2010) studied Caucasian and African American youth over four consecutive seasons in Georgia; they not only found an inverse relationship between obesity and serum vitamin D concentration, but also a positive relationship between serum vitamin D concentration and increased physical activity. In a different 16-week randomized clinical trial involving daily vitamin D_3 supplementation in African American youths, Dong, Stallmann-Jorgensen, et al. (2010) showed that the treatment group (2,000 IU) reached significantly higher calcifediol concentrations at 8 and 16 weeks compared to the control group (400 IU). Moreover, this study also demonstrated that total body fat mass at baseline was significantly inversely associated with calcifediol concentrations in response to the 2,000 IU supplement over time. Alemzadeh et al. (2008) also found an inverse relationship between obesity and vitamin D concentration in obese, Caucasian, Hispanic, and African American children and adolescents living in a high latitude

environment. Thus, the research findings over the past 15 years support the notion that the current recommended daily allowance is not high enough to achieve a sufficient serum vitamin D concentration in an obese individual.

To combat obesity and reduce morbidity and mortality, individuals are turning to gastric restrictive and malabsorptive surgeries. In their literature review, Compher et al. (2008) found that vitamin D deficiency was common in obese individuals before surgery and that serum vitamin D concentrations did not markedly improve after surgery. Thus, these authors concluded that significant weight loss did not improve the serum vitamin D concentration and vitamin D concentration may be further reduced post-surgery due to malabsorption issues.

While the recommended vitamin D supplement dosages may be enough for normal-weight individuals to acquire a sufficient serum vitamin D concentration, the recommended dosages may not be the amounts needed for overweight and/or obese individuals (Arunabh et al., 2003; Dong, Stallmann-Jorgensen, et al., 2010; Drincic et al., 2012; Harel et al., 2011; Rajakumar et al., 2008). Furthermore, researchers and clinicians do not fully understand the mechanisms of vitamin D metabolism in obese and overweight individuals (Earthman et al., 2012; Drincic et al., 2012; Wortsman et al., 2000) or why the serum vitamin D concentration does not improve when an obese individual approaches normal weight (Compher et al., 2008). Thus, the present secondary analysis included overweight and obese individuals to investigate these gaps in vitamin D research and knowledge.

Race. Between 2001 and 2006, approximately 51% more African Americans than Caucasian Americans exhibited deficient or inadequate serum vitamin D concentrations (Looker et al., 2011). Forrest and Stuhldreher (2011) analyzed the 2005-20006 NHANES data and found that while vitamin D insufficiency was common in American adults, this condition existed more in minorities: 82.1% of African American

adults and 69.2% of Hispanic adults were vitamin D deficient compared to 30.9% of Caucasian adults. Dawson-Hughes (2004) surmised that African American and Caucasian adults had the same capability to absorb and synthesize vitamin D through the skin, but the synthesis process was less efficient in an African American person. Melanin (the pigment giving human skin its color) acts as a highly effective sun screen that minimizes vitamin D absorption and production in the skin. Individuals with darker skin color, such as many African Americans, have more melanin in their skin compared to light-skinned individuals such as many Caucasian Americans. Therefore, African American individuals with darker skin require up to 5-10 times longer sun exposure than do light-skinned individuals to produce adequate vitamin D_3 in their skin (Holick, 2005).

Park and Johnson (2005) found that race and ethnic origin determined skin pigmentation, which in turn affected one's vitamin D status, when they compared Caucasian, Hispanic, and African American adults. Their findings showed that inadequate vitamin D status was two times greater in Hispanic than Caucasian adults, and four times greater in African American adults. When these researchers examined sun-protective behaviors and sun exposure via occupation, they found that Hispanic adults (more than Caucasian adults) worked in high sun-exposure jobs such as farming, forestry, and fishing industries without wearing sunscreen. Furthermore, Park and Johnson (2005) found that Hispanic and African American adults had a lower vitamin D dietary intake than Caucasian adults. However, this finding may be due to 75% of African American and 60% of Hispanic adults exhibiting lactose maldigestion that prevents them from ingesting fortified dairy products. Lastly, these researchers found that Hispanic adults were the least likely to take vitamin D supplements compared to Caucasian and African American adults.

When investigating adolescents of different races, Harel et al. (2011) had different results: no difference in baseline serum vitamin D concentration was found

between Caucasian, Hispanic or African American adolescents. However, these researchers found a significant difference in treatment response among the three groups of adolescents: serum vitamin D concentrations significantly increased to the sufficient status in Caucasian adolescents after an initial treatment of 50,000 IU once a week for 6-8 weeks compared to their Hispanic and African American counterparts. Dong, Pollock, et al. (2010) found significantly lower serum vitamin D concentrations in African American youth compared to Caucasian youth in each of the four seasons over one year. Lastly, Alemzadeh et al. (2008) found that even with children of various races exhibiting no difference in BMI, lower serum vitamin D concentrations were more prevalent in Hispanic and African American children as compared with Caucasian children. Also, Caucasian children reported a higher intake of vitamin D and calcium compared to Hispanic and African American children.

In their meta-analysis investigating the link between insufficient serum vitamin D concentration and chronic disease, Grant and Peiris (2010) concluded that sufficient vitamin D would decrease the risk of chronic diseases in the African American population. These authors suggested that vitamin D deficiencies were pandemic in the African American population and that larger dosages than recommended in either the 2011 IOM report or 2011 Endocrine Society Clinical Guideline were needed to increase serum vitamin D concentrations to sufficient status. Also, Harris et al. (2011) found that taking a monthly vitamin D supplementation dosage of 60,000 IU (i.e., 2,000 IU/day) for four months effectively improved vascular endothelial function in African American adults, which potentially could improve cardiovascular function. Lastly, Forrest and Stuhldreher (2011) found significant associations between race and characteristics such as skin pigmentation, a need for long duration of sun exposure, dietary patterns with low dietary vitamin D intake, low socioeconomic status, poor access to medical services, and low education status.

When investigating healthy postmenopausal African American women over a three-year period, Talwar, Aloia, Pollack, and Yeh (2007) found that these women needed a vitamin D supplement dosage of 2,800 IU/day to obtain a serum vitamin D concentration > 75 nmol/L when their baseline serum vitamin D concentration was > 45 nmol/L; women with a baseline serum vitamin D concentration < 45 nmol/L needed a vitamin D dosage of 4,000 IU/day. Similarly, Ng et al. (2014) found African American adults needed 1,640 IU/day to achieve 50 nmol/L and 4,000 IU/day to reach ≥ 75 nmol/L, which has been the suggested recommendation for reducing risk of cancer and other chronic diseases.

As previously mentioned, African American adults have a high rate of obesity that combined with dark pigmented skin, poor intake of vitamin D-rich foods and supplements, increases the risk of vitamin D insufficiency in this population. Since the literature presented in this review supports the notion that all individuals receive the same recommended daily allowance without regard to race, the present secondary analysis included African American individuals to investigate these gaps in the current vitamin D research.

Age. ***Infants, children, adolescents.*** Physical changes over the lifespan influence the vitamin D status of an individual. In utero, vitamin D crosses the placental barrier providing a sufficient amount to the fetus. If the mother has maintained a sufficient vitamin D status during pregnancy, then the infant remains vitamin D sufficient for 2-3 weeks after birth (Holick et al., 2011). If the mother is deficient or insufficient in vitamin D during pregnancy, the infant is at great risk for rickets and difficulty standing or walking during growth into childhood. Thus, women are encouraged to take vitamin D supplements during pregnancy and breastfeeding, particularly if their infant has dark-skinned pigmentation (Holick et al., 2011).

Historically, children received most of their vitamin D from spending time in the sun and drinking milk fortified with vitamin D (Holick et al., 2011). Today, children sustain a higher risk of vitamin D deficiency due to drinking less fortified milk and not spending enough time playing outdoors. Children may not play outdoors due to playing indoors with computerized video games or lacking a safe outdoor playground. Mothers also now have their children apply sunscreen lotion and/or wear protective clothing when playing outdoors, which limits the positive effects of the sun such as vitamin D absorption (Holick et al., 2011). Lastly, recent research has demonstrated a link between the tripling of adolescent obesity in the United States and a high prevalence of insufficient serum vitamin D concentration in adolescent children (Arunabh et al., 2003; Compher et al., 2008; Dong, Pollock, et al., 2010; Goldner et al., 2008; Harel et al., 2011).

When examining the 2001-2004 NHANES data, Kumar et al. (2009) found that vitamin D insufficiency was common in children aged 1-21 years and associated with negative cardiovascular risks due to hypertension and low HDL cholesterol levels. These researchers also found that vitamin D insufficiency in this age group was characterized by older children, girls, African Americans, Hispanic Americans, low economic status, and those born outside the United States and who spent a great amount of time indoors with playing video games, watching television, and using computers.

Children and adolescents often experience periodic growth spurts between the ages of 9 and 18 years, which increases the demand for calcium and phosphorus for skeletal mineralization. In response to this growth spurt, an increase in the metabolism of 25(OH)D to $1,25(OH)_2D$ occurs that results in an increase of dietary calcium and phosphorus absorption by the intestine. However, no scientific evidence currently exists to suggest that this age group needs additional vitamin D for enhancing this mineralization process (Holick et al., 2011).

Adults. The adult populations aged 19-50 years are typically outdoors less due to their employment and/or attention to protecting their skin from harmful sun rays to prevent skin cancer. Consequently, adults in this age group decrease their chances of obtaining vitamin D per the sun and their skin. To date, scientific research is lacking regarding the relationship of vitamin D needs and health outcomes in this population (Holick et al., 2011).

Elderly. The older adults > 51 years old have the same lack of sunlight exposure as discussed with the other age groups. However, the aging individual experiences a progressive decline in their ability to produce cholecalciferol that is associated with cancer, heart disease, chronic illness, and skeletal disease (Holick et al., 2011). Furthermore, vitamin D insufficiency in the elderly promotes the development of osteomalacia that causes generalized bone and muscle pain, weakness, increased unsteady mobilization, frequent falls and bone fractures. Aloia et al. (2005) conducted a randomized, placebo-controlled, double blind study of postmenopausal African American women given either a placebo or 800 IU of vitamin D over 3 years. These researchers found no observed effect on bone loss or bone turnover markers in these women, despite also given calcium supplements as a part of this study.

Telomeres are the caps at the end of the DNA that protect the chromosomes; thus, telomeres are important structures in the aging process. Without telomeres, chromosomes become damaged and may malfunction (Grossman & Porth, 2014). Zhu et al. (2012) investigated the relationship between aging and vitamin D, finding that vitamin D supplementation significantly increased telomerase activity in overweight/obese African American adults. These researchers concluded that vitamin D supplementation improved telomere maintenance, prevented cell senescence, and counteracted obesity-induced acceleration of cellular aging. Later, Sohl et al. (2014) developed a profile tool for identifying those elderly people living in the Netherlands at

risk for vitamin D insufficiency. These researchers found 13 easily-assessable predictors that accurately predicted inadequate and deficient serum vitamin D concentrations of < 50 nmol/L and/or < 30 nmol/L, respectively. These predictors included older age, sex (female), higher BMI, smoking, less alcohol use, season (winter), no vitamin supplement use, no bicycling, no sporting, no gardening, medication use, poor appetite, and not having a partner. In addition to these predictors, those elderly individuals with a serum vitamin D concentration < 30 nmol/L experienced limitations in the use of transportation and inability to remember the year.

To date, scientific knowledge is lacking regarding the relationship of vitamin D needs in specific age groups such as the elderly (Holick et al., 2011). Therefore, the present secondary analysis included subjects 13-45 years of age to investigate the age-related gap in vitamin D research. The secondary analysis did not include anyone > 46 years since the original study did not due to the confounding influence of age. However, future vitamin D research is recommended with an aged population to determine the effect of age and age-related comorbidities on vitamin D and its supplementation.

Sex. Worldwide, men generally have a higher serum vitamin D concentration than women (Lips, 2010). In the United States, approximately 6% more women than men had inadequate or deficient serum vitamin D concentration (Looker et al., 2011). Inadequate vitamin D concentration has been shown to promote colon, prostrate, and/or esophageal cancers in men and breast cancer in women (Harel et al., 2011). When examining the 2001-04 NHANES data, Kumar et al. (2009) found that adolescent girls exhibited a higher occurrence of vitamin D insufficiency and consumed less vitamin D from food compared to adolescent boys. These findings were verified by Dong, Pollock, et al. (2010) who found that boys had higher serum vitamin D concentrations than girls during all four seasons within one year. This sex difference in serum vitamin D concentration is not well understood, especially as the sexes are also different in how

they develop diseases related to vitamin D deficiency (Harel et al., 2011). Therefore, the present secondary analysis included both male and female participants to investigate this knowledge gap with vitamin D and explore the implications of treatment with supplemental vitamin D.

Region of residence. A popular assumption is that serum vitamin D concentrations are lower in people living in higher latitude geographical regions such as the northern regions of the United States and northern European countries (Lips, 2010; Park & Johnson, 2005). However, a literature review by Lips (2010) did not find consistent evidence supporting this assumption. Rather, this investigator concluded that while a country may be in a low latitude geographical region, cultural habits precluding sun exposure or skin types preventing them from acquiring an abundance of sunshine to absorb into their skin greatly fostered vitamin D insufficiency. For example, vitamin D insufficiency is common in the Middle East where people stay out of the hot sun and wear an abundance of clothing to stay cool. In the African continent, people living in the low latitudes have darker skin pigmentation and stay out of the hot sun. Thus, wearing clothing, having dark skin, and staying out of the hot sun prevents these populations from producing sufficient vitamin D.

Hispanic Americans are the largest minority group in the United States with 50.5 million people comprising 16.3% of the total population (Ennis, Ríos-Vargas, & Albert, 2011). While most Hispanic Americans live in the western and southern regions of the United States, this population also exhibits major vitamin D insufficiency (Park & Johnson, 2005; Ennis et al., 2011). Both Park and Johnson (2005) and Dong, Pollock, et al. (2010) found that living in southern Florida and eastern Georgia did not guarantee that Hispanic or African American individuals could maintain a sufficient serum vitamin D concentration, regardless of the time of year. Furthermore, Parikh et al. (2012) found a

relationship between vitamin D concentration and cardiometabolic risk in adolescent individuals despite living in a region with sunshine all year long.

By the mid-1900s, research evidence showed that people living in the northern latitudes of the United States had higher morbidity and mortality rates due to cancer, autoimmune disease, hypertension and/or cardiovascular disease compared to people living in the southern latitudes (Apperley, 1941; Garland, Garland, Gorham, & Young, 1990; Grant, 2002; Hanchette & Schwartz, 1992). Current research evidence now suggests that these conditions are influenced by vitamin D insufficiency (Holick, 2005). The theoretical supposition is that vitamin D receptors are found in most tissues and cells of the body and thus can produce calcitriol. Exposure to UV light or ingestion of vitamin D in food or supplements increases the production of calcifediol, which is then utilized by the cells to produce calcitriol. The calcitriol keeps the cells from over-proliferating and becoming malignant, thus explaining the relationship between vitamin D, region of residency, and chronic disease (Chen & Holick, 2003; Garland et al., 1985; Garland et al., 1990; Holick, 2004, 2005; Krause et al., 1998; Mathieu & Adorini, 2002).

Besides geographical latitude, Lips (2010) reported that socioeconomic status exerted a major influence on one's vitamin D status. Populations in the Middle East and India have the lowest serum vitamin D concentrations; the populations of Mongolia, India, and Ethiopia have a high incidence of rickets. Also, Lips (2010) found that elderly individuals, especially those who were institutionalized, had higher rates of vitamin D insufficiency. Exploring the socioeconomic and geographical residency moderators on serum vitamin D concentration was beyond the scope of the original study and thus the present secondary analysis too. To control for these moderators and their possible confounding influence, all participant data used for the present secondary analysis originated from African Americans living in the same southeastern region of the United States with a similar socioeconomic status.

Vitamin D and the Immune System

Until the 1980s, the scientific community considered the major role of vitamin D was for calcium, phosphorus and bone metabolism (Deluca & Cantorna, 2001). This classic perception changed with the identification of vitamin D receptors and 1,25-(OH)2D3 on cells other than those associated with bone, calcium, and phosphorus metabolism (Baeke et al., 2008). For example, vitamin D receptors were found in promyelocytes, monocytes, lymphocytes, macrophages, and thymus tissue suggesting a relationship with the immune system and cancers characterized by inflammation (Deluca & Cantorna, 2001; Tanaka et al., 1982; van Etten & Mathieu, 2005). After reviewing the literature, Bikle (2009) concluded that vitamin D also had a role in the inhibition of adaptive immunity and promotion of innate immunity. The following discussion delineates the current understanding of the anatomy, physiology, and pathophysiology of the immune system as well as its relationship with vitamin D.

Anatomy and physiology. The immune system consists of the cells, tissues, and organs that coordinate together to protect the body from harmful foreign pathogens (Grossman & Porth, 2014). The first line of immunological defense (i.e., innate immunity) consists of the epithelial cells of the skin and the mucosa lining the gastrointestinal and respiratory tracts, along with the associated flora, tears, sebaceous glands, gastric acid, and pancreatic enzymes (Grossman & Porth, 2014). These organs act as barriers to pathogens as well as trap, destroy, and eliminate them. The innate immune system may initiate the inflammatory process if prolonged, activating the second line of defense (i.e., adaptive immunity) to further protect the internal environment of the body.

Defending against extracellular and intracellular foreign pathogens, the internal defense consists of the white blood cells (leukocytes) that are manufactured in the bone marrow and matured in the lymphoid tissue of the thymus, spleen, and lymph nodes (Grossman & Porth, 2014). The differentiation process splits the leukocytes into

lymphocytes, which further differentiates into T-cells, B-cells, phagocytes, granulocytes, and dendritic cells. T-cells then migrate to the thymus where they mature and differentiate into the helper cells (CD4), cytotoxic cells (CD8), suppressor T-cells, and/or memory T-cells (Grossman & Porth, 2014). T-cells are programmed to recognize and remember the self cells of the body and invasive non-self cells that they encounter while maturing in the thymus (Barron, 2011). Activation of helper CD4 T-cells marks the beginning of the adaptive immunity process by identifying invading pathogens, which activates B-cells, other T-cells, CD8 cells and macrophages to attack the foreign invaders. Thus, CD4 cells are considered the “master regulators of the immune system” (Grossman & Porth, 2014, p. 298).

Cytokines are the chemical protein messengers that mediate interaction between the immune cells and body tissues during the innate immune defense process, while also stimulating such processes as inflammation (Grossman & Porth, 2014). Interleukins are a group of cytokines that promote the development and differentiation of T-, B-, and hematopoietic cells. Synthesized from lymphocytes, interleukins adhere to various target cells that trigger immunomodulatory functions including cell proliferation, maturation, migration, adhesion, differentiation and activation of immune processes (Grossman & Porth, 2014). Interleukin-6 (IL-6) is the major cytokine that has several functions including regulating “the immune response, hematopoiesis, the acute phase response, and inflammation” (Ishihara & Hirano, 2002, p. 357). The normal IL-6 serum concentration in healthy individuals without evidence of chronic disease is ~ 5 picograms/milliliter (pg/ml; Faulkner et al., 2014; Wang et al., 2017).

Manufactured predominately by T lymphocytes, IL-6 causes B lymphocytes to increase antibody production that in turn increases body temperature resulting in a fever. Besides promoting T-cell differentiation, IL-6 is also essential for CD4 function. The presence of IL-6 at sites of acute and chronic inflammation stimulates a transcriptional

inflammatory response (Grossman & Porth, 2014). Thus, IL-6 has been associated with inflammatory diseases such as diabetes, rheumatoid arthritis, chronic inflammatory proliferative disease, osteoporosis, psoriasis, and multiple myeloma (Deluca & Cantorna, 2001; Ishihara & Hirano, 2002). The National Center for Biotechnology Information (NCBI, 2017) maintains a website detailing the genetic code of IL-6, since the synthetic version of IL-6 is now used as a biological response modifier to boost the immune system when treating various cancers and autoimmune diseases (Liu et al., 2017).

Pathophysiology. The immune system protects an individual from foreign pathogens, microorganisms, toxins, and proliferation of cancer cells, while also aiding with wound healing (Grossman & Porth, 2014). Immunodeficiency diseases, allergy or hypersensitivity reactions, and autoimmune diseases all stem from immune malfunctions. These pathologies all promote the inflammatory process and stimulate IL-6 production. Malfunction of the immune system may occur at any stage during the protective process, and although the detailed the pathophysiological processes are unknown, treatment frequently focuses on symptom control. The immune system is known to become less effective as one ages such that immunological-associated inflammatory diseases are becoming more common with the lengthening life expectancy (Grossman & Porth, 2014). Consequently, additional research is needed to reveal the specific pathophysiological mechanisms of the malfunctioning immune system for improving treatment options and patient outcomes.

Vitamin D and immune system link. The link between vitamin D and the immune system was initially found with the discovery of vitamin D receptors in the immune system (Adams & Hewison, 2008). For example, Yang, Smith, Prahl, Luo, and Deluca (1993) found that vitamin D deficient mice showed an impaired immune reaction to pathogen exposure. Later, Deluca and Cantorna (2001) found that vitamin D receptors were highly concentrated on CD8 cells in the thymus of their animal models.

Eventually, high doses of 1,25(OH)2D3 were found to cause this impaired immune response suggesting the role of vitamin D in autoimmune diseases and organ transplantation (van Etten & Mathieu, 2005; Yang, Smith, & DeLuca, 1993; Yang, Smith, Prahl, et al., 1993;). Canning, Grotenhuis, de Wit, Ruwhof & Drexhage (2001) found that (in vitro) 1,25(OH)2D3 inhibited the maturation of dendritic cells, which are part of the innate immunity of the respiratory and gastrointestinal tract. When mature, dendritic cells initiate the adaptive immune response and thus T-cell proliferation (Grossman & Porth, 2014). Müller and Bendtzen (1996) found literature evidence that 1,25(OH)2D3 inhibited T-cell proliferation resulting in immunosuppression and inhibition of IL-6 production, which was later verified by Canning et al. (2001). This important finding led to the current notion that IL-6 encourages the inflammation process and possibly is an indicator of autoimmune diseases, particularly since suppression of IL-6 is known to suppress inflammation and autoimmune disease (Grossman & Porth, 2014).

In their review of the literature involving in vivo experimentation, Deluca and Cantorna (2001) concluded that hypercalcemia, when combined with the active form of vitamin D, was required to sufficiently suppress the effects of autoimmune disease such as encephalomyelitis, rheumatoid arthritis, lupus, Type 1 diabetes, and inflammatory bowel disease. Furthermore, these researchers surmised that dosing with 1,25(OH)2D3 supplementation may prevent most of these diseases (except rheumatoid arthritis) if combined with a high calcium diet. Deluca and Cantorna (2001) postulated that “It was not clear whether the suppression of delayed type hypersensitivity response seen during vitamin D deficiency is due to the secondary effect of hypocalcemia or a direct action of vitamin D on certain components of the immune system” (p. 2584).

Schleithoff et al. (2006) also suggested that vitamin D supplementation could be utilized to suppress the inflammatory process after conducting a double-blind, randomized, placebo-controlled clinical trial on 93 individuals with congestive heart

failure. To determine the effect of vitamin D supplementation on survival rates and associated serum concentrations, these researchers designed their study with an intervention group receiving 2,000 IU/day of vitamin D with 500 mg/day of calcium, and a control group receiving a placebo with 500 mg/day of calcium for 9 months. Serum vitamin D concentrations were obtained at the baseline and 9-month time points with survival rates assessed at 15 months. Both groups of these heart failure participants had insufficient serum vitamin D concentrations at baseline as well as high levels of tumor necrosis factor-alpha (TNFα), a pro-inflammatory cytokine. The intervention group also had low levels of interleukin-10, an anti-inflammatory cytokine. After vitamin D supplementation, the serum vitamin D concentrations significantly increased for the intervention group compared to the control group (26.8 ng/mL and 3.6 ng/mL, respectively), and the serum interleukin-10 level increased 43% for the intervention group (the control group had no significant change). Also, the TNFα level remained constant for the intervention group after 9 months, while the control group had a 12% increase in this cytokine. The survival rate did not differ significantly between the groups.

The study by Pittas, Harris, Stark and Dawson-Hughes (2007) produced different results compared to previous studies examining vitamin D and inflammation biomarkers. These researchers conducted a double-blind, randomized clinical trial with 314 healthy Caucasian participants, aged ≥ 65 years, to determine the effects of a calcium/vitamin D combination supplementation on serum glucose and inflammation markers. The intervention group received 700 IU/day calcium and 500 mg/day vitamin D for 3 years; IL-6 and other biomarkers were measured at baseline and 3 years. No statistical difference was found in serum IL-6 concentrations between the intervention and control groups after 3 years. However, Shea et al. (2008) reported that the relationship between vitamin K, vitamin D, and inflammation was enhanced with vitamin D supplementation when participants suffered from chronic diseases. Thus, the findings from the Pittas et

al. (2007) study may have been due to using healthy individuals without chronic diseases rather than unhealthy individuals with chronic inflammation-related diseases.

Although obesity is not considered a chronic disease per se, it has been linked to coronary artery disease, stroke, hypertension, Type 2 diabetes, sleep apnea, osteoarthritis, and infertility as well as endometrial, breast and colon cancer, liver and gallbladder disease, and mental illness (CDC, 2017a). Obesity has also been associated with pathological processes that involve the inflammatory response (Doumatey et al., 2010; Hevener, Febbraio, & Stock Conference Working Group, 2009; Petty et al., 2010; Tilg & Moschen, 2006; Zanetti, Harris, & Dawson-Hughes, 2013). In addition, Petty et al. (2010) found that serum IL-6 concentrations in overweight/obese adolescents differed between the sexes: females had significantly higher serum IL-6 concentrations than their male counterparts.

In their literature review, Zanetti et al. (2013) found conflicting results among the seven studies examined concerning the relationship of vitamin D status and inflammatory biomarkers in adults without illness or injury. Universally, these researchers found a significant inverse correlation between serum 25(OH)D concentrations and inflammatory biomarkers, particularly with individuals with a deficient serum 25(OH)D concentration < 51 nmol/L. However, two clinical trials showed no significant change in serum inflammatory biomarkers after a vitamin D supplementation of 200 to 5,714 IU/day. Zanetti et al. (2013) attributed this finding to the fact that neither clinical trial included individuals with serum 25(OH)D concentrations < 51 nmol/L or a high level of inflammation markers. Therefore, the link between vitamin D and the immune system still needs to be more clearly delineated, albeit vitamin D has been shown to suppress pro-inflammatory cytokines such as IL-6 and increase the anti-inflammatory cytokines. Also, research evidence suggests that vitamin D may be an adjunct treatment for autoimmune diseases whereby inflammation is a characteristic.

Therefore, this secondary analysis research project included IL-6 as a variable to investigate this gap in vitamin D research.

Part 2: Theoretical Framework

Vulnerable Populations Conceptual Model

For the optimum health of all clients, nurse scientists rely on theories to guide their healthcare research. These theories should describe the nursing phenomenon, define the relationships among the variables, offer a framework for interventions, and predict outcomes (Polit & Beck, 2017). A theoretical framework provides the basic structure to organize the concepts focused on specific questions (Meleis, 2018). The *Vulnerable Populations Conceptual Model* (VPCM; Flaskerud & Winslow, 1998) was used as the theoretical framework for this secondary analysis since the original D-SUNNY study (Dong, 2012) used the vulnerable populations of women, children, and African American adults for its subjects.

In their seminal publication, Flaskerud and Winslow (1998) conceptualized the relationship(s) between resource availability, relative risk, and health status of vulnerable populations. These researchers defined vulnerable populations as “social groups who have an increased relative risk or susceptibility to adverse health outcomes” (p. 69), resource availability as “the availability of socioeconomic and environmental resources” (p. 69), and relative risk as “the ratio of the risk of poor health among groups who do not receive resources and are exposed to risk factors compared with those groups who do receive resources and are not exposed to these risk factors” (p. 69). Health status referred to the disease prevalence, morbidity and mortality rates within the community. This community health perspective views the community as responsible for the health and wellbeing of its citizens, ensuring that everyone receives the necessary resources to maintain their health. Flaskerud and Winslow (1998) considered people associated with vulnerability as “the poor persons subjected to discrimination, intolerance, subordination

and stigma; and those who are politically marginalized, disenfranchised, and denied human rights" (p. 69). Thus, vulnerable populations defined by the VPCM included women, children, racial and ethnic groups (e.g., African Americans), immigrants, gay men and women, the homeless, and the elderly.

Flaskerud and Winslow (1998) further described socioeconomic resources as human capital such as the person's income, employment, education, and housing as well as their social connectedness. Marginalized persons and/or those lacking family support have little socioeconomic resources available to them. Social status may also influence resource availability since people in a lower social status have little power or control over the distribution of resources. Lastly, a person's environment may affect access to, and quality of, health care resources (e.g., lack of public transportation in a rural community).

Flaskerud and Winslow (1998) classified relative risk factors into three groups: (1) lifestyle, behaviors, and choices such as dietary behaviors, weight, physical activity, sexual behaviors, unintended pregnancy, tobacco use, and/or alcohol/drug use; (2) lack of using health promotion services such as prevention screening and immunizations; and (3) injuries resulting from failure to use safety belts while driving, drinking alcohol and driving, and firearm use. The CDC routinely tracks these risk factors and supports the link between them and lack of available resources across all age groups, races, income brackets, and genders (Flaskerud & Winslow, 1998).

Health status concerns age- and gender-specific morbidity and mortality. Morbidity refers to the incidence of illness caused by disease, while mortality denotes the proportion of deaths in population (CDC, 2017b). Flaskerud and Winslow (1998) described the relationship between health status, risk factors, and resource availability as interactive and complex. For example, morbidity may promote development of additional risk factors such as when decreased exercise leads to obesity then diabetes

and heart disease. Poverty, discrimination, subordination, and stigmatization also influence those populations exposed to unhealthy risk factors. Higher mortality rates occur in populations who lack societal and environmental resources due to an increase in violence, homicide and injury, particularly in men, African Americans, the poor, American Indians, Puerto Ricans, Hawaiians, Central and South Americans (Flaskerud & Winslow, 1998).

The VPCM further defines the relationship between available resources and relative risk factors as an inverse one; if little resources are available, then the risk of disease, morbidity and mortality increases that in turn diminishes health status (Flaskerud & Winslow, 1998). A feedback loop further exacerbates these adverse relationships. For example, the high prevalence of poor nutrition, obesity, and lack of exercise exists in adult minority females living in poverty – a relationship that is associated with use of drugs, alcohol, cigarettes, and drinking while driving.

Nursing research and practice could intervene and break the adverse feedback loop. Nurses have the potential to influence resource availability, relative risk, and health status directly or indirectly via their healthcare interventions (Flaskerud & Winslow, 1998). The VPCM allows nursing community intervention to intercede at any stage and between any interaction. Primary prevention activities are most appropriate at the interaction between social and environmental resources to minimize risk factors. Secondary prevention activities would be most beneficial in the link between risk factors and disease, and tertiary prevention activities should focus on chronic illness. All concentrations of prevention should be considered when focusing on changing/improving public policy (Flaskerud & Winslow, 1998).

Figure 2 illustrates the relationship between resource availability, relative risk, and health status associated with vitamin D insufficiency. If resources are not available for obtaining sufficient vitamin D, then the relative risk factors may further decrease

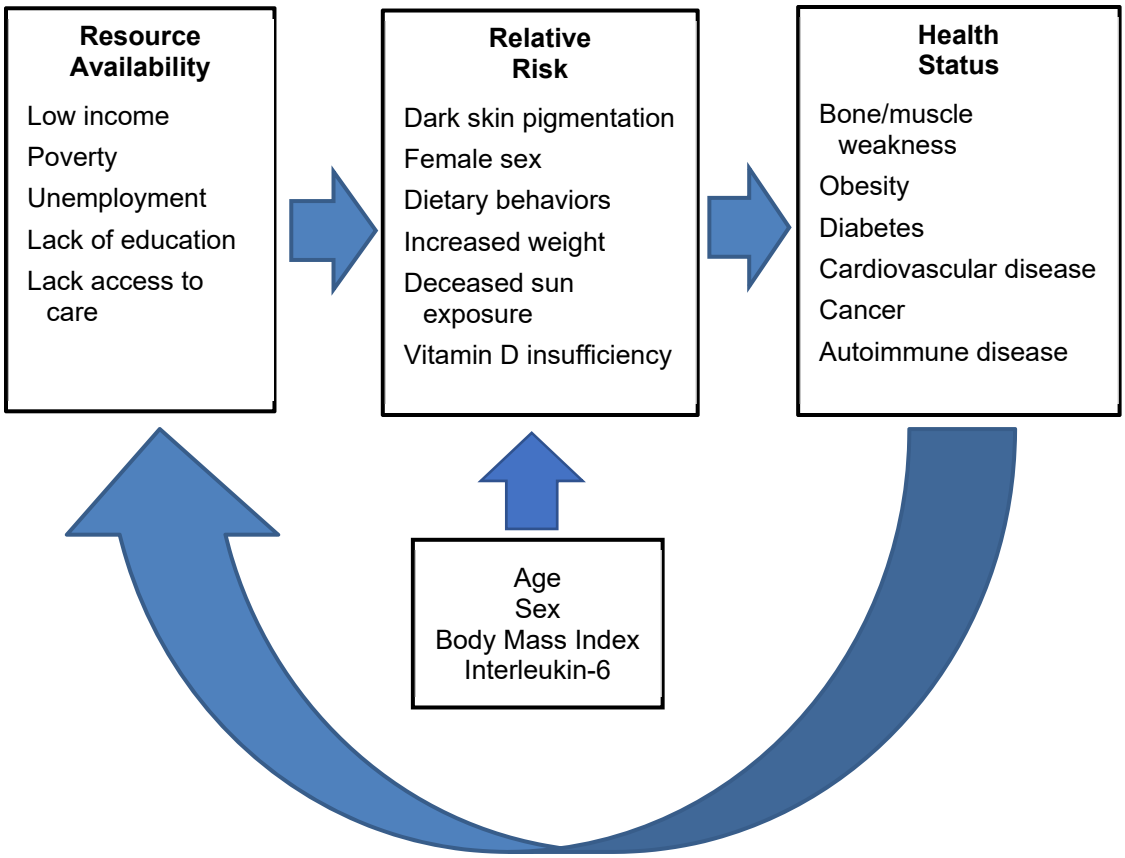

Figure 2. Application of Vulnerable Populations Conceptual Model as the theoretical framework for this secondary analysis research project. Original artwork by R. Havens influenced by Flaskerud and Winslow (1998).

health status in a potentially adverse cyclic pattern. The VPCM guided this research project by providing a nursing perspective as to how age, sex, BMI, and IL-6 may impact the vitamin D status of a vulnerable population. This knowledge will ultimately guide development of nursing interventions for resolving vitamin D insufficiency in vulnerable populations.

Flaskerud and Winslow (1998) suggested that population-based nursing research be used to examine each concept delineated in the VPCM, while adhering to ethical principles of cultural sensitivity. This project controlled for race and geographical location, while examining the influence of age, sex, BMI, and IL-6 on vitamin D status.

Age, sex, and BMI are particularly culturally sensitive variables that require thoughtful consideration when collecting data from individuals in a vulnerable population. Flaskerud and Nyamathi (2002) also suggested that a nurse researcher should reduce health disparities rather than just study them. These researchers suggested that nursing research should reflect income, education, access to care, social and political power, and human rights rather than focusing only on behaviors and risk factors.

Flaskerud and Winslow (1998) provided three recommendations for a successful study among vulnerable populations: (1) use members of the target population to recruit, retain and communicate with participants; (2) collaborate with other community programs to share information and resources; and (3) foster pride and empowerment to enhance constructive responses to their own health needs. Flaskerud and Nyamathi (2002) further explained that by involving the members of vulnerable group(s) in the entire research process, the group members can identify the relevant questions and share the products of the research collaboration such as gaining economic resources and political power. Flaskerud et al. (2002) recommended community-based intervention studies that provided “tangible resources and methodologic approaches that involve participants in the research process” (p. 74). By providing the vulnerable populations with tangible resources, researchers may determine the effect on health disparities.

Nursing knowledge of vulnerable populations and health disparities has steadily increased since the late 1980s, particularly with the development of the VPCM. For example, Flaskerud and Lee (2001) examined the health problems of informal caregivers of people experiencing HIV/AIDS and age-related dementias. Previous research had examined the personal and interpersonal characteristics of these caretakers. However, Flaskerud and Lee (2001) used the VPCM to explain the relationship between resource availability and relative risk exposure to the health status of female informal caregivers. With this sample of caregivers, the findings suggested that

income and race/ethnicity were the major variables impacting health status. These caregivers had few resources and more personal health issues due to being unable to obtain assistance with the arduous demands of caring for a chronically ill person. In another study, Dyer (2003) identified the health problems and disparities specific to Florida. Using the VPCM to pinpoint disparities, Dyer suggested approaches to improve health care in Florida by effectively intervening using nursing research, practice, and political activity. A few years later, Carr (2006) used the VPCM to examine African American grandmother caregivers of grandchildren and found that limited household income correlated with the relative risk of their personal health problems.

Despite increasing nursing research concerning health disparities, NeSmith (2006) found in her literature review that little research had been done regarding racial/ethnic health disparities even though trauma was the leading cause of death in minority populations aged 15-44 years. NeSmith further suggested that VPCM would be a valuable theoretical framework to use for this type of research. Similarly, Bay, Kreulen, Shavers, and Currier (2006) used the VPCM to examine traumatic brain injury and suggested that primary, secondary, and tertiary prevention interventions with specific research-based objectives be employed at the community level to reduce the prevalence of these injuries. Rodehorst, Wilhelm, and Stepans (2006) organized their study of rural children screened for asthma using the VPCM framework as did Gonzalez-Guarda, Peragall, Vasquez, Urrutia, and Mitrani (2009) in their study of intimate partner violence and depression as it related to resource availability in Hispanic women. Lastly, the VPCM was the theoretical foundation for a study conducted by Spears, Stein, and Koniak-Griffin (2010) in their investigation of cigarette, alcohol and marijuana use among Latina and African American adolescents during pregnancy and the postpartum period.

These studies demonstrated the usefulness of the VPCM as a theoretical framework for investigating the health of vulnerable populations. Therefore, the VPCM

was an appropriate framework to use for this present secondary analysis since it used adolescent, obese, and African American women as subjects who are all considered members of a vulnerable population. Many African American women experience increased rates of low vitamin D, obesity, and poverty (Flegal et al., 2010; Semega, Fontenot, & Kollar, 2017). This vulnerable population may experience limited resource availability such as low income, unemployment, lack of education, and lack of access to quality care. This decrease in resource availability – coupled with a decreased ability to absorb vitamin D through their dark pigmented skin, low vitamin D diet, increased weight, and decreased sun exposure – could increase their relative risk for acquiring chronic diseases and lower their overall health status (Holick et al., 2011; Lips, 2006; Park, & Johnson, 2005; Semega, Fontenot, & Kollar, 2017). This present secondary analysis used an established database to examine variable relationships based on the VPCM principles. The relationships between vitamin D status and 1) adolescent and adults, 2) female and male, and 3) overweight and obese African Americans subjects were investigated to determine how these variables affected serum vitamin D concentrations that may have treatment implications.

Summary

Vitamin D deficiency is a global health concern that may foster a variety of diseases. Factors affecting an individual's vitamin D status include age, sex, BMI, race, and region of residence. Individuals with darker skin pigmentation require 5-10 times more sun exposure to produce adequate vitamin D_3 than light-skinned individuals due to the increased amount of melanin in their skin. BMI is a moderator of vitamin D since body fat content is inversely related to serum vitamin D concentration; individuals with higher BMIs need a higher intake of vitamin D supplementation than their leaner counterparts to attain a sufficient vitamin D status. Age and sex are also moderators of vitamin D status due to the physical changes that occur as one grows older and 6%

more American women have vitamin D deficiency than American men. While research evidence suggests that serum vitamin D concentrations are lower in higher latitude geographical regions, these findings may be spurious due to cultural practices of wearing abundant clothing in hot, dry climates. Recent research findings suggest that an inverse correlation exists between the serum inflammatory biomarkers (e.g., IL-6) and serum vitamin D concentration. Therefore, vitamin D supplementation may be useful in treating autoimmune diseases since high doses of vitamin D_3 have been shown to impair the immune inflammatory response. Overall, this literature review revealed insufficient knowledge still exists regarding the health effects of deficient vitamin D and its underlying mechanisms. More research evidence is needed to provide clinicians with suitable guidelines for effectively identifying symptom clusters and treating low vitamin D concentrations and/or those diseases linked to vitamin D insufficiency, while accounting for race, sex, age, BMI, and geographical regions of residence. Using the VPCM as a theoretical framework, this secondary analysis should provide scientific evidence to help resolve the dispute between the 2011 IOM report and Endocrine Society's clinical guidelines regarding the definition of vitamin D deficiency and the supplemental dosages needed for its treatment.

Chapter 3: Methodology

This chapter is composed of two parts. Part 1 briefly discusses the methodology used for the original D-SUNNY study by Dong (2012) and described in Bhagatwala et al. (2015). Part 2 discusses the methodology used to conduct this secondary analysis of the D-SUNNY database.

Part 1: D-SUNNY Study

Research Design

The original D-SUNNY study was a clinical trial that followed a double-blinded, randomized, placebo-controlled experimental design with four randomized groups. The four experimental groups consisted of (1) a control group that received a placebo; (2) a group that received monthly supervised doses of 18,000 IU (equivalent to 600 IU/day); (3) a group that received monthly supervised doses of 60,000 IU (equivalent to 2,000 IU/day); and (4) a group that received monthly supervised doses of 120,000 IU (equivalent to 4,000 IU/day). Monthly dosing was used instead of daily dosing to improve participant compliance. All questionnaires, physiological and physical measurements were completed at the baseline, 8-week, and 16-week time points. The questionnaires requested information concerning the participants' family medical history, diet and smoking histories, demographics, activity/exercise, sun exposure, and mood/mental health status. The physiological measurements obtained via venipuncture included serum vitamin D, PTH, calcium, interleukin-6, adiponectin, leptin, osteocalcin, fasting glucose and A1C, lipid profile, and renal function. The physical measurements obtained from the participants included blood pressure, pulse wave velocities (carotid-radial,

carotid-femoral, and carotid-dorsalis-pedis), carotid artery compliance, flow mediated dilation, pulmonary function, exhaled nitric oxide concentration, maximum exercise capacity, and DEXA scan for bone density.

Setting and Sample

The setting for distributing the vitamin D supplements and collecting data was a research laboratory structured to collect human data located on the health sciences campus of a university. Generally, the D-SUNNY participants were healthy male and female African Americans, 13 to 45 years old, who were not taking any medications or vitamin/mineral supplementation. The inclusion criteria for D-SUNNY also required participants to have

1) no difficulty swallowing pills,
2) could provide blood samples via a brachial venipuncture,
3) no chronic disease, and
4) a baseline serum vitamin D concentration ≤ 50 nmol/L.

The exclusion criterion for D-SUNNY was women who were pregnant. These criteria were chosen because serum vitamin D concentrations are affected by extreme age (e.g., children and elderly people), chronic diseases, and medications (Wallace et al., 2013). Thus, these inclusion/exclusion criteria controlled for the confounding influence that these variables may exert on the collected data. Pregnant women were excluded due to the unknown nature pregnancy exerts on serum vitamin D concentration and prenatal vitamins are usually taken during pregnancy (Holick et al., 2012).

Recruitment, Screening, and Consent

The D-SUNNY participants lived in the same southeastern geographical region of the United States. The participants were recruited through advertisements in the local newspapers, fliers distributed on bulletin boards and via email, online classified ads, and

by word of mouth. All fliers and ads were approved by university's internal review board (IRB) prior to distribution. Also, study team members visited local high schools to recruit eligible participants after obtaining permission from the school administrators to do so.

A two-step screening process was performed to determine the eligibility of participants for the D-SUNNY study. Step 1 utilized telephone screening: potential participants who called requesting study information or who had signed up to be called, were contacted via telephone by a study team member and asked questions pertaining to the inclusion/exclusion criteria. Step 2 utilized an in-person/in-house appointment: an appointment was made for the potential participant to visit the research laboratory if they met the inclusion/exclusion criteria during the telephone screening. Once the potential participant arrived at the research laboratory, a written informed consent was obtained as well as a parental informed consent for those participants younger than 18 years of age. If the potential participant met the inclusion/exclusion criteria during this meeting, they were formally invited to continue their participation in the study.

Protocol and Data Collection

The baseline data was collected during the initial in-house meeting after the informed consent was signed and the participants agreed to fully participate in the D-SUNNY study. Each participant was randomized to one of the four experimental groups. Every four weeks, the participants returned to the laboratory to have their vital signs taken by the research assistants, give blood samples, take their vitamin D supplementation pills, and complete health surveys related to the data collection portion of this study. The university's IRB approved all aspects of the D-SUNNY study prior to beginning the data collection procedures.

The vitamin D supplementation was administered on a monthly (i.e., every four weeks) basis in four different dosing capsules: (1) a placebo containing no vitamin D; (2) a capsule containing 18,000 IU (equivalent to 600 IU/day); (3) a capsule containing

60,000 IU (equivalent to 2,000 IU/day); and (4) a capsule containing 120,000 IU (equivalent to 4,000 IU/day). The university's clinical research pharmacy generated the randomization codes and dispensed the vitamin D capsules, which were provided by Bio-Tech Pharmacal (Fayetteville, AR). Each participant was given their vitamin D capsule once their blood sample was obtained by the research assistants via a brachial venipuncture. The concentration of the circulating form of vitamin D (i.e., 25(OH)D aka calcifediol) in each blood sample was measured via enzyme immunoassay (Immunodiagnostic Systems, Fountain Hills, AZ). The amount of IL-6 in each blood sample was determined using ELISA kits (R&D Systems, Inc., Minneapolis, MN).

Part 2: Secondary Analysis

As described in Bhagatwala et al. (2015), a total of 129 potential participants were screened with 70 eligible participants enrolled in the original D-SUNNY study who were then randomized into four experimental groups. For this doctoral research study, the data records of these 70 participants were examined as a retrospective secondary analysis project to achieve two specific aims: 1) determine if age, sex, and/or BMI affected serum vitamin D concentration at differing dosages of supplementation in a sample of African Americans, and 2) determine if vitamin D supplementation affected serum IL-6 levels in the same sample of African Americans. Few studies exist with adequate data to achieve these aims. Therefore, the population size of 70 was accepted for this project with the intention of using the results as preliminary data to inform and design future studies. The doctoral-student investigator conducting this secondary analysis of the D-SUNNY database obtained IRB approval, including a waiver of participant consent.

Secondary analysis is the examination of previously collected data from another larger study; this type of research allows the investigator to explore different aspects of the data that may not have been considered for the original study (Burns & Grove,

2001). For this project, a de-identified database was securely obtained from the D-SUNNY statistician that contained all the variable data of the original 70 D-SUNNY participants; this database was stored on an encrypted device with an ample memory storage drive. A separate database was created after each data record was examined, using only the variable data needed to complete this secondary analysis project. The inter-rater reliability was tested by re-entering data from 10% of records until a 100% inter-rater reliability score was achieved to reinforce data fidelity.

Variables and Measurements

Table 1 shows the variables chosen for this secondary analysis. The vitamin D supplemental dosages and time points were determined by Dong (2012), the primary investigator of the original D-SUNNY study. The race and region of residence moderating variables were controlled for in the original D-SUNNY study; hence, they were also controlled for in this secondary analysis project. The moderating variables used for this secondary analysis were age, sex, and BMI to determine if they influenced the amount of serum vitamin D concentration already enhanced by the supplemental vitamin D dosages given to the participants over time. Furthermore, these variables operationalized the concepts denoted in the VPCM theoretical framework. For example, the age, sex, and BMI moderating independent variables may adversely impact the serum vitamin D concentration (despite vitamin D supplementation) that in turn may enhance the relative risk of vitamin D insufficiency and promote a detrimental health status. Age, sex, and BMI may also reduce resource availability, adding to the relative risk of vitamin D insufficiency and a poor health status. While IL-6 is not a moderating variable of serum vitamin D concentration, its inverse relationship with vitamin D may potentially foster a declining health status due the development of cardiovascular or autoimmune diseases.

Table 1

Independent and Dependent Variables

Variable	Definition
Independent	
Vitamin D Supplement Dosage	Supplements regular diet; pills distributed as either a placebo containing no vitamin D, 600 IU/day, 2,000 IU/day, or 4,000 IU/day
Time	Blood samples obtained at the baseline, 8-week, and 16-week time points.
Age	Date of birth: ≤ 21 years old or ≥ 21 years old
Sex	Gender: male or female
Body Mass Index (kg/m^2)	Overweight: adult BMI 25-29.9 kg/m^2 or adolescent 85-94th percentile Obese: adult BMI ≥ 30 kg/m^2 or adolescent ≥ 95th percentile
Dependent	
Serum Vitamin D Concentration (nmol/L)	Amount of calcifediol [25-hydroxyvitamin D, 25(OH)D] in blood sample
Interleukin-6 (pg/ml)	Amount of inflammatory biomarker in blood sample

Note: IU = International Units; BMI = Body Mass Index

The participants' self-reported birthdate and sex (as male or female) served as the age and sex measurements, respectively, for the D-Sunny study as well as this secondary data analysis. BMI is considered the gold standard for establishing whether a person is underweight, healthy weight, overweight, and/or obese (Flegal et al., 2010). The BMI was calculated for each participant using the weight and height measurements obtained during the baseline data collection. For this secondary analysis, each adult participant was categorized as overweight if their calculated BMI was 25.0-29.9 kg/m^2

and obese if their calculated BMI was ≥ 30 kg/m^2 (CDC, 2017a). For the adolescent participants, their BMI was expressed as a percentile. For this secondary analysis, the adolescent participants in the 85^{th}-95^{th} percentile were categorized as overweight, and those in the ≥ 95^{th} percentile as obese (CDC, 2017a).

Dosage is the amount of a substance taken at a given time, while dosage effect is the result of an independent variable interacting with a dependent variable (Potter, Perry, Stockert, & Hall, 2013). Thus, dosage effect is the phenomenon resulting from when a larger dose of a pharmaceutical agent produces a greater effect than the smaller dosage did of the same agent. Time effect was defined as a design used to explore the sequence, changes, growth, and/or trends over time (Burns & Grove, 2001). This secondary analysis sought to determine the interaction (moderation) effects of the four dosages of vitamin D supplementation, time, age, sex, and BMI on the serum vitamin D concentration as well as the dosage and time interaction effect on serum IL-6 concentration. The resulting data should indicate whether age, sex, and/or BMI (the moderating variables) influence the measured serum vitamin D concentration (outcome) affected by dosage and time. The results should also indicate if vitamin D supplementation reduced serum IL-6 concentration, an outcome indicative of diminished tissue inflammation. Guided by the VPCM, this information may suggest nursing strategies as available resources that would reduce the relative risk of vitamin D insufficiency and improve health status in a vulnerable population.

Data Analysis

All data statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS©) version 25 (2017; IBM, Armonk, NY). The level of significance level was set at 0.05. The demographic data were described as frequency, percentage, mean, and standard deviations depending on the nominal or continuous nature of the data. A repeated-measures analysis of variance (ANOVA) was performed to determine

the interaction (i.e., moderating) effects that the independent variables (dosage, time, age, sex, and BMI) exerted on the dependent ones (serum vitamin D concentration, serum IL-6 level). Specifically, the repeated-measures ANOVA used the General Linear Model technique with time (baseline, 8 weeks, and 16 weeks) as the within-subject factor by dosage (placebo, 600 IU/day, 2,000 IU/day, 4,000 IU/day) as the between-subject factor with age, sex, and BMI as the moderating variables. For the IL-6 data, the repeated measures ANOVA used the General Linear Model technique only to determine if an interaction (moderation) effect existed with time (baseline and 16 weeks) and dosage (placebo, 600 IU/day, 2,000 IU/day, 4,000 IU/day).

The repeated-measures ANOVA was used to test the hypotheses of Specific Aim 1 because a statistically significant interaction would indicate that a third variable (age, sex, or BMI) moderated the relationship between the independent variable (dosage and/or time) and the dependent variable (serum vitamin D concentration). As explained by Mackinnon and Luecken (2008), mediation is when an independent variable causes a mediating variable that in turn causes a dependent variable. Sophisticated statistical techniques such as Structural Equation Modeling are employed to determine the existence of mediating variables, their relationships and effects within a model. The primary purpose of this secondary analysis was to determine if age, sex, and BMI moderated (i.e., influenced/affected/changed) serum vitamin D concentration, not mediate (i.e., cause) it. Therefore, statistically testing for mediation effects was beyond the scope of this research project.

A one-way ANOVA was also performed with the overall serum vitamin D concentration and IL-6 data to further determine if the four vitamin D supplementation dosage groups significantly differed with time (i.e., which supplemental vitamin D dosage most effectively moderated/influenced/changed the serum vitamin D concentration and serum IL-6 level over time). In other words, performing the one-way ANOVA statistical

procedure further assessed which vitamin D supplemental dosage over time best treated the insufficient vitamin D status of the African American participants. If statistical group differences were found, then the Scheffé post hoc test was used to establish which specific group pairs were significantly different from each other. The Scheffé test is more conservative (i.e., narrower confidence intervals) compared to the Tukey or Bonferroni methods. Therefore, the Scheffé was the preferred statistical post hoc test when making unplanned pairwise comparisons during this data analysis procedure for this project (https://www.statsdirect.com/help/analysis_of_variance/multiple_comparisons.htm).

Chapter 4: Results

Out of the 129 potential African American participants living in the CSRA who were screened, 70 of them met the inclusion/exclusion criteria and fully participated in the original D-SUNNY investigation. For this secondary analysis, the data records of these 70 participants were reviewed to determine if they each had a baseline serum vitamin D concentration ≤ 50 nmol/L, BMI in the overweight or obese categories, and a complete data set containing no missing values. The retrospective examination of the D-SUNNY database found that 60 participants met these inclusion criteria. Table 2 shows the demographic characteristics for these 60 participants, including how many were in each dosage group.

Table 3 and Figure 3 show the overall mean ± standard deviation (sd) serum vitamin D concentration over time for each dosage group. This data demonstrated that the mean baseline serum vitamin D concentration for all participants was well below the minimum values for a sufficient vitamin D status per the IOM report (50 nmol/L) and Endocrine Society Clinical Practice Guideline (75 nmol/L). After 8 weeks, the vitamin D supplementation increased the mean serum vitamin D concentration to the sufficient status per the IOM report for participants receiving each vitamin D supplementation dosages. However, only the 4,000 IU/day dosage increased the mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines by the 8-week time point. After 16 weeks, only the 2,000 IU/day and 4,000 IU/day dosages effectively increased the serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines.

Table 2

Demographic Characteristics (n = 60)

Variable	Group Size # (% of total)	Mean ± Standard Deviation
Age		
≤ 21 years	28 (47%)	17.5 ± 2.3
≥ 21 years	32 (53%)	33.0 ± 6.7
Sex		
Male	10 (17%)	
Female	50 (83%)	
Body Mass Index		
Overweight (25-29.9 kg/m^2)	12 (20%)	27.6 ± 1.3
Obese (≥ 30 kg/m^2)	48 (80%)	37.9 ± 6.7
Dosage		
Placebo	16 (27%)	
600 IU/day	13 (21%)	
2,000 IU/day	16 (27%)	
4,000 IU/day	15 (25%)	

Note: kg = kilograms; m = meter; IU = international unit

Table 3

Values of Overall Serum Vitamin D Concentration per Dosage Over Time

Time	Placebo (n = 16)	600 IU/day (n = 13)	2,000 IU/day (n = 16)	4,000 IU/day (n = 15)	*p* value
Baseline	36.1 ± 9.2	32.1 ± 7.9	34.2 ± 8.7	31.6 ± 9.0	.475
8 weeks	40.0 ± 10.4	53.3 ± 9.6	73.2 ± 17.8	91.8 ± 36.3	.0001*
16 weeks	42.0 ± 12.1	55.7 ± 11.9	85.0 ± 25.3	89.4 ± 26.1	.0001*

* signifies a statistically significant group difference

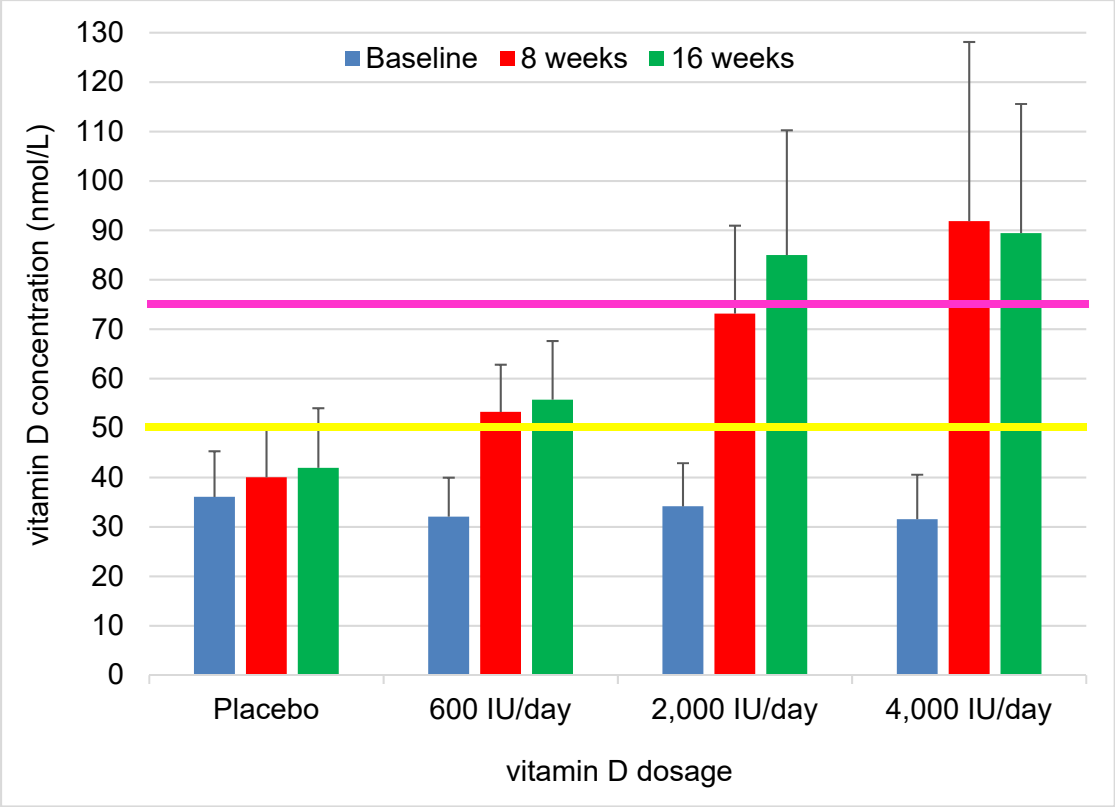

Figure 3. Overall serum vitamin D concentration per dosage over time for the 60 participants. Yellow line denotes sufficient vitamin D status (50 nmol/L) per the Institute of Medicine (2011) report. Pink line denotes sufficient vitamin D status (75 nmol/L) per the Endocrine Society Clinical Practice Guideline (Holick et al., 2011).

A one-way ANOVA was also performed to determine if the mean serum vitamin D concentration values for the four dosage groups were significantly different from one another at each time point. No significant group differences were found at the baseline time point (p = .475). However, significant group differences were found at both the 8-week and 16-week time points (p = .0001). Post hoc testing showed that at 8 weeks, the mean serum vitamin D concentration for the placebo group was significantly different from both the 2,000 and 4,000 dosage groups ($p \leq .001$). Also, the mean serum vitamin D concentration for the 600 dosage group was significantly different from the 4,000 dosage group (p = .0001). Post hoc testing showed that at 16 weeks, the mean serum

vitamin D concentration values among the four dosage groups were all significantly different from each other ($p \leq .004$) except for comparisons between placebo and 600 dosage groups ($p = .354$), and 2,000 and 4,000 dosage groups ($p = .945$).

Table 4 shows the number of participants in each age group by dosage group. Figure 4 shows the mean ± sd serum vitamin D concentration values per age group for each dosage group. After 8 weeks, both age groups showed an increase in mean serum vitamin D concentration to the sufficient status per the IOM report when participants received one of the three vitamin D supplementation dosages. While the younger participants had an increased mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines with the 2,000 IU/day and 4,000 IU/day dosages by the 8-week time point, only the 4,000 IU/day dosage had this effect on the older participants. After 16 weeks, the 2,000 IU/day and 4,000 IU/day dosages increased the mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines for both age groups.

A repeated-measures ANOVA was performed to determine if age affected serum vitamin D concentration at the different supplementation dosages for these participants. Since the Mauchly's Test of Sphericity did not meet the assumption of sphericity ($p = .0001$), the Huynh-Feldt correction statistic was used to determine that age and dosage did not exhibit a significant within-subject interaction effect ($F [1.779, 94.275] = 3.072$, $p = .057$) with an effect size $\eta^2 = .055$. When testing for between-subject effects, however, the mean serum vitamin D concentration values for the dosage groups significantly differed with age ($F [1, 53] = 5.814$, $p = .019$) with an effect size $\eta^2 = .099$. The decision to accept or reject the hypothesis that the younger participants required higher dosages of vitamin D supplementation than the older participants to achieve a sufficient vitamin D status remains elusive due to the inconclusive nature of these statistical results.

Table 4

Number of Participants per Age Group by Dosage

Age Group	Placebo	600 IU/day	2,000 IU/day	4,000 IU/day
< 21 years (n = 28)	n = 5	n = 5	n = 9	n = 9
> 21 years (n = 32)	n = 11	n = 8	n = 7	n = 6

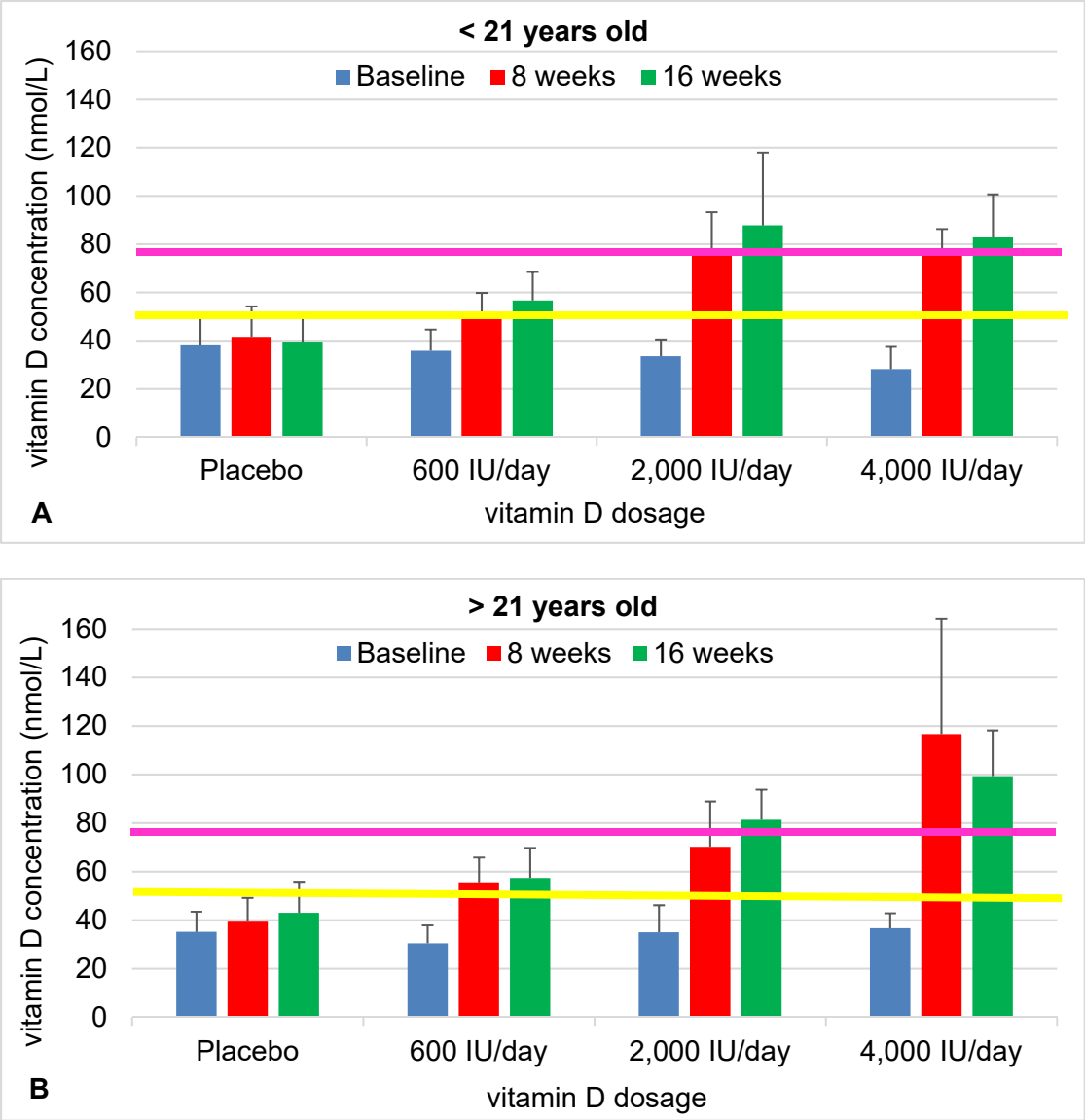

Figure 4. Histograms showing < 21 years old (A) and > 21 years old (B) serum vitamin D concentration per dosage over time. Yellow line denotes sufficient vitamin D status (50 nmol/L) per the Institute of Medicine (2011) report. Pink line denotes sufficient vitamin D status (75 nmol/L) per the Endocrine Society Clinical Practice Guideline (Holick et al., 2011).

Table 5 shows the number of participants in each sex group by dosage group. Figure 5 shows the mean ± sd serum vitamin D concentration values per sex group for each dosage group. Interestingly, the men showed an increase in mean serum vitamin D concentration to the sufficient status per the IOM report for all four dosage groups after 8 weeks – the placebo and each dosage of vitamin D supplementation dosages. The women only had an increase in serum vitamin D concentration with the supplemental vitamin D dosages. The men also had an increased mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines with the 2,000 IU/day and 4,000 IU/day dosages by the 8-week time point, whereas the women only had this effect with the 4,000 IU/day dosage. After 16 weeks, the 2,000 IU/day and 4,000 IU/day dosages increased the mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines for both sex groups.

A repeated-measures ANOVA was performed to determine if sex affected serum vitamin D concentration at the different supplementation dosages for these participants. Since the Mauchly's Test of Sphericity did not meet the assumption of sphericity (p = .0001), the Huynh-Feldt correction statistic was used to determine that sex and dosage exhibited a significant within-subject interaction effect (F [1.779, 94.275] = 4.865, p = .012) with an effect size η^2 = .084. When testing for the between-subject effects, the mean serum vitamin D concentration values for the dosage groups significantly differed with sex (F [1, 53] = 7.112, p = .010) with an effect size η^2 = .118. Given these findings, the decision could be made to accept the hypothesis that the female participants required higher dosages of vitamin D supplementation than the male participants to achieve a sufficient vitamin D status per the Endocrine Society's clinical guidelines. However, more research is needed to verify this decision since so few male participants were included in this secondary analysis.

Table 5

Number of Participants per Sex Group by Dosage

Sex Group	Placebo	600 IU/day	2,000 IU/day	4,000 IU/day
Male (n = 10)	n = 4	n = 2	n = 2	n = 2
Female (n = 50)	n = 12	n = 11	n = 14	n = 13

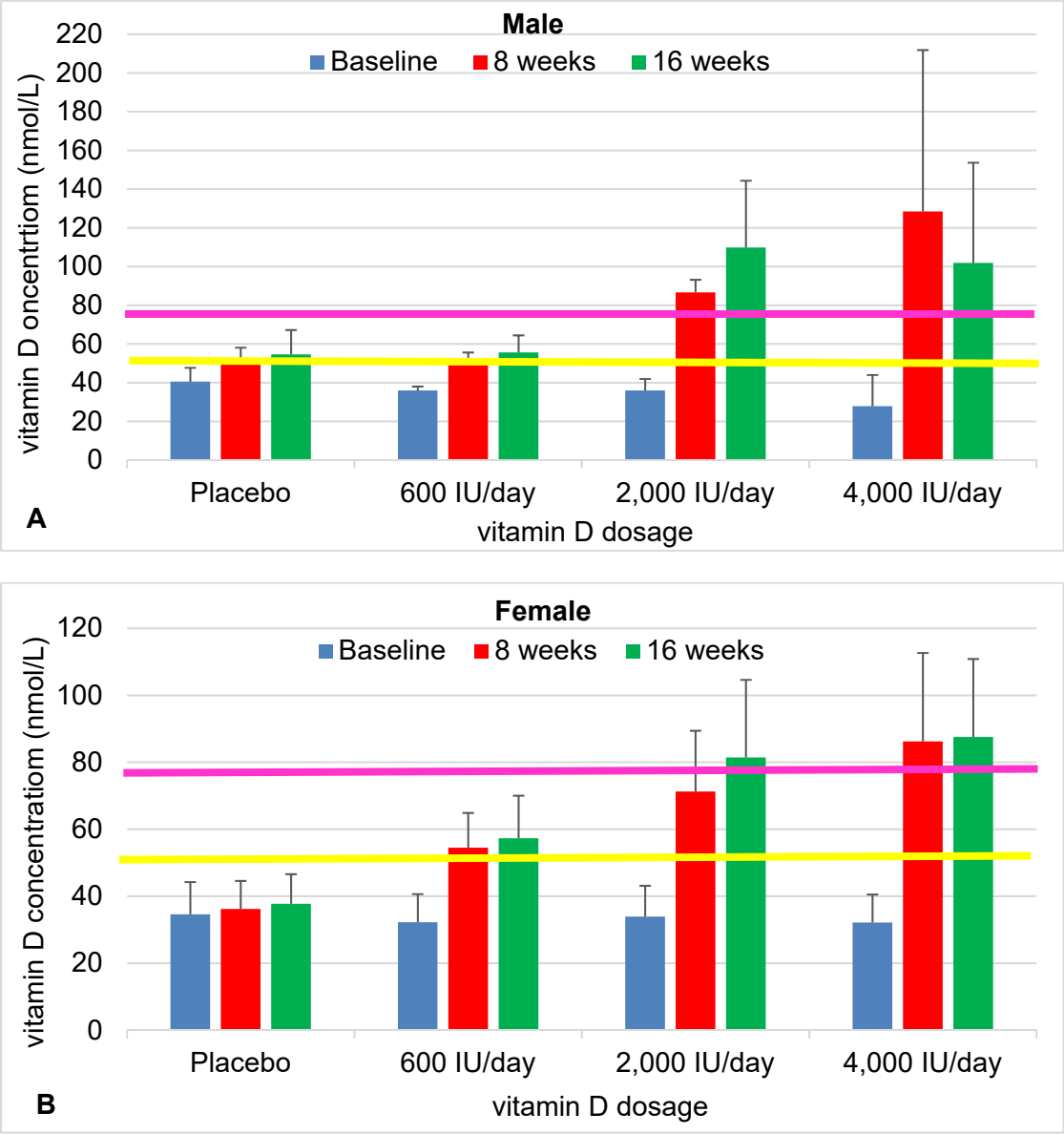

Figure 5. Histograms showing Male (A) and Female (B) serum vitamin D concentration per dosage over time. Yellow line denotes sufficient vitamin D status (50 nmol/L) per the Institute of Medicine (2011) report. Pink line denotes sufficient vitamin D status (75 nmol/L) per the Endocrine Society Clinical Practice Guideline (Holick et al., 2011).

Table 6 shows the number of participants in each BMI group by dosage group. Figure 6 shows the mean ± sd serum vitamin D concentration values per BMI group for each dosage group. Both BMI groups showed an increase in mean serum vitamin D concentration to the sufficient status per the IOM report by 8 weeks when taking one of the three vitamin D supplementation dosages. Both BMI groups also showed an increased mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines with the 4,000 IU/day dosage by the 8-week time point. After 16 weeks, the 2,000 IU/day and 4,000 IU/day dosages increased the mean serum vitamin D concentration to the sufficient status per the Endocrine Society's clinical guidelines for both BMI groups.

A repeated-measures ANOVA was performed to determine if BMI affected serum vitamin D concentration at the different supplementation dosages for these participants. Since the Mauchly's Test of Sphericity did not meet the assumption of sphericity (p = .0001), the Huynh-Feldt correction statistic was used to determine that BMI and dosage showed no within-subject interaction (F [1.779, 94.275] = 0.048, p = .939) and no effect η^2 = .001. When testing for the between-subject effects, the mean serum vitamin D concentration values for the dosage groups did not differ with BMI (F [1, 53] = 3.032, p = .087), effect size η^2 = .054. Given these findings, the decision could be made to reject the hypothesis that the obese participants required higher dosages of vitamin D supplementation than the overweight participants to achieve a sufficient vitamin D status. However, more research is needed to verify this decision since so few overweight participants were included in this secondary analysis.

Table 6

Number of Participants per Body Mass Index Group by Dosage

BMI Group	Placebo	600 IU/day	2,000 IU/day	4,000 IU/day
Overweight (n = 12)	n = 4	n = 3	n = 2	n = 3
Obese (n = 48)	n = 12	n = 10	n = 14	n = 12

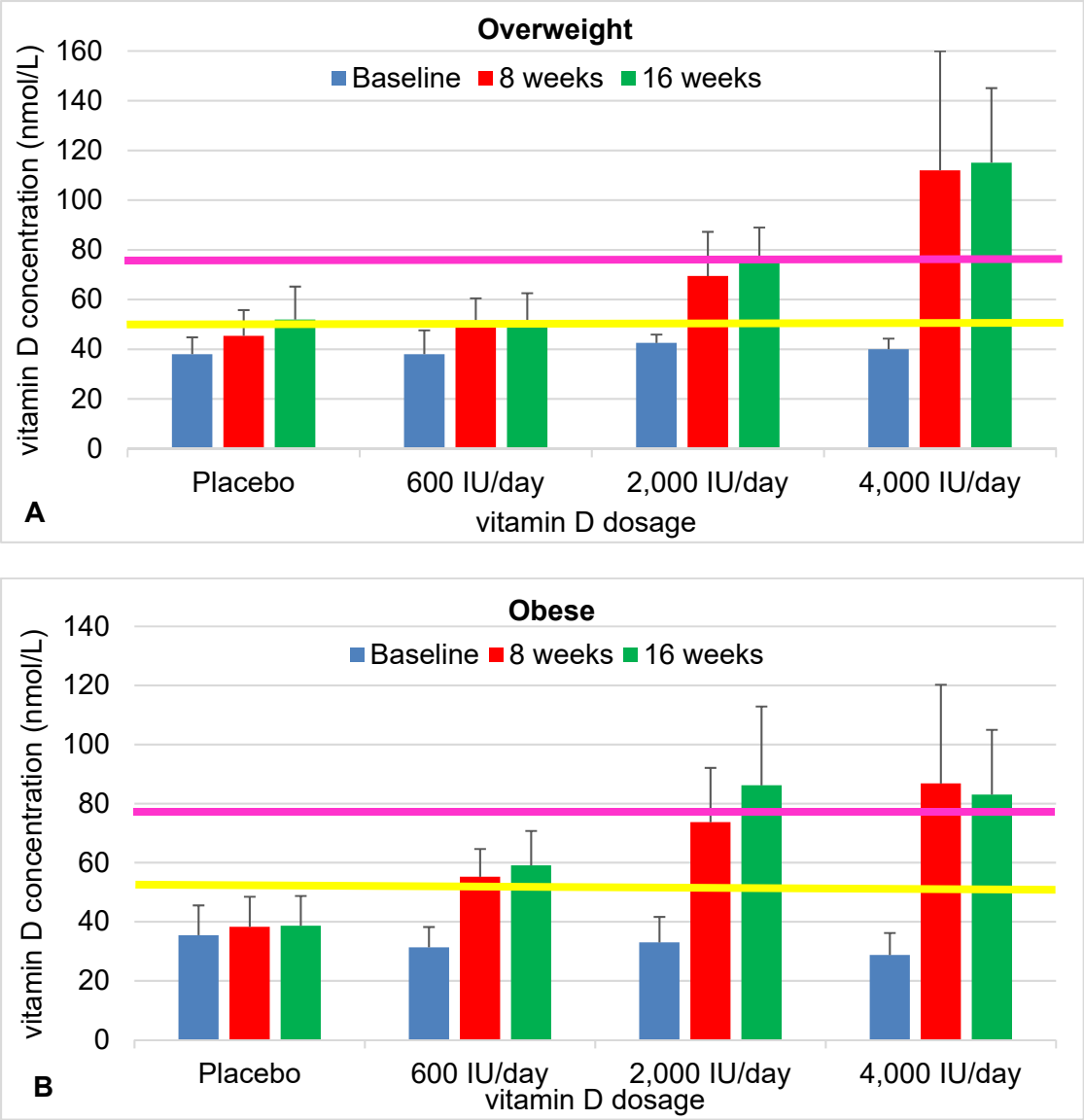

Figure 6. Histograms showing Overweight (A) and Obese (B) serum vitamin D concentration per dosage over time. Yellow line denotes sufficient vitamin D status (50 nmol/L) per the Institute of Medicine (2011) report. Pink line denotes sufficient vitamin D status (75 nmol/L) per the Endocrine Society Clinical Practice Guideline (Holick et al., 2011).

Table 7 and Figure 7 show the serum IL-6 concentration values over time for each dosage group. This data demonstrated that the mean baseline serum IL-6 concentration for all participants was below the minimum value for healthy individuals without chronic disease. Only the placebo and 4,000 IU/day vitamin D supplementation groups showed a decreased serum IL-6 concentration after 16 weeks. The serum IL-6 concentration values did not change over time for 600 IU/day group and increased after 16 weeks for the 2,000 IU/day group.

A repeated-measures ANOVA was performed to determine if IL-6 exhibited an interaction between time and dosage. As with the other results, the Mauchly's Test of Sphericity did not meet the assumption of sphericity (p = .0001). The Huynh-Feldt correction statistic showed that with IL-6, time and dosage did not exhibit a significant within-subject interaction effect (F [3, 53] = 2.439, p = .075), effect size η^2 = .121, nor a between-subject effect (F [1, 53] = 1.31, p = .281), effect size η^2 = .069.

A one-way ANOVA was also performed to determine if the mean serum IL-6 concentration values for the four dosage groups were significantly different from one another at each time point. No significant group differences were found at the baseline time point (p = .753). A significant group difference was found at the 16-week time point (p = .038). However, this statistical finding may be a spurious one due to mathematics. The post hoc testing showed no significant groups differences after 16 weeks; e.g., the mean IL-6 values for the 2,000 and 4,000 dosage groups were not significantly different from each other (p = .082). Given these statistical findings, the decision could be made to reject the hypothesis that vitamin D supplementation decreases serum IL-6 concentration. However, more research is needed to verify this decision due to the small sample size used for this secondary analysis.

Table 7

Values of Serum Interleukin-6 Concentration per Dosage Over Time

Time	Placebo (n = 16)	600 IU/day (n = 13)	2,000 IU/day (n = 16)	4,000 IU/day (n = 15)	*p* value
Baseline	3.3 ± 1.9	2.8 ± 2.1	3.6 ± 2.9	2.9 ± 1.5	.753
16 weeks	2.5 ± 1.6	2.8 ± 2.0	4.4 ± 3.4	2.3 ± 1.3	.038*

* signifies a statistically significant group difference

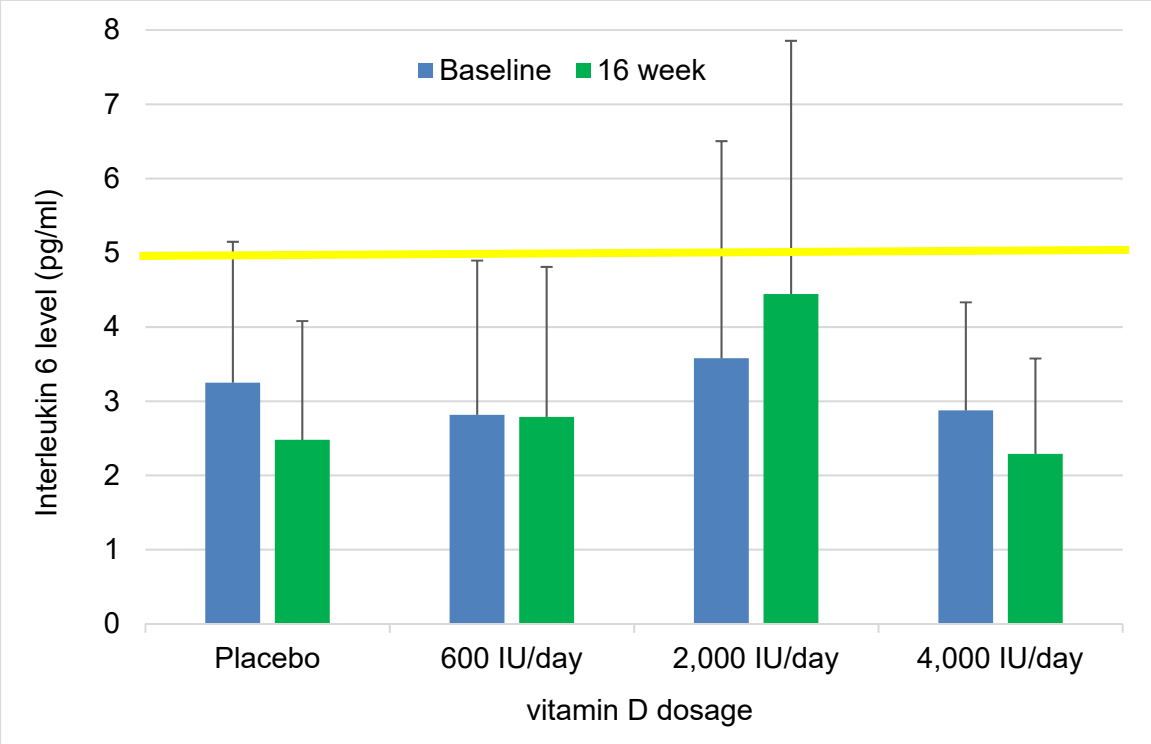

Figure 7. Serum Interleukin-6 concentration per dosage over time for the 60 participants. The normal serum interleukin-6 (IL-6) concentration in healthy individuals without evidence of chronic disease is ~ 5 pg/ml (Faulkner et al., 2014; Wang et al., 2017). The laboratory at the medical center sends blood samples to the ARUP Laboratories for IL-6 analysis (personal communication). ARUP Laboratories uses an IL-6 reference level ≤ 5 pg/ml (http://ltd.aruplab.com/Tests/Pub/0051537).

Chapter 5: Discussion

This chapter discusses the findings from this secondary analysis of the original D-SUNNY data and their meaning via a comparison with relevant literature. The findings are also discussed within the context of the VPCM as well as nursing clinical implications for improving patient outcomes. Lastly, the strengths and limitations of this secondary analysis research project are discussed with suggestions for future research.

Discussion

As with the D-SUNNY findings reported by Bhagatwala et al. (2015), the dosages of vitamin D supplementation successfully increased the serum vitamin D concentration for the 60 participants included in this secondary analysis (Table 3, Figure 3, $p = .0001$). By 8 weeks, the three dosages containing vitamin D had increased serum vitamin D concentration to the sufficient status (50 nmol/L) established by the 2011 IOM report. Compared to the placebo dosage, the 4,000 IU/day dosage by 8 weeks had also significantly increased the serum vitamin D concentration to the sufficient status (75 nmol/L) established by the Endocrine Society Clinical Practice Guideline ($p \leq .001$). By 16 weeks and compared to the placebo group, both the 2,000 IU/day and 4,000 IU/day dosages significantly increased the serum vitamin D concentration to the sufficient status established by the Endocrine Society's guidelines ($p \leq .004$). Holick et al. (2012) discussed a variety of reasons for sustaining higher serum vitamin D concentrations in both healthy and chronically-ill people via high dosages of vitamin D supplementation. One major reason for doing so is the reduction in mortality. For example, Grant (2011)

calculated a 7.6% reduction in all-cause mortality rates for African women and 17.3% for European women with a two-year increase in life expectancy if serum vitamin D concentrations were maintained at ≥ 110 nmol/L.

The purpose of Specific Aim 1 was to determine whether age, sex, and BMI moderated the serum vitamin D concentration at the differing dosages of vitamin D supplementation over time in a sample of healthy African American individuals. Although the statistical results were inconclusive, the histogram graphs visually revealed trends that raises provocative questions. For example, Figure 4 revealed that age may moderate serum vitamin D concentration. In the younger < 21 years old age group, the 600 IU/day vitamin D supplement barely raised the serum vitamin D concentration to the sufficient 2011 IOM status – event after 16 weeks. Furthermore, only the 2,000 and 4,000 IU/day dosages increased serum vitamin D concentration by 16 weeks to that recommended by the Endocrine Society's guidelines. Harel et al. (2011) found similar results when examining 68 male and female, Hispanic, African American, and Caucasian obese adolescents. In this study, the adolescents who were deficient in vitamin D were given 50,000 IU per week for 6 to 8 weeks, while those who were insufficient were given 400 IU per day for 3 months. These investigators found that only 12 adolescents (28%) reached sufficient levels of vitamin D by the end of their treatment.

Figure 5 revealed that sex moderated the serum vitamin D concentration in these participants. After 16 weeks, both sexes taking the 600 IU/day vitamin D supplement reached the of 50 nmol/L serum vitamin D concentration, but the females on the 2,000 IU/day dosage barely reached 75 nmol/L by 16 weeks. When taking 4,000 IU/day, the female participants reached 75 nmol/L at the 8-week time point; the male participants accomplished this feat when only taking 2,000 IU/day of vitamin D supplement. Both sexes who took the 4,000 IU/day dosage reached 75 nmol/L by 8 weeks, which

remained unchanged by 16 weeks. The statistical results verified that sex moderated serum vitamin D concentration, significantly interacting with dosage over time. These results concur with the data results reported by Talwar et al. (2007) when investigating the effect of two different vitamin D supplement dosages in 208 healthy postmenopausal African American women over three years. These investigators found that to achieve vitamin D concentrations above 75 nmol/L in all the participants, a dosage of 2,800 IU/day was necessary for those women with a baseline vitamin D concentration > 45 nmol/L. A dosage of 4,000 IU/day was required for women with a baseline serum vitamin D concentration < 45 nmol/L.

While Figure 6 revealed that BMI may have affected the serum vitamin D concentrations, the statistical results showed no interaction/moderation at all between BMI and dosage over time. Both the overweight and obese participants did not reach 75 nmol/L until the 16-week time point when taking the 2,000 IU/day dosage. The obese individuals showed similar results with 4,000 IU/day dosage, the overweight participants achieved a serum vitamin D concentration ≥ 100 nmol/L by 16 weeks taking this higher dosage. Harel et al. (2011) administered vitamin D supplement dosages as high as 50,000 IU/week (7,143 IU/day) to their study participants. These researchers found that 72% of obese Hispanic, Caucasian, and African American adolescents still failed to reach a sufficient vitamin D status even after repeated treatment.

To test the hypotheses of Aim 1 for this research project, three additional variables were analyzed along with dosage and time to determine if they influenced (moderated) serum vitamin D concentration. Consequently, the sample size for the age, sex, and BMI groups was extremely small, e.g., n = 2 for the male and overweight 2,000 IU/day dosage groups. These limited group sizes may explain why only sex exhibited a

statistical interaction/moderation effect and not age or BMI. Studies are needed with more African American participants (i.e., increase sample size) to verify these findings, especially since the female and obese group sizes were disproportionately higher than their male and overweight counterparts, respectively.

The purpose of Specific Aim 2 was to determine whether vitamin D supplementation affected (moderated) serum levels of the IL-6 inflammatory biomarker in a sample of African American participants. No statistical significant interaction was found with dosage and time regarding vitamin D supplementation moderating serum IL-6 concentration levels for these African American participants. Thus, supplemental vitamin D did not affect serum IL-6 concentration. Figure 7 shows that the serum IL-6 concentration levels were below 5 pg/ml at both the baseline and 16-week time points, indicating that these healthy participants had no evidence of systemic inflammation. This finding was a bit surprising considering that 80% of the participants were categorized as obese with baseline serum vitamin D concentrations < 50 nmol/L. Previous research has shown both conditions to be associated with an increased inflammatory response (Doumatey et al., 2010; Hevener et al., 2009; Petty et al., 2010; Tilg & Moschen, 2006; Zanetti et al., 2013). However, most research studies designed similar to the original D-SUNNY study and this secondary analysis included chronically-ill participants, e.g., the study by Schleithoff et al. (2006) used individuals diagnosed with heart failure. The participants in this present research project were healthy without evidence of chronic disease, who exhibited serum IL-6 concentration levels similar to the healthy subjects in the studies by Faulkner et al. (2014) and Wang et al. (2017).

Other differences in research design may explain the different findings when comparing this secondary analysis to the study by Schleithoff et al. (2006). The

investigation by Schleithoff et al. (2006) used a dosage of 2,000 IU/day of vitamin D supplement plus a calcium supplement as a treatment intervention and measured serum IL-10 (an anti-inflammatory cytokine) levels. These researchers found that vitamin D supplementation increased serum IL-10 levels, which deterred progression of systemic inflammation. The original D-SUNNY study and this secondary analysis did not include a calcium supplement and measured serum IL-6 (a pro-inflammatory cytokine) levels.

Another study, however, reported results similar to this secondary analysis. Shea et al. (2008) obtained inconsistent data results when examining the relationship between vitamin D and inflammation in healthy participants, which they explained as due to using healthy participants rather than ones with chronic illness. These investigators suggested that most studies reporting a benefit from vitamin D supplementation used participants who experienced chronic diseases, not healthy individuals as they did.

Another explanation for the results found in this secondary analysis is that the African American participants were recruited for the original D-SUNNY study during the winter and summer months. Sunlight absorbed through the skin provides 50-90% of vitamin D (Lips, 2010). Also, serum vitamin D concentrations may be lower in northern geographical regions (Park & Johnson, 2005). The data for this secondary analysis was collected during the winter months, then into the summer months, on participants living in the southern region of the country. Therefore, the moderating effects of vitamin D supplementation to alter serum IL-6 concentration may not have been as apparent compared to individuals living in the northern regions of the United States. Therefore, a larger, more homogeneous sample of healthy African American individuals need to be examined that may produce different outcomes.

The results of this secondary analysis showed that the African American, overweight, and obese male and female participants aged 13-45 years were unable to achieve, after 16 weeks of supplementation, the vitamin D serum concentration of 50 nmol/L on the vitamin D supplemental dosage of 600 IU/day – which is the recommended daily allowance for these ages (NIH, 2017). But after the same time interval, those participants receiving the 2,000 and 4,000 IU/day of vitamin D supplements achieved the vitamin D serum concentration of 50 nmol/L recommended by the IOM (2011) for bone health. Therefore, the findings of this secondary analysis imply that overweight/obese, African American adults and adolescents need much more vitamin D than the 600 IU/day dosage recommended by the researchers at the NIH (2017) for individuals aged 1-70 years.

Limitations

Several limitations constrained this secondary analysis research project. First, because only African Americans were recruited, the results cannot be generalized to other vulnerable populations or races. Second, research evidence suggests that serum vitamin D concentrations are higher in lower latitude regions and during the summer season due to the prevalence of sunlight over a longer time interval (Lips, 2010; Park & Johnson, 2005). Unfortunately, participant recruitment for the original D-SUNNY study occurred during the winter and summer months. However, the baseline serum vitamin D concentration for all participants was well below 50 nmol/L regardless of when the participant was recruited, and their baseline blood sample given. Third, women were over-represented in this secondary analysis, although the sex distribution between dosage groups was not different. Fourth, the sample size was small that possibly produced a Type II error. A Type II error occurs when the null hypothesis is accepted

when it is actually false (Polit & Beck, 2017). Given the increased number of variables for this secondary analysis, it is also possible that a sample size of 60 did not provide sufficient power to detect a consistent interaction and/or moderating relationship among the variables. As this research project is a doctoral dissertation representing emerging science, and few studies existed with which to assess an adequate sample size, the sample size of 60 was accepted for this secondary analysis. The knowledge generated with this doctoral research project is intended to inform adequately-powered sample sizes for future vitamin D-related studies.

Several potential methodological limitations also existed for this secondary analysis. The first limitation is related to data quality. Retrospective, secondary analysis using data from previous vitamin D investigation was the design method for this present doctoral research project. Secondary analysis is the examination of previously collected data from another study (Burns & Grove, 2001). The advantages of conducting a secondary analysis are that this method is an effective, economic, and expedient mechanism for providing research data for research, plus it provides researchers access to large data sets containing multiple variables and sample sizes (Garmon Bibb, 2007). Unfortunately, a secondary analysis has several inherent disadvantages, including potential issues regarding data quality, since the variables used in a secondary analysis were not originally collected for the purpose and primary aims of the subsequent secondary study using data (Garmon Bibb, 2007).

The following actions were taken to minimize potential issues with data quality as a limitation to this secondary analysis. This doctoral researcher worked with the investigators of the original D-SUNNY study and assisted with collecting the data. Thus, she was very familiar with the D-SUNNY database and its variables. Working with the

original D-SUNNY team gave her an opportunity to observe the investigators as data quality was assessed and its characteristics examined by the investigators. The purpose, content, development, and target population in the original D-SUNNY study were well-defined and subsequently examined for validity and reliability. The process of assessing the validity of a data set allows the data to be analyzed to the degree that the data set contained all the variables required to address the research questions (Garmon Bibb, 2007). Thus, this doctoral researcher was able to observe the assessment of the primary data set, which was determined by the research team to contain all the variables needed to address the specific aims of the original study as described in Bhagatwala et al. (2015).

Similarly, reliability refers to the process of how the data was collected, coded, and entered in the database (Garmon Bibb, 2007). Again, this doctoral researcher was a member of the research team for the original D-SUNNY study. All team members were familiar with the study design, aims, procedures, and protocols and trained on all measurements procedures. Measurement data were reviewed by other team members for accuracy. Data were collected and arranged in an accurate and consistent manner that was replicable. The data were recorded onto forms and kept separate from participant identification files. Data was then transcribed onto an electronic spreadsheet (MS Excel® 2013, Microsoft, Seattle, WA) by two members of the research team; one of which was this doctoral researcher. Inter-rater reliability was assessed at the end of each transcription session. Therefore, this doctoral researcher is confident that the data collected for the primary data set is of high quality for this secondary analysis, and met the requirements to answer the specific aims of this present research project.

To further assure data quality after collecting it for the primary data set, this doctoral researcher reviewed the data for the present secondary analysis regarding inter-rater reliability. Data quality can best be assured when the statistical analysis of the new study is handled with as much care as the initial study data (Garmon Bibb, 2007). To ensure that the data were correct, inter-rater reliability for this secondary analysis was tested by re-entering 10% of the sample records. Correctness was acceptable at 90%. When re-entering 10% of the data, this researcher found 100% of the data were correct, falling within the chosen range of acceptability. If 90% correctness had not been obtained upon first testing, she would have re-entered an additional 10% of the sample records until 90% was obtained before conducting the secondary data analysis.

Finally, the secondary data set may not represent as well as desired the target population of the subsequent study. However, the aims of this secondary analysis were closely aligned with the aims of the original D-SUNNY study, including the target population. Furthermore, this researcher participated in recruiting and screening the African American subjects for original study. Participants were recruited from the community through advertisements in the local newspapers, distribution of IRB approved study fliers on the bulletin boards and via email with IRB approved flier attachment, online classifieds, and by word of mouth. Additionally, several study team members, including this doctoral researcher, visited local high schools to recruit eligible participants with permission from the local school administration. The present doctoral researcher has firsthand knowledge that the population of the original study did indeed match the intended population of interest in the present secondary analysis research project.

Strengths

Despite the limitations, this secondary analysis had strengths as a research project. The original D-SUNNY research project provided the database for this secondary-analysis project based on the examination of variables concerning serum vitamin D and calcium concentrations, vascular measures, telomerase activity, arterial physiology, and renal function in healthy, overweight and obese African Americans with vitamin D insufficiency residing in southeastern region of the United States (Dong, 2012). This present secondary analysis built upon the original D-SUNNY study by exploring the possible relationships between vitamin D status, sex, age, and BMI as well as the possible influence of vitamin D supplementation on the immune system via IL-6 in this vulnerable population.

The original D-SUNNY study used a double-blind, randomized, placebo-controlled, experimental design. This design strived to ensure a rigorous study by preventing bias from awareness, chance rather than design, and a baseline for which to measure the vitamin D supplementation treatment. This secondary analysis used a database that had successfully recruited African American participants who were overweight or obese with a vitamin D deficiency, even in the summer months. This doctoral researcher worked as an assistant in the original D-SUNNY study, witnessing firsthand the data collection procedures. Thus, this doctoral research can attest to the original database's validity and reliability. The vitamin D dosages used in the original D-SUNNY study were selected purposely to test the effectiveness of the IOM (2011) recommendations, along with other suggested dosages derived from the research literature. Also, the D-SUNNY study employed monthly dosing of vitamin D supplementation, which increased participant compliance due to lowering the burden of

weekly or daily dosing. Participant compliance was also assured by requiring that participants come to the research laboratory to take their vitamin D supplement in the presence of a research team member.

Lastly, this is the first study to use the VPCM as the theoretical framework for a vitamin D-related investigation. The age, sex, and BMI moderating variables could adversely influence the resources available to a vulnerable African American population, which would eventually increase the relative risk of acquiring a chronic disease and negatively impact health status. For example, older African American women may lack access to stores selling vitamin D-rich food and/or they may not have the money to purchase vitamin D supplements. The data results of this secondary analysis showed that the female African American participants exhibited an insufficient vitamin D status at baseline. Thus, nursing interventions need to be developed and tested that would improve African American women's access to available resources and/or educate them on the importance of taking vitamin D supplements. Within the VPCM framework, these nursing interventions could to be tested to determine if they interventions improved the vitamin D status for the African American women and lowered their risk of chronic disease while maintaining a healthy status. Furthermore, all nurse clinicians (regardless of practice setting) could intervene with primary, secondary or tertiary levels of prevention to improve the vitamin D status and overall health of vulnerable populations. Nurse practitioners are a prime resource to educate their patients about the benefits of vitamin D, screen them for vitamin D insufficiency, and prescribe safe and effective doses of vitamin D supplementation to achieve the vitamin D status recommended by the IOM (2011), NIH (2011) and/or the Endocrine Society (Holick et al., 2011) for health promotion and disease prevention.

Research Implications and Clinical Recommendations

In the last 60 years, researchers have established how vital vitamin D is for maintaining overall health. Nurses need to be educated regarding the benefits of vitamin D, its sources, risk factors for deficiency, screening procedures, and treatment regimens recommended by the IOM, NIH, and Endocrine Society. As leaders in health education, knowledgeable nurses need to teach the public about the significance of vitamin D, its influence on overall health, and the importance of getting enough vitamin D via sunshine exposure or oral supplementation. Nurse practitioners need to identify those individuals at risk for vitamin D insufficiency and treat them before the irreversible occurrence of chronic disease. Also, nurse practitioners need to be aware of the moderating effect age, sex, and BMI have on vitamin D status so to prescribe appropriate dosages of vitamin D supplements. The findings from this secondary analysis suggest that the national clinical guidelines for vitamin D supplementation may warrant revision to at least 2,000 IU/day to be an effective treatment insufficient vitamin D in vulnerable populations. The results of this secondary analysis provided data that nurse practitioners and other clinicians should use as evidence for prescribing higher vitamin D dosages to their vulnerable clients, e.g., obese female African Americans. Nursing studies are needed to test nursing interventions in a clinical trial with large sample sizes that would improve vitamin D status and resolve health disparities. Nurse researchers are needed to conduct studies that examine the impact of nursing interventions on biological variables such as cardiac and renal function to elucidate best practice for prevention of chronic diseases related to vitamin D insufficiency such as cardiovascular and autoimmune diseases.

Conclusions

Although not statistically significant, the data results of this secondary analysis research project showed that the 2,000 or 4,000 IU/day dosages of vitamin D supplementation was needed for the overweight and obese African American participants to achieve a sufficient serum vitamin D concentration > 50 nmol/L as recommended in the IOM (2011) report. These results also suggest that the overweight/obese, African American adolescents and adults needed much more vitamin D supplementation than the 600 IU/day recommended by NIH (2017) researchers for individuals aged 1-70 years. Lastly, the findings suggest that the national clinical guidelines published by the Endocrine Society (Holick et al., 2011) may warrant revision to at least 2,000 IU/day to be effective for individuals in vulnerable populations. Future research is needed to further elucidate the role vitamin D plays in maintaining overall good health and the benefits of vitamin D supplementation.

Summary

Vitamin D insufficiency is a global and American health concern. Insufficient vitamin D promotes the development of cardiovascular and autoimmune diseases. The results of this secondary analysis of the D-SUNNY database provided evidence that healthy African American participants, who showed no evidence of chronic disease, improved their vitamin D status with the 2,000 and 4,000 dosages of supplemental vitamin D. Obese female African American participants especially needed the higher vitamin D dosages to achieve a sufficient vitamin D status. Nursing research is needed to determine the best techniques for assessing vitamin D insufficiency and its symptom clusters as well as identifying appropriate nursing interventions for improving the vitamin D status of individuals in a vulnerable population.

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