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ABSTRACT

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Americans are not reaching sufficient levels of serum 25-Hydroxy Vitamin D$_3$ (25(OH)D$_3$), and some groups are at a higher risk of developing vitamin D deficiency. Having a sufficient level of 25(OH)D$_3$ can help prevent diseases like osteoporosis, certain types of cancers, cardiovascular disease, and hypertension.

The data of 7479 American participants 18 years of age and older were derived from the NHANES 2001-2002 and NHANES 2003-2004 surveys. These participants were then analyzed to assess serum 25(OH)D$_3$ levels. The variables used were age, gender, ethnicity, Body Mass Index (BMI), and smoking status. The statistical software, SUDDAN was used to control the weights of the samples, and least square means were calculated to control the confounding factors.

The mean serum 25(OH)D$_3$ levels (ng/ml) for Mexican males, Black males, White males and Mexican females, Black females and White females were 22.0 +/-0.6, 15.7 +/-0.5, 26.4 +/-0.5 and 19.0 +/-0.4, 14.7 +/-0.3, 26.5 +/-0.4. The percent of Mexican males, Black males, White males and Mexican females, Black females and White females falling into the deficient category were 37.6%, 72.7%, 19.5% and 55.4%, 80.6%, 23.7%. Overall, the percent of males falling into the deficient category was 27.2% and for the females 33.9%. The mean serum 25(OH)D$_3$ level for individuals greater than 70 years of age was the lowest overall, with the males’ mean serum 25(OH)D$_3$ level at 23.6 +/-0.4 and the females’ mean serum 25(OH)D$_3$ level at 22.7 +/-0.5. The mean serum 25(OH)D$_3$ level for the obese BMI group was the lowest overall, with the males’ mean serum 25(OH)D$_3$ level at 23.2 +/-0.5 and the females’ mean serum 25(OH)D$_3$ level at
21.0 +/- 0.5. The mean serum 25(OH)D₃ level for the smokers was the lowest overall, with the male mean serum 25(OH)D₃ level at 24.6 +/- 0.5 and the female mean serum 25(OH)D₃ level at 23.2 +/- 0.6. In conclusion, the mean serum 25(OH)D₃ level of Americans fell below the sufficient cut-off level of greater than 30 ng/ml, with only a small percentage of the population reaching this level.
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# Table of Contents

## CHAPTER I: INTRODUCTION
- Statement of the Problem ................................................................. 1
- Significance of the Study ................................................................. 1
- Objectives of the Study ................................................................. 1

## CHAPTER II: REVIEW OF THE LITERATURE ......................................................... 3
- Vitamin D Sources ........................................................................... 3
  - Synthesis of Cholecalciferol within the Skin .................................. 3
  - Structures of Vitamin D .............................................................. 4
- Absorption and Vitamin D Binding Protein ........................................ 5
- Metabolism of Vitamin D .................................................................. 5
  - Vitamin D₃ to 25-Hydroxyvitamin D₃ to 1,25-Dihydroxyvitamin D₃ .... 5
- Transport and Vitamin D Receptor .................................................. 6
  - Active Vitamin D: 1,25-Dihydroxyvitamin D₃ ................................ 6
- Storage and Excretion ..................................................................... 7
- Roles of Vitamin D .......................................................................... 7
  - Bone Mineral Homeostasis ......................................................... 7
  - Muscle, Balance, and Gait .......................................................... 8
  - Cancer ....................................................................................... 9
  - Hypertension and Cardiovascular Disease .................................. 10
- Assessment of Vitamin D Status ...................................................... 11
  - Serum 25(OH)D₃ ........................................................................ 11
- Populations At Risk ........................................................................ 14
  - Vitamin D and Aging ................................................................. 15
  - Vitamin D and Gender ............................................................... 17
  - Vitamin D and Ethnicity ............................................................ 18
  - Vitamin D and Body Mass Index .............................................. 19
  - Vitamin D and Smoking ............................................................ 20

## CHAPTER III: METHODS ............................................................................... 23
- Subjects .......................................................................................... 23
- Data Collection ................................................................................ 23
- Design ............................................................................................ 24
- Statistical Methods ......................................................................... 24

## CHAPTER IV: RESULTS ................................................................................. 25

## CHAPTER V: DISCUSSION ............................................................................ 38
- Conclusion ...................................................................................... 46
List of Tables

Estimates of Minimum Serum 25(OH)D₃ Levels Optimal for Fracture Prevention ..........14

Studies of Serum 25(OH)D₃ Levels Among the American Population .........................21

Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity and Gender: NHANES 2001-2002 and 2003 to 2004 .................................................................25

Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity, Gender and Age: NHANES 2001-2002 and 2003 to 2004 .................................................................27

Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity, Gender and Body Mass Index (BMI): NHANES 2001-2002 and 2003 to 2004 .........................................................29

Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity, Gender and Smoking Status: NHANES 2001-2002 and 2003 to 2004 .........................................................30

List of Figures

Structure of Erogcalciferol ..........................................................5
Structure of Cholecalciferol ..........................................................5

Figure 1 Mean Serum 25-Hydroxy Vitamin D₃ by Race and Gender .................26
Figure 2 Mean Serum 25-Hydroxy Vitamin D₃ by Race and Age .........................27
Figure 3 Mean Serum 25-Hydroxy Vitamin D₃ by Race and Body Mass Index ...........29
Figure 4 Mean Serum 25-Hydroxy Vitamin D₃ by Race and Smoking Status ...........30

Figure 5 American Adults with Deficient, Insufficient, Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ by Gender ..............................................................33
Figure 6 American Adults with Deficient, Insufficient, Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ by Race .................................................................34
Figure 7 American Adults with Deficient, Insufficient, Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ by Age .................................................................35
Figure 8 American Adults with Deficient, Insufficient, Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ by Body Mass Index ...................................................36
Figure 9 American Adults with Deficient, Insufficient, Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ by Smoking Status ......................................................37
CHAPTER I: INTRODUCTION

Statement of the Problem

Americans are not reaching sufficient levels of serum 25-Hydroxy Vitamin D$_3$ \((25(OH)D_3)\) and some groups may be at a higher risk of developing vitamin D deficiency.

Significance of the Study

There is growing evidence that many American’s have low levels of serum vitamin D$^1,2,3,4,5,6$ As evidenced from the literature, there are groups that are at a particularly greater risk of developing vitamin D deficiency due to physiological factors and lifestyle habits. Having a sufficient vitamin D status can help prevent diseases like osteoporosis$^7,8,9$ certain types of cancers,$^{10,11,12,13}$ cardiovascular disease,$^{14,15,16}$ and hypertension.$^1$

Objectives of the Study

The main purpose of this study is to assess vitamin D status using serum 25(OH)D$_3$ levels of the American population, 18 years of age and older based on the National Health and Nutrition Examination Survey (NHANES) 2001 - 2002 and NHANES 2003 - 2004.$^{17}$ The secondary purpose is to identify groups of Americans that are at risk for developing vitamin D deficiency. The objectives of the study are:

1. To determine the mean serum 25(OH)D$_3$ status in different age (18-30, 31-50, 51-70, + 70 years), gender, and race/ethnicity groups (Mexicans, non-Hispanic Blacks, non-Hispanic Whites).

2. To determine the mean serum 25(OH)D$_3$ status in different Body Mass Index groups (<18.5 kg/m$^2$, 18.5 - 24.9 kg/m$^2$, 25 to < 30 kg/m$^2$, 30 $\geq$ kg/m$^2$).

3. To determine the mean serum 25(OH)D$_3$ status in non-smoker and smoker groups.
4. To determine the percent of the American population with deficient, insufficient and sufficient levels of serum 25(OH)D\textsubscript{3} using the cut-off levels < 20 ng/ml, 20-30 ng/ml and > 30 ng/ml.
CHAPTER II: REVIEW OF THE LITERATURE

Vitamin D Sources

Vitamin D is at least 500 million years old, originating from photosynthesis by phytoplankton exposed to sunlight. Today, humans obtain this fat soluble vitamin from two sources: the diet and through synthesis by the skin. Sources coming from the diet are found either in the form of D₂ (ergocalciferol) or D₃ (cholecalciferol). Cholecalciferol is obtained through the diet from animal sources, which includes oily fish, egg yolks, and liver. Ergocalciferol is obtained through the diet from manufactured plants. The manufacturing of ergocalciferol occurs through ultraviolet (UV) irradiation of yeast and plant sterols. Ergocalciferol has also been found naturally in some sun exposed wild mushrooms. Most vitamin D in supplements, fortified foods and beverages contain the plant form, ergocalciferol. It wasn’t until about 20 years ago that fortification of foods included cholecalciferol. Fortified foods and beverages found in the United States include milk, milk products, breakfast cereals, grain products, pastas, infant formula, and margarine. Cholecalciferol can also be made in the skin when exposed to the sunlight. The sun provides the UV rays that initiate the synthesis of vitamin D. The majority of cholecalciferol comes from the human body through synthesis by the skin. Thus, exposure to UV rays is an essential source to maintain a healthy vitamin D status.

Synthesis of Cholecalciferol within the Skin

Cholecalciferol synthesis within the skin begins with exposure to the sun. Photolysis is initiated by UV photons with at least a 297 nm spectra hitting the skins surface. The UV rays are absorbed by 7-dehydrocholesterol which is a provitamin found in the skin’s basal and suprabasal layers. This absorption causes a rearrangement of double bonds, opening up the B
ring of 7-dehydrocholesterol producing previtamin D$_3$.\textsuperscript{20} Previtamin D$_3$ takes on two conformationer forms which include $s$-$cis$, $s$-$cis$ (czc) and $s$-$trans$, $s$-$cis$ (tzc).\textsuperscript{11} Out of these forms, cholecalciferol can only be produced from the czc conformer. Finally, catalyzed by body heat of 37 degrees Celsius, previtamin D$_3$ in the czc form is isomerized to yield cholecalciferol.\textsuperscript{10, 18} Synthesis of vitamin D by the skin can be affected by several different factors which include season, latitude, skin pigmentation, sunscreen use, clothing, and aging.\textsuperscript{24, 25} Synthesis of cholecalciferol is photoregulated which is the body’s way of preventing vitamin D intoxication.\textsuperscript{18, 26} When sun exposure is prolonged, 7-dehydrocholesterol will continue to convert to previtamin D$_3$ only for the first few minutes.\textsuperscript{18} When the production of previtamin D$_3$ ends, it begins to degrade through isomerisation. Previtamin D$_3$ is isomerized to the inert isomers lumisterol and tachysterol.

\textit{Structures of Vitamin D}

The structures of ergocalciferol and cholecalciferol are similar except for a few small differences. Ergocalciferol has a carbon side chain which includes double bonds between carbon 22, carbon 23, and has an additional methyl group on carbon 24,\textsuperscript{18, 27} and cholecalciferol has a cholesterol side chain.\textsuperscript{18, 19} Below, figures 1 and 2 show the structures of ergocalciferol and cholecalciferol. Ergocalciferol is reported to be 30 to 50\% as effective as cholecalciferol to humans.\textsuperscript{24} Recommendations have been made to include cholecalciferol to properly utilize calcium.\textsuperscript{25} The biological difference between these two forms of vitamin D is small and they are actually considered to be equivalent.\textsuperscript{27} This equivalency supports the research that cholecalciferol and ergocalciferol are both adequate sources of vitamin D.\textsuperscript{24}
Absorption and Vitamin D Binding Protein

Vitamin D binding protein (DBP) is the major carrier protein for all vitamin D (when vitamin D is written without a subscript it represents D₂, D₃) metabolites, with its main functions being to bind, solubilize, and transport. Depending on the source of vitamin D, binding to DBP will occur at different points during metabolism. Vitamin D from dietary sources are absorbed in the small intestine and are incorporated into chylomicrons that enter the lymphatic system. From the lymphatic system, dietary vitamin D is bound to the DBP, allowing it to enter into the venous circulation. Vitamin D from the diet has a short half life due to the fact that only two thirds is actually bound to DBP. Unlike vitamin D from the diet, vitamin D made in the skin has a longer half life because almost 100% is bound to DBP. Vitamin D made in the skin is forced out of the skin’s plasma membrane into the extracellular space, and is picked up directly by DBP. After this, it is carried straight to the venous circulation.

Metabolism of Vitamin D

*Vitamin D₃ to 25-Hydroxyvitamin D₃ to 1,25-Dihydroxyvitamin D₃*

In the body, vitamin D₃ is changed to 25-hydroxyvitamin D₃ (25(OH)D₃, calcidiol) and from it to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, calcitriol). The first hydroxylation occurs
in the liver. Hydroxylation begins at carbon 25, catalyzed by vitamin D-25-hydroxalase. This reaction produces 25(OH)D$_3$, which is the less active metabolite. The metabolite 1,25(OH)$_2$D$_3$ is the major circulating form of vitamin D and is considered to be more active. Bound to the DBP, 25(OH)D$_3$ is carried from the liver to the kidneys where megalin cells recognize 25(OH)D$_3$-DBP complex. Megalin is an important regulator for the transport of this complex into the kidney cells. When the 25(OH)D$_3$-DBP complex enters the kidney cell, it goes straight into the mitochondria. Here, it is hydroxylated for the second time at the carbon one alpha position by the 25(OH)D$_3$-1alpha-hydroxylase, which is regulated tightly by the parathyroid hormone (PTH), serum calcium, and serum phosphorous. 25(OH)D$_3$-1alpha-hydroxylase is mainly found in the kidney, but is also found in other cells throughout the body. Some of these cells include, human bone, keratinocytes, lungs, colon, prostate, and breast. The localized 25(OH)D$_3$-1alpha-hydroxylase does not actually enter the circulation, but specifically acts locally. This final hydroxylation process yields the most active form of vitamin D, which is 1,25(OH)$_2$D$_3$. 1,25(OH)$_2$D$_3$ then, enters the blood, where it is rarely found free floating and is tightly bound to the DBP or to albumin.

Transport and Vitamin D Receptor

Active Vitamin D: 1,25-Dihydroxyvitamin D$_3$

The 1,25(OH)$_2$D$_3$-DBP complex can be carried to various target tissues, but in order to enter into a cell, vitamin D needs to be detached from DBP. Vitamin D receptors (VDR) are important regulators of 1,25(OH)$_2$D$_3$ actions in target organs. VDRs can be found in several different tissues, such as brain neurons, skin, breast, bone, testes, ovaries, parathyroid gland, cardiac muscles, kidney, liver, and colon to name a few. The regulation of 1,25(OH)$_2$D$_3$ by the VDRs helps to prevent vitamin D toxicity and hypercalcemia.
Storage and Excretion

When there is a sufficient amount of 1,25(OH)2D3, 24,25(OH)2D (citroic acid), a water soluble, inactive excretory product is made in the kidney.11, 30 Citroic acid is then excreted by the kidney into the urine. If the body has enough vitamin D, it can also be stored in the fat for later use.18 The storage of vitamin D in the fat is not large enough to prevent variations in the serum plasma concentrations of 25(OH)D3.31

Roles of Vitamin D

Bone Mineral Homeostasis

Vitamin D is important to maintain bone health and homeostasis of calcium and phosphorous. Calcium and phosphorous are essential for bone formation through the life span.7, 12 Without these two minerals, impaired bone mineralization and compromised osteoblast formation would occur. Osteoblasts are important since they are responsible for building up the bone.30

Vitamin D plays a role in the transport and absorption of calcium and phosphorous and keeping them at normal serum levels.32 When calcium intake does not meet the necessary requirements, serum levels drop, which stimulates the PTH levels to increase. The high levels of PTH stimulate vitamin D-25-hydroxalase activity in the kidney, producing more 1,25(OH)2D3.20 1,25(OH)2D3 acts as a catabolic hormone,11 breaking down the bone which releases calcium into the blood restoring homeostasis.30 Also, absorption of both calcium and phosphorous is enhanced in the small intestine by 1,25(OH)2D3, and renal reabsorption is stimulated.30, 33

Vitamin D promotes the opening and closing of important calcium and chloride ion channels. For this to occur, 1,25(OH)2D3 is recognized by the vitamin D receptors located in the
small intestine. This increases the expression of the epithelial calcium channel and results in the increased absorption of calcium in the small intestine, mainly in the duodenum.

Vitamin D and bone research has become important with the increasing aging population and the rise of osteoporosis. Osteoporosis is a metabolic disorder that reduces bone mass and changes bone turnover, and is often referred to as the silent illness. The National Osteoporosis Foundation states that osteoporosis is a health threat to 44 million people in the United States, with 10 million already diagnosed and 34 million with low bone mineral density (BMD). In older adults with osteoporosis, minimal trauma can cause a fracture leading to a decrease in one’s quality of life. World-wide, 1.66 million hip fractures occur each year. It has been found that patients with osteoporosis tend to have inadequate levels of vitamin D and this has become an established risk factor. Many studies show that serum 25(OH)D$_3$ levels are related to BMD. Arya et al found that in healthy Asian Indians BMD at the femoral neck and Ward’s triangle correlated with serum 25(OH)D$_3$. Low levels of 25(OH)D$_3$ resulted in decreased BMD. The economic burden that osteoporosis will cost the health care services is forecasted to be billions of dollars every year. The National Osteoporosis Foundation stated that in 2005 the cost of health care was $19 billion, and they estimate that in 2025, the cost will be $25.3 billion. Not only is this disease costly, but once a osteoporotic fracture has occurred, it can cause morbidity and disability.

**Muscle, Balance, and Gait**

Vitamin D is needed for muscle, balance, and gait, which is important to prevent falls and fractures. Research on the role of vitamin D in relation to this topic is still not completely clear. There is evidence showing that vitamin D is needed for proper muscle growth and development and to maintain the integrity and function of the muscle cell. Vitamin D is also needed for
active transport of calcium into the sarcoplasma reticulum by calcium-ATPase\textsuperscript{12, 32} which increase the calcium ion pool for muscle contractions.

Vitamin D receptors have been found on muscle cells, explaining its possible role in improving muscle strength.\textsuperscript{20} Vitamin D deficiency has been found in people with osteomalacic myopathy, which causes muscle weakness and impaired gait.\textsuperscript{32} Individuals with osteomalacic myopathy have shown through muscle biopsies to have atrophy of type II muscle fibers. Type II muscle fibers tend to be fast and strong, and are the first to respond when someone begins to fall.\textsuperscript{8} Body sway which can affect balance and gait was observed in two groups of elderly women supplemented with either calcium and vitamin D or calcium only.\textsuperscript{20} The group that was supplemented with both vitamin D and calcium had a decrease in body sway compared to the calcium only group. It seems that vitamin D affects the lower extremities that are used in walking and balance.\textsuperscript{8} This can possibly be linked to the location of vitamin D receptors in the brain that may have an affect on the central nervous system which controls balance and gait.\textsuperscript{35}

Not all studies support vitamin D’s role in muscle strength and physical performance. Sixty men who were community-dwelling with age ranges between 65 to 87 years completed a study involving vitamin D supplementation.\textsuperscript{36} One group was given 1000 IU/day of cholecalciferol and the other group was given a placebo for six months. The results from this study showed no gains in muscle strength or physical performance.

\textit{Cancer}

Vitamin D synthesis by the skin and cancer prevention has become an important area of research. In 1941, the first observation was made that people who lived in northern latitudes in the United States were more likely to die from cancer when compared to people living in the southern latitudes.\textsuperscript{13} This observation was not studied further until 1980’s. Through the years, the
link between increased rate of cancer of the breast, colon, ovaries, and prostate\textsuperscript{12,37} in individuals who live at higher latitudes has been well documented.\textsuperscript{10,11,12,13} Individuals living in the southern part of California had less of an incidence of colon cancer. There has been no solid link, however to dietary intake of vitamin D and its role in cancer prevention.\textsuperscript{10} The established level of serum 25(OH)D\textsubscript{3} needed to reduce prostate, breast, and colon cancers by 30 to 50\% was reported in several studies to be 20 ng/mL.\textsuperscript{13}

Research has been carried out further on many tissues and organs that show the link between vitamin D and cancer. One major discovery was the location of vitamin D receptors on tissues not involved in calcium metabolism.\textsuperscript{13,23} This was an important discovery because these receptors are involved with the binding of 1,25(OH)\textsubscript{2}D\textsubscript{3}. The second discovery showing that these tissues express the 25(OH)D\textsubscript{3}-1alpha-hydroxylase, makes it possible for local tissues to convert 25(OH)D\textsubscript{3} to 1,25(OH)\textsubscript{2}D\textsubscript{3}.\textsuperscript{13} With these discoveries, research went further and found that 1,25(OH)\textsubscript{2}D\textsubscript{3} does in fact play several roles in cancer prevention by being a potent inhibitor of cell proliferation.\textsuperscript{38}

\textit{Hypertension and Cardiovascular Disease}

Vitamin D may play a role in hypertension and cardiovascular disease. Active vitamin D has the ability to inhibit rennin secretion.\textsuperscript{14} Rennin starts a cascade of events that lead to the increase in blood pressure when it produces angiotensin I and II. Angiotensin II is a vasoconstrictor that stimulates aldosterone productivity which in turn stimulates the sodium uptake by the kidney. 1,25(OH)\textsubscript{2}D\textsubscript{3} vitamin D receptors have been found on smooth muscle cells.\textsuperscript{14,15} It acts as a vasoactive and pro-oxidative substance on smooth muscle cells.\textsuperscript{16}

Lind et al conducted a study on hypertension and other related cardiovascular risk factors involving 34 middle-aged men.\textsuperscript{15} Results from the measurements taken on these subjects showed
that serum levels of 1,25(OH)₂D₃ was inversely correlated to blood pressure, VLDL triglycerides, and triglyceride removal at the intravenous fat tolerance test. 25(OH)D₃ was also associated with insulin sensitivity, lipoprotein lipase activity, and skeletal muscle. A study using NHANES III data found that there was an inverse relationship between blood pressure and serum 25(OH)D₃.¹ Vascular calcification in high or moderate risk populations for coronary heart disease was inversely related to serum levels of active vitamin D.³⁹ Research on congestive heart failure patients has found that low vitamin D status leads to alterations in mineral metabolism and myocardial dysfunction which may contribute to the cause of the disease.⁴

Assessment of Vitamin D Status

Measuring vitamin D status is difficult to do through dietary records alone. Many foods are now fortified with vitamin D, and there are no specific standards for the amounts that are added to the foods, making it difficult to measure the amount.⁵, ²⁶ Sources also come from synthesis by the skin when exposed to sunlight adding to the complication of measuring vitamin D status. Production of cholecalciferol can be affected by latitude where one lives, sunscreen use, amount of clothing worn, and skin pigmentation making it difficult to measure exactly how much is produced.¹¹, ²⁶, ⁴¹ These are the reason behind using serum metabolites since they take into account all of the sources of vitamin D and the amounts coming from these sources.⁹, ²⁶

Serum 25(OH)D₃

In 1997, the Food and Nutrition Board (FNB) of the Institute of Medicine defined 25(OH)D₃ as the functional indicator of vitamin D status.²⁶ Serum 25(OH)D₃ is the widely used assay for studies including NHANES 2001-2002 and NHANES 2003-2004. The idea of using 1,25(OH)₂D₃ was considered, but for several reasons serum 25(OH)D₃ was selected instead. First, serum 25(OH)D₃ has a half-life of two weeks and serum 1,25(OH)₂D₃ has a half-life of 2 to
6 hours. Since serum 1,25(OH)\(_2\)D\(_3\) is tightly regulated by calcium, phosphorus, PTH, and other hormones many fluctuations occur in a short period of time. This can lead to an inaccurate analysis of vitamin D status. Serum 25(OH)D\(_3\) however, remains stable in the body making it a more accurate measure of status. Another reason behind the accuracy of serum 25(OH)D\(_3\) is that levels of serum 1,25(OH)\(_2\)D\(_3\) can remain normal or even elevated in a deficient state. The last important difference between these metabolites is that concentrations of serum 1,25(OH)\(_2\)D\(_3\) are a thousand times less than 25(OH)D\(_3\) making it a less valuable measure of vitamin D status.

Assays to measure serum 25(OH)D\(_3\) include radioimmunoassays (RIAs), competitive vitamin D binding protein (CBP), high-performance liquid chromatography (HPLC), and HPLC with tandem mass spectroscopy (LC-MS). The assays used by NHANES 2001-2002 and NHANES 2003-2004 to analyze serum 25(OH)D\(_3\) are the RIAs.

Determining a serum 25(OH)D\(_3\) cut-off level to measure deficient, insufficient, and sufficient vitamin D status is yet to be established. Complicating this establishment is the many different physiological processes and pathological events that vitamin D is involved in. It is important to establish a specific level that can lead to the multiple positive health and disease prevention outcomes. At this time, researchers are inconsistent when defining a cut-off level of serum 25(OH)D\(_3\) due to the lack of a clear consensus. At this time, most researchers are basing the cut-off levels on optimal bone health. Heaney reviewed several studies that focused on bone markers showing reduced fractures, increased calcium absorption, and increased neuromuscular function in the lower extremities with serum 25(OH)D\(_3\) levels at 32 ng/ml (80 nmol/L). Also, there was even an increase in BMD at serum 25(OH)D\(_3\) levels at higher than 40 ng/ml (100
Despite this finding, calcium absorption seems to reach its maximum when serum 25(OH)D₃ levels are at about 32 ng/ml (80 nmol/L). The National Osteoporosis Foundation recommends that serum 25(OH)D₃ be at 30 ng/ml (75 nmol/L) or higher to be considered optimal. Osteomalacia, which is the softening of the bones, has been observed in individuals with serum 25(OH)D₃ levels below 8 ng/ml (20 nmol/L). Another way researchers have determined the level of adequacy for serum 25(OH)D₃ is through CBP studies. Looker et al used NHANES III to study adolescents and adults in two seasonal subpopulations and set serum 25(OH)D₃ cut-off levels based on the CBP. The cut-off levels were a serum 25(OH)D₃ level of less than 7 ng/ml (17.5 nmol/L) to define deficiency. Also, four serum 25(OH)D₃ levels were used as cut-offs to define insufficiency, and these levels were less than 10 ng/L (25 nmol/L), less than 15 ng/ml (37.5 nmol/L), less than 20 ng/ml (50 nmol/L), and less than 25 ng/ml (62.5 nmol/L). The reason behind using several serum 25(OH)D₃ levels was the fact that there is no set value to define deficiency, insufficiency, and sufficiency, and multiple values would benefit other researchers with their studies. However, comparing the serum cut-off levels that were set by Looker et al in the above NHANES III study, they are much lower than Holick’s report of vitamin D deficiency developing when levels are less than 20 ng/ml (50 nmol/L).

During a round table discussion at the Fifth International Symposium on the Nutritional Aspects of Osteoporosis, held in Lausanne, Switzerland, in May 2003, a consensus was made between six investigators. They considered bone health markers to be an accurate way to determine adequate serum 25(OH)D₃ levels. The markers included maximum suppression of PTH, greatest calcium absorption, highest BMD, reduced rates of falling, and reduced fracture rates. The investigators determined that optimum serum 25(OH)D₃ for bone health to be between
20 and 32 ng/ml (50 and 80 nmol/L), which is shown in Table 1. Five out of six of these investigators felt that serum 25(OH)D₃ should be between 28 and 32 ng/ml (70 and 80 nmol/L).

The conclusion can be drawn from the above research that serum 25(OH)D₃ levels above 30 ng/ml (75 nmol/L) is a sufficient goal for Americans to reach. As levels fall below 30 ng/ml (75 nmol/L), vitamin D insufficiency develops, and with a further drop below 20 ng/ml (50 nmol/L), vitamin D status is considered to be deficient.

**Table 1** Estimates of minimum serum 25(OH)D₃ levels optimal for fracture prevention.

Converted from nmol/L to ng/ml. Equation: nmol/L multiplied by .4= ng/ml

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Optimal 25(OH)D₃ Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lips</td>
<td>20 ng/ml (50 nmol/L)</td>
</tr>
<tr>
<td>Holick</td>
<td>30 ng/ml (75 nmol/L)</td>
</tr>
<tr>
<td>Heaney</td>
<td>32 ng/ml (80 nmol/L)</td>
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<td>Vieth</td>
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<td>Dawson-Hughes</td>
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</table>

Populations At Risk

Vitamin D deficiency has become a pandemic with 1 billion people worldwide falling into this category.²⁴ Many Americans have low levels of serum 25(OH)D₃ based on numerous studies and findings which are listed in Table 2.¹,²,³,⁴,⁵,⁶ Looker et al used 18,875 NHANES III subjects from two different seasonal subpopulations, lower latitude/winter and higher latitude/summer.² In both seasonal subpopulations, less than 1% were considered to be deficient (<7 ng/ml). However, when the serum cut-off level was set at less than 25 ng/ml, the lower
latitude/winter subpopulation had 25-57% and the higher latitude/summer had 21-49% in the insufficient category.

Americans major source of vitamin D is by synthesis through the skin which requires exposure to sunlight. The amount of exposure to sunlight can be affected by the use of sunscreen, the amount of clothing worn, latitude, season, and time of day. NHANES III and the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998, found that intakes of vitamin D from food sources and dietary supplements was not meeting the recommended daily intakes. On top of the entire US population being at risk for developing vitamin D deficiency due to lower intake and decreased sun exposure, there are a few other factors that may actually put some individuals at greater risk. These factors include age, ethnicity, gender, body mass index, and smoking.

**Vitamin D and Aging**

Cross-sectional and longitudinal studies have shown that with increasing age there is a decrease in serum 25(OH)D$_3$. Using The New Mexico Aging Process Study, a longitudinal study, serum 25(OH)D$_3$ samples were obtained from both men and women subjects with the average age around 70 years old at the baseline of the study. The subjects were divided into two groups, one group was taking oral vitamin D supplements and the other was taking none. Serum 25(OH)D$_3$ samples were obtained during the same seasons to control for varied amounts of sun exposure from 1980 to 1982 and in 1989 to 1994. The final results from the study showed that the group without vitamin D supplements had a decline in serum 25(OH)D$_3$. This decline occurred at a rate of 4.3 ng/ml per decade. Using the cross-sectional Zutphen Study, which is the Dutch contribution to the Seven Countries Study, serum 25(OH)D$_3$ dropped as age increased by .6 ng/ml (1.5 nmol/L) for each year of age. This would be a 6 ng/ml drop per decade,
which is a greater drop than the results from The New Mexico Aging Process Study. However, most but not all of the studies support the decreasing serum levels in the aging population. Tangpricha et al found that the age group with the most seasonal variation and lowest percentage falling at an insufficient level was the 18 to 29 year olds.5

There are several reasons linked to the low levels of serum 25(OH)D3. Some of these factors are related to lifestyle changes that occur as one ages, and others are biological. One lifestyle factor that leads to the aging population’s low serum 25(OH)D3 levels is, as people age, they lack in the variety of foods that they consume and there is a decreased consumption of foods fortified with vitamin D like milk.22, 31, 48, 57 A study using NHANES III and CSFII (1994-96, 1998) showed that less then 10% of adults ages 51 to 70 years meet the requirement for vitamin D through the diet.22 The adults that were older then 70 years of age had less than 2% meeting the requirement for vitamin D through the diet. Furthermore, this study showed that even with supplementation, 90% of individuals older than 70 years were not meeting the recommended requirements. Brock et al studied Vietnamese elderly immigrants and that one of the best predictors of vitamin D deficiency was low dairy intake.49 Another possible reason for low levels serum 25(OH)D3 with aging is that with increasing age comes a decrease in physical and outdoor activities. This decrease in activity can possibly lead to decreased sun exposure.7, 31, 38, 57 The lack of exposure to the UVB rays causes the decreased synthesis of vitamin D by the skin. Brock et al found that like low dairy intake among the Vietnamese elderly immigrants, no vigorous exercise was a strong predictor of vitamin D deficiency, again linked to the possible decrease in sun exposure.49
Several biological factors lead to the decrease of serum 25(OH)D₃. The biggest factor is the atrophy that occurs with the skin, interfering with the ability of the skin to produce vitamin D when exposed to the sun. These atrophic changes that occur to the skin cause a decrease in the amount of the precursor present to produce vitamin D. This age-dependant skin atrophy can actually result in a decrease of cholecalciferol production to about one fifth of young individuals. Another biological factor affecting metabolism of vitamin D is the decline in liver and kidney functioning. This has an effect on the hydroxylation process that is needed to produce 1,25(OH)D₃. Elderly with low serum 25(OH)D₃ concentrations often experience hip fractures, have high PTH levels, and are suspected of having an accelerated rate of bone loss increasing their risk for fractures.

**Vitamin D and Gender**

Research shows that serum 25(OH)D₃ tends to be lower in women than in men. Bolland et al looked at the effects of several variables on serum 25(OH)D₃. One of the variables that they looked at was gender. They found that out of 50 women who were greater than 55 years old, had an average serum 25(OH)D₃ of 26.8 ng/ml (67 nmol/L) and that out of 50 men who were greater than 40 years and older, the average was 36.4 ng/ml (91 nmol/L). A cross-sectional study that was done on community-dwelling postmenopausal women from several different countries (Northern, Central, and Southern Europe, the Middle East, Latin America, the Pacific Rim, and Asia) to evaluate their vitamin D status. Results from this study showed that out of 2589 women, 64% had vitamin D inadequacy which was defined as levels below 30 ng/ml (75 nmol/L). Scragg et al used subjects from NHANES III and found that the mean serum 25(OH)D₃ levels in men to be 31.2 ng/ml compared to 29.2 ng/ml in women. However, Guardia et al found that 80% of men compared to 72% of women fell
below 30 ng/ml with 17% of men and 15% of women reaching the deficient level (<15 ng/ml).\textsuperscript{6}

Several lifestyle and biological factors have been used to explain the lower serum 25(OH)D\textsubscript{3} levels that are found in women. After reviewing several studies, Rizzoli et al reports that from postmenopausal women with vitamin D inadequacy, lifestyle factors identified as being associated with inadequacy were low sun exposure and low vitamin D intake from both diet and supplements.\textsuperscript{53} Supporting these reports, Bolland et al states that sun exposure tends to be different between men and women due to the behavioral and cultural differences of the two groups.\textsuperscript{52} When looking at Americans age 1 year and older, dietary intakes of vitamin D were lowest in female teenagers and female adults according to a study using NHANES III and CSFII (1994-96, 1998).\textsuperscript{22} Intakes of college students show that 22% of female and 47% of male students attained the adequate intake (AI) for vitamin D.\textsuperscript{58} A biological factor that has been identified and may possibly play a role in the difference in serum 25(OH)D\textsubscript{3} levels has to do with hormones. Total serum 25(OH)D\textsubscript{3} levels have been found to be higher in women who are taking oral contraceptives or hormone replacement therapy containing estrogen.\textsuperscript{52} This observation is thought to be due to the effect that estrogen has on the vitamin D binding protein, which rises in response to this hormone.

\textit{Vitamin D and Ethnicity}

Serum 25(OH)D\textsubscript{3} levels have been found to be lower in certain ethnic groups, putting them at a greater risk for deficiency. Results from a study using NHANES III showed that the mean serum 25(OH)D\textsubscript{3} for non-Hispanic whites was 31.6 ng/ml (79 nmol/L), for Mexican Americans it was 27.2 ng/ml (68 nmol/L), and for non-Hispanic blacks it was 19.6 ng/ml (49 nmol/L).\textsuperscript{1} Similar results were found in a study looking at two seasonal
subpopulations ranging from adolescents to adults using NHANES III to analyze vitamin D status. The results showed that non-Hispanic whites had mean serum 25(OH)D3 levels 1.2 to 1.7 times higher than the Mexican Americans and the non-Hispanic blacks in all of the age groups. It is interesting to mention that even though non-Hispanic blacks have a lower vitamin D status, they still have a high bone mineral density genetically and have a low bone turnover as compared to whites. This finding contradicts the findings that bone mineral density correlates with serum 25(OH)D3 which is found in many studies. In a study done in Queensland, Australia where sun exposure is high, individuals with black hair/olive skin or brown eyes had lower serum 25(OH)D3 levels when compared to brown or fair hair, fair skin, or people with blue/green eyes. Looking at the Asian Indians, one study set the cut off for vitamin D insufficiency at 20 ng/ml, with 78.3% of the subjects falling below this level. The researchers also associated their vitamin D status to the possibility of skin pigmentation. Low levels of serum 25(OH)D3 maybe affected by the skin pigmentation in the different ethnic groups. Darker skin from tanning or from natural pigmentation contains increased amounts of melanin which absorbs and protects the skin from UV wave lengths. Thus, melanin interferes with the production of pre-vitamin D which is needed to make cholecalciferol. Another reason for the possible difference of serum 25(OH)D3 levels among ethnic groups according to some studies is that blacks tend to have higher levels of parathyroid hormone. Increased PTH levels has been inversely associated with decreased levels of serum 25(OH)D3.

*Vitamin D and Body Mass Index (BMI)*

Body mass index has been found in several studies to affect serum 25(OH)D3. The results have shown that BMI has an inverse affect on serum 25(OH)D3.
al found that children and adolescents with serum 25(OH)D$_3$ levels below 30 ng/ml (75 nmol/L) had higher BMI and fat mass, and lower ratios of fat-free mass.$^4$ It is hard to decide on the cause of low levels of serum 25(OH)D$_3$ in individuals with a high BMI. It could be linked to lifestyle factors such as a lack of sun exposure from avoiding activity outdoors or covering up more areas of the skin due to self conscious feelings.$^{59}$ Another lifestyle factor could be poor dietary choices leading to low vitamin D intake. Alemzadeh et al found that children and adolescents with serum 25(OH)D$_3$ levels below 30 ng/ml (75 nmol/L) reported having a lower intake of vitamin D and had a lower percentage of the adequate intake (AI) than the vitamin-D sufficient group.$^4$ Some research mentions that the increase in fat mass creates a larger pool to distribute vitamin D coming from both the diet and sunlight.$^1,11,24,47$ This pool of vitamin D is not bioavailable to the body leading to low serum 25(OH)D$_3$ levels.

**Vitamin D and Smoking**

Few studies have included smoking as a variable when studying serum 25(OH)D$_3$. There is evidence that smoking is detrimental to bone health, but specifics surrounding vitamin D are unclear and findings are inconsistent.$^{55,56}$ There is evidence that serum 1,25(OH)$_2$D$_3$ is lower in smoking women than in non-smoking women.$^{56,60}$ This is important because adequate levels of 1,25(OH)$_2$D$_3$ is needed for adequate intestinal calcium absorption. However, when it comes to measuring serum 25(OH)D$_3$, Kimlin et al reports that smoking had no association with low levels.$^{59}$ Brock et al actually found smoking to have a protective factor for the Vietnamese elderly immigrant population.$^{49}$ The thought behind this protective factor is that smokers go outside to smoke, increasing the synthesis of vitamin D by the skin.
Table 2 Studies of Serum 25(OH)D₃ Levels Among the American Population

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study</th>
<th>Variable</th>
<th>Subjects (n)</th>
<th>Cut-Off Value Serum 25(OH)D₃</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Looker et al</td>
<td>1988-1994</td>
<td>NHANES III</td>
<td>Gender, Ethnicity, Age, Latitudes, Seasons, Serum 25(OH)D₃</td>
<td>Age ≥ 12 years n=18,875</td>
<td>7 ng/ml (Deficiency)</td>
<td>Both subpopulations had less than 1% at deficient level. At a cut-off of 25 ng/ml lower latitude/winter had 25-57% and the higher latitude/summer had 21-49% fall under the insufficient level.</td>
</tr>
<tr>
<td>Guardia et al</td>
<td>January 1997 to December 2001</td>
<td>Cross-Sectional Study In Detroit, MI</td>
<td>Gender, Ethnicity (Black/White), Age, Serum 25(OH)D₃</td>
<td>Patients Seeking Advice for Osteoporosis is in the Bone and Mineral Hospital n=2,924</td>
<td>Under 15 ng/ml Under 20 ng/ml Under 30 ng/ml</td>
<td>Under 15 ng/ml: Men (17%), Women (15%), Whites (12%), Blacks (35%), Total (15%); Under 20 ng/ml: Men (38%), Women (31%), Whites (28%), Blacks (60%), Total (32%); Under 30 ng/ml: Men</td>
</tr>
</tbody>
</table>
Continued- Studies of Serum 25(OH)D₃ Levels Among the American Population

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Participants</th>
<th>Data Collection</th>
<th>Measures</th>
<th>n (winter)</th>
<th>n (summer)</th>
<th>Insufficiency&lt;br&gt;(&lt; 20 ng/ml)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tangpricha et al</td>
<td>March/April 1999 (end of winter) and September/October (end of summer) 1999</td>
<td>Boston University Medical Center</td>
<td>Age, Season, Milk Consumption, Supplement Use, PTH Levels, Serum 25(OH)D₃</td>
<td>Hospital Visitors, Employees, Medical Students Ages ≥ 18 years</td>
<td>n (winter)=165</td>
<td>n (summer)=142</td>
<td>≤ 20 ng/ml&lt;br&gt;(Insufficiency)</td>
<td>The most seasonal variation was found in 18-29 year olds from the end of winter to the end of summer with serum levels rising by 30%. The oldest age group which was ≥ 50 was least likely in summer (4%) and winter (16%) to have insufficiency compared to all other age groups which had 10% in summer and 30% in winter at insufficient serum levels.</td>
</tr>
<tr>
<td>Perry et al</td>
<td>Published: 1999</td>
<td>Longitudinal Study Using the New Mexico Aging Process Study</td>
<td>Age, Supplement Use, Dietary Intake, Month of Year, Body Weight, Physical Activity, Serum 25(OH)D₃</td>
<td>n=136</td>
<td>No Cut-Off Values</td>
<td>Mean Serum 25(OH)D₃ (ng/ml) Group A (no supplementation) at baseline mean was 26.2 and at final 21.5. Group B (supplementation) at baseline mean was 27.4 and 27.7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alemzadeh et al</td>
<td>Published: 2008</td>
<td>Children’s Hospital of Wisconsin</td>
<td>Gender, Age, BMI, Weight, Fat Mass, Fat Free Mass, Glucose, Insulin, HbA1C, Dietary Intake, Serum 25(OH)D₃</td>
<td>Children with BMI &gt; 95th Percentile Ages 6 to 17.9 years</td>
<td>n=127</td>
<td>&lt; 20 ng/ml&lt;br&gt;(Deficient) 20-30 ng/ml&lt;br&gt;(Insufficient) ≥ 30 ng/ml&lt;br&gt;(Sufficient)</td>
<td>Mean Serum 25(OH)D₃ (ng/ml) for the enter group with an average BMI of 37.1 was 24. 74% of the children had insufficient/deficient levels with 32.3% of children at the deficient level.</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER III: METHODS

Subjects

The sample for this study included 7479 participants 18 years of age and older derived from the NHANES 2001-2002 and NHANES 2003-2004 surveys. The participants were excluded from the study if they had kidney or liver disorders, were pregnant, or were lactating. The purposes of these exclusions were due to the fact that these conditions affect normal vitamin D metabolism or dietary habits. The participants were grouped according to age, gender, ethnicity, Body Mass Index (BMI), and smoking status.

Within the sample of 7479 participants, there were 3825 males, and 3654 females. The age group of 18-30 years included 2232 participants; 31-50 years included 2373 participants; 51-70 years included 1839 participants; and ≥ 70 years included 1035 participants. For the ethnicity groups, 1866 participants were classified as Mexican; 1719 were classified as non-Hispanic Blacks; 3894 were classified as non-Hispanic Whites. For BMI (kg/m²), 153 participants belonged to the < 18.5 group, 2459 participants belonged to the 18.5-<25 group, 2586 participants belonged to the 25-<30 group, and 2281 participants belonged to the > 30 group. For smoking status, 5460 participants belonged to the non-smoking group and 2019 participants belonged to the smoking group which is based on cotinine levels in the blood (< 14 ng/ml is a non-smoker and ≥ 14ng/ml is a smoker).

Data Collection

The data were obtained from the NHANES 2001-2002 and NHANES 2003-2004 surveys. Information on age, gender, ethnicity, pregnancy and lactating status, medical conditions, BMI, cotinine levels, and serum 25(OH)D₃ were selected from the data.
Design

Factors, such as age, gender, ethnicity, BMI, and smoking status that might influence serum 25(OH)D$_3$ were studied.

The age was divided into groups of 18-30 years, 31-50 years, 51-70 years, and $\geq$ 70 years. The ethnicity groups included: Mexicans, non-Hispanic Blacks, and non-Hispanic Whites. BMI groups included < 18.5 kg/m$^2$, 18.5-<25 kg/m$^2$, 25-<30 kg/m$^2$, and $>$ 30 kg/m$^2$. Smoking status was determined by using cotinine levels in the blood. Subjects were in the non-smoking group if cotinine levels were $<$ 14 ng/ml and were in the smoking group if cotinine levels were $\geq$ 14ng/ml.

Serum 25(OH)D$_3$ levels were used to determine vitamin D status. Serum 25(OH)D$_3$ groups were $<$ 20 ng/ml showing deficiency, 20-30 ng/ml showing insufficiency, and $>$ 30 ng/ml showing sufficiency.

Statistical Methods

The data were analyzed by the Statistical Consulting Center at Bowling Green State University using the SUDAAN program. This statistical software program specifically analyzes data from complex sampling designs, such as NHANES 2001-2002 and NHANES 2003-2004, which were stratified multistage probability design.

Analysis of covariance was used to compare the mean serum 25(OH)D$_3$ among various groups using age, gender, ethnicity, BMI, and smoking status as the covariates. If differences were found, then Tukey’s multiple comparison procedure was used to find where the differences were. Both the analysis of covariance and Tukey’s multiple comparison procedure were run with $\alpha=0.05$ showing statistical significance.
CHAPTER IV: RESULTS

Table 1 and figure 1 shows the mean serum 25-Hydroxy Vitamin D₃ levels of American adults by ethnicity and gender. In males, the mean values were 22.0 +/-0.6, 15.7 +/-0.5 and 26.4 +/-0.5 in Mexican, Black and White Americans, respectively. In Females, the values were 19.0 +/-0.4, 14.7 +/-0.3 and 26.5 +/-0.4 in Mexican, Black, and White Americans, respectively. Between males and females, there was a significant difference among the Mexican Americans and Black Americans. Mexican American males and Black American males had a mean serum vitamin D₃ level significantly higher than the females. Among White American males and females there was no significant difference when it comes to gender. Overall, the results for the ethnic groups showed that the Black Americans had the lowest level of serum vitamin D₃.

Table 1 Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity and Gender: NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Ethnicity Gender</th>
<th>Mexican Mean + SE (n)</th>
<th>Blacks* Mean + SE (n)</th>
<th>Whites** Mean + SE (n)</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Total</td>
<td>22.0 ± 0.6 (982)</td>
<td>15.7 ± 0.5 (860)</td>
<td>26.4 ± 0.5 (1983)</td>
<td>24.7 ± .4</td>
<td>0.0000</td>
</tr>
<tr>
<td>Female Total</td>
<td>19.0 ± 0.4 (884)</td>
<td>14.7 ± 0.3 (859)</td>
<td>26.5 ± 0.4 (1911)</td>
<td>24.4 ± .4</td>
<td>0.0000</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.9332</td>
<td>0.1811</td>
<td>*****</td>
</tr>
</tbody>
</table>

*Black: Non Hispanic Blacks
**White: Non Hispanic White

Superscript: the same letter means there is no difference.
Table 2 and figure 2 show the mean serum 25-Hydroxy Vitamin D₃ levels of American adults by ethnicity, gender and age. Results show that there is no significant age effect among Mexican American males. However, in Black American males the difference was significant in the age groups of 18 to 30 and 31 to 50 years when compared to the 51 to 70 and greater than 70 years age group. Black American males in the 18 to 30 and 31 to 50 year age groups had lower levels of serum vitamin D₃ than the older two age groups. White American males also showed that there was a significant difference with the greater than 70 years age group. White American males greater than 70 years had lower levels of serum vitamin D₃ than the other younger age groups. In Mexican American females, age significantly affected the level of serum vitamin D₃. Mexican American females in the 31 to 50 and greater than 70 year age groups had a lower level of serum vitamin D₃ than the other two age groups. Black American females
in the age groups of 18 to 30 and 31 to 50 years had significantly lower values than in the age
groups of 51 to 70 and greater than 70 years. White American females in the 51 to 70 and
greater than 70 years age groups had significantly lower levels than 18 to 30 and 31 to 50
years age groups.

Table 2 Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity,
Gender and Age: NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Ethnicity Age, Male (Years)</th>
<th>Mexican Mean ± SE (n)</th>
<th>Blacks* Mean ± SE (n)</th>
<th>Whites** Mean ± SE (n)</th>
<th>Total P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 to 30</td>
<td>21.8 ± 0.7 (372)</td>
<td>14.3 ± 0.5 (321)</td>
<td>26.2 ± 0.6 (485)</td>
<td>24.2 ± 0.5</td>
</tr>
<tr>
<td>31 to 50</td>
<td>22.1 ± 0.6 (292)</td>
<td>15.2 ± 0.5 (291)</td>
<td>26.7 ± 0.5 (645)</td>
<td>25.0 ± 0.5</td>
</tr>
<tr>
<td>51 to 70</td>
<td>22.0 ± 0.8 (225)</td>
<td>18.2 ± 0.6 (190)</td>
<td>26.4 ± 0.6 (515)</td>
<td>25.3 ± 0.6</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>20.9 ± 1.8 (93)</td>
<td>18.5 ± 1.0 (58)</td>
<td>25.0 ± 0.4 (338)</td>
<td>23.6 ± 0.4</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.4065</td>
<td>0.0000</td>
<td>0.0220</td>
<td>0.0159</td>
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</table>

<table>
<thead>
<tr>
<th>Ethnicity Age, Female (Years)</th>
<th>Mexican</th>
<th>Blacks*</th>
<th>Whites**</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 to 30</td>
<td>20.5 ± 0.7 (314)</td>
<td>14.1 ± 0.7 (302)</td>
<td>28.5 ± 0.9 (438)</td>
<td>25.4 ± 0.7</td>
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</tr>
<tr>
<td>31 to 50</td>
<td>18.6 ± 0.5 (281)</td>
<td>14.2 ± 0.4 (288)</td>
<td>26.8 ± 0.6 (576)</td>
<td>24.6 ± 0.5</td>
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</tr>
<tr>
<td>51 to 70</td>
<td>20.2 ± 0.9 (201)</td>
<td>17.0 ± 0.5 (194)</td>
<td>24.8 ± 0.6 (514)</td>
<td>23.4 ± 0.5</td>
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</tr>
<tr>
<td>&gt; 70</td>
<td>19.2 ± 1.1 (88)</td>
<td>17.00 ± 0.8 (75)</td>
<td>24.9 ± 0.5 (383)</td>
<td>22.7 ± 0.5</td>
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<tr>
<td>P-Value</td>
<td>0.0284</td>
<td>0.0045</td>
<td>0.0022</td>
<td>0.0176</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Mean Serum 25-(OH) Vitamin D by Race and Age

*: P < 0.05
**: P < 0.001
Table 3 and figure 3 show the mean serum 25-Hydroxy Vitamin D₃ levels of American adults by ethnicity, gender and BMI. BMI was significantly associated with the serum vitamin D₃ levels in Mexican American males. Mexican American males in the BMI category of less than 18.5 had the lowest serum vitamin D₃ level and those in the 18.5 to 24.9 had the highest level. BMI was not associated with the vitamin D status in Black American males. In White American males, BMI was significantly associated with the serum vitamin D₃ levels. White American males in the BMI category of greater than 30 had the lowest serum vitamin D₃ level. White American males with a normal BMI had the best vitamin D status like the Mexican American Males. In Mexican American females, BMI was significantly associated with the serum vitamin D₃. For Mexican American females, the underweight group had a significantly lower mean serum vitamin D₃ level than the rest of the BMI groups. In Black females, the best serum vitamin D₃ level was found in the normal BMI group of 18.5 to 24.9 followed by 25 to less than 30, less than 18.5 and greater than 30. In White American Females, the best serum vitamin D₃ level was found in the normal BMI group followed by less than 18.5, 25 to less than 30 and greater than 30. Overall in American females, the lowest serum vitamin D₃ level was found in the obese BMI group.
Table 3 Mean Serum 25-Hydroxy Vitamin D3 Levels of American Adults by Ethnicity, Gender and Body Mass Index (BMI): NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>BMI, Male (Kg/m²)</th>
<th>Mexican Mean ± SE (n)</th>
<th>Blacks* Mean ± SE (n)</th>
<th>Whites** Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td></td>
<td>13.1 ± 3.0 (11)</td>
<td>14.6 ± 1.3 (25)</td>
<td>26.7 ± 2.1 (24)</td>
<td>25.0 ± 1.6</td>
<td>0.0000</td>
</tr>
<tr>
<td>18.5 to 24.9</td>
<td></td>
<td>23.6 ± 0.7 (305)</td>
<td>15.8 ± 0.6 (322)</td>
<td>27.7 ± 0.6 (612)</td>
<td>26.1 ± 0.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>25 to &lt; 30</td>
<td></td>
<td>21.9 ± 0.6 (419)</td>
<td>16.9 ± 0.6 (282)</td>
<td>26.6 ± 0.5 (799)</td>
<td>24.9 ± 0.4</td>
<td>0.0000</td>
</tr>
<tr>
<td>30 +</td>
<td></td>
<td>20.7 ± 0.8 (247)</td>
<td>14.9 ± 0.5 (231)</td>
<td>24.8 ± 0.5 (548)</td>
<td>23.2 ± 0.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0186</td>
<td>0.0879</td>
<td>0.0000</td>
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<td>0.0000 ****</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>BMI, Female (Kg/m²)</th>
<th>Mexican Mean ± SE (n)</th>
<th>Blacks* Mean ± SE (n)</th>
<th>Whites** Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td></td>
<td>16.5 ± 3.2 (16)</td>
<td>13.8 ± 1.5 (17)</td>
<td>26.8 ± 1.0 (60)</td>
<td>24.2 ± 0.9</td>
<td>0.0000</td>
</tr>
<tr>
<td>18.5 to 24.9</td>
<td></td>
<td>20.2 ± 0.6 (255)</td>
<td>15.2 ± 0.6 (206)</td>
<td>29.7 ± 0.6 (759)</td>
<td>27.1 ± 0.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>25 to &lt; 30</td>
<td></td>
<td>18.8 ± 0.8 (297)</td>
<td>14.9 ± 0.5 (234)</td>
<td>26.7 ± 0.5 (555)</td>
<td>24.7 ± 0.4</td>
<td>0.0000</td>
</tr>
<tr>
<td>30 +</td>
<td></td>
<td>17.5 ± 0.7 (316)</td>
<td>12.4 ± 0.4 (402)</td>
<td>22.6 ± 0.7 (537)</td>
<td>21.0 ± 0.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0289</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000 *****</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Mean Serum 25-(OH) Vitamin D by Race and BMI

Table 4 and figure 4 shows the mean serum 25-Hydroxy Vitamin D₃ levels of American adults by ethnicity, gender and smoking status. There was no difference in the
serum vitamin D₃ levels in the non-smokers and smokers in all three male ethnic groups. However, in females there was significant difference in the serum vitamin D₃ in all three ethnic groups.

Table 4 Mean Serum 25-Hydroxy Vitamin D₃ Levels of American Adults by Ethnicity, Gender and Smoking Status: NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Ethnicity Smoking Status, Male</th>
<th>Mexican Mean ± SE (n)</th>
<th>Blacks* Mean ± SE (n)</th>
<th>Whites** Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Smoker Cotinine &lt; 14 ng/ml</td>
<td>21.8 + 0.5 (759)z</td>
<td>16.0 + 0.5 (501)z</td>
<td>26.4 + 0.4 (1312)z</td>
<td>24.9 + 0.4z</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Smoker Cotinine &gt; 14 ng/ml</td>
<td>22.7 + 0.9 (223)z</td>
<td>15.2 + 0.6 (359)z</td>
<td>26.4 + 0.6 (671)z</td>
<td>24.6 + 0.5z</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>P-Value</td>
<td>0.4018</td>
<td>0.3136</td>
<td>0.5450</td>
<td>0.5415</td>
<td>******</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity Smoking Status, Female</th>
<th>Mexican Mean ± SE (n)</th>
<th>Blacks* Mean ± SE (n)</th>
<th>Whites** Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>Total Mean ± SE (n)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Smoker Cotinine &lt; 14 ng/ml</td>
<td>18.8 + 0.4 (786)</td>
<td>15.6 + 0.3 (632)</td>
<td>26.7 + 0.5 (1470)z</td>
<td>24.7 + 0.4</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Smoker Cotinine &gt; 14 ng/ml</td>
<td>22.3 + 1.5 (98)a</td>
<td>12.5 + 0.5 (227)</td>
<td>25.8 + 0.8 (441)za</td>
<td>23.2 + 0.6</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0457</td>
<td>0.0000</td>
<td>0.0410</td>
<td>0.0162</td>
<td>******</td>
<td></td>
</tr>
</tbody>
</table>
Table 5 presents the percent of the population with deficient, insufficient, or sufficient levels of serum 25-hydroxy vitamin D$_3$ among adult Americans. Figure 5 shows that from the total population of 3825 males, 27.2% had a deficient level, 46.0% had insufficient levels and only 26.9% sufficient levels. Figure 6 shows the results broken down based on ethnicity. In Mexican Americans, 37.6 %, 48.7 %, and 13.8 % had deficient, insufficient, or sufficient levels, respectively. In Black Americans, 72.7%, 23.9% and 3.3% had deficient, insufficient, or sufficient levels, respectively. In White Americans, 19.5%, 48.7% and 31.8% had deficient, insufficient, or sufficient levels, respectively. These findings based on ethnicity were found to be significant. Overall, the Black American males had the worst serum vitamin D status with the highest percentage at the deficient level and the lowest percentage at sufficient level. The Mexican American males vitamin D status fell between the Black American males and White American males. The White Americans had the best vitamin D status with the highest percentage at the sufficient level and the lowest percentage at the deficient level. Figure 5 also shows the total population of 3654 females, 33.9% had deficient level, 38.6% had insufficient levels, and 27.5% had sufficient levels. Figure 6 shows the individual female ethnic groups. In the Mexican Americans, 55.4%, 34.8%, and 9.9% had deficient, insufficient, and sufficient levels, respectively. In Black Americans 80.6%, 17.2%, and 2.2% had deficient, insufficient, and sufficient levels, respectively. In White Americans, 23.7%, 42.6% and 33.6% had deficient, insufficient, and sufficient levels, respectively. These findings based on ethnicity were found to be significant. Like Black American Males, Black American females had the worst vitamin D status followed by the Mexican American females. Out of the three groups, White American females had the best vitamin D status.
Table 5 Percent of the Population with Deficient, Insufficient, or Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ among Adult Americans: NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Percent of Population with Deficient, Insufficient, or Sufficient Levels of Serum 25-Hydroxy Vitamin D₃</th>
<th>Ethnicity, Male</th>
<th>Deficient &lt; 20 ng/ml % (n)</th>
<th>Insufficient 20 to 30 ng/ml % (n)</th>
<th>Sufficient &gt; 30 ng/ml % (n)</th>
<th>Total % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexican</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blacks*</td>
<td>72.7 (629)</td>
<td>23.9 (203)</td>
<td>3.3 (28)</td>
<td>100 (860)</td>
<td></td>
</tr>
<tr>
<td>Whites**</td>
<td>19.5 (407)</td>
<td>48.7 (983)</td>
<td>31.8 (593)</td>
<td>100 (1983)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.2 (1459)</td>
<td>46.0 (1626)</td>
<td>26.9 (740)</td>
<td>100 (3825)</td>
<td>p = .0000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity, Female</th>
<th>Mexican</th>
<th>Blacks*</th>
<th>Whites**</th>
<th>Total % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexican</td>
<td>55.4 (499)</td>
<td>34.8 (309)</td>
<td>9.9 (76)</td>
<td>100 (884)</td>
</tr>
<tr>
<td>Blacks*</td>
<td>80.6 (688)</td>
<td>17.2 (149)</td>
<td>2.2 (22)</td>
<td>100 (859)</td>
</tr>
<tr>
<td>Whites**</td>
<td>23.7 (472)</td>
<td>42.6 (807)</td>
<td>33.6 (632)</td>
<td>100 (1911)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age, Male</th>
<th>18 to 30</th>
<th>30.8 (511)</th>
<th>46.5 (473)</th>
<th>22.7 (194)</th>
<th>100 (1178)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 to 50</td>
<td>25.7 (449)</td>
<td>44.9 (521)</td>
<td>29.4 (258)</td>
<td>100 (1228)</td>
<td></td>
</tr>
<tr>
<td>51 to 70</td>
<td>26.3 (341)</td>
<td>46.5 (405)</td>
<td>27.2 (184)</td>
<td>100 (930)</td>
<td></td>
</tr>
<tr>
<td>&gt; 70</td>
<td>25.5 (158)</td>
<td>49.2 (227)</td>
<td>25.4 (104)</td>
<td>100 (489)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.2 (1459)</td>
<td>46.0 (1626)</td>
<td>26.9 (740)</td>
<td>100 (3825)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age, Female</th>
<th>18 to 30</th>
<th>34.7 (520)</th>
<th>35.0 (314)</th>
<th>30.3 (220)</th>
<th>100 (1054)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 to 50</td>
<td>34 (543)</td>
<td>38.5 (380)</td>
<td>27.5 (222)</td>
<td>100 (1145)</td>
<td></td>
</tr>
<tr>
<td>51 to 70</td>
<td>34.1 (388)</td>
<td>40.7 (353)</td>
<td>25.3 (168)</td>
<td>100 (909)</td>
<td></td>
</tr>
<tr>
<td>&gt; 70</td>
<td>31.1 (208)</td>
<td>42.6 (218)</td>
<td>26.3 (120)</td>
<td>100 (546)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.9 (1659)</td>
<td>38.6 (1265)</td>
<td>27.5 (730)</td>
<td>100 (3654)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI, Male</th>
<th>&lt; 18.5</th>
<th>31.7 (30)</th>
<th>36.7 (20)</th>
<th>31.7 (10)</th>
<th>100 (60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5 to 24.9</td>
<td>24 (437)</td>
<td>44.3 (514)</td>
<td>31.7 (288)</td>
<td>100 (1239)</td>
<td></td>
</tr>
<tr>
<td>25 to &lt; 30</td>
<td>26.9 (550)</td>
<td>45.0 (648)</td>
<td>28.1 (302)</td>
<td>100 (1500)</td>
<td></td>
</tr>
<tr>
<td>30 +</td>
<td>30.7 (442)</td>
<td>49.4 (444)</td>
<td>19.9 (140)</td>
<td>100 (1026)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.2 (1459)</td>
<td>46.0 (1626)</td>
<td>26.9 (740)</td>
<td>100 (3825)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI, Female</th>
<th>&lt; 18.5</th>
<th>21.5 (33)</th>
<th>46.4 (37)</th>
<th>32.1 (23)</th>
<th>100 (93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5 to 24.9</td>
<td>20.6 (397)</td>
<td>40.1 (457)</td>
<td>39.2 (366)</td>
<td>100 (1220)</td>
<td></td>
</tr>
<tr>
<td>25 to &lt; 30</td>
<td>31.3 (459)</td>
<td>41.4 (415)</td>
<td>27.3 (212)</td>
<td>100 (1086)</td>
<td></td>
</tr>
<tr>
<td>30 +</td>
<td>52.0 (770)</td>
<td>33.8 (356)</td>
<td>14.2 (129)</td>
<td>100 (1255)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.9 (1659)</td>
<td>38.6 (1265)</td>
<td>27.5 (730)</td>
<td>100 (3654)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5 Continued- Percent of the Population with Deficient, Insufficient, or Sufficient Levels of Serum 25-Hydroxy Vitamin D₃ among Adult Americans: NHANES 2001-2002 and 2003 to 2004

<table>
<thead>
<tr>
<th>Smoking Status, Male</th>
<th>Non-Smoker Cotinine &lt; 14 ng/ml</th>
<th>25.5 (944)</th>
<th>48.5 (1146)</th>
<th>26.1 (482)</th>
<th>100 (2572)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker Cotinine ≥ 14 ng/ml</td>
<td></td>
<td>30.4 (515)</td>
<td>41.3 (480)</td>
<td>28.3 (258)</td>
<td>100 (1253)</td>
</tr>
<tr>
<td>Total</td>
<td>p = .0041</td>
<td>27.2 (1459)</td>
<td>46.0 (1626)</td>
<td>26.9 (740)</td>
<td>100 (3825)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smoking Status, Female</th>
<th>Non-Smoker Cotinine &lt; 14 ng/ml</th>
<th>32.4 (1275)</th>
<th>40.2 (1042)</th>
<th>27.4 (571)</th>
<th>100 (2888)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker Cotinine ≥ 14 ng/ml</td>
<td></td>
<td>38.6 (384)</td>
<td>33.6 (223)</td>
<td>27.8 (159)</td>
<td>100 (766)</td>
</tr>
<tr>
<td>Total</td>
<td>p = .0335</td>
<td>33.9 (1659)</td>
<td>38.6 (1265)</td>
<td>27.5 (730)</td>
<td>100 (3654)</td>
</tr>
</tbody>
</table>

*Black: Non Hispanic Blacks  
**White: Non Hispanic White

![Figure 5: American Adults with Deficient (< 20ng/ml), Insufficient (20-30ng/ml) and Sufficient (> 30ng/ml) Levels of Serum 25-(OH) Vitamin D by Gender](image-url)
Figure 7 shows the percent of American adults with deficient, insufficient and sufficient levels of serum 25-Hydroxy Vitamin D₃ by age. Unlike ethnicity, age had no significance when it comes to vitamin D status. However, in American males, the 18 to 30 year age group had the highest percent at the deficient level and lowest percent at the sufficient level. In American females, 18 to 30 years had highest percentage at the deficient level.
Figure 8 shows the percent of American adults with deficient, insufficient and sufficient levels of serum 25-Hydroxy Vitamin D₃ by BMI. American males with a normal BMI of 18.5 to 24.9, had the lowest percentage at the deficient level. American males with an underweight BMI of less than 18.5, had the highest level of deficiency followed by American males with an obese BMI of 30 plus. The highest percentage of sufficiency was found in the American males with a BMI in underweight and normal weight group. American males in the obese BMI group had the lowest percentage in the sufficient level. American females, in the obese group had 52.0% with a deficient level of serum 25-Hydroxy Vitamin D₃ and only 14.2% had a sufficient level. American females with a normal BMI, had the lowest percentage with a deficient level of serum 25-Hydroxy Vitamin D₃ and highest percentage at the sufficient level.
Figure 9 shows the percent of American adults with deficient, insufficient and sufficient levels of serum 25-Hydroxy Vitamin D₃ by smoking status. In American males, 30.4% of smokers and 25.5% of non-smokers had deficient levels and 41.3% of smokers and 48.5% of non-smokers had insufficient levels of serum 25-Hydroxy Vitamin D₃. This shows that 71.7% of smokers and 74% of non-smokers are below sufficient levels. In American females, 38.6% of smokers and 32.4% of non-smokers had deficient levels and 33.6% of smokers and 40.2% of non-smokers had insufficient levels of serum 25-Hydroxy Vitamin D₃. This shows that 72.2% of smokers and 72.6% of non-smokers are below sufficient levels.

Overall, the smokers had a higher percentage of both males and females at the deficient level, but the non-smokers had lower percentage than smokers achieving sufficient levels of serum 25-Hydroxy Vitamin D₃. In American males, only 26.1% of non-smokers and 28.3% of smokers had sufficient serum 25-Hydroxy Vitamin D₃. In American females, only 27.4% of
non-smokers and 27.8% of smokers had sufficient serum 25-Hydroxy Vitamin D₃ levels.

Figure 9: American Adults with Deficient (< 20ng/ml), Insufficient (20-30ng/ml) and Sufficient (> 30ng/ml) Levels of Serum 25-(OH) Vitamin D by Smoking Status
CHAPTER V: DISCUSSION

Measuring vitamin D status and finding an accurate serum marker to determine sufficiency, insufficiency and deficiency is difficult. Vitamin D sources include dietary sources and the amount that is synthesized by the skin. Many foods such as milk, milk products, breakfast cereals, grain products, pastas, infant formula, and margarine are fortified with vitamin D. 22 The problem with fortification is that there are no specific standards for the amounts that are added to the foods, making it difficult to measure.26 These factors combined make it hard to use dietary records alone to determine vitamin D status. The serum marker 1,25(OH)2D3 was considered, but for several reasons serum 25(OH)D3 was selected instead. First, serum 25(OH)D3 has a half-life of two weeks and serum 1,25(OH)2D3 has a half-life of 2 to 6 hours. 42 Since serum 1,25(OH)2D3 is tightly regulated by calcium, phosphorus, PTH, and other hormones many fluctuations occur in a short period of time. This can lead to an inaccurate analysis of vitamin D status. Serum 25(OH)D3 however, remains stable in the body making it a more accurate measure of status.43 Another reason behind the accuracy of serum 25(OH)D3 is that levels of serum 1,25(OH)2D3 can remain normal or even elevated in a deficient state.12, 43 The last important difference between these metabolites is that concentrations of serum 1,25(OH)2D3 are a thousand times less than 25(OH)D3 making it a less valuable measure of vitamin D status. 11, 42 This is why researchers have determined that measuring serum 25(OH)D3 is the gold standard for determining vitamin D status.1, 2, 3, 4, 5, 6, 11, 24, 26, 41, 46 However, the exact cut off levels of serum 25(OH)D3 for determining sufficiency, insufficiency, and deficiency are yet to be determined. Studies are not consistent with the levels that they use to determine vitamin D status. Each researcher has used serum cut-off levels based on other studies’ findings. For this study, the serum levels that were set
to define sufficiency, insufficiency, and deficiency were based on a round table discussion at the Fifth International Symposium on the Nutritional Aspects of Osteoporosis, held in Lausanne, Switzerland, in May 2003, where a consensus was made between six investigators and from The National Osteoporosis Foundation. The investigators considered bone health markers to be an accurate way to determine adequate serum 25(OH)D$_3$ levels. The markers included maximum suppression of PTH, greatest calcium absorption, highest BMD, reduced rates of falling, and reduced fracture rates. The investigators determined that optimum serum 25(OH)D$_3$ for bone health to be between 20 and 32 ng/ml (50 and 80 nmol/L). The National Osteoporosis Foundation recommends that serum 25(OH)D$_3$ be at 30 ng/ml (75 nmol/L) or higher to be considered optimal. Based off the above recommendations, the cut-off levels for this study were above 30 ng/ml for sufficiency, below 30 ng/ml to 20 ng/ml indicating insufficiency, and below 20 ng/ml indicating deficiency.

The results from the current study show that many Americans were not reaching sufficient levels of serum 25(OH)D$_3$ and many are experiencing vitamin D insufficiency or deficiency. These results are consistent with previous studies on vitamin D status of Americans. Looker et al found that few Americans (less than 1%) from the lower latitude/winter and higher latitude/summer subpopulations had serum 25(OH)D$_3$ levels below 7 ng/ml (17.5 nmol/L) which was the level set for deficiency. Compared to the current study, the level set for deficiency by Looker et al was much lower. The current study’s results showed that 27.2% of males and 33.9% of females were deficient. When Looker et al set the serum cut-off level at less than 25 ng/ml (62.5 nmol/L) to indicate insufficiency, it was closer to the current studies cut-off level. At this level, the lower latitude/winter
subpopulation had 25-57% and the higher latitude/summer had 21-49% fall into the insufficient category compared to the current study which found 46% of males and 38.6% of females had insufficient levels. Guardia et al conducted a cross-sectional study on vitamin D status using the older American population. This study revealed that 15% of the population fell below 15 ng/ml, 32% fell below 20 ng/ml, and 72% fell below 30 ng/ml. These cut-off levels for serum 25(OH)D₃ were close to the current study, and the results were similar with the majority of the population falling below 30 ng/ml.

The current study found mean serum 25(OH)D₃ levels to be 24.7 ng/ml for males and 24.4 ng/ml for females, respectively. Scragg et al found the mean serum 25(OH)D₃ levels for males to be 31.2 ng/ml (78 nmol/L) and 29.2 ng/ml (73 nmol/L) for females. Both studies results showed that the males had higher mean serum 25(OH)D₃ levels, but there was a larger difference between the males and females in the study done by Scragg et al. Compared to Scragg et al, the current study had much lower mean serum 25(OH)D₃ levels. Guardia et al found that 80% of males and 72% of females had serum 25(OH)D₃ levels below 30 ng/ml, showing that there is a large percent difference between males and females, with the males having a higher percentage below 30 ng/ml. In the current study, 73.1% of men and 72.5% of females fell below 30 ng/ml. The current study shows that there is only a slight difference in the percentage of males and females reaching serum 25(OH)D₃ levels above 30 ng/ml, with the males also having a higher percentage below 30 ng/ml.

In the current study, white American males and females had lower serum 25(OH)D₃ levels as age increased. White American males greater than 70 years had lower levels of serum 25(OH)D₃ than the other younger age groups, and white American females in the 51 to 70 and greater than 70 years age groups had lower levels than 18 to 30 and 31 to 50 year age
groups. Scragg et al found similar results when subjects were broken into the age groups of 20-30 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years and greater than 70 years, with the mean serum 25(OH)D₃ levels at 32.4 ng/ml (81 nmol/L), 31.2 ng/ml (78 nmol/L), 29.2 ng/ml (73 nmol/L), 28.8 ng/ml (72 nmol/L), 28 ng/ml (70 nmol/L) and 26.8 ng/ml (67 nmol/L). Guardia et al conducted a cross sectional study and also found that from 1997 to 2001, the serum 25(OH)D₃ levels actually fell over time. However, not all ethnic groups had decreasing serum 25(OH)D₃ levels with increasing age in the current study. Black American males and females in the 18 to 30 and 31 to 50 year age groups had lower levels of serum 25(OH)D₃ than the older two age groups. Tangpricha et al also found that in subjects 18 to 29 years of age had 20% lower serum 25(OH)D₃ levels than the ≥ 50 year age group during the winter months. The 18 to 29 year old subjects also had the greatest seasonal variation in serum 25(OH)D₃ levels. In Mexican American females, there was no consistent pattern with the 31 to 50 and greater than 70 year age groups with lower level of serum vitamin D₃ than the other two age groups.

The current study found that Blacks had the lowest serum 25(OH)D₃ level followed by the Mexicans and Whites. Scragg et al found the mean values for Non-Hispanic Blacks, Non-Hispanic-Mexicans, and Non-Hispanic Whites were 19.6 ng/ml (49 nmol/L), 27.2 ng/ml (68 nmol/L), and 31.6 ng/ml (79 nmol/L). These mean serum 25(OH)D₃ levels from NHANES III also show that Blacks and Mexicans had lower mean serum 25(OH)D₃ levels than Whites. However, the mean serum 25(OH)D₃ levels from NHANES III are higher compared to the current study, showing that over time, Americans from all ethnicities are experiencing a more insufficient level of vitamin D. Guardia et al found that among older Americans, the black population had a higher percentage of the population (88%) with serum
25(OH)D₃ levels below 30 ng/ml compared to the white population (70%). Alemzadeh et al found that among the obese American adolescent population deficiency was higher in Hispanics and African Americans than in Caucasians.

The current study found that the obese BMI group had the lowest serum 25(OH)D₃ levels, with the males having a mean of 23.2 ng/ml and the females having a mean of 21 ng/ml. Scragg et al also found that with an increase in BMI, there was a decrease in serum 25(OH)D₃ levels. The subjects with an obese BMI of greater than or equal to 30.7 had a mean serum 25(OH)D₃ level of 26.8 ng/ml (67 nmol/L). Alemzadeh et al studied the obese adolescent American population and found that 32.3% had deficient levels, 41.7% had insufficient levels, and only 26% had sufficient levels of serum 25(OH)D₃.

The growing concern and the current study’s findings on the vitamin D status of Americans can be linked to several possible factors. First, exposure to the sun seems to have a major influence on serum 25(OH)D₃ levels. Tangpricha et al obtained serum 25(OH)D₃ levels from Americans 18 years and older during two different seasons. Serum 25(OH)D₃ levels for all groups regardless of age were higher during the end of summer and lower at the end of winter. It was also mentioned that the group with the most seasonal variation was the age group of 18 to 29 years, which may be explained by the high amount of medical students falling under this age group. These individuals tend to be indoors during the day attending classes in the fall and spring months. Leisure-time physical activity is another factor that seems to have an association with serum 25(OH)D₃ levels. Scragg et al found that individuals participating in no leisure-time activity to have a mean serum 25(OH)D₃ level of 27.6 ng/ml (69 nmol/L) and those participating in moderate leisure-time activity (greater than 12 times per week) to have a mean serum 25(OH)D₃ level of 31.2 ng/ml (78 nmol/L). The link
between leisure-time physical activities may be related to the amount of outdoor sun exposure, but studies do not determine if the activities were done indoors or outdoors making it difficult to come to this conclusion. However, this thought should be considered since nearly one in four adults in 2004 report no leisure-time physical activity.\textsuperscript{61} Dong et al used data from the National Human Activity Pattern Survey which took place in 1992-1994 and found that the largest contributor to energy expenditure was driving a car, office work and watching television.\textsuperscript{62} The current study found that overall, the mean serum 25(OH)D\textsubscript{3} level was lowest for the obese BMI group which maybe linked to the amount of activity that these individuals do throughout the day. Sun et al used subjects from CSFII-1994-1998, NHANES III and NHANES 1999-2002 and found that increased television time and lower amounts of physical activity had an impact on obesity.\textsuperscript{63} These two findings show that obesity may actually lead to less time out in the sun and more time indoors. The use of sunscreen and sun-protective clothing has been mentioned in previous studies to have a possible affect on serum 25(OH)D\textsubscript{3} levels.\textsuperscript{26} Evidence that too much UV exposure can cause skin cancer\textsuperscript{10,11} and wrinkles, has led to the promotion of sun screen protection.\textsuperscript{11} Sunscreen with an SPF as low as 8 blocks the synthesis of cholecalciferol in the skin, a major source of vitamin D. Another protective measure is the use of clothing which acts as a barrier between the skin and the sun absorbing 100\% of the UV radiation. Despite the many warnings Americans are not practicing these preventative measures as much as they should be. These protective factors may actually have less of an affect on the serum 25(OH)D\textsubscript{3} levels than thought. According to the 2005 National Health Interview Survey, infrequent use of regular sunscreen with an SPF of greater then 15 was high among all age groups.\textsuperscript{64} The percent of 18 to 29 year olds that did not wear sun-protective clothing on a regular basis was 86.7\% with this
percentage declining with age. Results did show that the older age groups of 65 years and older tended to avoid the sun when they were outdoors on a sunny day with only 19.8% remaining in the sun. Comparing this finding to the 18 to 29 year old age group, the percentage of subjects that remained in the sun was much higher, with 35.4% remaining in the sun. This may help to explain the declining serum 25(OH)D₃ levels in the older age groups among the White Americans. Low levels of serum 25(OH)D₃ may also be explained by the skin pigmentation in the different ethnic groups. Darker skin from tanning or from natural pigmentation contains increased amounts of melanin, which absorbs and protects the skin from UV wave lengths. Thus, melanin interferes with the production of pre-vitamin D which is needed to make cholecalciferol. This may help to explain the current studies findings that Black Americans had the lowest mean serum 25(OH)D₃ levels followed by the Mexican Americans and then the White Americans. The dietary intake of vitamin D is another possible factor that can affect the serum levels of Americans. Evidence shows that Americans are not meeting the daily requirements of 200 IU for adults under 70 years old and 600 IU for adults older then 70 years of age. Reaching the recommended levels may be difficult since few foods naturally contain vitamin D. In order to get enough through the diet one must eat fortified foods. Some of the products include milk products like yogurt, breakfast cereals, grain products, pastas, infant formulas, and margarines. Milk is the main source of vitamin D for Americans, and adults could meet the daily recommended allowance if they would just consume three eight ounce glasses of milk per day. However, Block found that subjects from NHANES III and NHANES 1999-2000 were getting most of their daily energy intake from regular soft drinks and cakes, sweet rolls, doughnuts and pastries. This study included 144 different foods and dairy foods did not even make the top ten list of
energy contributors. A study using NHANES III and CSFII (1994-96, 1998) showed that less than 10% of adult’s ages 51 to 70 years met the requirement for vitamin D through the diet.22 The adults that were older then 70 years of age had less than 2% meeting the requirement for vitamin D through the diet. From the same study, intakes of college students showed that 22% of female and 47% of male students attained the adequate intake (AI) for vitamin D.58 It is clear that all age groups are falling short of meeting the requirement for vitamin D with the older population having the lowest percentage meeting the requirement. This may explain the current study’s results that among white Americans, serum 25(OH)D₃ levels based on age were lowest in the older groups. The National Dairy Council has reported that African Americans are coming up short on their intake of dairy products.67 The United States Department of Agriculture (USDA) found that African American girls ages 12 to 19 years consume less than one glass of milk per day. A study on dairy consumption was done using subjects from NHANES 1999-2000 and CSFII (1994-96, 1999), and this study showed that African Americans of all ages did not meet the recommended 2005 United States Dietary Guidelines. Compared to non-African Americans, they consumed fewer mean servings of all dairy products per day.68 The study also showed that as age increased, the mean levels of dairy servings decreased, with a drastic drop in the average mean at age 9 to 18 years for females and 19 to 30 years for males. According to the National Medical Association, this can possibly be linked to the increased consumption of soda and the cultural belief that African Americans experience more lactose intolerance.67 It is estimated that 75% of the African American population is lactose intolerant; however, this may actually be overestimated.68 The decreased consumption of dairy products among African Americans may help to explain the low levels of serum 25(OH)D₃. Also, the drastic drop-off of dairy
products seen in the adolescent to middle age African American adults may help to explain the results from this study which showed low serum 25(OH)D₃ levels in the younger population of black Americans.

Conclusion

In conclusion, the results of this study showed that many Americans were not meeting a sufficient serum 25(OH)D₃ level, with some groups of Americans at a greater risk of deficiency. The Black Americans had the lowest mean serum 25(OH)D₃ level and had the highest percent at the deficient level compared to the other ethnic groups. The Mexican Americans had the second lowest mean serum 25(OH)D₃ level with, the highest mean serum 25(OH)D₃ level among the White Americans. The highest percent of sufficiency was also found among the White Americans. There was a slight difference between the mean serum 25(OH)D₃ level of males and females, with the males having a higher mean serum 25(OH)D₃ level than the females. The highest percent of deficiency was among females. Depending on ethnicity, age had a different association with the mean serum 25(OH)D₃ level. The White Americans had a decline in the mean serum 25(OH)D₃ level with increasing age, and the Black Americans had a decline in the mean serum 25(OH)D₃ level with decreasing age. Regardless of gender and ethnicity, the highest percent of deficiency was found in the 18 to 30 year old age group. Among the BMI groups, the lowest mean serum 25(OH)D₃ level for both genders was found in the obese BMI group. Despite the mean serum 25(OH)D₃ level, gender differences did occur with the males having the highest percent of deficiency in the underweight BMI group and the females having the highest percent of deficiency in the obese BMI group. Like age, smoking status had a different association with the mean serum 25(OH)D₃ level based on the ethnic group. For the Mexican Americans, the smokers had a
higher mean serum 25(OH)D₃ level than non-smokers. For all other ethnic groups, non-smokers had a higher mean serum 25(OH)D₃ level than smokers. Regardless of ethnicity, the smokers for both males and females had the highest percent at the deficient level. Overall, Americans mean serum 25(OH)D₃ level fell below the sufficient cut-off level set for this study. Americans may not be obtaining enough vitamin D in their diets, and synthesis by the skin may be decreased due to a lack of sun exposure. In the future, increased fortification of foods and supplementation among certain groups, and even the entire population should be considered.
References


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