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ACCEPTANCE

This thesis, DEMOGRAPHIC, DIETARY, AND LIFESTYLE DETERMINANTS OF VITAMIN D STATUS IN THE U.S. POPULATION: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 2005-2006, by Shalini Patel, was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

DEMOGRAPHIC, DIETARY, AND LIFESTYLE DETERMINANTS OF VITAMIN D STATUS IN THE U.S. POPULATION: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 2005-2006

by

SHALINI PATEL

Background: Determinants of vitamin D status are of interest when studying the epidemiology of disease in population groups because vitamin D is now recognized to decrease the risk of diseases such as osteoporosis, cancer, and cardiovascular disease. Understanding modifiable determinants of vitamin D status are important for managing vitamin D deficiency at the individual level and for addressing this issue at population level.

Objective: The objective of this study was to evaluate the associations between serum vitamin D status (deficiency and insufficiency) and distinct demographic, dietary, and lifestyle characteristics of adults in the United States using a large, nationally representative sample survey, the National Health and Nutrition Examination Survey (NHANES) 2005-2006.

Methods: The study sample consisted of 2340 adults aged 20-59 who had serum 25(OH)D measured and who had completed various questionnaires concerning dietary intake of vitamin D and other lifestyle factors. Multivariate logistic regression was used to estimate the odds ratio (OR) of vitamin D deficiency, insufficiency, and sufficiency in



adults based on distinct demographic, dietary, and lifestyle characteristics. Statistical significance was set at $\alpha < 0.05$.

Results: The prevalence of vitamin D deficiency was higher in obese adults than in underweight to normal weight adults ($50.9\% \pm 4.57$ vs. $29.3\% \pm 3.57$), higher in adults who reported no sunburns than in adults who reported ≥ 3 sunburns (49.9% ± 3.82 vs. $18.0\% \pm 3.07$), and higher in adults who use sun protective measures regularly than in adults who do not ($48.4\% \pm 3.93$ vs. $27.0\% \pm 3.75$). The prevalence of vitamin D deficiency increased as dietary intake of vitamin D decreased. Non-Hispanic black adults were significantly more likely to be vitamin D deficient (OR = 45.27, 95% CI = 17.27-118.64) and insufficient (OR = 9.37, 95% CI = 3.43-25.61) than non-Hispanic white adults. Significant positive associations were found between vitamin D deficiency and several characteristics, namely obesity (OR = 7.43, 95% CI = 4.33-12.77), physical inactivity (OR = 1.63, 95% CI = 1.03-2.58) poor dietary vitamin D intake (OR = 2.34, 95% CI = 1.44-3.81, non-supplement use or supplement use with a low amount of vitamin D (OR = 1.75, 95% CI = 1.05-2.89), and activities that decrease exposure to sunlight (from OR = 2.97, 95% CI = 2.14-4.13 to OR = 5.30, 95% CI = 3.17-8.85). **Conclusion:** The results of this nationally representative study demonstrate that obesity, physical inactivity, poor dietary intake of vitamin D, and low sunlight exposure increases the risk for vitamin D deficiency in U.S adults. Future studies are needed to investigate

efficient in correcting vitamin D deficiency and insufficiency among these groups.

whether vitamin D supplementation, sunlight exposure, and vitamin D-fortified foods are



DEMOGRAPHIC, DIETARY, AND LIFESTYLE DETERMINANTS OF VITAMIN D STATUS IN THE U.S. POPULATION: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 2005-2006

By

SHALINI PATEL

A Thesis Presented in Partial Fulfillment of Requirements for the Degree of

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TABLE OF CONTENTS

ACKNOWLEGEMENTSii
LIST OF TABLESvi
LIST OF FIGURES vii
ABBREVIATIONS
CHAPTER I: INTRODUCTION
CHAPTER II: REVIEW OF LITERATURE
VITAMIN D
Vitamin D Biosynthesis and Metabolism
Figure 1: Vitamin D Metabolism4
Sources of Vitamin D5
Table 1: Selected Sources of Vitamin D ₂ and Vitamin D ₃ 5
Determination of Vitamin D Status
Table 2: Classification of Vitamin D Status by Serum 25-Hydroxyvitamin D7
Factors Influencing Vitamin D Status7
Solar Zenith Angle7
Pollution
Clothing



Sunscreen
Melanin9
Age9
Adiposity10
Medication Use11
Malabsorption11
Prevalence of Vitamin D Deficiency12
Biological Functions of Vitamin D13
Definition of Optimal Vitamin D Status for Skeletal Health14
SELECTED METHODS OF DIETARY ASSESSMENT14
Food Frequency Questionnaire14
24-Hour Recall16
Conclusions17
CHAPTER III: METHODOLOGY18
NHANES Survey Design18
NHANES 2005-2006 Study Sample19
Description of Demographic Study Variables19
Description of Dietary Study Variables20
Description of Lifestyle Study Variables21
Biochemical Measurements



	Current Study Sample	22
	Figure 2: Derivation of Study Sample	24
	Statistical Analysis	25
Cl	HAPTER IV: RESULTS	26
	Sample Characteristics	26
	Table 3: Characteristics of the Study Sample	27
	Distribution of Vitamin D Deficiency in the Study Sample	30
	Table 4: Prevalence of Vitamin D Deficiency in the Study Sample	32
	Likelihood of Vitamin D Deficiency	37
	Table 5: Multivariate Odds Ratio and 95% Confidence Interval for Vitamin D Deficiency According to Characteristics of the Study Sample	39
Cl	HAPTER V: DISCUSSION AND CONCLUSIONS	44
	Strengths and Limitations	49
	Conclusions	50
RI	EFERENCES	52
A	PPENDIX	58



LIST OF TABLES

Table 1: Selected Sources of Vitamin D2 and Vitamin D3	5
Table 2: Classification of Vitamin D Status by Serum 25-Hydroxyvitamin D	,
Table 3: Characteristics of the Study Sample	7
Table 4: Serum Vitamin D Status According to Characteristics of the Study Sample32	2
Table 5: Multivariate Odds Ratio and 95% Confidence Intervals for Vitamin D Deficiency According to Characteristics of the Study Sample)



LIST OF FIGURES

Figure 1: Vitamin D Metabolism	4
Figure 2: Derivation of Study Sample	24



ABBREVIATIONS

1,25(OH) ₂ D	1,25-dihydroxyvitamin D (calcitriol)
24-HR	24-hour recall
25(OH)D	25-hydroxyvitamin D (calcidiol)
ACE	angiotensin-converting enzyme
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CI	confidence interval
DBP	vitamin D binding protein
DV	Daily Value
FFQ	food frequency questionnaire
HDL	high-density lipoprotein
IU	International Unit
kg	kilogram
L	liter
m	meter
mcg	microgram
MEC	mobile examination center
mL	milliliter
n	sample size
NCHS	National Center for Health Statistics



NHANES	National Health and Nutrition Examination Survey
ng	nanogram
nm	nanometer
nmol	nanomole
OR	odds ratio
OZ	ounce
р	probability value
РТН	parathyroid hormone
Ptrend	probability value for trend
RANK	receptor activator of nuclear factor-κB
RANKL	receptor activator of nuclear factor-kB ligand
RDA	Recommended Dietary Allowance
RIA	radioimmunoassay
SAS	Statistical Analysis Software
SE	standard error
SPF	sun protection factor
SZA	solar zenith angle
tbsp	tablespoon
U.S.	United States
UVB	ultraviolet B
VDR	vitamin D receptor



CHAPTER I

INTRODUCTION

Vitamin D deficiency is a common condition, especially among adults (1). New research has demonstrated that serum vitamin D concentrations previously considered in the normal range are not sufficient for optimal health, thereby increasing the risk of bone disease. In their consensus report for dietary reference intakes for calcium and vitamin D, the Institute of Medicine recognizes concentrations of serum 25-hydroxyvitamin D $[25(OH)D] \le 50 \text{ nmol/L}$ as "inadequate for bone and overall health in healthy" individuals." Furthermore, concentrations of serum 25(OH)D < 30 nmol/L are associated with vitamin D deficiency, rickets in infants and children, and osteomalacia in adults (2). It is well known that vitamin D plays a role in decreasing the risk of age-related osteoporosis (1) and therefore, determinants of vitamin D status have been of interest when studying the epidemiology of bone-related disease. However, the functions of vitamin D are now recognized to extend beyond skeletal health. Emerging research has demonstrated vitamin D to play a role in decreasing the risk of some types of cancer, type 1 and type 2 diabetes mellitus, multiple sclerosis, infectious diseases, cardiovascular disease, myocardial dysfunction, and hypertension in middle to older-aged women (3–5).

Because the risk of disease increases with age, maintaining vitamin D adequacy, especially during the teenage and early adult years, is recommended by health professionals in order to improve long-term health outcomes (4). Nonetheless, a high



prevalence of vitamin D deficiency persists among adults in the United States, especially in certain subgroups. Data from several large, nationally representative surveys indicate that serum 25(OH)D concentrations are declining on the population level (6,7). One possible explanation for this decline is an increase in sun protective behaviors due to heightened awareness of skin cancer prevention. Because direct exposure of the skin to sunlight is the main source of vitamin D in this country (3,8,9), behaviors that decrease or impede sunlight exposure should be considered as possible determinants of vitamin D status.

Understanding modifiable determinants of vitamin D status are important for managing vitamin D deficiency at the individual level and for addressing vitamin D deficiency at the population level. There is a lack of comprehensive population-based studies that investigate modifiable determinants, such as sun protective measures, in relation to serum vitamin D status. Therefore, the objective of this study was to evaluate the associations between serum vitamin D status (deficiency and insufficiency) and distinct demographic, dietary, and lifestyle characteristics of adults, to see if other behaviors as compared to dietary data are better able to predict vitamin D status in the U.S. using a large, nationally representative survey, the National Health and Nutrition Examination Survey (NHANES), 2005-2006.



CHAPTER II

REVIEW OF LITERATURE

VITAMIN D

Vitamin D Biosynthesis and Metabolism

Vitamin D, a general term for the fat-soluble vitamin, may refer to vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), or its metabolites. Both vitamin D_2 and D_3 are metabolized in a similar fashion (8). The biosynthesis of cholecalciferol occurs in the skin upon exposure to ultraviolet B (UVB) radiation from sunlight. In the epidermis and dermis, UVB rays react with 7-dehydrocholesterol in the plasma membrane of the skin cell to form vitamin D_3 (10,11). Once vitamin D_3 is formed, it travels into the extracellular space where vitamin D binding protein (DBP) transports it into the dermal capillary bed. Vitamin D_3 is then transported to the liver where it is hydroxylated to 25(OH)D or calcidiol (11). Although biologically inactive, this is the major circulating form of vitamin D and is used as a determinant of vitamin D adequacy (8,11). Calcidiol is then converted to 1,25-dihydroxyvitamin D [1,25(OH)₂D] or calcitriol, the biologically active form of vitamin D. This conversion takes place under the influence of 1α hydroxylase in the proximal renal tubule of the kidney and is tightly regulated by several factors including serum phosphorous and parathyroid hormone (PTH) levels (9,11). Once this conversion takes place, calcitrol acts on various organs of the body including the intestine, bone, kidney, and parathyroid glands (10).



The biosynthesis of ergocalciferol is similar to that of cholecalciferol. Ergosterol, a form of vitamin D present in plants and a precursor to ergocalciferol, undergoes the same hydroxylation reactions in the liver and kidney (8,11). Vitamin D metabolism is described in Figure 1.



Figure 1: Vitamin D Metabolism (8)

Cholecalciferol is formed after the absorption of UVB radiation in the skin or after ingestion of dietary vitamin D. In the liver, cholecalciferol is hydroxylated on carbon 25 to form 25(OH)D or calcidiol, the biologically inactive form of vitamin D. In the kidneys, the biologically active form of vitamin D, calcitriol, is formed after another hydroxylation. Activation of calcitriol is regulated by PTH.



Sources of Vitamin D

Vitamin D is obtained through cutaneous synthesis after exposure to sunlight, through diet, and through dietary supplements. Cutaneous synthesis of vitamin D₃ occurs after exposure to ultraviolet radiation in wavelengths between 290 and 315 nm (9). Twenty minutes of sun exposure in this wavelength range during the summer months can produce the equivalent of up to 20,000 International Units (IU) of vitamin D₃ (3,8).

Few dietary sources naturally contain vitamin D (3). Vitamin D_2 is produced through the irradiation of yeast and is found in some plant foods (8). This form is used to fortify certain foods such as cereal, milk, and orange juice (3). Vitamin D_3 is manufactured through the irradiation of 7-dehydrocholesterol from lanolin and is found in animal sources such as oily fish, egg yolk, and liver (3,8). Both forms are used in prescription and over-the-counter supplements (8). Selected sources of vitamin D_2 and vitamin D_3 are found in Table 1.

Source	Vitamin D content ²	% DV ³
Salmon, fresh, wild, 3.5 oz	600-1000 IU	150-250
Sardines, canned, 3.5 oz	300 IU	75
Tuna, canned, 3.6 oz	230 IU	57.5
Cod liver oil, 1 tbsp	1360 IU	340
Egg yolk, 1 whole	20 IU	5
Breast milk ⁴ , 1 L	20 IU	5
Milk, 8 oz	100 IU	25
Orange juice, 8 oz	100 IU	25

Table 1: Selected Sources of Vitamin D_2 and Vitamin $D_3^{-1}(3,12)$



Cheeses, 3 oz	100 IU	25
Margarine, 3.5 oz	430 IU	107.5
Ergocalciferol, 1 capsule	50,000 IU	12,500
Calcitriol [Rocaltrol], 1 capsule	0.25 or 0.5 mcg	2.5 or 5
Multivitamin	400 IU	100
Cholecalciferol, 1 tablet	400, 800, or 1000 IU	100, 200, or 250

 $^{1}IU =$ International Unit

²Primarily vitamin D_3 , except egg yolk (D_2 or D_3)

 3 DV = Daily Value

⁴In vitamin D sufficient lactating women

Determination of Vitamin D Status

Although 1,25(OH)₂D is the biologically active form of vitamin D, it is not used to determine vitamin D status due to its short half-life and low circulating levels. Circulating levels of 25(OH)D are a thousand fold more than 1,25(OH)₂D and its half-life is approximately 2 to 3 weeks (8,13,14). Therefore, measurement of 25(OH)D will represent a steady concentration of vitamin D produced from both the diet and UVB exposure up to several months (11).

There are several assay methodologies used to measure 25(OH)D in the serum. The most commonly used assays include the DiaSorin radioimmunoassay (RIA), the Nichols Advantage competitive binding protein assay, and the Immunodiagnostic Systems RIA (15). Recently, the Centers for Disease Control and Prevention (CDC) discontinued their use of the DiaSorin RIA, which identifies both 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ as total serum 25(OH)D in NHANES. A new method that



will independently measure 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 was adapted starting with NHANES 2007-2008 (16). Classification of vitamin D status by serum 25(OH)D concentration is given in Table 2.

 Table 2: Classification of Vitamin D Status by Serum 25-Hydroxyvitamin D (13)

ng/mL ¹	nmol/L	Classification
≤ 20	≤ 50	Deficient
21-30	51-75	Insufficient
> 30	>75	Sufficient

¹Multiply by 2.496 to convert ng/mL to nmol/L

Factors Influencing Vitamin D Status

Solar Zenith Angle

The amount of UVB radiation absorbed through human skin is influenced by a number of factors including the solar zenith angle (SZA) (17). The SZA, established by time of day, season, and latitude, influences the intensity of UVB radiation (9). Oblique SZAs increase the path of UVB radiation through the ozone layer allowing increased ozone absorption of UVB photons (18). As a result, fewer UVB photons strike the skin leading to inefficient conversion of 7-dehydrocholesterol to vitamin D₃ (9,17,18). It has been reported that very little vitamin D₃ synthesis occurs at latitudes above 37° during the winter months because the number of UVB photons striking the earth and skin is extremely decreased. However, latitudes closer to the equator provide more opportunity for vitamin D₃ synthesis throughout the year (18).



Pollution

Pollution can also lower the biosynthesis of vitamin D_3 by decreasing the number of UVB photons available for absorption through the skin (19,20). This is particularly common in highly urbanized areas with low-level air pollution (17) and in areas where fossil fuel and biomass combustion occurs (9,21). For example, a study conducted in the rainforests of Brazil revealed UVB radiation reductions up to 81% due to smoke from biomass burning (21).

Clothing

Clothing may interfere with UVB exposure and decrease the photosynthesis of vitamin D_3 in the skin (9). Fabric quality such as fiber, color, and presence of dyes influence the transmission of UVB through clothing. In a comparative study, Davis et al (22) measured UVB transmission through 28 different types of fabric. As expected, results indicated that lightweight fibers such as cotton and linen allowed more UVB transmission than heavier fibers such as wool and polyester. Certain dress styles also have the ability to impede photosynthesis of vitamin D_3 in the skin. Several studies have suggested that women who wear veils or clothing that covers the entire body (usually for religious purposes) exhibit low serum concentrations of 25(OH)D (23,24).

Sunscreen

Sunscreen agents impede UVB-7-dehydrocholesterol interactions by absorbing UVB radiation before it enters the skin (9,18). Sunscreens with a sun protection factor (SPF) up to 15 have the ability to reduce cutaneous synthesis of vitamin D_3 by greater than 98% (25). The application of sunscreen also prevents sun burning, wrinkles, and melanoma (25,26). Therefore, regular sunscreen application, avoidance of UVB



exposure, and other sun protective measures are highly encouraged despite the potential of these practices to decrease vitamin D_3 synthesis in the skin (26).

Melanin

Melanin is a natural substance produced by melanocytes in the skin through the action of α -melanocyte-stimulating hormone in response to ultraviolet radiation (9,26). It is often referred to as "natural sunscreen" because of its tendency to compete with 7-dehydrocholesterol for UVB photons (11,18,27). Individuals will exhibit varying pigmentation depending on the type of melanin and size and shape of melanosomes (pigment granules) in the skin. Individuals with large melanosomes have higher concentrations of melanin and darkly pigmented skin while those with small melanosomes have lower concentrations of melanin and lightly pigmented skin (26). Persons with lower concentrations of melanin require less UVB exposure to generate the same amount of vitamin D₃ compared to their dark-skinned counterparts (28). Therefore, variations in serum 25(OH)D concentrations among different ethnicities may partly be explained by differences in skin color (23).

Age

The cutaneous production of vitamin D_3 declines with age due to decreased concentrations of 7-dehydrocholesterol in the skin (9,23). MacLaughlin et al (29) confirmed this age-related decrease in the ability of human skin to synthesize vitamin D_3 in a comparative study. Skin samples obtained from individuals aged 8 to 92 years were exposed to ultraviolet radiation and after which levels of vitamin D_3 were determined. The authors of this study found a significant decline in the ability of skin obtained from the 77- and 82-year-old subjects to synthesize vitamin D_3 when compared to skin



obtained from the 8- and 18-year-old subjects. The findings of this study, however, are limited in their extrapolation since the skin samples were obtained from Caucasian subjects only. Low serum 25(OH)D concentrations among older adults is common regardless of season (30) and can be further exacerbated by confined living conditions and decreased dietary intake (1,23).

Adiposity

Vitamin D obtained through cutaneous synthesis after exposure to sunlight, through diet, and through dietary supplements can be stored by adipocytes for later use, such as in the winter when little cutaneous synthesis occurs (18,31). A high level of adiposity, however, appears to be inversely related to vitamin D status (9). Wortsman et al (32) demonstrated this inverse relationship in a comparative study; obese individuals (body mass index [BMI] \geq 30 kg/m²) were found to have lower vitamin D₃ concentrations compared to normal weight control subjects (BMI \leq 25 kg/m²). Therefore, the authors of this study concluded that obesity increases the risk of vitamin D deficiency.

Several mechanisms for suboptimal levels of vitamin D in obesity have been proposed. Obesity has been associated with diminished bioavailability of vitamin D due to the sequestration of vitamin D in larger amounts of adipose tissue (3,9). It has also been suggested that obese individuals avoid UVB exposure, which is necessary for cutaneous synthesis of vitamin D (32). This is most likely due to a sedentary lifestyle (12). Lastly, it has been proposed that $1,25(OH)_2D$, the biologically active form of vitamin D, is synthesized at a higher rate and therefore has a negative feedback control on the production of 25(OH)D in the liver (33).



Medication Use

Medication use has the ability to interfere with the catabolism and bioavailability of vitamin D. It has been suggested that certain medications such as glucocorticoids, antiretroviral therapy, and antirejection drugs have the capacity to increase catabolism of 25(OH)D and $1,25(OH)_2D$ to calcitroic acid, an inactive metabolite of vitamin D (3). Anticonvulsant therapy may also play a role in the development of vitamin D deficiency by similar mechanisms (3); however, there is conflicting evidence confirming this association (34,35). Bile-acid binding medications such as cholestyramine and colestipol, often used in the treatment of hypercholesterolemia, have the capacity to impair vitamin D absorption (3,9).

Recently, a study by Lee et al (36) demonstrated the impact of medication use on vitamin D status in subjects aged 55 to 88 years. Results indicated the use of oral antidiabetics, calcium-channel blockers, and angiotensin-converting enzyme (ACE) inhibitors lowered the serum 25(OH)D concentration of medication users by 7.4 nmol/L (p = 0.04), 7.7 nmol/L (p = 0.01), and 7.6 nmol/L (p = 0.01), respectively. The results of this study demonstrate the ability of common medications to influence vitamin D status in older adults. This, compounded with the use of multiple medications in older adults, may warrant vitamin D supplementation in individuals with chronic disease.

Malabsorption

Following cutaneous synthesis or oral consumption, vitamin D is incorporated into bile salt micelles and absorbed into the proximal small intestine (9). Vitamin D is a fat-soluble vitamin and therefore, intestinal absorption of this vitamin may be impaired in individuals with fat malabsorption syndromes and various gastrointestinal disorders



(11,37–39). Vitamin D insufficiency has been observed in post-gastrectomy, celiac disease, inflammatory bowel syndromes such as Crohn's disease and ulcerative colitis, pancreatic insufficiency, bariatric surgery (39), Whipple's disease, and cystic fibrosis (11). In addition, because vitamin D is implicated in skeletal health, malabsorption can lead to bone disease such as osteoporosis and osteomalacia (39).

Prevalence of Vitamin D Deficiency

Vitamin D deficiency is often underreported (1). Results of NHANES 2001-2004 found approximately 30% of the study population to be insufficient or deficient in vitamin D. In this study of over 20,000 U.S. individuals, vitamin D insufficiency was defined as a serum 25(OH)D concentration between 25 and 75 nmol/L and vitamin D deficiency was defined as a serum 25(OH)D concentration less than 20 to 25 nmol/L (40). Furthermore, data from several NHANES cycles indicate serum 25(OH)D levels are decreasing on the population level. The 1988-1994 cycle (n = 18,883) showed a mean serum 25(OH)D level of 75 nmol/L followed by a mean level of 60 nmol/L in the 2001-2004 cycle (n = 13,369) (7). The most recent data from the 2005-2006 cycle (n =4995) show a mean serum 25(OH)D level of 49.8 nmol/L (6).

Levels of 25(OH)D present in the serum vary depending upon ethnicity, age, health status, and various lifestyle factors. Forrest et al (6) analyzed data from NHANES 2005-2006 and found 82.1% (95% CI = 76.5-86.5) of African American adults to have serum 25(OH)D levels below 20 mg/mL. Hispanic adults followed with a prevalence rate of 62.9% (95% CI = 53.2-71.7). Other factors associated with a high prevalence rate of vitamin D deficiency in this study population included obesity, low high density lipoprotein (HDL) cholesterol, hypertension, smoking, and college education.



Biological Functions of Vitamin D

The main biological function of vitamin D is to maintain serum levels of calcium in the body (11). Once $1,25(OH)_2D$, the biologically active vitamin D metabolite, binds to the nuclear vitamin D receptor (VDR), intestinal absorption of both calcium and phosphorous is triggered. In a vitamin D deficient state, intestinal absorption of dietary calcium is reduced up to 15%, which is inadequate for proper bone metabolism and neuromuscular function. As the circulating level of ionized calcium declines, the parathyroid glands begin to produce and release PTH (1). PTH then functions in normalizing the circulating levels of calcium by increasing the amount reabsorbed in the renal tubules, mobilizing calcium from the bone, and stimulating renal production of $1,25(OH)_2D$ (1,41).

Vitamin D also plays a role in bone metabolism by indirectly influencing osteoclast (cells that resorb bone) maturation. During times of low intestinal calcium absorption, both calcium and phosphorous are pulled from the bone by the interaction of $1,25(OH)_2D$ with its VDR in the osteoblast (cells that form bone). $1,25(OH)_2D$ enhances the expression of receptor activator of nuclear factor- κ B ligand (RANKL) on the cell surface of the osteoblast (11,42,43). RANKL binds to its receptor, receptor activator of nuclear factor- κ B (RANK), on the cell surface of the immature osteoclast thereby initiating osteoclastogenesis (osteoclast maturation). The mature osteoclasts release hydrochloric acid and collagenases to dissolve bone mineral and matrix (1,11). As a result, calcium and phosphorous are pulled from the bone and deposited into the extracellular space (11).



Definition of Optimal Vitamin D Status for Skeletal Health

Currently, there is no consensus on the classification of vitamin D status by serum 25(OH)D concentration (3,13,44). Vitamin D deficiency is defined by most experts as a 25(OH)D level less than 10 to 20 ng/mL (25 to 50 nmol/L) (3,8,9,12,13,45). As discussed previously, low serum 25(OH)D levels can impair calcium metabolism and cause an increase in PTH. Excessive release of PTH due to hypocalcemia (otherwise known as secondary hyperparathyroidism) coupled with the release of calcium from bone after osteoclast maturation will promote increases in skeletal resorption and eventually, bone loss (41). Because serum 25(OH)D levels are inversely related to PTH levels, some researchers define the level of vitamin D needed for optimal skeletal health as the level of 25(OH)D that maximally suppresses PTH (1,41). Several studies propose optimal vitamin D status as serum 25(OH)D between 75 and 80 nmol/L (44,46–48).

SELECTED METHODS OF DIETARY ASSESSMENT

Food Frequency Questionnaire

The FFQ is a method of dietary assessment that attempts to estimate usual intake (49). It is based on grouping foods into categories and uses the frequency of consumption of listed foods as an index of diet pattern. The frequency of consumption of the listed foods will vary depending on whether the FFQ is collecting information on short- or long-term intake. Examples of common frequency of consumption terminology include "times per day," "times per week," and "times per month" (50). In addition, the FFQ may also attempt to collect information regarding portion size, such as the quantitative FFQ or the semiquantitative FFQ (51).



The FFQ was originally devised to serve as a self-administered method of dietary assessment. A limited number of food items were included to test a single hypothesis or diet-disease relationship (49). More recently, longer variations of the FFQ have emerged and are commonly used in large epidemiological studies to test several hypotheses (49,51,52). In addition, it is not uncommon for the FFQ to be administered by a trained interviewer (49).

There are several advantages associated with the use of FFQs to assess dietary intake. First, the FFQ often serves as an inexpensive method of dietary assessment, especially when self-administered. (Interviewer-administered FFQs are more costly due to interviewer training expenses.) Costs are further reduced if the data collected is scanned directly into a computer thereby eliminating the need for manual data entry. Second, because FFQs collect intake information for the preceding year, they are more representative of usual intake than a short diet record or 24-HR. This reduces the chance of misclassifying subjects into categories of nutritional status and ultimately increases the accuracy of information concerning diet-disease relationships (49). Finally, if selfadministered, the risk of interviewer or measurement bias is decreased (52).

The FFQ is considered to be the dominant nutrition assessment tool, especially in large epidemiological studies (53). However, it is not without limitations. Even a very short, nonquantitative, self-administered FFQ requires a certain degree of literacy. Very short FFQs that list a limited number of foods will only be able to address one or two very specific hypotheses. Listing specific foods therefore makes the FFQ a very culturally specific nutrition assessment tool. Limiting the number of foods will also increase the chances of excluding certain dietary habits. Because the FFQ usually



collects information for the preceding year, it is subject to variations in seasonality and recall. Finally, self-administered FFQs are at best semiquantitative because fixed definitions of portion size (such as small, medium, and large) are subject to individual interpretation (49).

24-Hour Recall

The 24-HR is a method of dietary assessment that requires respondents to describe in detail their food and beverage intake for the preceding 24 hours (50). If correctly administered, this method can provide accurate, quantitative information concerning recent nutrient intake. Correct administration of the 24-HR includes the use of food models, containers, and measuring devices to assess quantity. A trained dietitian should perform the interview, which typically lasts 30 to 60 minutes (49). Subjects are asked to recall the last food item eaten during the last 24 hours and work backwards (54).

Compared to the FFQ, the 24-HR requires short-term memory (49) and less time and effort from the participant (50). It has also been suggested that memory of recent intake is more precise and portions are estimated with greater accuracy with the 24-HR (55). This method of dietary assessment is also applicable to most age groups and literacy levels (50). Furthermore, the training effect is eliminated because the 24-HR is obtained only once from an unprepared participant (56).

One of the 24-HR's greatest disadvantages is its limited ability to represent usual intake (56). However, it has been suggested that variability in usual intake can be captured by repeated administration of the 24-HR (57). The 24-HR is considerably more expensive than the FFQ. Because the intake of vitamins and minerals will vary from day to day, the 24-HR is not meant to detect actual deficiency states in individuals (49) but it



has been suggested that the 24-HR can provide an estimate of the average nutrient intake of a group (56).

Conclusions

Estimating vitamin D status proves difficult due to the many factors influencing vitamin D status. This is particularly true regarding data derived from the U.S. and other nations where the majority of the population's vitamin D pool is cutaneously synthesized upon exposure to sunlight. Therefore, investigators attempting to estimate serum 25(OH)D status should consider information regarding sun protective measures and supplement use, if available. In the U.S., NHANES collects information regarding sun protective measures in the Dermatology Questionnaire and information regarding supplement use in the Dietary Supplements and Prescription Medication Questionnaire. However, to date, there is a lack of comprehensive population-based studies that investigate modifiable determinants, such as sun protective measures, dietary behaviors, and physical activity, in relation to serum vitamin D status. Therefore, the objective of this study was to evaluate the associations between serum vitamin D status and distinct demographic, dietary, and lifestyle characteristics of adults, to see if other behaviors as compared to vitamin D supplementation and vitamin D intake as determined by a 24-HR, are better able to predict vitamin D status in the U.S. using a large, nationally representative survey, the National Health and Nutrition Examination Survey (NHANES), 2005-2006.



CHAPTER III

METHODOLOGY

NHANES Survey Design

NHANES, an annual representative survey of the U.S. civilian noninstitutionalized population aged 2 years and older, was conducted by the National Center for Health Statistics (NCHS) of the CDC. The sample is selected using a complex, stratified, multistage, probability cluster sampling design. NHANES is unique in that it combines interviews, physical examinations, and laboratory tests. Survey participants are interviewed in their homes and are invited to a mobile examination center (MEC) to undergo physical examinations, blood and urine sample collection, and additional computer assisted interviews. The interview portion of the survey consists of demographic, socioeconomic, dietary, and health-related questions while the examination and laboratory portions include medical, dental, and physiological measurements. Informed consent is obtained from each participant for the interview and examination components (58).

NHANES has historically oversampled certain subgroups including low-income individuals, adolescents, individuals 60 years or older, African Americans, and Mexican Americans in order to conduct more accurate analyses of these groups. Since the U.S. population has been experiencing a dramatic growth in the number of older people, particular attention and extensive examination is performed for this population in



question. Detailed descriptions of NHANES survey designs and methodologies have been described elsewhere (59).

NHANES 2005-2006 Study Sample

NHANES 2005-2006 was conducted between January 2005 and December 2006. Examination data in the northern part of the U.S. was collected between May 1st and October 31st and examination data in the southern part of the U.S. was collected in between November 1st and April 30th. The study sample included 12,862 civilian, noninstitutionalized individuals aged 2 months and older. Among this sample, 6351 were male, 6509 were female, and 9950 were both interviewed and MEC examined.

Description of Demographic Study Variables

For this study, data from NHANES 2005-2006 demographic, dietary, examination, laboratory, and questionnaire files were used. The demographic file provides family-level and individual-level information. All survey participants who have a household interview record have a demographic file record. The demographic file record also includes the language used in the household and examination interviews, information about household reference person, proxy respondent codes, and demographic variables about each survey participant. For the purposes of this study, demographic variables included age, gender, race/ethnicity, education level, pregnancy status, and six month time period in which each participant was surveyed and examined.

Gender, age, race/ethnicity, education level, and time of examination were considered as determinants of vitamin D status as these variables are known to affect serum 25(OH)D concentrations (9,17,18,25). Age was calculated using the survey participants' actual or imputed date of birth and classified into groups according to



NHANES guidelines (20-29; 30-39; 40-49; 50-59). Race/ethnicity and education level were based on responses to the Demographic Questionnaire. In the analysis, education level was classified into five groups: 1 = < 25 years of age; 2 = less than high school; 3 = high school; 4 = some college; 5 = college graduate. Six-month time period was based on when each survey participant was examined. In the analysis, a value of '1' indicated November 1st through April 30th (fall/winter) and a value of '2' indicated May 1st through October 31st (spring/summer).

Description of Dietary Study Variables

The dietary file provides data collected from participants on their dietary intake, which includes foods, beverages, and dietary supplements. For the purposes of this study, the Dietary Supplements and Prescription Medication Questionnaire and total nutrient intakes as determined by dietary interviews (24-HRs) were used to estimate dietary intake of vitamin D. Although NHANES administers a FFQ, dietary data from this assessment method was not included in the analysis because portion size information is not collected and because the NHANES FFQ is not intended to derive estimate of absolute intake for either nutrients or foods (60). Vitamin D supplement use was determined based on ingredient information reported by participants in the Dietary Supplements and Prescription Medication Questionnaire. In the analysis, dietary vitamin D from supplementation was classified into tertiles of intake: $1 = \le 200$ IU; 2 = 201-400IU; 3 = > 400 IU. Dietary vitamin D from diet was classified into quartiles of intake: $1 = \le 22$ IU; 2 = 73-125 IU; 3 = 126-193 IU; 4 = > 193 IU.


Description of Lifestyle Study Variables

The questionnaire file provides data collected from participants on various healthrelated topics. Data is collected in a MEC via personal interview with a trained interviewer and via computerized interviews. For the purposes of this study, the Dermatology Questionnaire and the Physical Activity and Physical Fitness Questionnaire were used to determine participant sun protective measures and level of activity, respectively. In the current study, variables obtained from these questionnaires plus the variable for BMI were considered as "lifestyle variables." Below is a list of variables obtained from the dermatology file and included in the analysis.

- DEQ034A: "When you go outside on a very sunny day, for more than one hour, how often do you stay in the shade?"
- 2. DEQ034B: "When you go outside on a very sunny day, for more than one hour, how often do you wear a hat that shades your face, ears, and neck?"
- DEQ034C: "When you go outside on a very sunny day, for more than one hour, how often do you wear a long sleeved shirt?"
- DEQ034D: "When you go outside on a very sunny day, for more than one hour, how often do you use sunscreen?"
- DEQ038G/DEQ038Q: "How many times in the past year have you had a sunburn?"

Variables indicating participant use of sun protective measures on a very sunny day [shade (DEQ034A); hat that shades the face, ears, and neck, (DEQ034B); long sleeved shirt (DEQ034C)] were combined in the analysis. Possible answers in the survey included: always, most of the time, sometimes, rarely, or never. In order to have stable



estimates, responses were collapsed into three frequency categories: regularly (always and most of the time), occasionally (sometimes), and rarely to never (rarely or never). Participants were also categorized based on their use of sunscreen (DEQ034D). In the analysis, this variable was classified into three groups: 1 = regular user; 2 = occasional user; 3 = scant user to non-user. Also included in the analysis were variables indicating incidence and frequency of sunburns (DEQ038G/DEQ038Q). Participants were classified into three groups based on their response: 1 = 0 sunburns; 2 = 1-2 sunburns; 3 = ≥ 3 sunburns. Data for BMI was the only variable obtained from the examination file and was calculated as weight in kilograms divided by height in meters squared. In the analysis, this variable was categorized as underweight to normal (≤ 24.9 kg/m²), overweight (25.0-29.9 kg/m²), and obese (≥ 30.0 kg/m²).

Biochemical Measurements

Data for serum 25(OH)D was obtained from the laboratory file. Blood samples were collected by venipuncture from participants in the MECs according to standard protocols. Detailed specimen collection and processing methods have been described elsewhere (61). Serum 25(OH)D concentrations were analyzed using DiaSorin RIA, which identifies both 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 as total serum 25(OH)D (16).

Current Study Sample

The current study sample included data from NHANES 2005-2006 and initially consisted of 12,862 participants. Only those survey participants who were aged 20-59 were eligible to answer the Dermatology Questionnaire and for this reason, participants younger than 20 years and older than 59 years were excluded from the analysis (n =



8432). A further number of participants were excluded due to missing values for vitamin D concentration, demographic, dietary, and lifestyle variables, or if pregnant at the time of examination (n = 440). After applying the above exclusion criteria, the final sample consisted of 2340 participants representing approximately 144 million U.S. non-institutionalized civilians aged 2 years and older. A detailed derivation of the current study sample is depicted in Figure 2.





Figure 2: Derivation of Study Sample

The final study sample consisted of 2340 participants (weighted sample = 144, 129, 696).

Participants were excluded due to missing values for vitamin D concentration,

demographic, dietary, and lifestyle variables, or if pregnant at the time of examination.



Statistical Analysis

Statistical analysis was performed using Statistical Analysis Software (SAS) to account for the complex survey design of NHANES. The survey analysis procedures accounted for stratum, cluster, and observation weight in variance estimation. Sampling errors were estimated using the Taylor series (linearization) method. Data were sorted by SDMVSTRA (stratum) and SDMVPSU (primary sampling units). Detailed guidelines on the sample weighting and the proper variance estimation procedures are outlined in the NHANES Analytic and Reporting Guidelines (59).

Using cutoff values proposed by Holick et al (1,13) and Bischoff-Ferrari et al (62), serum 25(OH)D was divided into three categories: $1 = \le 50$ nmol/L (deficient); 2 = 51-75 nmol/L (insufficient); 3 = > 75 nmol/L (sufficient). Chi-squared tests were used to identify associations between demographic, dietary, and lifestyle characteristics among categories of vitamin D status.

Logistic regression was used to evaluate the odds ratio (OR) and 95% CI for vitamin D deficiency and vitamin D insufficiency. Data were adjusted with energy and fat intake which were included in the analysis as continuous variables. All other variables were categorized as described above. Statistical significance was set at $\alpha < 0.05$ in all analyses.



CHAPTER IV

RESULTS

Sample Characteristics

After applying exclusion criteria such as pregnancy and missing data for any study variable, the final sample consisted of 2340 participants who had dietary interview and serum 25(OH)D data. Of those, 49.7% (n = 1176) were male and 50.3% (n = 1164) were female. Participants were fairly distributed across age groups: 20-29 (22.7%, n =587); 30-39 (25.7%, n = 562); 40-49 (27.3%, n = 666); 50-59 (24.3%, n = 525). The majority of the study sample was non-Hispanic white (70.4%, n = 1110), followed by non-Hispanic black (12.2%, n = 538), Hispanic/Mexican American (8.9%, n = 505), and 'other' (8.6%, n = 187). The 'other' category included non-Hispanics from racial groups not already categorized. The largest percent of the population (56.5%, n = 1255) was sampled in the northern part of the U.S. during the spring/summer. Participants were fairly distributed across categories of BMI: $\leq 24.9 \text{ kg/m}^2$ (33.8%, n = 707); 25.0-29.9 kg/m² (32.2%, n = 776); ≥ 30.0 kg/m² (34.0%, n = 857). The majority of participants reported regular use of sun protective measures (38.5%, n = 970), scant to non-use of sunscreen (49.1%, n = 1382), and no sunburns during the last year (52.4%, n = 1434). Approximately 38.3% (n = 780) of the study sample reported vitamin D supplement use during the 30 days prior to the survey. The majority of the population (75.4%, n = 1846) reported no supplement use or supplement use providing ≤ 200 IU/day of vitamin D.



Daily vitamin D intake from diet was fairly distributed across quartiles: \leq 72 IU (21.5%, n = 599); 73-125 IU (24.8%, n = 553); 126-193 IU (24.9%, n = 590); > 193 IU (28.7%, n = 598). Unweighted values and weighted percentages of selected variables are presented in Table 3.

	n^2	°⁄0 ³
Gender		
Male	1176	49.7 ± 1.03
Female	1164	50.3 ± 1.03
Age		
20-29	587	22.7 ± 1.37
30-39	562	25.7 ± 1.86
40-49	666	27.3 ± 1.00
50-59	525	24.3 ± 1.44
Race/ethnicity		
Non-Hispanic white	1110	70.4 ± 3.28
Non-Hispanic black	538	12.2 ± 2.29
Hispanic/Mexican American	505	8.9 ± 1.06
Other	187	8.6 ± 1.19
Education level		
Age < 25 years	301	11.8 ± 0.91
Less than high school	433	10.9 ± 1.35
High school	452	20.6 ± 1.03



Some college	657	29.8 + 1.07
Some conege	057	29.0 ± 1.07
College graduate	497	26.9 ± 2.30
Time of examination ⁴		
Fall/winter	1085	43.5 ± 7.16
Spring/summer	1255	56.5 ± 7.16
BMI ⁵		
Underweight to normal	707	33.8 ± 1.94
Overweight	776	32.2 ± 1.47
Obese	857	34.0 ± 2.46
Physical activity		
No activity	769	27.2 ± 2.03
Moderate to vigorous	1338	63.8 ± 2.39
Vigorous	233	9.0 ± 1.11
Sunscreen user		
Regular user	499	27.6 ± 1.35
Occasional user	459	23.2 ± 1.10
Scant user to non-user	1382	49.1 ± 1.62
Use of sun protective measures ⁶		
Regularly	970	38.5 ± 1.32
Occasionally	856	37.8 ± 0.94
Rarely to never	514	23.7 ± 0.82
Frequency of sunburn during last year		
0	1434	52.4 ± 2.33



1-2	725	37.9 ± 2.02
\geq 3	181	9.8 ± 0.93
Vitamin D supplement use ⁷		
Yes	780	38.3 ± 1.60
No	1560	61.7 ± 1.60
Vitamin D intake from supplementation (IU/day)		
\leq 200	1846	75.4 ± 1.52
201-400	416	19.8 ± 1.22
> 400	78	4.8 ± 0.74
Vitamin D intake from food sources (IU/day) ⁸		
≤ 72	599	21.5 ± 1.50
73-125	553	24.8 ± 1.32
126-193	590	24.9 ± 1.18
> 193	598	28.7 ± 1.15
Serum vitamin D concentration (nmol/L)		
\leq 50	1175	38.4 ± 3.25
51-75	839	41.7 ± 2.01
> 75	326	19.9 ± 2.39

¹Study sample = 2340 (weighted sample = 144, 129, 686)

²Unweighted values

³Weighted percentages ± standard error (SE)

⁴Data collected during November 1-April 30 (fall/winter) and May 1-October 31

(spring/summer)



⁵Underweight to normal ($\leq 24.9 \text{ kg/m}^2$), overweight (25.0-29.9 kg/m²), and obese ($\geq 30.0 \text{ kg/m}^2$)

⁶Data collected on the use of shade and/or use of hat that shades face, ears, and neck and/or use of long sleeved shirt when participant is outside ≥ 1 hour on a very sunny day ⁷Participants who took supplements during the past 30 days prior to the survey ⁸Data represents average dietary vitamin D intake as determined by 2 dietary interviews (24-HRs)

Distribution of Vitamin D Deficiency in the Study Sample

In this study, 80.1% of the population had either deficient ($\leq 50 \text{ nmol/L}$) or insufficient (51-75 nmol/L) concentrations of serum 25(OH)D. The prevalence of vitamin D deficiency, insufficiency, and sufficiency according to characteristics of the study sample are presented in Table 4. Prevalence rates of deficiency were highest among non-Hispanic black adults (84.1%, n = 449) followed by Hispanic/Mexican American adults (59.4%, n = 318). Non-Hispanic white adults ranked last with a prevalence of vitamin D deficiency of 25.6% (n = 300). Based on serum vitamin D status, the prevalence of vitamin D deficiency and insufficiency was fairly distributed among men and women. Participants aged 20-29 years had the highest prevalence of vitamin D deficiency (40.4%, n = 301) and sufficiency (23.9%, n = 100) when vitamin D deficient and sufficient persons were stratified by age. Participants who reported highest education level completed as 'less than high school' ranked first with a prevalence of vitamin D deficiency of 52.5% (n = 260). The prevalence of vitamin D deficiency was higher among participants who were examined during the fall and winter months (51.1%, n = 679) than participants who were examined during the spring and summer months



(28.6%, n = 496). Vitamin D deficiency increased with BMI. In underweight and normal weight persons, the prevalence of vitamin D deficiency was 29.3% (n = 274), while overweight persons had a prevalence of 34.7% (n = 360), and obese persons had a prevalence of 50.9% (n = 541). Vitamin D deficiency was also highest among participants who reported physical inactivity (52.3%, n = 462). Scant to non-users of sunscreen, participants that reported regular use of sun protective measures, and participants that reported no sunburns in the past year had the highest prevalence of vitamin D deficiency. The prevalence of vitamin D deficiency was lower among participants who reported vitamin D supplement use within 30 days prior to the survey than those who reported no vitamin D supplement use. Participants who were in the lowest tertile of vitamin D intake from supplementation (≤ 200 IU/day) and the lowest quartile of vitamin D intake from diet (≤ 72 IU/day) had the highest prevalence of deficiency (42.2%, n = 1002; 52.4%, n = 370).



	Serum vitamin D status, $n (\%)^2$					
	Deficiency ³	Insufficiency ³	Sufficiency ³			
Gender						
Male	569 (37.2 ± 3.40)	464 (45.1 ± 2.12)	143 (17.7 ± 2.45)			
Female	$606 (39.6 \pm 3.58)$	375 (38.4 ± 2.70)	183 (22.0 ± 2.69)			
Age						
20-29	301 (40.4 ± 4.98)	186 (35.7 ± 2.85)	100 (23.9 ± 3.56)			
30-39	270 (34.9 ± 3.32)	219 (45.4 ± 2.89)	73 (19.8 ± 3.48)			
40-49	347 (39.6 ± 4.33)	247 (43.6 ± 3.53)	72 (16.7 ± 2.87)			
50-59	257 (38.8 ± 3.46)	187 (41.4 ± 3.56)	81 (19.8 ± 3.86)			
Race/ethnicity						
Non-Hispanic white	$300 (25.6 \pm 3.10)$	531 (47.9 ± 2.39)	279 (26.5 ± 2.70)			
Non-Hispanic black	449 (84.1 ± 2.61)	82 (15.0 ± 2.72)	7 (0.9 ± 0.33)			
Hispanic/Mexican American	318 (59.4 ± 7.04)	163 (35.4 ± 5.76)	24 (5.2 ± 1.63)			

Table 4: Prevalence of Vitamin D Deficiency in the Study Sample¹



Other	108	(56.8 ± 3.80)	63	(35.8 ± 3.06)	16	(7.4 ± 2.03)	
Education level							
Age < 25 years old	153	(41.2 ± 6.40)	95	(36.0 ± 3.13)	53	(22.8 ± 5.08)	
Less than high school	260	(52.5 ± 5.79)	147	(37.9 ± 4.24)	26	(9.6 ± 3.37)	
High school	232	(41.4 ± 3.97)	159	(41.0 ± 3.76)	61	(17.6 ± 2.61)	
Some college	334	(38.1 ± 3.91)	221	(38.2 ± 3.04)	102	(23.7 ± 3.62)	
College graduate	196	(29.4 ± 2.39)	217	(50.3 ± 2.03)	84	(20.3 ± 1.71)	
Time of examination ⁴							
Fall/winter	679	(51.1 ± 4.80)	322	(35.8 ± 2.65)	84	(13.1 ± 1.50)	
Spring/summer	496	(28.6 ± 2.69)	517	(46.3 ± 3.42)	242	(25.1 ± 2.63)	
BMI ⁵							
Underweight to normal	274	(29.3 ± 3.57)	263	(38.9 ± 2.01)	170	(31.8 ± 3.32)	
Overweight	360	(34.7 ± 3.53)	315	(46.4 ± 2.93)	101	(18.8 ± 3.43)	
Obese	541	(50.9 ± 4.57)	261	(40.1 ± 4.08)	55	(9.0 ± 2.34)	
Physical activity							



	No activity	462	(52.3 ± 4.08)	240	(34.5 ± 2.95)	67	(13.1 ± 2.10)
	Moderate to vigorous	602	(32.3 ± 3.01)	509	(45.1 ± 2.27)	227	(22.6 ± 2.83)
	Vigorous	111	(39.4 ± 5.31)	90	(39.9 ± 4.10)	32	(20.7 ± 4.22)
S	unscreen user						
	Regular user	185	(28.7 ± 0.71)	210	(47.3 ± 1.07)	104	(24.0 ± 0.97)
	Occasional user	174	(29.7 ± 0.88)	199	(47.1 ± 0.75)	86	(23.2 ± 0.50)
	Scant user to non-user	816	(47.9 ± 2.27)	430	(36.1 ± 1.36)	136	(16.0 ± 1.53)
U	se of sun protective measures ⁶						
	Regularly	576	(48.4 ± 3.93)	316	(40.1 ± 2.99)	78	(11.5 ± 2.07)
	Occasionally	409	(35.3 ± 3.10)	308	(43.2 ± 2.37)	139	(21.5 ± 2.87)
	Rarely to never	190	(27.0 ± 3.75)	215	(42.0 ± 2.77)	109	(30.9 ± 3.33)
F	requency of sunburn during last year						
	0	875	(49.9 ± 3.82)	429	(35.5 ± 2.90)	130	(14.7 ± 1.65)
	1-2	254	(27.7 ± 3.48)	317	(46.7 ± 2.55)	154	(25.6 ± 3.42)
	\geq 3	46	(18.0 ± 3.07)	93	(56.3 ± 4.41)	42	(25.7 ± 3.91)



Vitamin D supplement use ⁷			
Yes	291 (28.4 ± 0.92)	336 (46.9 ± 1.41)	153 (24.8 ± 1.23)
No	884 (44.6 ± 2.55)	503 (38.6 ± 1.33)	173 (16.8 ± 1.55)
Vitamin D intake from supplements (IU/day)			
≤ 200	1002 (42.2 ± 3.67)	617 (39.5 ± 2.26)	227 (18.3 ± 2.40)
201-400	148 (27.8 ± 3.58)	187 (47.8 ± 3.57)	81 (24.4 ± 3.33)
> 400	25 (22.1 ± 4.98)	35 (52.6 ± 8.63)	18 (25.3 ± 6.77)
Vitamin D intake from food sources (IU/day) ⁸			
\leq 72	370 (52.4 ± 4.19)	178 (35.3 ± 3.51)	51 (12.3 ± 2.19)
73-125	290 (42.1 ± 4.43)	176 (34.6 ± 2.82)	87 (23.3 ± 3.64)
126-193	288 (34.4 ± 3.54)	220 (46.1 ± 2.64)	82 (19.5 ± 3.06)
> 193	227 (28.2 ± 3.35)	265 (48.9 ± 2.66)	106 (22.8 ± 3.18)

¹Study sample = 2340 (weighted sample = 144, 129, 686)

²Unweighted values, *n*, and weighted percentages \pm SE in parentheses

³Deficient = $25(OH)D \le 50 \text{ nmol/L}$; insufficient = $51 \le 25(OH)D \le 75 \text{ nmol/L}$; sufficient = 25(OH)D > 75 nmol/L

⁴Data collected during November 1-April 30 (fall/winter) and May 1-October 31 (spring/summer)



⁵Underweight to normal ($\leq 24.9 \text{ kg/m}^2$), overweight (25.0-29.9 kg/m²), and obese ($\geq 30.0 \text{ kg/m}^2$)

⁶Data collected on the use of shade and/or use of hat that shades face, ears, and neck and/or use of long sleeved shirt when participant

is outside ≥ 1 hour on a very sunny day

⁷Participants who took supplements during the past 30 days prior to the survey

⁸Data represents average dietary vitamin D intake as determined by 2 dietary interviews (24-HRs)



Likelihood of Vitamin D Deficiency

The likelihood of vitamin D deficiency according to determinants of vitamin D status is presented in Table 5. When stratified by race/ethnicity, non-Hispanic black adults were the most likely to be vitamin D deficient (OR = 45.27, 95% CI = 17.27-118.64) and vitamin D insufficient (OR = 9.37, 95% CI = 3.43-25.61). Adults classified as 'other' and Hispanic/Mexican American adults were 6 times more likely to be vitamin D deficient (OR = 6.29, 95% CI = 2.72-14.57 [other]; OR = 6.17, 95% CI = 2.78-13.70[Hispanic/Mexican American]) than non-Hispanic white adults. The likelihood of vitamin D deficiency in participants who were examined during the fall and winter months was significantly higher (OR = 2.81, 95% CI = 1.07-7.43) relative to persons examined during the spring and summer months. The odds of vitamin D deficiency and vitamin D insufficiency increased as BMI increased. Overweight persons were significantly more likely to be deficient (OR = 2.11, 95% CI = 1.20-3.71) and insufficient (OR = 1.84, 95%) CI = 1.25-2.70) in vitamin D than underweight to normal weight persons. Similarly, obese persons were 7 times more likely to be deficient (OR = 7.43, 95% CI = 4.33-12.77) and 4 times more likely to be insufficient (OR = 4.33, 95% CI = 2.36-7.94) in vitamin D than adults with an underweight or normal BMI. In this study, inactive adults were more likely to be vitamin D deficient than moderately to vigorously active adults. Regular and occasional use of sun protective measures (shade and/or hat that shades face, ears, and neck and/or long sleeved shirt) significantly increased the likelihood of vitamin D deficiency and insufficiency. The odds of vitamin D deficiency increased as the number of reported sunburns decreased. Compared to adults who reported ≥ 3 sunburns in the past year, adults that reported 1-2 sunburns were almost 2 times more likely to be vitamin D



deficient (OR = 1.68, 95% CI = 1.01-2.81) and adults that reported 0 sunburns were almost 3 times more likely to be vitamin D deficient (OR = 2.73, 95% CI = 1.50-4.96). The odds of vitamin D deficiency were highest among adults who were in the lowest tertile of vitamin D intake from supplementation (OR = 1.75, 95% CI = 1.05-2.89) and the lowest quartile of vitamin D intake from food sources (OR = 2.34, 95% CI = 1.44-3.81).



Table 5: Multivariate Odds Ratio and 95% Confidence Interval for	Vitamin D Deficiency According to Characteristics of the
Study Sample ¹	

	Serum vitamin D status	Serum vitamin D status, OR (95% CI) ²					
	Deficiency ³	Insufficiency ³	Sufficiency ³				
Gender							
Male	0.95 (0.58-1.57)	1.21 (0.74-1.96)	0.84 (0.59-1.21)				
Female ⁴	1.00	1.00	1.00				
Age							
20-29	0.86 (0.49-1.52)	0.88 (0.49-1.57)	1.19 (0.72-1.97)				
30-39	0.85 (0.38-1.91)	1.22 (0.78-1.90)	0.94 (0.57-1.56)				
40-49	1.27 (0.71-2.27)	1.40 (0.76-2.61)	0.73 (0.42-1.25)				
50-59 ⁴	1.00	1.00	1.00				
Race/ethnicity							
Non-Hispanic white ⁴	1.00	1.00	1.00				
Non-Hispanic black	45.27 (17.27-118.64) ⁵	9.37 (3.43-25.61) ⁵	$0.04 (0.02 - 0.10)^5$				



Hispanic/Mexican American	6.17	$(2.78-13.70)^5$	3.19	$(1.98-5.14)^5$	0.24	$(0.14-0.42)^5$
Other	6.29	$(2.72-14.57)^5$	2.91	$(1.13-7.52)^5$	0.24	$(0.10-0.58)^5$
Education level						
Age < 25 years old	1.10	(0.38-3.24)	0.88	(0.45-1.75)	1.05	(0.49-2.24)
Less than high school	0.69	(0.28-1.73)	0.74	(0.31-1.76)	1.37	(0.58-3.25)
High school	0.96	(0.54-1.71)	0.75	(0.47-1.22)	1.25	(0.78-1.98)
Some college	0.70	(0.38-1.28)	0.53	(0.33-0.86) ⁵	1.76	$(1.14-2.71)^5$
College graduate ⁴	1.00		1.00		1.00	
Time of examination ⁶						
Fall/winter	2.81	(1.07-7.43) ⁵	1.36	(0.86-2.14)	0.56	(0.31-1.01)
Spring/summer ⁴	1.00		1.00		1.00	
BMI ⁷						
Underweight to normal ⁴	1.00		1.00		1.00	
Overweight	2.11	(1.20-3.71) ⁵	1.84	$(1.25-2.70)^5$	0.55	(0.38-0.80) ⁵
Obese	7.43	(4.33-12.77) ⁵	4.33	(2.36-7.94) ⁵	0.20	$(0.12-0.34)^5$



Physical activity						
No activity	1.63	$(1.03-2.58)^5$	1.18	(0.77-1.81)	0.74	(0.50-1.08)
Moderate to vigorous ⁴	1.00		1.00		1.00	
Vigorous	1.30	(0.76-2.14)	0.93	(0.55-1.57)	0.91	(0.54-1.54)
Sunscreen user						
Regular user	0.61	(0.36-1.02)	1.01	(0.59-1.75)	1.18	(0.75-1.87)
Occasional user	0.79	(0.46-1.35)	0.83	(0.57-1.21)	1.25	(0.96-1.64)
Scant user to non-user ⁴	1.00		1.00		1.00	
Use of sun protective measures ⁸						
Regularly	5.30	(3.17-8.85) ⁵	2.37	(1.57-3.57) ⁵	0.35	(0.24-0.50) ⁵
Occasionally	2.97	(2.14-4.13) ⁵	1.67	(1.26-2.22) ⁵	0.52	(0.41-0.66) ⁵
Rarely to never ⁴	1.00		1.00		1.00	
Frequency of sunburn during last year						
0	2.73	(1.50-4.96) ⁵	0.85	(0.53-1.36)	0.86	(0.57-1.30)
1-2	1.68	(1.01-2.81) ⁵	0.77	(0.51-1.16)	1.11	(0.74-1.66)



$\geq 3^4$	1.00	1.00	1.00
Vitamin D intake from supplementation (IU/day) ⁹			
\leq 200	1.75 $(1.05-2.89)^5$	1.25 (0.93-1.67)	$0.70 (0.51 - 0.97)^5$
201-400 ⁴	1.00	1.00	1.00
> 400	0.45 (0.10-2.10)	1.23 (0.47-3.22)	0.93 (0.40-2.14)
Vitamin D intake from food sources (IU/day) ¹⁰			
≤ 72	2.34 $(1.44-3.81)^5$	1.56 $(1.12-2.18)^5$	$0.60 (0.44 - 0.80)^5$
73-125	1.02 (0.55-1.90)	$0.63 (0.42 - 0.94)^5$	1.40 (0.98-2.01)
126-193	1.07 (0.61-1.87)	0.99 (0.71-1.38)	1.01 (0.71-1.44)
> 193 ⁴	1.00	1.00	1.00

¹Study sample = 2340 (weighted sample = 144, 129, 686)

²All values represent OR and 95% CI in parentheses

³Deficient = $25(OH)D \le 50 \text{ nmol/L}$; insufficient = $51 \le 25(OH)D \le 75 \text{ nmol/L}$; sufficient = 25(OH)D > 75 nmol/L

⁴Referent category

⁵Significantly different from the referent category

⁶Data collected during November 1-April 30 (fall/winter) and May 1-October 31 (spring/summer)



⁷Underweight to normal ($\leq 24.9 \text{ kg/m}^2$), overweight (25.0-29.9 kg/m²), and obese ($\geq 30.0 \text{ kg/m}^2$)

⁸Data collected on the use of shade and/or use of hat that shades face, ears, and neck and/or use of long sleeved shirt when participant

is outside ≥ 1 hour on a very sunny day

⁹Participants who took supplements during the past 30 days prior to the survey

¹⁰Data represents average dietary vitamin D intake as determined by 2 dietary interviews (24-HRs)



CHAPTER V

DISCUSSION AND CONCLUSIONS

To our knowledge, this is one of the most comprehensive studies that investigates the associations between serum vitamin D status and distinct demographic, dietary, and lifestyle characteristics of adults, to see if other behaviors as compared to vitamin D supplementation and vitamin D intake as determined by a 24-HR, are better able to predict vitamin D status in the U.S. using a large, nationally representative survey. The overall prevalence rate of suboptimal serum vitamin D concentration was 80.1%. Vitamin D deficiency was fairly distributed among males and females, higher in younger adults, adults with an obese BMI, inactive adults, and in adults without a college degree. In general, these results were expected and are similar to results that other investigators have found (6,40,63,64). In our adjusted models, the highest odds of vitamin D deficiency were for non-Hispanic black adults, adults with an obese BMI, adults who were regular users of sun protective measures, adults who reported no sunburns during the past year, and adults who fell into the lowest quartile of vitamin D intake from food sources.

Several studies have reported a high prevalence rate of vitamin D deficiency among non-Hispanic blacks (6,40,63–67). A high prevalence rate of deficiency among this subgroup persists even though different cutoff values have been used to define vitamin D status. Using the definition of serum 25(OH)D concentrations \leq 50 nmol/L,



we found that 84.1% of non-Hispanic black adults, both male and female, were vitamin D deficient. When stratified by race/ethnicity, our findings agree with the findings of Forrest et al (6) who investigated correlates of vitamin D deficiency in the same NHANES cycle. In both studies, non-Hispanic black adults had the highest prevalence rate and odds for vitamin D deficiency. Using the same classification method of vitamin D status as the present study, Forrest et al reported a deficiency prevalence rate of 82.1% among this subgroup. Although the likelihood of vitamin D deficiency was highest among non-Hispanic blacks in both studies, Forrest et al reported a much lower odds ratio of vitamin D deficiency. In relation to non-Hispanic whites, we found that non-Hispanic blacks were approximately 45 times more likely to be deficient (OR = 45.27, 95% CI = 17.27-118.64) compared to an odds ratio of approximately 9 (OR = 9.6, 95%) CI = 6.3-14.5). A possible explanation for this discrepancy could be attributed to differences in age range among study participants. Our sample was restricted to individuals aged 20-59 who had data on use of sun protective measures while Forrest et al sampled individuals aged 20 years or older and included adults aged 60 years and older.

When compared to non-Hispanic whites, other minorities also had higher prevalence rates of and a higher risk for vitamin D deficiency, which is consistent with the findings of previous studies (6,40,63,67). The association between minority groups and vitamin D deficiency may be related to several factors. It is well known that melanin, often referred to as "natural sunscreen," competes with 7-dehydrocholesterol for UVB radiation (11,18,27). Persons with higher concentrations of melanin have more pigmented skin and require more UVB exposure to generate the same amount of vitamin



D compared to their light-skinned counterparts (26,28). Therefore, darkly pigmented people are at particularly high risk for vitamin D deficiency (18). Moreover, several studies suggest that individuals of non-Hispanic black descent and of Hispanic/Mexican American descent seek out shade frequently (68–70). This is particularly true for non-Hispanic blacks. This, compounded with lower rates of vitamin D formation (from UVB radiation) in darkly pigmented individuals, will undoubtedly increase the likelihood of developing a suboptimal concentration of serum 25(OH)D.

Other studies suggest cultural differences in diet, lower socioeconomic status among minority groups, and lower educational attainment among minority groups as possible explanations for the high prevalence rates seen in these subgroups (68–70). Even though direct exposure of the skin to UVB radiation from sunlight is the main source of vitamin D in this country, it may be beneficial to require a higher oral intake of vitamin D from food sources and supplements specifically for these subgroups.

When stratified by time of examination, the incidence of vitamin D deficiency was higher during the fall and winter months. This finding was unexpected since examination data during this time period was collected in southern regions of the U.S., where more opportunity for vitamin D synthesis is possible year round. As mentioned previously, surveys and exams were not collected simultaneously in northern and southern regions of the U.S. Therefore, this result is most likely due to different timings in blood sample collection. Therefore, it is possible that the prevalence rate of vitamin D deficiency in persons whose serum 25(OH)D was sampled during the spring and summer months (in northern regions of the U.S.) is underestimated.



Due to the number and complexity of dermatology variables analyzed, the association between vitamin D deficiency and participant sun protective measures requires a separate discussion for each variable. When stratified by use of sun protective measures (shade and/or hat that shades face, ears, and neck and/or long sleeved shirt), the highest prevalence of vitamin D deficiency was found among participants who reported regular use (48.4%), followed by participants who reported occasional use (35.3%), and participants who reported rare to no use (27.0%). In addition, multivariate logistic regression showed a significant association between vitamin D deficiency and regular use of sun protective measures. Similarly, scant or non-users (36.1%) had a lower prevalence rate of vitamin D insufficiency compared to regular users of sunscreen (47.3%), followed by occasional users (47.1%). These associations are dose-response in nature and agree with the idea that sun protective measures may impede UVB-induced vitamin D synthesis in the skin (25,63).

A similar relationship, however, was not observed between the prevalence of vitamin D deficiency and sunscreen use. Participants who were identified as scant to non-users of sunscreen had a higher deficiency prevalence rate. This association may be explained by confounding factors such as application of sunscreen before intentional prolonged exposure to the sun. For example, in a study that examined behaviors associated with sunscreen use, Thieden et al (68) found the use of sunscreen to be highly correlated with sunbathing with the intention to tan indicating that sunscreens were used as tanning aids to avoid sunburn.

When stratified by frequency of sunburns, the prevalence rate of vitamin D deficiency increased as sunburns decreased with approximately 50% of vitamin D



deficient persons reporting no sunburns during the past year. This association, although dose-response in nature, may be explained by several confounding factors such as less time spent outdoors, increased use of shade or other sun protective measures on a very sunny day, or applying a liberal amount of high SPF sunscreen on a very sunny day. However, the same relationship was not apparent in vitamin D insufficient persons. The results of the present study are consistent with the findings of Linos et al (63) who found a significant positive association between vitamin D deficiency, staying in the shade, and wearing a long sleeved shirt, but not between vitamin D deficiency and frequent sunscreen use. Linos also found non-Hispanic whites that frequently stayed in the shade or wore long sleeved shirts to have double the odds of vitamin D deficiency compared to others who rarely did so (OR = 2.16, 95% CI = 1.41-3.32, p_{trend} = 0.001 [shade]; OR = 2.11, 95% CI = 1.48-3.00, p_{trend} = 0.02 [long sleeved shirt]).

In this study, approximately 38% of participants reported vitamin D supplement use. The Recommended Dietary Allowance (RDA) for vitamin D is 600 IU (2). However, only 5% of participants reported an intake of \geq 400 IU/day while 13% of participants reported an intake of \leq 200 IU/day from supplementation. Intake of vitamin D from food was also low and more than 2/3 study participants consumed < 193 IU/day. In order to meet the RDA, adults in the highest end of this tertile of intake would either have to spend a considerable amount of time outdoors (if conditions were not optimum for maximal UVB absorption) or consume an additional 400 IU of vitamin D per day from food sources (equivalent to four 8 oz cups of milk). As expected, a higher prevalence rate of deficiency was observed in participants who fell into the lowest tertile of vitamin D intake from supplementation. A similar relationship was observed between



vitamin D deficiency and participants who were classified into the lowest quartile of vitamin D intake from food sources. These associations, in general, were expected and confirm the results of other studies (6,64). What is interesting is that participants in the lowest tertile of intake from supplementation were 1.75 times more likely to be deficient (OR = 1.75, 95% CI = 1.05-2.89) while participants in the lowest quartile of intake from food sources were 2.34 times more likely to be deficient (OR = 2.34, 95% CI = 1.44-3.81) when compared to their referent groups. Yet another interesting finding is that regular users of sun protective measures were more likely to be vitamin D deficient than participants consuming ≤ 72 IU of vitamin D per day from food sources. Although other studies demonstrate that factors such as race, season, and sun exposure are better predictors of serum 25(OH)D concentrations than dietary intake (69,70), our results suggest that less use of sun protective measures on very sunny day and vitamin-D fortified foods may be as effective as vitamin D supplementation in correcting vitamin D deficiency.

Strengths and Limitations

The current study has several strengths, one being a fairly large study sample from a nationally representative survey. This allowed for increased precision in estimating serum 25(OH)D which in turn increased the likelihood that subjects were correctly classified into categories of vitamin D status. Also, dietary interviews were performed twice and this allowed for increased precision in estimating the average dietary vitamin D intake of the study sample. The statistical method used in this study was capable of handling the complex survey design of NHANES and account for the different probability of selection and overrepresentation of certain subgroups. Finally,



the findings of this study can be extrapolated to the general U.S. adult population due to the probability sample survey design of NHANES.

A limitation of this study was due to the cross-sectional nature of the NHANES survey design. Therefore, cause and effect relationships between variables in the current study could not be established. Also, it is possible that the incidence of vitamin D deficiency was underestimated in this study due to seasonal variations in data and blood sample collection in the northern and southern regions of the country. Unfortunately, it was not possible to adjust for this and other potential confounding variables such as latitude of the participant's home in the analysis. In addition, dietary intake of vitamin D estimated by the dietary interviews may be over- or underreported due to subjects' inability to recall intakes accurately (51).

Conclusions

In conclusion, the results of this nationally representative study demonstrate that obesity, physical inactivity, poor dietary intake of vitamin D, and behaviors that decrease skin exposure to direct UVB radiation increases the risk of vitamin D deficiency in U.S. adults. The prevalence of vitamin D deficiency was highest among non-Hispanic blacks followed by Hispanic/Mexican Americans. Regularly staying in the shade; wearing a hat that shades the face, ears, and neck; and/or wearing a long sleeved shirt on a sunny day were associated with the vitamin D deficient state and increased the odds for vitamin D deficiency. Lower frequencies of sunburns followed the same pattern. In particular, non-Hispanic black and Hispanic/Mexican American adults may require higher oral intake of vitamin D than the currently recommended 600 IU. Further studies are needed to investigate whether less use of sun protective measures, vitamin D supplementation, and



vitamin D-fortified foods are efficient in correcting vitamin D deficiency and insufficiency among these groups.

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NHANES 2005-2006 Dermatology Questionnaire

2005-06 Questionnaire

DERMATOLOGY - DEQ TARGET GROUP: 8P 20-58

DEQ.031 Next are some general questions about (your/SP's) skin.

If after several months of not being in the sun, (you/SP) then went out in the sun without sunscreen or protective clothing for a half hour, which one of these would happen to (yourhis/her) skin?

HAND CARD DEQ2

GET A SEVERE SUNBURN WITH	
BLISTERS	1
A SEVERE SUNBURN FOR A FEW DAYS	
WITH PEELING	2
MILDLY BURNED WITH SOME TANNING	3
TURNING DARKER WITHOUT A	
SUNBURN	4
NOTHING WOULD HAPPEN IN HALF AN	
HOUR	5
HOUR	5
HOUR OTHER REFUSED	5 6 7

DEQ.034 When {you go/SP goes} outside on a very sunny day, for more than one hour, how often {do you/does SP}. a/b/c/d ...

HAND CARD DEQ2A

a. Stay in the shade? Would you say ...

always,	1	
most of the time,	2	
sometimes,	3	
rarely, or	4	
never?	5	
DON'T GO OUT IN THE SUN	6	(DEQ.038)
REFUSED	7	
DON'T KNOW	9	

SP_DEQ



b. Wear a hat that shades (your/his/her) face, ears and neck? Would you say ...

always,	1
most of the time,	2
sometimes,	3
rarely, or	4
never?	5
REFUSED	7
DON'T KNOW	9

CAPI INSTRUCTION:

INCLUDE THE FOLLOWING HELP SCREEN AT THIS SCREEN. HELP SCREEN:

Include any wide-brimmed hat that shades (yourhis/her) face, ears and neck from the sun. Do NOT Include visors, baseball caps, or hats that do not shade the ears and neck.

c. Wear a long sleeved shirt? Would you say ...

always,	1
most of the time,	2
sometimes,	3
rarely, or	4
never?	5
REFUSED	7
DON'T KNOW	9

d. Use sunscreen? Would you say ...

always,	1	
most of the time,	2	
sometimes,	3	
rarely, or	4	
never?	5	(DEQ.038)
REFUSED	7	(DEQ.038)
DON'T KNOW	9	(DEQ.038)





المتسارات

CAPI INSTRUCTION:

3

DEQ.053 [Have you/Has 3P] ever been told by a health care provider that [you/s/he] had psoriasis (sore-eye-asis)?

YE8	1	
NO	2	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

99

DEQ.055 On a scale of 1 to 10, how much of a problem has (yourihis/her) psoriasis been in (yourihis/her) everyday life, where 1 means no problem at all and 10 means a very large problem?

CAPI INSTRUCTION: BUILD HARD EDITS AS 1-365.

HAND CARD DEQ3

ONLY ALLOW ENTRY OF 1 THROUGH 10 (NO '0' ALLOWED).

1.1 ENTER NUMBER

REFUSED

DON'T KNOW

DON'T KNOW

. 99

How many times in the past year (have you'has SP) had a sunburn?

DON'T KNOW

ENTER NUMBER OF TIMES

NEVER	000
REFUSED	777
DON'T KNOW	999

CAPI INSTRUCTION: BUILD HARD EDITS AS 1-50. INCLUDE THE FOLLOWING HELP SCREEN: HELP SCREEN: By SPF, we mean the "Sun Protection Factor"; the number on the label of the sunscreen that tells you how much protection against the sun it has.

DEQ.036 What is the SPF number of the sunscreen (you/s/he) use most often?

READ IF NECESSARY:

DEQ.038

G/Q

If you use more than one or different ones, pick the one you use most often.

ENTER NUMBER OF SPF

60

DEQ.057 {Do you/Does SP} currently have ...

HAND CARD DEQ4

little or no psoriasis,	1
only a few patches (that could be covered	
by one or two paims of (your/his/her)	
hand),	2
scattered patches (that could be covered	
between three and ten paims of (your/	
his/her} hand), or	3
extensive psoriasis (covering large areas of	
the body, that would be more than ten	
paims of (your/his/her) hand)?	4
REFUSED	7
DON'T KNOW	9

SP_DEQ

