UNDERSTANDING THE RELATIONSHIP BETWEEN SKIN COLOR, VITAMIN D, AND BLOOD PRESSURE

by

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To Mom

Thank you for always being in my corner.

Also, thank you for putting up with me.

I know I can be a handful. ☺

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Understanding the intersection of skin color, behavioral factors and chronic disease risk is important in addressing underlying causes of race/ethnic disparities in blood pressure. Literature suggests that vitamin D impacts the renin-angiotensin system, vascular remodeling, and insulin resistance and thus, by extension, blood pressure. Previous research has identified that skin color is important to the cutaneous production of vitamin D, with the higher melanin content in darker skin reducing the amount produced from sun exposure. It is hypothesized that the high burden of hypertensive disease among African Americans as compared to Caucasians may be due in part to low serum vitamin D owed to darker skin color.

This dissertation extends the research about health-related effects of vitamin D by examining its influence on blood pressure over time and quantifying its mediating effect on the relationship between skin color and blood pressure. This research used data from

a population-based sample of mid-life African American and Caucasian women in southeastern Michigan enrolled in the Study of Women's Health Across the Nation (SWAN) and followed annually from 1996 to 2010.

The study found that independent of other variables, skin color was an important contributor to circulating serum vitamin D in mid-life women over a 14-year period, with lighter skin color associated with higher vitamin D levels and higher odds of optimal vitamin D, defined as 75 nM. Independent of race, a skin color threshold for optimal vitamin D was identified. Additionally, vitamin D was an important correlate with baseline systolic blood pressure in African American and Caucasian women, however the change in vitamin D was not associated with systolic blood pressure over the study period. No association was observed between vitamin D and diastolic blood pressure. There was evidence that 11% of the relationship between skin color and systolic blood pressure was mediated by vitamin D.

The relationship between skin color and systolic blood pressure was mediated by vitamin D. Establishing the effect of vitamin D on systolic blood pressure identifies a modifiable target for interventions aimed at ameliorating the high prevalence of hypertensive disease in the United States and the disproportionate burden among African Americans.

CHAPTER I

Introduction

Overview

Health disparities are the differences in incidence, prevalence, mortality, disease burden, and health status among different population groups, often defined by race/ethnicity, sex, educational level, socioeconomic status, or geographic location of residence. A key factor in health disparities is that they are created by contextual factors and thus potentially reversible. Therefore, eliminating health disparities became a central tenet of the Healthy People National US Public Health agenda. (1) Because of sensitivities in the US to its troubling national history of racism both inside and outside the context of health, the exploration of racial health disparities has been largely premised on socioeconomic position, to the exclusion of obvious biological factors that are not reversible. The irreversible biological components of race have been avoided, even where they may be appropriate, because historically they have been promulgated with less than scholarly pursuits to justify discrimination. (2, 3) These factors give rise to the question: what if a key element to the etiology of health disparities is at the intersection of unexplored biological components of race and factors such as geography and aging?

The importance of the unexplored realm of race, geography and aging may be greatest for blood pressure where the racial disparity is overwhelming. (4, 5) Current literature suggests that vitamin D measured as serum 25(OH)D plays a key role in vascular remodeling, insulin sensitivity, and renin expression, all of which regulate blood pressure. (6-8) There is also strong evidence that the primary source of 25(OH)D in humans is cutaneous synthesis where its production depends largely on melanin, the contributory factor to skin color. (9, 10) Studies have determined that during the winter season, geographic locations above and below 35° latitude north and south lack ultraviolet-blue exposure which is the necessary prerequisite for 25(OH)D synthesis in

1

the skin. (11-14)   Further, researchers have reported that the precursor to cutaneous 25(OH)D synthesis decreases with age. (15, 16) Consolidating and synthesizing these disparate findings forms the basis for the hypothesis that an important "missing component" to understanding the racial disparity in blood pressure regulation is that African Americans, who largely have darker skin than whites, may be getting insufficient sunlight due to geographical location and sun exposure. This, combined with insufficient dietary intake, may generate an inadequate amount of 25(OH)D to sustain healthy regulation of blood pressure; and, this relationship may be exacerbated by the effect of aging. This dissertation research evaluated this hypothesis longitudinally over 14 years amongst women to determine whether there was evidence to support the "missing component" theory in a population that, due to its age, is at imminent risk for hypertension and adverse cardiovascular events.

Specific Aims

The goal of this study was to characterize the effect of skin color on serum 25(OH)D and blood pressure among women as they age using data from the Michigan site of the longitudinal Study of Women's Health Across the Nation (SWAN) comprised of African American and Caucasian women aged 42-52 years at the 1996 recruitment.

Aim 1: To evaluate whether lower serum 25(OH)D levels over a 10-year period would be associated with increased systolic and diastolic blood pressure levels and to determine if there was a declining trajectory for serum 25(OH)D with advancing age that was greater for African American women than for Caucasian women, using repeated annual measures acquired over a 10-year period from the Michigan site of the SWAN.

Aim 2: To evaluate differences in the 14-year longitudinal trajectory of serum 25(OH)D by skin reflectance, a proxy measure of melanin content, to establish if there is a skin reflectance threshold that is predictive of optimal serum 25(OH)D (defined as a value ≥ 75 nM) and to compare the predictive probability of race and the skin reflectance threshold in the Michigan SWAN population.

Aim 3: To characterize the cross-sectional relationship between skin color and systolic and diastolic blood pressure and to determine the extent to which these associations are explained or mediated by serum 25(OH)D in the Michigan SWAN population.

Public Health Importance

Previous epidemiological studies of 25(OH)D have been limited in that they utilize self-reported racial and ethnic classifications in lieu of direct measurements of skin color. (11, 13, 17, 18) Further, the existing studies that have measured skin color lack a broad range of skin pigmentations, longitudinal data to explore changes with age, or measures of serum 25(OH)D). (9, 10, 19-27)   The SWAN cohort study was used to assess the onset and progression of hypertension in women with respect to three fundamental factors—age, skin color, and 25(OH)D, and to add nuance to the risk factor profile with a greater understanding of 25(OH)D trends. Because the population is comprised of African American and Caucasian women, a broad range of skin colors were available to examine the impact of coloration at all levels of the color spectrum. Further, understanding the intersection of biological skin color, behavioral factors and chronic disease is important in addressing the underlying cause of race/ethnic health disparities. While it is necessary to elucidate the contribution of immutable risk factors such as skin color to hypertension, an important contribution is deconstructing immutable factors to find modifiable aspects such as 25(OH)D that can promote healthy blood pressure regulation.

Background

The term "vitamin D" refers to a group of prohormones which are precursors to their hormonally-active counterparts. By definition these compounds are not vitamins, but secosteroids which arise from the cleavage of the steroid bond through ultraviolet sun exposure. Cholecalciferol (vitamin D3), the naturally occurring form which originates from cholesterol, has a side chain equivalent to 2-methylheptane. (28) Serum 25(OH)D is largely derived from cutaneous generation. Figure 1.1 illustrates the stages of vitamin D synthesis. Provitamin D, a precursor to vitamin D3, known as 7-dehydrocholesterol (7-DHC) is distributed throughout the epidermis and dermis, but largely concentrated in the stratum spinosum and stratum basale. (29) To begin the process, UVB exposure from sunlight in the range of (290-315 nm) serves as a catalyst to the conversion of 7-DHC to through photolysis of the B-ring bonds resulting in tacalciol (previtamin D3). Immediately following this step, spontaneous thermal isomerization of previtamin D3 into cholecalciferol (vitamin D3) occurs via the movement of sigma double bonds known

as a sigmatropic shift. Vitamin D3 then binds to vitamin D-binding globulin and is transported via dermal capillaries to the systemic circulation and liver. (30) Next, the hepatocytes release 25-hydroxylase, a liver enzyme, which hydroxylates vitamin D3, converting it to 25-hydroxycholecalciferol (25(OH)D), the circulating compound which is assayed to estimate bodily stores. The last stage involving further hydroxylation by 1-α hydroxylase in the renal tubules, is required to convert 25(OH)D into the primary biologically active hormone, 1-25 dihydroxycholecalciferol. (31)

Optimal 25(OH)D is popularly defined as serum concentrations of 25(OH)D equal to or greater than 75 nM. (32-35) The classification is based on evidence that measures above 75 nM optimize calcium absorption and parathyroid hormone levels, and decrease the likelihood of rickets, osteomalacia, periodontal disease, colorectal cancer, lower extremity dysfunction, myocardial infarction, and all-cause mortality. (31, 34, 36-41)

Skin Color and 25(OH)D

The origin and function of diverse skin color in the humans was largely related to protection from sun exposure. (42) It is postulated that melanin, which causes darker skin pigment, is protective against the adverse effects of ultraviolet-blue (UVB) from prolonged sun exposure. (43) It follows that proximity to the equator, the region with the greatest amount of UVB year-round, was highly correlated with skin color. Because 25(OH)D does not naturally exist in copious quantities in most human foodstuffs—notable exceptions being oily fish—over thousands of years, humans evolved a photosynthetic mechanism to produce cholecalciferol (Vitamin D3) from UVB. (44) The abatement of UVB absorption by melanin in darker pigmented populations slowed the production of 25(OH)D, but did not occlude its adequate production for bodily function because of the high frequency of sun exposure in equatorial regions. (42) When human populations emigrated from sub-Saharan Africa 50,000 years ago to northern latitudes, their adaptation involved loss of skin pigmentation. (45, 46) Thus, among populations further from the equator, lighter skin prevailed because limited UVB exposure at higher latitudes minimized the need for protection from UVB and increased the need to produce more 25(OH)D with lesser UVB exposure. (47) With the rapid onset of industrialization

and globalization and more rapid shifts in population geography, the interaction of skin color with environment altered more quickly than is accommodated by evolutionary adaptation. Alterations in this relationship may have deleterious effects including deregulation of 25(OH)D, a potential superintendent of blood pressure. (12, 29)

To corroborate this hypothesis, studies have demonstrated that skin color is of great import in the determination of 25(OH)D status. The rate-limiting step in 25(OH)D synthesis is controlled by skin color. (15) Melanin in the skin competes with 7-DHC for ultraviolet photons by absorbing them and thereby limiting the amount of provitamin D converted to previtamin D per UVB exposure. A minimal erythemal dose (MED) of 215 J/m^2, the amount of UV required to produce erythema (reddening) of a person in the lightest skin category, can lead to a vitamin D intake from 10,000 - 25,000 IU in light skinned individuals; (48) among the darkest pigmented individuals the intake for the same dose can be as low as 1,000 – 2,500 IU, values 4-10 orders of magnitude lower than those found among light skinned individuals. Other studies found similarly strong correlations between skin color and 25(OH)D, with individuals with lighter skin having higher measures of 25(OH)D. (10, 14, 15, 49) In studies of UVB irradiation, findings suggest that the rate of increase in 25(OH)D is higher among the lightest skin tones, with a graded responses for darker complexions. These studies found that 80% of the variation in 25(OH)D response to UVB was attributable to UVB dose and natural skin color, (49) and that the percentage increase in 25(OH)D among Caucasians was 210%, whereas among black participants the increase was 40%. (15) Thus, there is evidence linking lighter skin pigmentation to more efficient production of 25(OH)D for a given UVB dose than that in darker pigmentation. There is no evidence however, that the rate of conversion of provitamin D to previtamin D and subsequent steps along the 25(OH)D cascade differs by skin color, but rather that the amount of UVB reaching provitamin D to begin the cascade differs. Thus, research has demonstrated that all skin types are able to produce sufficient levels of 25(OH)D with appropriate, albeit distinct, exposure to UVB. (9, 48, 50) However, research has not addressed the extent to which the relationship between skin color and 25(OH)D changes over time. Additionally, previous research has not addressed the extent to which other factors associated with serum 25(OH)D contribute to, reduce, or exacerbate the relationship between skin color and

25(OH)D. This research examined skin color and serum 25(OH)D over a 14-year period in a population with variable skin color to address some of the gaps in the literature.

Factors associated with Circulating Serum 25(OH)D

The UV irradiation primarily responsible for 25(OH)D production is determined by the solar zenith angle (SZA). SZA is the angle between the sky directly overhead and the position of the sun. Values range from 0-90°, with larger values indicating a lower position of the sun in the sky and lesser UVB absorption because UVB has to travel a longer optical path length to reach the earth's surface. (15) The earth's rotation changes SZA for a location throughout the year. The largest fluctuations are experienced furthest from the equator where the tilt of the earth has greatest bearing on the angle from the sun. SZA becomes smallest in summer and in lower latitudes illustrating the difficulty in achieving optimal 25(OH)D at higher latitudes, even during summer months. Further compounding this issue is the higher ozone in northern areas, which also serves to deflect UVB. (48) Requisite for the onset of the 25(OH)D synthesis cascade, the combination of season and location are important in human production. (29)

Dietary intake can be an important source of vitamin D if foods with high content are consumed in generous portions. (37) The only foods that naturally contain vitamin D3 are seafood, mushrooms and egg yolks. The highest concentration of naturally occurring vitamin D3 is found in the fatty oils of salmon, oysters, catfish, bluefish, mackerel, trout, sardines, halibut, tuna cod and shrimp. (43) The largest contributor to dietary vitamin D is wild salmon with 1000 IU per 3oz serving. Milk and multivitamins are fortified to supplement the dietary intake and ensure adequate intake and have been demonstrated to increase serum 25(OH)D. (51-53) However, the 2011 Institute of Medicine recommendation for vitamin D intake is 400 IU/d up for infants up to 12 months, 600 IU/d for males12 months or older and females until age 70, and 800 IU/d for women 71 years or older. (125)   The fact that the 25(OH)D level in many populations, is drastically lower than the 75 nM recommendation, combined with the observation that higher doses have been only shown to raise vitamin D by small amounts, indicates that the current dietary recommendation may increase serum 25(OH)D, but be too low to achieve optimal levels. (29, 33, 54, 55)

The process of aging alters the function of many bodily processes that affect serum 25(OH)D concentration. Of critical importance is the decreased production of 7-DHC with age. (31) It is well established there are substantive changes in the composition and structure of the skin after age 20. Principally, the thickness decreases linearly with age, in addition to elastic fiber structure shrinkage in the dermis, and the reduction of the epidermal papillary body. (16, 37) There is an age-related decline in epidermal but not dermal 7-DHC content. As the precursor to 25(OH)D, decreases in the concentration in the skin reduce the net production of 25(OH)D per UVB unit exposure. Additionally, final stages of 25(OH)D synthesis involve liver and kidney enzymatic processes which decrease in efficiency with aging. Further, the impact of dietary sources of 25(OH)D which circumvent the initial stages of vitamin D production in the skin may be altered with age because absorption of vitamin D from food and supplements is reduced from the gut, a result of decreased motility. (43) The aggregated effects of aging combine to create the perfect storm of factors restricting 25(OH)D synthesis.

Obesity has been implicated in low serum 25(OH)D. Research has demonstrated that 25(OH)D has an affinity for deposition in adipose tissue. Sequestration and storage in adipose tissue decreases circulating availability in serum and the ability to be converted to other metabolites including bioactive vitamin D. (56) However, during states of deprivation where there is accelerated fat turnover, these stores have been released and are associated with increases in circulating serum 25(OH)D. Population based studies have corroborated these findings, demonstrating that irrespective of season, individuals with higher body fat content have lower 25(OH)D and prospective UV irradiation studies demonstrated that the amount of 25(OH)D measured after UV radiation is lower in obese individuals. (57, 58) Because body mass index (BMI) is a population proxy measure for body fat, the study results suggest that 25(OH)D is more strongly correlated with total body fat, not BMI, indicating a link to fat mass not body mass.

Smoking has also been identified as a risk factor for suboptimal 25(OH)D and bioactive vitamin D. (41, 51, 59, 60) Toxicity from heavy metals such as hydroxyquinones present in tobacco smoke affect liver function, accumulation of cadmium in the kidney, and altered hepatic metabolism of 25(OH)D. (61, 62)

Serum 25(OH)D and Blood Pressure

There are three primary mechanisms that possibly explain the link between 25(OH)D deficiency and blood pressure: renin-angiotensin system, insulin resistance, and vascular remodeling. (63) Each of the 25(OH)D-blood pressure mechanisms relies heavily on the discovery of a wide distribution of vitamin D receptors (VDR) throughout diverse tissue types, especially those related to cardiovascular regulation. In addition to the relative ubiquity of VDR, the hydroxylase enzyme required to convert 25(OH)D to bioactive vitamin D is also widely distributed, which leads to multiple systemic effects of 25(OH)D that may work in concert to affect blood pressure. (64)

Renin-Angiotensin Pathway: Inactivation of VDR or the enzyme that converts vitamin D3 to bioactive vitamin D appears to disrupt the renin-angiotensin regulatory cascade, the primary function of which is to maintain vascular resistance and extracellular homeostasis. (65) Renin is a protease produced in the juxtaglomerular apparatus of the nephron where it cleaves angiotensin I (Ang I) from angiotensinogen in the liver. A converting enzyme then, in turn, converts Ang I to Ang II which binds to receptors and increases blood pressure. (8)

An inverse relationship between the plasma bioactive vitamin D concentration and blood pressure or plasma renin activity has been established in studies of both normotensive and hypertensive individuals. (66-69) Furthermore, ultraviolet exposure has been demonstrated to be inversely related to the rise of blood pressure and the prevalence of hypertension in the general population and was also shown to have blood pressure-lowering effects. (70, 71) It has also been reported that vitamin D3 supplementation reduces blood pressure, plasma renin activity and Ang II levels in patients with hyperparathyroidism, a condition caused by suboptimal 25(OH)D. (72, 73) In explanation of these findings, one study found that bioactive vitamin D is a negative regulator of renin expression and that disruption of the vitamin D signaling pathway leads to deregulated elevation of renin expression; similarly, an increase in serum 25(OH)D levels lead to suppression of renin. (74) Another study found that plasma 25(OH)D concentrations are inversely associated with blood pressure in Dahl salt-sensitive rats. (75)

Insulin Resistance Pathway: Insulin resistance, characterized as the inability of circulating insulin to stimulate the uptake and metabolism of glucose by muscle, is a metabolic abnormality that may be an etiologic factor in the pathogenesis of hypertension. (7) In the normal fasting state, plasma glucose decreases from an increase in plasma insulin. This balance lessens the insulin-mediated uptake and metabolism of glucose by insulin-sensitive cells in the ventromedial hypothalamus. The low levels of glucose stimulate the inhibitory pathway between the hypothalamus and the brain stem, resulting in decreased sympathetic nervous system activation. Conversely, the hyperglycemia and hyperinsulinemia associated with insulin resistance, deactivates the inhibitory pathway, resulting in increased sympathetic nervous system activity, contributing to hypertension by stimulating the heart, vasculature and kidneys. Both animal and human studies have demonstrated that 25(OH)D is essential for normal insulin secretion; (76, 77) 25(OH)D is associated with the biosynthetic capacity of β cells in the Islets of Langerhans and accelerates the conversion of proinsulin to insulin in the pancreas. (78) Human studies have demonstrated a positive association between 25(OH)D and insulin sensitivity and appropriate β cell function; suboptimal 25(OH)D has similarly been associated with insulin resistance and dysfunctional β cell activity. (79)

Vascular Remodeling Pathway: In addition to the pancreas, VDRs are prevalent among vascular smooth muscle cells (VSMC). (80) In the rat model, bioactive vitamin D suppresses thymidine (a substance used in proliferation studies to measure the amount of incorporation into the cell medium and thus cell reproduction) uptake and propagation in VSMCs. (6) Bioactive vitamin D was also found to suppress the mitogenic effect of epidermal growth factor (EGF) on VSMC proliferation by blocking the ability of mitogens to trigger signal transduction pathways that lead to mitosis. (81) Evidence that bioactive vitamin D inhibits the growth of VSMCs in vitro, gives plausibility to the theory that 25(OH)D deficiency allows the continued growth of VSMCs by activating EGF which leads to a decreased lumen and increased blood pressure. (82) The importance of genes regulating VDR has also been implicated in the relationship between 25(OH)D and blood pressure. (83, 84) Because VSMCs and endothelial cells have VDRs, they have the ability to convert 25(OH)D to 1,25(OH)$_2$D. Additionally, there is

substantiation that 25(OH)D deficiency triggers secondary hyperparathyroidism to maintain serum and body calcium by mobilizing calcium from bone. High parathyroid hormone affects blood pressure by leading to insulin resistance and by causing an autoimmune response. The inflammation resulting from the release of myocytes in the endothelial cells causes vascular remodeling. (64)

Observations from epidemiologic studies have given credence to the plausibility of these mechanistic pathways. The National Health and Nutrition Examination Survey (NHANES III) found that systolic and diastolic blood pressure were lower for participants in the lowest 25(OH)D quintiles compared to the highest, a relationship which was exacerbated by age.(17) Further evidence was found in a study of plasma 25(OH)D and the risk of incident hypertension among young women in the Nurses' Health Study which found that 25(OH)D levels were inversely and independently associated with the risk of developing hypertension. When evaluated continuously, this amounted to an 8% increase in odds of hypertension for every 12.5 nM increase in 25(OH)D. Studies have also determined that vitamin D dietary and supplement intake is inversely associated with the risk of hypertension.(85, 86) These findings have prompted a clinical recommendations to increase 25(OH)D. (87-90) However, all studies have not yielded similar results. Some studies have been unable to establish an association between serum 25(OH)D and blood pressure or between vitamin D intake and blood pressure. (91-95) These disparate findings merit further study to understand this relationship. (96) This study will examine the relationship between serum 25(OH)D and blood pressure over at ten-year period in a population-based cohort of mid-life women to contribute to the understanding of this relationship.

Skin Color and Blood Pressure

Hypertension occurs more often in African American adults than in white or Hispanic American adults. In national studies, among African American men and women, the prevalence of hypertension is 40.6 and 43.5% respectively; among white men and women (the population group with the lowest prevalence) the proportion is 27.6 and 28.5% respectively. (1) In relation to these groups, African Americans tend to have hypertension earlier in life, experience greater severity, have less success in achieving target control levels with treatment, and have higher rates of premature death from

hypertension-related complications, such as coronary heart disease, stroke, and kidney failure. (97) Conversely, however, African Americans are more likely to be aware of elevated blood pressure levels and to be treated for the condition. (4) Age is an important factor in hypertension, with the prevalence and disparities in hypertension being greatest in persons over the age of 55; women comprise two-thirds of hypertension cases in this age group and the black prevalence becomes two-fold higher than whites. (98)

The potential influence of social and psychological factors on the risk of hypertension within the US black community has been explored in relation to the community's history and culture. The socioeconomic disadvantage that is disproportionately experienced by blacks in the US underscores an epidemiologic model in which poor health is associated with a chronic struggle to achieve and maintain valued social and personal goals. (99) Persistent stress is specifically associated with higher blood pressure and also compounds blood pressure elevation. (100, 101) Mechanistically, chronic stress may cause hypertension through the continual elevation of cortisol which stimulates sympathetic nerve activity and causes sensitivity to epinephrine and norepinephrine resulting in vasoconstriction related to regulation by renin expression. (102)

Other risk factors for hypertension have been explored including behavior and diet,(103) body size,(104) and salt processing,(105) socioeconomic position,(106, 107) neighborhood composition,(108) and genetics(26, 109). However, few factors have successfully accounted for the racial hypertension disparity.(110) Alternatively, social support structures in the black community, including the church and the extended family networks, serve as stress buffers which ameliorate the impact of blighted circumstances and lower the risk of hypertension. (111) Efforts to achieve the twin goals of lowering blood pressure therapeutically and preventing the onset of hypertension have thus been shaped by social and cultural context of African Americans and social and psychological processes associated with hypertension. (99)

Despite these efforts, inadequate progress has been made toward the eradication of the racial disparity in hypertension. (5) Because of the continuing unexplained racial variation in hypertension, it has been proposed that the high burden of hypertensive disease among African Americans may be due in part to low serum 25(OH)D via darker

skin color. Some studies have examined skin color and blood pressure and found that darker skin color is related to higher blood pressure irrespective of self-reported racial classification. (20-27) These studies have not, however, examined the extent to which the association between skin color and blood pressure is due to mediation by serum 25(OH)D. Figure 1.2 displays an overview of the mechanisms and influential factors in the relationship between skin reflectance, serum 25(OH)D and blood pressure. This research seeks to extend the current body of knowledge by contributing to our understanding of the relationships between skin color and 25(OH)D, between 25(OH)D and blood pressure, and by evaluating evidence to explain one of the mechanisms by which skin color exerts its effect on blood pressure.

Public Health Significance

The exploration of skin color and environment has largely been examined in the context of anthropology and evolution. In the discipline of epidemiology, the importance of skin color as a prominent etiologic factor began with studies of skin cancer. Previous research has focused on the relationship between skin color and 25(OH)D with very little examination of other health implications. It is of great import to understand the intersection of biological skin color and context as it relates to the risk of chronic disease, a concept that is central to identifying the underlying causes of race/ethnic health disparities. In this capacity, it may be necessary to conceptualize race/ethnicity as a crude proxy measure for constructs beyond socioeconomic position and culture. Blood pressure is a precipitating factor in cardiovascular disease mortality, which is the leading cause of death in the United States. (4) Thus, determining detailed risk factors that may influence 25(OH)D and subsequently blood pressure can provide substrate for public health actions to mitigate the risk of adverse cardiovascular events.

Overview of Study Population and Data Collection

This study used data from one of the seven sites of the Study of Women's Health Across the Nation (SWAN). (112) SWAN is an ongoing multi-ethnic, multi-site longitudinal community-based study of middle-aged women begun in 1996. Community-based samples were recruited at seven study sites (Ypsilanti and Inkster, Michigan; Boston, Massachusetts; Hackensack, New Jersey; Chicago, Illinois; Pittsburgh,

12

Pennsylvania; Los Angeles, California; and Alameda and Contra Costa County, California). The Michigan sample was recruited as a population-based sample developed from the census in two communities. Michigan SWAN study population was recruited using a two-stage design with different criteria for inclusion at each stage. In the first stage, conducted in 1995, potential Michigan SWAN enrollees were identified from a census of 24,283 households located in census tracts associated with two suburban communities (Ypsilanti and Inkster) near Detroit. This census identified eligible women, aged 40–55 years, residing in the pre-selected census tracts, by telephone (25% of contacts) or in-person contact (75%) and resulted in the successful cross-sectional interview of 2621 (65%) of these eligible women. The list of successfully interviewed women in the first stage served as the sampling frame for identification of women eligible for enrollment in the longitudinal cohort (second stage). For the Michigan site, Caucasian and African Americans women were recruited in a sampling ratio of 40:60.

Women eligible for the longitudinal cohort study were aged 42–52 years with intact uterus, not using hormones, had a menstrual period in the previous three months (premenopausal or early perimenopausal) and were either African American or Caucasian by self-definition. A longitudinal cohort of 543 (72% of those eligible from the first stage) women (325 African-American; 218 Caucasian) was enrolled in Michigan. This number was reduced in the first follow-up year to reduce study costs, but in the fifth follow-up, 62 enrollees with baseline data were re-recruited.

This study will be located in women at the Michigan SWAN site who participated anytime from 1996-2010. In 2010, 405 (75%) women were available for contact and data collection. Measurements of the initially recruited cohort may have been unavailable because women were deceased (n=28), lost-to-follow-up (n=58), refused participation (n=32), were unable to complete the examination (n=38) or participated via telephone in lieu of a clinical visit (n=22). Cohort retention was similar by race. African American women still comprised 60% of the active participants in 2010.

Study Population Rationale

The Michigan SWAN site population resides at 42°N indicating a minimal solar zenith angle (SZA) near 20° during summer months and a maximum SZA near 70°

during winter months, sufficient to preclude UVB exposure for the duration of winter. (14, 48) Additionally, the regional burden of cardiovascular disease morbidity and mortality is high in Michigan. According to the Centers for Disease Control and Prevention, between the years 2000-2006, the Michigan age-adjusted cardiovascular disease death rate for women aged 35 years and older was 409 per 100,000, exceeding the national rate of 351 per 100,000. (113) The Michigan SWAN site population is comprised of African American and Caucasian women, and the stratum-specific death rates in Michigan for these groups (580 per 100,000 and 387 per 100,000) also exceeded the national rates of (479 per 100,000 and 341 per 100,000) respectively. The geographic location of this research strongly influences its importance in examining the influence of skin color on the prevalence of suboptimal 25(OH)D and its relationship to blood pressure as women age.

Collection and Management of Skin Color and Serum 25(OH)D Data

During the 2009-2010 annual study visit, 365 (90.1%) of enrolled women had skin color measured with reflectance. A questionnaire was developed to gather additional data about participant sun exposure, sunscreen use, and vitamin D supplementation and approved by the University of Michigan Institutional Review Board (Appendix). The questionnaire was validated in previous vitamin D studies and clinic staff was trained for administration. The skin reflectance protocol and questionnaire were approved by the University of Michigan Institutional Review Board. Data collection began in May 2009 and was completed in June 2010. To minimize data entry errors, data were doubly entered into Epi Info version 6 and imported to Statistical Analysis Systems (SAS) version 9.2 for data management and data analysis.

To address the specific aims, we added 25(OH)D assays and digital reflectance measures of skin color to the 2010 data collection cycle in the Michigan SWAN cohort. Specimens of serum from 2010 and freezer archives were assayed for 25(OH)D. The 25(OH)D assays for each woman were performed on specimens collected in years 1, 2, 3, 5, 6, 7, 8, 9, 10, and 14. At corresponding time points, blood pressure and measures of additional covariates were related to the 25(OH)D data. Two assessments of skin color, natural (taken at a non-UV exposed site) and facultative (taken at a UV exposed site) were measured in 2009-2010 using digital reflectance. Because measures of natural skin

color are presumed to be immutable, they were linked to the aforementioned blood pressure levels and 25(OH)D assay results from all data collection years. Comparing to natural skin color to facultative skin color provides an assessment of UV-Tan from recent sun exposure; measures of UV-Tan skin color may vary over time and are only used in cross-sectional analyses for year 14. A depiction of the annual data collection scheme for each variable is available in Table 1.

Measurements

Skin Color: Skin color was measured in this study by exposing areas of the skin to controlled light and measuring the amount of light reflected. Reflectance was measured on a 0-100 scale with 0 indicating no reflectance and 100 indicating complete reflectance; reflectance is higher in persons of lighter skin color and lower for persons of darker skin color. This measure was performed using a narrow band PhotoVolt® digital reflectance meter which approximates melanin content in the skin based on the amount of light reflected into the instrument. (114)

Measures were taken with subjects at rest, away from direct sunlight. An alcohol wipe as used to clean the area prior to measurement to remove any dirt, topical medications or cosmetics. Natural skin reflectance, representing genetically-inherited skin color at a non-UV exposed site, was measured in the inner upper arm. Facultative skin reflectance, a measure of the skin color at a site modified by UV exposure, was measured at the glabella, the area between the eyebrows at the center of the forehead. (14)   The operator held the probe perpendicular to the skin area avoiding depressing the skin with pressure. Measurements were taken in duplicate and the correlation between the first and second reflectance readings for skin reflectance has been determined to be in excess of 0.95, demonstrating the precision of this measure. (115)

All reflectance measures are reported on the L* index scale using the Commission International d'Eclairage (CIE) L*a*b* system (1976). L* index measures the lightness-darkness axis and is highly correlated with the melanin index [$R^2 = 0.93$, $p<0.001$] in populations with a wide range of skin color values, demonstrating construct validity. (116)

25(OH)D: Phlebotomy has been performed at each subject visit. Serum has been separated using a refrigerated centrifuge within an hour of collection with minimal

15

exposure to light and stored at -80°C awaiting laboratory analyses. There is documentation of date, time of day, relation to time of menses, and fasting status. Serum 25(OH)D was measured using automated chemiluminescence immunoassay (CLIA) by Heartland Assays in Ames, IA to measure 25-hydroxycholecalciferol (25(OH)D). The efficacy of this technique in comparison to other methods has been previously described. (117) Measures of serum 25(OH)D were reported in nanomolar (nM). The assay has a 7.5 nM limit of detection, an 8% intra-assay coefficient of variation (CV), and an 11% inter-assay CV. Optimal 25(OH)D is defined as a serum measure greater than or equal to 75 nM.

Blood pressure: At each SWAN participant visit, an examiner has measured blood pressure twice in the right arm in millimeters of mercury (mm Hg) using a mercury column sphygmomanometer with a cuff bladder encircling at least 80% of the arm. Four cuffs are available to allow appropriate selection for the arm size of individual women. The measurement was taken after the subject rested in a seated position for a minimum of five minutes and each measurement is separated by a minimum of two minutes. Subjects were positioned with feet on the floor and arm supported at heart level. Systolic blood pressure (SBP) was defined as the commencement of sound at Korotkoff phase 1; diastolic blood pressure was defined as the cessation of sound at Korotkoff phase 5. The duplicate measures were averaged for analysis.

The operational definition of hypertension according to the Seventh Report of the Joint National Committee on Prevention, Detection, Treatment and Control of High Blood Pressure is a blood pressure level greater than 140mm Hg SBP or 90 mm Hg DBP or persons currently receiving antihypertensive therapy. (118) Antihypertensive medication use was obtained via self-report and verified by observation of medication containers at the examination.

Covariate Measurement

Additional variables for which *a priori* knowledge indicates confounding or effect modification of the relationship between the exposures and outcomes of interest were measured and included in statistical modeling where appropriate. These variables include age, race, smoking status, body mass index (BMI), body fat, supplement and dietary

vitamin D intake, UV season, sun exposure, sunscreen use, education, employment, financial hardship, and study retention.

Age was calculated to 0.1 precision by subtracting the examination date from the participant's date of birth. Race was determined by self-identification during recruitment as either African American or Caucasian. A dichotomous measure of tobacco use in the 12 months preceding the visit was self –reported at each annual visit and treated as a time-varying covariate.

Height in centimeters (cm) and weight in kilograms (kg) were measured at each examination and used to calculate Body Mass Index (BMI) in kg/m^2. BMI values of 30 kg/m^2 or greater are classified as representing obesity.

Body fat percentage was assessed in kilograms (kg) with bioelectrical impedance in which the greater transmission speed of electrical current in muscle tissue and fluids is compared to the slower conduction by fat tissue.

The use and dosage of vitamin D dietary supplements or multivitamin supplements containing vitamin D was assessed annually and treated as time varying covariate. The amount of dietary vitamin D intake was assessed at three examinations (baseline and follow-ups 5 and 9) and reported in international units (IU) using the National Cancer Institute food frequency questionnaire and provisional vitamin D data from the United States Department of Agriculture. Dietary information was combined with vitamin D values from supplements during years when both variables were collected.

Assignment to one of two proxies for season of data collection was based on the average monthly UV index for southeast Michigan. Data collected between April 1 and September 30 was assigned to a high UV category based on monthly UV index average values of five or higher, whereas measures taken between October 1 and March 31 were grouped into a low UV category because the UV index average values were four or lower, with most months averaging zero. In data analysis, this measure was treated as a time-varying covariate. To estimate sun exposure, participants were asked to report the average number of hours spent in the sun daily during warm weather, the parts of the body typically exposed to sunlight during warm weather, and if they used sunscreen with a sun protective factor (SPF) of 15 or greater.

Estimates of various constructs of socioeconomic position were evaluated with measures of education, employment and financial hardship. The highest level of education completed was assessed at recruitment in 1996 and is reported as a dichotomous variable. Women reporting their highest level of completed education as college or post-graduate studies were grouped and compared to women reporting less than high school, high school, or post-high school as their highest level of completed education. Current employment was assessed at each study visit. An annual interview question about the difficulty in paying for necessities in the preceding 12 months (food, shelter and health care) was used as a time-varying dichotomous indicator of financial hardship.

To account for the potential impact of cohort retention on study findings, study participation variable was created to compare deceased women or enrollees with 0-2 visits in the past five years to the active cohort of participants with 3-5 visits during the same time period. This dichotomous variable was included in longitudinal modeling with repeated measures.

Analytic Approach

Analyses began with univariate and frequency estimations for continuous and categorical variables, respectively. All continuous variables were evaluated for normality and log transformations were performed where appropriate to meet model assumptions of normality and constant variance. Log transformed means and regression model parameter estimates were back transformed in the results and discussion to ease interpretation. Log transformed means were back transformed by taking the anti-log of the log transformed mean and half of the log transformed standard error squared. When the response variable was log transformed, taking the anti-log of the estimate, subtracting 1 and multiplying by 100 yielded the percentage increase in the response variable associated with a one-unit increase in the explanatory variable. When both the response and explanatory variables were log transformed, the parameter estimate was interpreted as the percentage increase in the response variable associated with a 1% increase in the explanatory variable. (119)

We stratified descriptive measures by variables of interest, namely race category, serum 25(OH)D status, or hypertensive treatment where appropriate. Chi-square tests were used to evaluate differences in categorical variables by two-stratum stratification

variables; Fisher's exact test was used to compare categorical variables by stratification variables that result in small cell sizes. The student's t-test was used to determine if the means of continuous variables differed between the strata of binary variables, whereas the Kruskal-Wallis test was used to compare means for three-stratum variables. Measures of association are reported with p-values or 95% confidence intervals (CI); univariate statistics are reported with standard deviations (SD). All statistical tests performed were two-sided and significance was defined at $\alpha=0.05$. Data management and analyses were completed using SAS v9.2.

Statistical Power

The Michigan SWAN sample is a closed cohort with no new recruitment; therefore, the sample size was fixed at the onset of this study. Using the SAS glmpower procedure, we calculated power for each aim based on the known sample size and estimated effect size from previous literature. For all models in all aims tested, there was 80% power or more to detect a minimum effect size of 0.2. (120)

Longitudinal Repeated Measures Data Analyses

Mixed effects modeling was used to estimate the associations between variables over time while accounting for correlated errors within individuals over time. A mixed model is used to account for the two distinct variable effects, those that change within subjects over time and those that are invariant. Linear mixed models were fit to include time (linear) and time-squared (quadratic) random effects variables, representing linear and curvilinear trajectories over the study period. Regression analyses began with bivariate models of a single set of independent and dependent variables with later adjustment for potential confounders in multivariate models. Variables were selected for inclusion in final models with consideration for a priori hypotheses and statistical associations observed in the data. We used the spatial power covariance structure to account for correlated errors in closely spaced measurements and to account for unequally spaced time points. This structure has homogenous variances and correlations decline exponentially with increasing distance from the first measure, indicating constant measurement variability over time, with correlation between measures decreasing as they get further apart. The spatial power covariance function has the form:

(Equation 1) $\text{Cov}(\varepsilon_{ij}, \varepsilon_{i'j}) = \sigma_\varepsilon \rho^{d_{ii'}}$

where $d_{ii'}$ is the distance in time between times i and i′ , and ρ is a correlation parameter that measures the linear association between the errors measured 1 unit of time apart. Because the time variable representing study visit was an integer, this covariance structure reduces to the first-order autoregressive structure. Chi-square tests were used to compare the -2 log likelihood ratios of nested and full models, characterize model fit, and determine appropriate covariance structure.

Longitudinal mixed effects models were used to address Aims 1 and 2. In Aim 1, separate models were employed to evaluate whether serum 25(OH)D was associated with systolic and diastolic blood pressure and to determine if mean serum 25(OH)D changed over time and if that change was different for African American and Caucasian women. The models of systolic and diastolic blood pressure controlled for potential confounders including baseline age, body fat percentage, smoking status, hypertensive treatment, length of hypertensive treatment, and serum 25(OH)D by treatment status. The model for 25(OH)D controlled for baseline age, race, vitamin D intake, anti-hypertensive medication use, length of hypertensive treatment, body fat percentage, UV season, smoking status, and study participation. For Aim 2, a linear mixed model was used to evaluate the trajectory of serum 25(OH)D by skin reflectance. This model also included baseline age, UV season, body fat percentage and vitamin D supplement use. The longitudinal linear mixed effects models for Aims 1 and 2 were of the following form:

(Equation 2) $Y_{ij} = \beta_0 + \beta_1 time_{ij} + \beta_2 X_{ij} + \beta_3 X_{ij}*time_{ij} + b_{i0} + b_{i1}time_{ij} + \varepsilon_{ij}$

where $time_{ij}$ is the jth linear time point for the ith woman and X_{ij} is the value of a covariate for the ith woman at time j. Y_{ij} are the values of the outcome variable for the ith woman at time j; β_0 is the intercept, β_1 (time) coefficient estimates the annual linear change in the outcome variable. The β_2 (covariate) coefficient estimates the mean change in the outcome associated with a unit change in a covariate. A significant β_2 coefficient is interpreted as a difference in baseline outcome measure according to level of the covariate only if there is a corresponding β_3 interaction. Otherwise, the β_2 can be interpreted as the association between the covariate and the outcome at any time over the study period. β_3 ($X_{ij}*time_{ij}$) estimates the longitudinal linear change in the covariate per year (slope); a negative beta coefficient for this interaction term indicates a decrease in

the outcome variable with increases in time and the covariate, whereas a positive beta coefficient indicates an increase in the outcome over the same conditions. The b_{i0} is a random intercept term to account for correlated errors in repeated measures from the same woman (which results in deviation of the intercept of woman i from the population intercept β_0) and $b_{i1}time_{ij}$ is the random slope term to account for correlated errors in repeated measures over time from the same woman which results from deviation of the slope of woman i from the population slope β_1, and ε_{ij} is the independent (random) measurement error.

An additional goal of Aim 2 was to examine the risk of optimal 25(OH)D by skin reflectance. To address this aim, longitudinal repeated measures logistic regression was performed for the dichotomous optimal 25(OH)D outcome variable. This model was of the following form:

(Equation 3) Logit $Y_i = \beta_0 + \beta_1 time_{ij} + \beta_2 X_{ij} + b_{i0} + b_{i1}time_{ij} + \varepsilon_{ij}$

where the outcome is binary (1=Yes/0=No); therefore a repeated measures logistic regression model similar to the longitudinal linear mixed model was used to ensure appropriate model specifications. The outcome (Logit Y_i) is interpreted as the risk of outcome in i[th] subject over the 14 year study period. The coefficient β_2 is the overall effect of a covariate on the incidence of optimal 25(OH)D over time, adjusting for the random and fixed effects of the other covariates. The exchangeable covariance structure was used to account for correlated errors in the logistic regression model. This structure involves equal variances for random effects and one common pairwise covariance and was chosen because the dichotomous serum 25(OH)D status for the same individual appeared to be equally correlated, regardless of the distance between responses.

Cross-Sectional Data Analyses

In Aim 2, logistic regression was performed to determine the association between skin reflectance and optimal 25(OH)D, to establish a relevant skin reflectance threshold for optimal 25(OH)D, and to compare the predictive probability of race and the skin reflectance threshold. Covariates, including UV Tan reflectance, age, UV season, sunscreen use body fat percentage and vitamin D supplement use, were included in the model. The logistic regression model was of the form:

(Equation 4) Logit $Y = \beta_0 + \beta_1 X + \varepsilon$

The "odds" is the probability of an event divided by the probability of a non-event, assuming that only two outcomes are possible. In logistic regression, the logits, the natural logs of the odds, of the unknown binomial probabilities are modeled as a linear function of the X using maximum likelihood estimation. (121) The regression parameters (β) are estimated by maximum likelihood using a method common to all generalized linear models. The maximum likelihood estimates can be computed numerically by using iteratively reweighted least squares. The interpretation of the β_1 parameter estimate is as the additive effect on the log of the odds for a unit change in the covariate. Positive regression coefficients indicate an increase in the probability of the outcome whereas negative coefficients indicate a decrease. The magnitude of the regression coefficients indicate the strength of the association; a near-zero regression coefficient means that that risk factor has little influence on the outcome probability.

Receiver operator characteristic (ROC) curves were used to assess the adequacy of the skin reflectance in predicting optimal serum 25(OH)D by plotting sensitivity against one minus specificity. The summary statistic for the ROC curve is the area under the ROC curve (AUC) which was used to determine if measuring skin reflectance had a higher probability of identifying optimal serum 25(OH)D than would have occurred by chance (0.5); a perfect prediction would yield and AUC of 1.0. (122) The nonparametric approach was used to compare the AUC for ROC curves with different covariates. (123)

For Aim 3, linear regression using ordinary least squares estimation was used to characterize the independent relationships between skin reflectance, serum 25(OH)D and blood pressure and to determine the degree of mediation by serum 25(OH)D in the relationship between skin reflectance and blood pressure. Confounders included in the models were age, body fat percentage, smoking status, financial hardship, anti-hypertensive medication use, sunscreen use, and vitamin D supplementation. The model fit, as estimated by the variation in the outcome explained from the included covariates, was assessed with the coefficient of determination R^2 statistic. The linear regression models were of the form:

(Equation 5) $Y = \beta_0 + \beta_1 X + \varepsilon$

where β_1 is the mean change in the outcome variable Y associated with a 1-unit change in the covariate X. The model assumes linearity of the relationship between X and Y and independence, constant variance and a normal distribution of the errors. (121)

Mediation is a means of identifying an intermediate variable (25(OH)D) that is both caused by the explanatory variable (skin reflectance) and also affects the response variable (blood pressure) and explains (in total or in part) how the skin reflectance affects blood pressure. The direct effect of skin reflectance is its immediate effect on blood pressure; the indirect effect of skin reflectance is the effect on blood pressure that operates through serum 25(OH)D; and the total effect is a combination of the indirect and direct effects. A powerful test for mediation requires that there exists a primary effect to be mediated (i.e. a test of the total effect rejects the null hypothesis of no association) and that the indirect effect is significant in the predicted direction. The Preacher and Hayes measure of indirect effects uses bootstrap resampling to calculate the quantity difference between the parameter estimates for the total effect and the direct effect in order to examine the underlying contribution of 25(OH)D in the association of skin reflectance and blood pressure. (124) Bootstrapping is accomplished by taking a large number of samples (we used 1126) and computing the indirect effect in each sample. In traditional testing of the indirect path, the distribution is usually skewed, failing to meet model assumptions of normality. The bootstrap approach to characterizing mediation avoids reliance on assumptions of normality in the distribution of the mediation statistic because it is based on an empirical estimation of the sampling distribution, thereby providing a more robust estimation of variance. This test also is also more readily applied to smaller samples because it avoids reliance on large-sample theory.

Summary and Chapter Overview

This dissertation extends the current health disparities literature by providing an objective measure of skin color using reflectance in the examination of associations with serum 25(OH)D and blood pressure. Additionally, the availability of annual repeated measures of serum 25(OH)D and blood pressure in a population-based cohort of mid-life women allowed us to examine the trajectory of changes over time, reducing temporal ambiguity and questions of dose response, to strengthen arguments for causality.

Chapter II describes the ten-year trajectories of systolic and diastolic blood pressure in relation to the change in serum 25(OH)D over the same period. This chapter also describes the ten-year trajectory of serum 25(OH)D comparing African Americans to Caucasians. Comparing self-reported race to skin color, Chapter III examines whether the 14-year trajectory of serum 25(OHJ)D differs by skin reflectance, establishes a longitudinal risk of suboptimal 25(OH)D by skin reflectance, and compares the predictive probability for suboptimal serum 25(OH)D between a skin reflectance threshold and self-reported race. In Chapter IV, the relationship between skin reflectance, serum 25(OH)D and blood pressure were examined to understand the mediation effect of serum 25(OH)D. Lastly, the primary research findings, significance to public health and clinical practice, and future research directions are presented in Chapter V.

Figure 1.1. Stages of Vitamin D Synthesis

7-dehydrocholesterol
(7-DHC)
(provitamin D)

Ultraviolet Blue
(UVB)

Tacalciol
(previtamin D3)

Cholecalciferol
(vitamin D3)

25-hydroxylase

25-hydroxycholecalciferol
(25-OH-D)
(vitamin D)
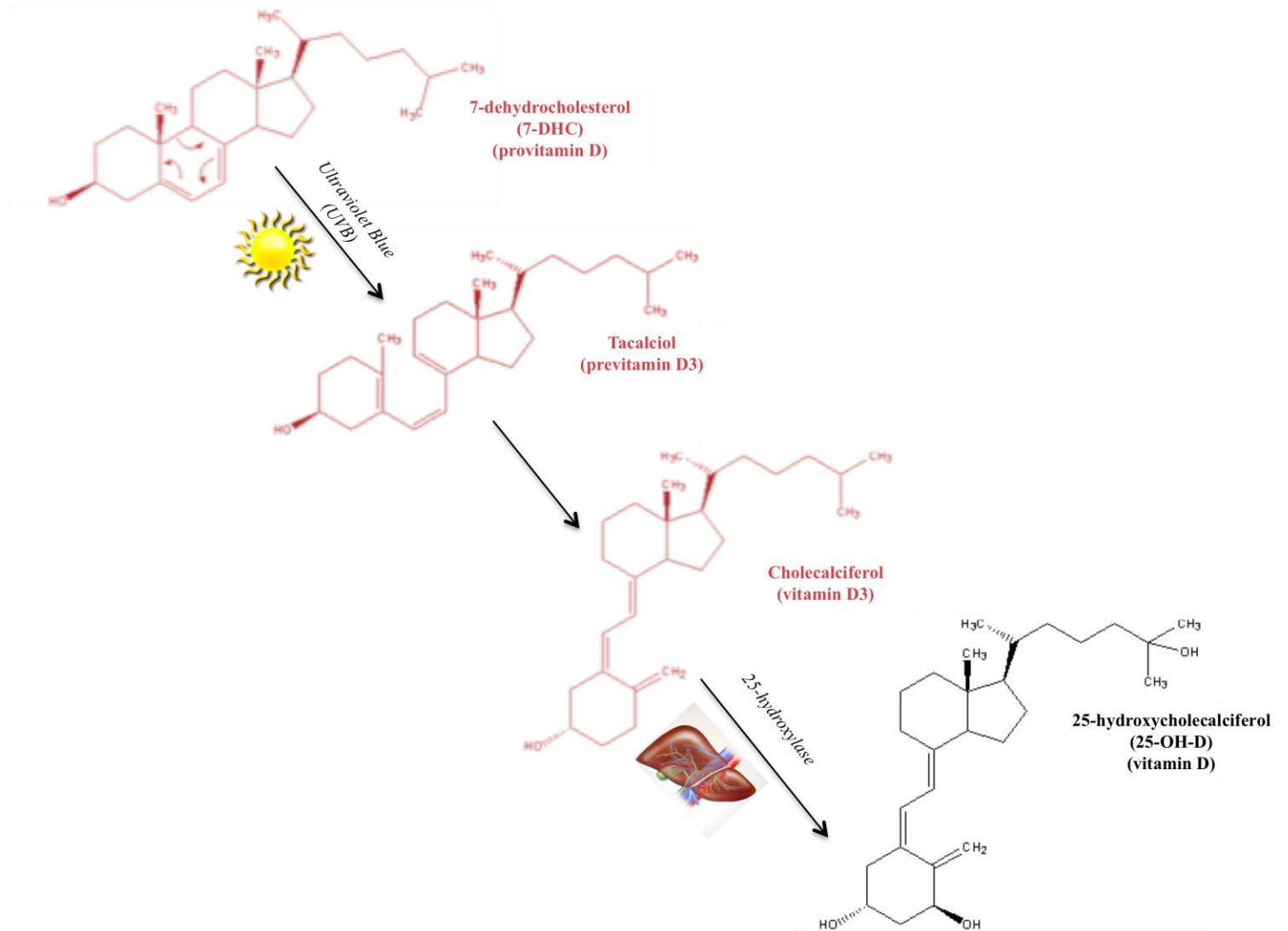
Figure 1.2. Schema for the relationship between Skin Reflectance, 25(OH)D, and Blood Pressure
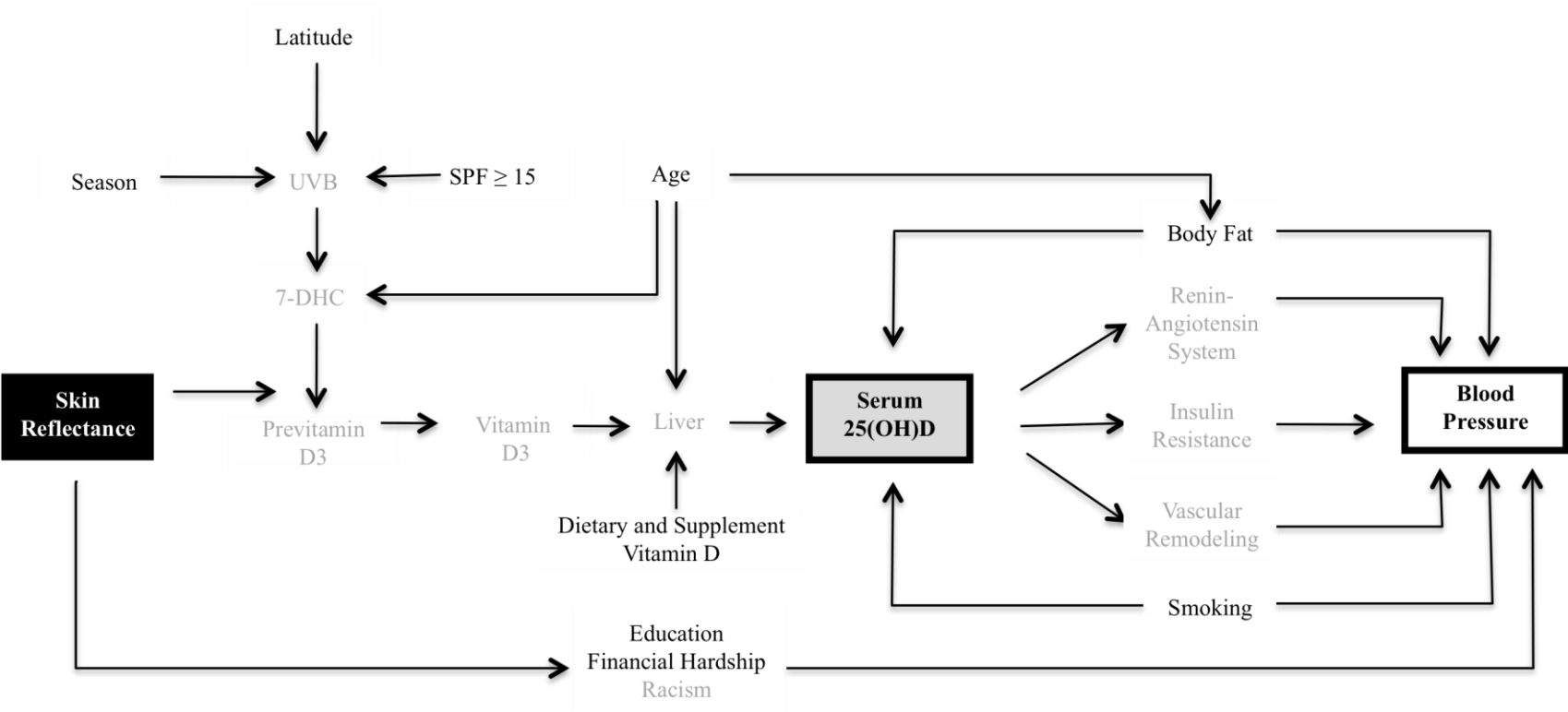
Figure 1.3. Michigan SWAN Annual Variable Data Collection
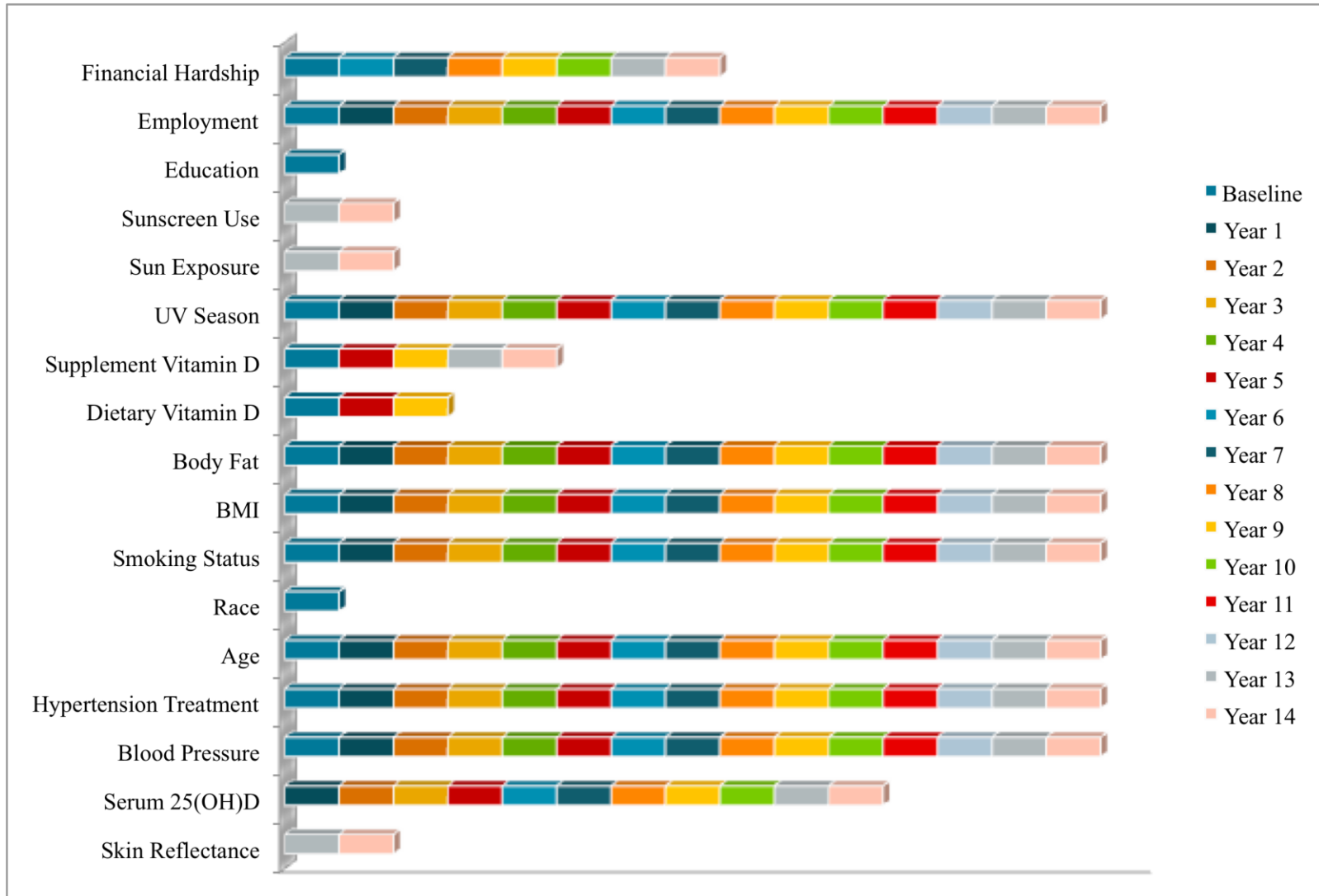
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CHAPTER II

The Longitudinal Trajectory of Serum 25(OH)D in Relationship to Blood Pressure in women at the mid-life

Abstract

The presence of vitamin D receptors in the cardiovascular system suggests that circulating vitamin D [25(OH)D] may contribute to blood pressure regulation. It has been suggested that 25(OH)D is a factor in blood pressure regulation and low 25(OH)D owed to darker skin among African Americans contributes to the often-reported race differences in hypertension between African-Americans and Caucasians. This study used repeated annual measures of serum 25(OH)D and blood pressure acquired between 1996 and 2007 in 543 participants of the Study of Women's Health Across the Nation (SWAN)-Michigan site to examine the longitudinal trajectory of vitamin D and blood pressure and to determine if the ten-year trajectory of vitamin D differed for African American and Caucasian women. Higher baseline serum 25(OH)D was associated with lower baseline systolic blood among African American[β=-.012 (p=0.02)] and Caucasian women [β=-0.08 (p=0.01)] but no relationship was observed for baseline diastolic blood pressure. Annual change in serum 25(OH)D over the ten-year follow up period was not associated with systolic or diastolic blood pressure. Baseline serum 25(OH)D values were 45% lower in African American women compared to Caucasian women (p<0.0001), however the annual change in serum 25(OH)D was 2% higher (p<0.0001)] for African American women than for Caucasian women. The results of this study suggest the importance of achieving recommended levels of serum 25(OH)D in early adulthood to modulate the likelihood of blood pressure increases with age and the need for targeted interventions to racial groups at differential risk for vitamin D deficiency.

Introduction

Vitamin D may be influential in blood pressure regulation due the vitamin D receptors found throughout the cardiovascular system which control renin expression, insulin resistance, and nervous system activation.(1-5) In cross-sectional and prospective epidemiologic studies, African Americans have lower serum 25(OH)D levels—a measure of the body's vitamin D stores—than Caucasians;(6, 7) It has been suggested that low levels of 25(OH)D are a factor in hypertension and contribute to the often-reported race differences in hypertension.(8-10)

It is well-documented that African Americans are differentially impacted by hypertension compared to Caucasians. The prevalence of hypertension among African Americans is two-fold higher than Caucasians and tends to occur earlier in life with greater severity. African-Americans have less success in achieving target control levels with treatment.(11, 12) African Americans also have higher rates of premature death from hypertension-related complications, such as coronary heart disease, stroke, and kidney failure.(13)

Numerous mechanisms have been the object of extensive research to identify causal factors in the race disparities associated with hypertension. Some of these include cortisol and stress, (14, 15) behavior and diet,(16) body size,(17) and salt processing,(18), socioeconomic position,(19, 20) neighborhood composition,(21) and genetics(22, 23). However, few factors have successfully accounted for the racial hypertension disparity.(24) Because of the continuing unexplained racial variation in hypertension, it has been proposed that African Americans, who generally have darker skin than Caucasians, experience hypertension more frequently because of diminished dermal conversion of 7-hydroycholesterol to 25(OH)D. This, in combination with inadequate dietary vitamin D intake, a higher prevalence of smoking, and higher prevalence of obesity may lead to an environment whereby 25(OH)D levels are insufficient to sustain adequate regulation of blood pressure. The impact of these factors may be exacerbated by increasing age.

Using repeated annual measures acquired over a 10-year period, we evaluated if lower serum 25(OH)D levels over time would be associated with increased systolic and diastolic blood pressure levels and if this inverse relationship between 25(OH)D and blood pressure would become more pronounced over a ten-year follow-up period. We

further evaluated if the trajectory for serum 25(OH)D in women at mid-life would decline with advancing age and if that the rate of the decline would be greater for African American women than for Caucasian women.

Study Population

This study uses data from the Study of Women's Health Across the Nation (SWAN) Michigan site. SWAN, begun in 1996, is an ongoing multi-ethnic, multi-site longitudinal community-based study of health and the menopause transition in middle-aged women. The two-stage design used to generate the study population at the Michigan SWAN site has been described. (25) In the first stage, study personnel contacted adults in 24,283 residences located in 40 Census tracts of Ypsilanti and Inkster, MI to identify those households that included women aged 40-55 years. In those households with age-eligible women, health interviews were conducted (n=2621 women) to provide cross-sectional health information and to generate a list of women eligible for participation in the longitudinal study. Women were eligible for the longitudinal study (the second stage) if they were aged 42–52 years, had an intact uterus and at least one ovary, were not using reproductive hormones, had a menstrual period in the 3 months prior to interview and self-identified as either African American or Caucasian. The Michigan SWAN site recruited 543 women, and by design, in a 60:40 ratio of African American to Caucasian women.

At the tenth annual follow-up (2007), 431 (80%) women were available for contact and data collection. Between 1997 and 2007, 18 (3%) women had died, 57 (11%) had been lost to follow-up, 27 (5%) of women refused participation, and 10 (2%) were unable to complete the examination. This analysis is based on serum 25(OH)D data from specimens acquired during annual visits from examination years 1-3 and 5-10.

Written informed consent was obtained annually from all participants, and approval for the conduct of the study was obtained from the University of Michigan Institutional Review Board.

Study Measures

Per protocol, blood pressure was measured twice in the right arm using a mercury column sphygmomanometer. The participant rested in a seated position for a minimum

of five minutes with feet positioned on the floor and arm supported at heart level. The two measures were separated by a minimum of two minutes and the results averaged for reporting. Systolic blood pressure (SBP) was defined as the commencement of sound at Korotkoff phase 1; diastolic blood pressure (DBP) was defined as the cessation of sound at Korotkoff phase 5. This report uses as its operational definition of hypertension a blood pressure level greater than 140mm Hg SBP or 90 mm Hg DBP or current use of antihypertensive therapy, consistent with the Seventh Report of the Joint National Committee on Prevention, Detection, Treatment and Control of High Blood Pressure. (26) The use of prescription anti-hypertensive medication was interviewer-assessed at each data collection time point. The report of antihypertensive medication use was corroborated by observation of medication containers at examination.

Blood was drawn annually and then separated using a refrigerated centrifuge within an hour of collection. Serum had minimal exposure to light and was stored at -80°C awaiting laboratory analyses. Serum 25(OH)D was measured singularly using automated chemiluminescence immunoassay by Heartland Assays in Ames, IA. The assay has a 7.5 nM limit of detection, an 8% intra-assay coefficient of variation (CV), and an 11% inter-assay CV. Optimal vitamin D is defined as serum 25(OH)D values ≥75 nM, insufficiency includes values ≥ 25 and <75 nM, and vitamin D deficiency consists of values <25 nM.

There were 3001 data points with concurrent serum 25(OH)D and blood pressure measures. Serum 25(OH)D was measured at least once in 498 (92%) of participants. Serum 25(OH)D data may be unavailable because women did not have phlebotomy (loss-to-follow-up, telephone interview in lieu of a clinical visit, or deceased) or the volume collected was inadequate to permit archival storage. Among women with at least one serum 25(OH)D measure, 224 (45%) had 8-9 annual 25(OH)D measures from serum; 172 (34.5%) had 5-7 annual 25(OH)D measures from serum, and 102 (20.5%) had 1-4 annual 25(OH)D measures from serum.

To examine the impact of differential cohort retention on study findings, we included an indicator for study participation and retention in the statistical models. Inactive cohort members, which included enrollees who are deceased or with 0-2 visits in

the past five years, were compared to members of the active cohort of participants with 3-5 visits during the same time period.

Covariate Measurement

Height in cm and weight in kg were measured at each examination and used to calculate Body Mass Index (BMI) in kg/m^2. Body fat percentage was assessed with bioelectrical impedance in which the greater speed of electrical current conduction in muscle tissue and fluids is compared to the slower conduction in fat tissue. A dichotomous measure of tobacco use in the 12 months preceding the visit was self – reported at each annual visit and treated as a time-varying covariate. The amount of dietary vitamin D intake was assessed at three examinations (baseline and follow-ups 5 and 9) using food lists from the National Cancer Institute food frequency questionnaire, whereas the use of vitamin D dietary supplements was assessed annually. Vitamin D intake was dichotomized to compare women with intakes of <400 IU per day to those with intakes ≥ 400 IU per day, according to the Food and Nutrition Board recommended daily intake in place at the time of analysis. (37)

Assignment to one of two proxies for season of data collection was based on the average monthly UV index for southeast Michigan. Data collected between April 1 and September 30 was assigned to a high UV category based on monthly UV index average values of five or higher, whereas measures taken between October 1 and March 31 were grouped into a low UV category because the UV index average values were four or lower, with most months averaging zero. In data analysis, this measure was treated as a time-varying covariate with high UV as the referent group.

Current employment was assessed at each study visit. An annual interview question about the difficulty in paying for necessities in the preceding 12 months (food, shelter and health care) was used as a time-varying dichotomous indicator of financial hardship.

Statistical Analysis

Analyses began with univariate and frequency estimations for continuous and categorical variables, respectively. The blood pressure variables were examined for normality to satisfy model assumptions; no transformations were required to meet these

assumptions. Vitamin D was log transformed to address the skewed distribution in calculating mean vitamin D or modeling the trajectory of vitamin D over time. Log transformed data are presented in tables, but were back transformed to ratio measures for ease of interpretation. We examined baseline variable distributions by race, hypertensive treatment status and vitamin D status. Chi-square tests were used to evaluate if categorical variables differed by race or hypertensive treatment; Fisher's exact tests were used to compare categorical variables by serum 25(OH)D categories because of small cell sizes. The student's t-test was used to determine if the means of continuous variables differed by race or hypertensive treatment, whereas the Kruskal-Wallis test was used to compare means according to the three-level variable representing adequacy of serum 25(OH)D. Measures of association are reported with p-values or 95% confidence intervals (CI); univariate statistics are reported with standard deviations (SD).

Mixed effects modeling was used to estimate the associations between variables over time while accounting for correlated errors within individuals over time. To account for correlated errors in closely spaced measurements we used the autoregressive covariance structure. Chi-square tests were used to compare the -2 log likelihood ratios of nested and full models, characterize model fit, and determine appropriate covariance structure. Linear mixed models were fit to include time (linear) and time-squared (quadratic) random effects variables, representing linear and curvilinear trajectories over the ten-year study period. In initial evaluations, the quadratic term (time-squared), representing rate of change, was not statistically significant and therefore was not included in final models for serum 25(OH)D or BP. Variables were selected for inclusion in final models with consideration for a priori hypotheses and statistical associations observed in the data.

To describe the change in serum 25(OH)D over the ten-year study period, the linear mixed models included baseline age, race, vitamin D intake, anti-hypertensive medication use, length of hypertensive treatment, body fat percentage, UV season, smoking status, study participation, and time. To characterize the race-specific change in SBP and DBP with serum 25(OH)D, data were stratified by race. Covariates for the blood pressure models—including serum 25(OH)D, baseline age, body fat percentage, current smoking status, hypertensive treatment—with significant p-values demonstrating

49

a longitudinal association with blood pressure, were included in the final models as main effects or in an interaction with the time variable.

The linear mixed models for blood pressure were of the following form:

$$BP_{ij} = \beta_0 + \beta_1 time_{ij} + \beta_2 X_{ij} + \beta_3 X_{ij}*time_{ij} + b_{i0} + b_{i1}time_{ij} + \varepsilon_{ij}$$

where $time_{ij}$ is the jth linear time point for the ith woman and X_{ij} is the value of a covariate (i.e., serum 25(OH)D) for the ith woman at time j. BP_{ij} are the values of blood pressure (systolic or diastolic) for the ith woman at time j; β_0 is the blood pressure intercept, β_1 (time) coefficient estimates the annual linear change in blood pressure. The β_2 (covariate) coefficient estimates the mean change in blood pressure associated with a unit change in a covariate such as serum 25(OH)D. A significant β_2 coefficient is interpreted as a difference in baseline BP according to level of the covariate only if there is a corresponding β_3 interaction. Otherwise, the β_2 can be interpreted as the association between the covariate and BP at any time over the ten-year study. β_3 ($X_{ij}*time_{ij}$) estimates the longitudinal linear change in the covariate per year (slope); a negative beta coefficient for this interaction term indicates a decrease in blood pressure with increases in time and the covariate, whereas a positive beta coefficient indicates an increase in blood pressure over the same conditions. The b_{i0} is a random intercept term to account for correlated errors in repeated measures from the same woman (which results in deviation of the intercept of woman i from the population intercept β_0) and $b_{i1}time_{ij}$ is the random slope term to account for correlated errors in repeated measures over time from the same woman which results from deviation of the slope of woman i from the population slope β_1, and ε_{ij} is the error.

All statistical tests performed were two-sided and significance was defined at $\alpha=0.05$. Data management and analyses were completed using SAS v9.2.

Blood Pressure Characteristics

At baseline, 23% of the population reported being treated for hypertension (Table 2.1). The mean SBP ($\pm$ SD) and DBP ($\pm$ SD) were 129 ($\pm$ 18) and 76 ($\pm$ 12) mm Hg in women treated for hypertension in comparison to 116 ($\pm$ 15) and 69 ($\pm$ 9) mm Hg for

women without treatment. Women being treated for hypertension were, on average, slightly older, had higher BMI and body fat percentages, and were more likely to report financial hardship in the previous year.

At baseline, 28% of African American women reported being treated for hypertension compared to 17% of Caucasian women. In the untreated, the mean SBP value in African American women was 120 (± 16) while the mean SBP in Caucasian women was 111 (± 14) mm Hg. DBP values for untreated African American women were not statistically distinguishable from Caucasian women, being 71 (± 10) mm Hg and 68 (± 9) mm Hg respectively. There were no statistically significant differences in BP among treated African American and Caucasians; in African-American women, the mean SBP and DBP were 131 (± 20) and 77 (± 12) mm Hg whereas among Caucasian women the values were 124 (± 12) and 74 (± 10) mm Hg.

Ten years after the baseline assessment, the prevalence of treated hypertension in the population had doubled to 48%. Among these women, the mean SBP and DBP were 130 (± 22) and 74 (± 12) mm Hg. Among untreated women, the racial difference in SBP first observed at baseline remained in 2007 (African American 124 (± 18); Caucasian 113 (± 17) mm Hg). Nevertheless, intergroup comparisons were observed in 2007. While there were no significant differences in SBP among treated African American women and Caucasian women at baseline, 10 years later treated African American women had significantly higher SBP values [134 (± 24)] than treated Caucasian women [119 (± 14) mm Hg].

Serum 25(OH)D Characteristics

At the 1996 baseline, the mean serum 25(OH)D level (± SD) was 28.4 (± 18.4) nM. Overall, 44% of women were classified as vitamin D deficient (<25 nM); 50% were vitamin D insufficient (≥ 25 and <75 nM); and, 6% of women were classified as having optimal vitamin D (≥75 nM). Compared to women with optimal serum 25(OH)D levels at baseline, women classified as being vitamin D insufficient or deficient at baseline had higher body size measures including BMI and body fat percentage (Table 2.1). Women in the vitamin D insufficiency or deficiency categories had lower dietary and supplement intakes of vitamin D, were more likely to have had their clinic visit during the winter, to

be current smokers, to report greater financial hardship in the previous 12 months, and to self-identify as African American.

Serum 25(OH)D levels were not statistically distinguishable according to anti-hypertensive use (p=0.5). However, serum 25(OH)D values differed by race (Table 2.1). The mean (± SD) 25(OH)D levels at baseline among African American and Caucasian women were 21.6 (± 11.9) and 42.2 (± 23.9) nM, respectively. Only one African American woman was classified as having optimal serum 25(OH)D; 37% of African American women were classified as being insufficient and 62% were classified as vitamin D deficient. In contrast, among Caucasian women, 14% were classified as having optimal vitamin D, 69% as vitamin D insufficient, and 17% as vitamin D deficient. Ten years later in 2006, the mean (± SD) 25(OH)D levels among African American and Caucasian women were 34.5 (± 17.8) and 50.4 (± 23.7) nM. At year 10, African American women experienced 6% adequacy, 69% insufficiency and 15% deficiency, whereas among Caucasian women there was 25% adequacy, 69% insufficiency, and 7% deficiency.

Table 2.2 depicts the association between the 10-year serum 25(OH)D trajectory and baseline age, race, vitamin D intake, anti-hypertensive medication use, length of hypertensive treatment, body fat percentage, UV season, smoking status, and study participation. While the mean serum 25(OH)D increased over the ten-year observation period, there was not a statistically significant increase in serum 25(OH)D after adjustment for the longitudinal effect other variables (p=0.65). Using the back transformed ratio measures of association for the log transformed serum 25(OH)D measure, we observed different serum 25(OH)D trajectories in African American and Caucasian women. Though African American women had baseline serum 25(OH)D measures 45% lower than those for Caucasian women, compared to Caucasian women, the linear increase per year in serum 25(OH)D levels was 2% higher among African American women. Additionally, compared to women not being treated for hypertension, serum 25(OH)D was 1% higher for every additional year spent on treatment.

Additional factors associated with lower serum 25(OH)D at baseline were older age at baseline, higher body fat percentage, and vitamin D intake < 400 IU/day, low UV season, smoking, and inactive study participation. Smoking and vitamin D intake <400

IU/day were associated with an annual decrease in serum 25(OH)D over time compared to non-smokers and those with vitamin D intakes ≥ 400 IU/day. Individuals with low UV season visits had an annual increase in serum 25(OH)D over the ten-year period compared to women with visits during the high UV season.

Associations of Serum 25(OH)D and Blood Pressure

Higher baseline serum 25(OH)D was associated with lower SBP among African American and Caucasian women not being treated for hypertension. For every 1 nM higher baseline serum 25(OH)D, baseline SBP was 0.12 mm Hg lower for African Americans and 0.08 mm Hg lower for Caucasians. However, the longitudinal effect of serum 25(OH)D was not related to SBP (Table 2.3). There were no statistically significant baseline or longitudinal relationships between serum 25(OH)D and SBP or DBP in African American or Caucasian women irrespective of hypertension treatment status.

Discussion

In a ten-year longitudinal cohort study we found that higher serum 25(OH)D was associated with having lower SBP in both African American and Caucasian women who were not undergoing hypertensive treatment. This study is the first population-based study to identify that the relationship between BP and serum 25(OH)D is not observed among women using anti-hypertensive medication. This is consistent with other studies that suggest serum 25(OH)D may play a role in renin expression, vascular remodeling, or sympathetic nervous system activation. (27-29) The absence of associations in treated women suggests that hypertensive treatments which target sodium and calcium channels, the renin-angiotensin-aldosterone system, and the sympathetic nervous system, override the normal biological processes which link circulating 25(OH)D with blood pressure.(26)

The identification of vitamin D receptors in cardiovascular tissue motivated research on serum 25(OH)D and blood pressure; this relationship gained credence because many of the factors associated with hypertension are co-factors of serum 25(OH)D inadequacy. Observational studies have provided support of a relationship between serum 25(OH)D and blood pressure, however reports of conflicting results are often explained by differences in study population, sample size, serum 25(OH)D

classification, and measurement timing.(10, 30, 31) A missing factor in identifying serum 25(OH)D as a contributing factor to blood pressure is temporality. To our knowledge this study is the first longitudinal study characterizing annual changes in serum 25(OH)D and blood pressure over time in a single population to establish the temporal sequence of events and the magnitude of changes within the population. Our findings confirm the cross-sectional relationship between serum 25(OH)D and SBP in women not receiving hypertensive treatment. While increases over the ten-year study period were observed for both serum 25(OH)D and blood pressure, changes in serum 25(OH)D were not associated with changes in SBP. Further, there was no observed association between serum 25(OH)D and DBP, which is consistent with previous null findings from cross-sectional studies.(8, 32)

The series of nine annual assays of serum 25(OH)D over a 10-year period allowed for the characterization of the longitudinal change in serum 25(OH)D. Unexpectedly, serum 25(OH)D, increased over the ten-year follow up period and older age was associated with a linear increase in higher serum 25(OH)D. While the higher mean values with older age and total mean increase over the study period were modest, it is noteworthy in that it is antithetical to expectations generated from previous studies which suggest that solar and dietary vitamin D production decrease with age due to changes in sun exposure and skin composition, hepatic and renal function, and motility of the gut.(9, 33-37) A possible explanation for this observation is that 40% of women in this study reported vitamin D supplementation of at least 400 IU/day in 2006, a statistically significant increase from the 26% reporting this level of supplementation at baseline. Furthermore, at each time period where supplement and vitamin D intake was assessed (baseline, years 5 and 9) older women had higher supplementation than younger women, though the lack of annual measures may also account for the null finding for the linear change variable.   Notably, even the 2011 Institute of Medicine recommended daily intake of 600 IU/day may require years to rectify tissue levels of deficiency. (38, 42) While vitamin D supplementation may have succeeded in elevating circulating levels of serum 25(OH)D and curtailing the expected age-related decline in serum 25(OH)D, the majority of the study population still failed to achieve 25(OH)D levels that could be

classified as optimal (≥ 75 nM).   The decrease in smoking prevalence may also contribute to the slight increase in serum 25(OH)D.

The increase in serum 25(OH)D levels over time was greater among African American women compared to Caucasian women. Previous studies have demonstrated that the predominant mechanism for vitamin D production is skin exposure to solar ultraviolet-blue (UVB);(38) melanin diffuses UVB and slows the rate of vitamin D synthesis, resulting in higher vitamin D deficiency among darker-skinned populations.(39-41) This study does not have annual measures of UVB exposure thereby precluding inferences about the dermal production of serum 25(OH)D. However, we observed the mean dietary vitamin D intake significantly increased in African American women over time whereas no such increase was observed for Caucasian women. Whether this observation reflects a greater knowledge and awareness about the emerging health benefits of vitamin D supplementation among mid-life African American women is unknown; however, medical and public health practitioners should provide reinforcement for this practice.

Among women treated for hypertension, serum 25(OH)D is not associated with blood pressure. In women without hypertensive treatment, serum 25(OH)D appears to have a consistent correlation with SBP, such that irrespective of small changes over time, serum 25(OH)D at mid-life correlates with SBP. Because the mean increase in serum 25(OH)D in this population was small (approximately 10 nM over the ten-year follow-up) it may be of a lesser biological consequence to BP than achieving vitamin D adequacy or other more rapidly changing factors associated with BP, including body size. The strength of the associations between BP and serum 25(OH)D at baseline combined with no statistically significant impact of increasing 25(OH)D trajectory on BP over ten years, suggest that the most powerful public health impact for BP can be achieved by establishing practices to assure that women achieve optimal serum 25(OH)D levels in early adulthood.

This study has strengths which generate confidence in the results. For the first time, a population-based study has been conducted which includes annual assays of serum 25(OH)D over a ten-year period. Because of the large number of assays and high retention rate, the study population is of sufficient size to examine additional covariates

of interest. Further, this population includes African American and Caucasian women allowing for discernible differences in serum 25(OH)D and blood pressure among these women. We stratified analyses on hypertensive treatment use recognizing its overwhelming impact on blood pressure levels relative to the modest effects that would have been expected of vitamin D.

Our study has some limitations. Cohort studies are vulnerable to the bias generated by loss-to-follow up; however, this study was largely successful in minimizing the potential bias with high retention. Because of the concern that lost participants may not be missing completely at random we used data prior to censoring to account for the impact of differential loss on study findings. Another limitation is that all variables of interest were not assessed annually. Of particular interest are measures of vitamin D dietary and supplement intake which were asked intermittently. Because the observed effects largely occurred at baseline, additional measures will likely not affect the validity of observed associations. In this population-based study, the number of study participants with optimal vitamin D ($\geq$ 75 nM) was small, suggesting the significant burden of low serum 25(OH)D in these women above the 35th parallel. The small number of women achieving optimal levels combined with a small mean increase in serum 25(OH)D (10 nM in ten years) may have generated an environment in which it was difficult to detect subtle increases in serum 25(OH)D to clinically significant changes in blood pressure over time.

Perspectives

This study found an inverse effect of baseline 25(OH)D and systolic blood pressure among African American and Caucasian women not receiving pharmacologic hypertensive treatment. This longitudinal cohort study also found that the change in serum 25(OH)D over time was not associated with blood pressure irrespective of hypertensive treatment status. Given the prevalence of vitamin D inadequacy (<75 nM) in this population was 94% at baseline, the results of this study suggest the importance of achieving recommended levels of serum 25(OH)D in early adulthood to modulate the likelihood of BP increases with age. Future studies should be powered to examine longitudinal trajectories of blood pressure among early adult women from various racial groups with a well-represented spectrum of serum 25(OH)D values at baseline. Given

the population burden of hypertension and vitamin D deficiency, efforts to further characterize the timing and dosage of dietary and behavioral interventions should be a component of hypertension research.

Table 2.1. Baseline (1996-1997) Population Characteristics by Hypertensive Treatment Status and Self-Reported Racial Category: Michigan SWAN

Independent Variable	Hypertension Treatment			Self-Reported Race			Vitamin D Status			
	No	Yes	p	African American	Caucasian	p	Deficient < 25 nM	Insufficient 25 ≤ nM < 75	Optimal ≥ 75 nM	p
N	340	102		254	188		170	194	23	
(%)	(76.9%)	(23.1%)		(57%)	(42.5%)		(43.6%)	(50.1%)	(5.9%)	
% African American	54.1%	68.6%	*	-	-		84.7%	44.3%	4.4%	*
Systolic Blood Pressure (mm Hg)	116 ±15	129 ± 18	*	123 ±18	113 ± 14	*	122 ± 17	118 ± 17	104 ± 13	*
Diastolic Blood Pressure (mm Hg)	69 ± 9	75 ± 12	*	72 ± 11	69 ± 9	*	72 ± 10	70 ± 11	68 ± 9	
% Anti-hypertensive treatment	-	-		27.6%	17.0%	*	24.3%	24.4%	4.4%	
25(OH)D (nM)	28.5± 19.2	28.1±15.7		21.6 ± 11.9	42.2 ± 23.9	*	15.5 ± 5.4	41.6 ±12.8	90.7 ± 10.8	*
Dietary Vitamin D (IU)	137 ± 120	141 ± 125		117 ± 102	169 ± 140	*	111 ± 94	154 ±140	199 ± 138	*
Supplement Vitamin D (IU)	116 ± 169	104 ± 166		104± 164	127± 173		87 ± 153	127 ± 175	181 ± 189	*
% of visits during high UV	56.8%	57.9%		50.0%	66.5%	*	38.5%	62.7%	82.6%	*
Age (years)	47.0 ± 2.7	47.8 ± 2.8	*	47.1 ± 2.9	47.2 ± 2.6		46.8 ± 2.8	47.5 ± 2.7	48.0 ± 2.9	*
Body Mass Index (kg/m2)	31.4 ± 7.5	34.9 ± 7.5	*	32.4 ± 7.7	32.0 ± 7.5		33.5 ± 8.2	31.6 ± 7.0	26.2 ± 5.0	*
Percentage Body Fat (kg)	40.0 ± 8.5	42.6 ± 8.6	*	41.4 ± 8.9	39.5 ± 8.0	*	42.1 ± 8.6	40.0 ± 8.5	34.1 ± 7.1	*
% Current Smoker	23.3%	30.4%		29.2%	20.1%	*	32.7%	20.7%	17.4%	*
% Currently Employed	75.6%	68.6%		70.9%	77.8%		76.2%	73.2%	73.9%	
% Financial Hardship	44.9%	51.3%		50.6%	39.9%	*	54.7%	38.3%	34.8%	*

Categorical data for the hypertensive treatment and race were compared using the χ^2 test and continuous data were compared using the student's t-test. Categorical data for the vitamin D categories were compared using Fisher's exact test and the continuous data were compared using the Kruskal-Wallis test.

* $p < 0.05$

Table 2.2. Baseline effect and ten-year linear change in log transformed serum 25(OH)D

Independent Variables	Baseline Effect			Linear Change		
	β	SE	p	β	SE	p
Baseline Age (years)	**0.01**	**0.01**	**0.10**	0.0008	0.0009	0.38
Percentage Body Fat (kg)	**-0.006**	**0.002**	**0.003**	-0.0002	0.0003	0.56
African American	**-0.60**	**0.05**	**<0.0001**	**0.02**	**<0.0001**	**<0.0001**
Vitamin D Intake	**-0.15**	**0.04**	**0.0002**	-0.0006	0.006	0.91
UV season	**-0.19**	**0.03**	**<0.0001**	**0.01**	**0.005**	**0.03**
Current Smoker	-0.05	0.05	0.32	**-0.01**	**0.006**	**0.03**
Study participation	**-0.12**	**0.05**	**0.02**	-	-	-
Hypertensive Treatment	-0.03	0.03	0.36	**0.01**	**0.005**	**0.03**

"African American", "Vitamin D Intake", "UV Season", "Current Smoker", "Study Participation" and "Hypertensive Treatment" are dichotomous categorical variables with respective reference categories of "Caucasian", "≥ 400 IU/day", "High UV Season", "Non-smoker", "Active", and "Untreated".

"Study Participation" was defined retroactively and is not a time-varying covariate.

Table 2.3. Model Coefficients from Linear Mixed Models with Systolic and Diastolic Blood Pressure as the Dependent Variable stratified by Self-Reported Racial Category

| | Systolic Blood Pressure | | | | | | Diastolic Blood Pressure | | | | | |
| | Baseline Effect | | | Linear Change | | | Baseline Effect | | | Linear Change | | |
Independent Variables	β	SE	p	β	SE	p	β	SE	p	β	SE	p
African American												
25(OH)D (nM)	**-0.12**	**0.05**	**0.02**	-0.01	0.01	0.37	-0.003	0.03	0.94	-0.002	0.005	0.65
Baseline Age (years)	**1.1**	**0.42**	**0.01**	-0.11	0.05	0.31	0.06	0.23	0.81	-0.05	0.03	0.07
Percentage Body Fat (kg)	-0.09	0.11	0.43	0.004	0.02	0.82	0.03	0.06	0.64	-0.01	0.009	0.19
Current Smoker	**4.3**	**2.4**	**0.01**	-0.51	0.32	0.11	0.57	1.3	0.6	-0.20	0.18	0.27
Hypertensive Treatment	2.6	1.5	0.09	0.28	0.26	0.28	1.4	0.89	0.12	-0.11	0.15	0.46
25(OH)D & Hypertensive Treatment	0.09	0.08	0.27	-0.01	0.01	0.24	0.47	1.7	0.78	0.21	0.28	0.46
Caucasian												
25(OH)D (nM)	**-0.08**	**0.03**	**0.02**	-0.002	0.005	0.65	-0.01	0.03	0.79	-0.004	0.004	0.30
Baseline Age (years)	**0.90**	**0.41**	**0.03**	-0.04	0.05	0.41	0.34	0.26	0.19	-0.05	0.03	0.11
Percentage Body Fat (kg)	**0.26**	**0.11**	**0.03**	-0.02	0.02	0.12	0.16	0.08	0.04	-0.01	0.01	0.46
Current Smoker	**7.1**	**2.5**	**0.006**	-0.60	0.35	0.09	1.1	1.7	0.51	-0.23	0.24	0.34
Hypertensive Treatment	-0.16	1.5	0.92	-0.35	0.27	0.20	-0.60	1.0	0.56	-0.12	0.18	0.49
25(OH)D & Hypertensive Treatment	0.23	0.07	0.10	-0.03	0.12	0.20	-2.8	2.5	0.25	-0.37	0.44	0.40

"Current smoker" and "hypertensive treatment" are dichotomous categorical variables with reference categories of non-smoker and untreated, respectively.

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CHAPTER III

Skin color and serum 25(OH)D over a 14-year period in women at mid-life

Abstract

Previous research has identified that skin color is important to the cutaneous production of vitamin D, with the higher melanin content in darker skin associated with lower circulating vitamin D levels. However, research has not addressed if skin color is associated with changes in vitamin D over time. Further, little work has been done to quantify the impact of skin color on the risk profile for contemporary definitions of optimal vitamin D. This research reports the longitudinal changes in vitamin D over a 14-year period in relation to skin reflectance, a proxy of dermal melanin content, and evaluates the skin reflectance threshold that was predictive of concurrent optimal serum 25(OH)D.

Longitudinal data from 1996-2010 in 543 women (60% African American and 40% Caucasian) aged 42-52 at baseline enrolled at the Michigan site of the Study of Women's Health Across the Nation (SWAN) were analyzed. Skin reflectance, an approximation of melanin content, was measured using the PhotoVolt® 577-A where the lowest reflectance score is 0, the darkest possible value, and 100 is the lightest or highest reflectance. Vitamin D was assayed as 25(OH)D in serum; the optimal value was defined as ≥ 75 nM. Longitudinal linear and logistic regression modeling for repeated measures were used to relate annual serum 25(OH)D measured over time to skin color, adjusting for covariates including time varying measures of body fat percentage, age, season of visit, smoking status, and vitamin D intake. Logistic regression and receiver operator characteristic (ROC) curves were used to evaluate and identify predictability of the association between natural skin color and optimal serum 25(OH)D.

The baseline (1996) serum 25(OH)D was 2% higher for every one-unit higher skin reflectance (p=0.0001). Women with lower reflectance measures had a 0.1%

greater rate of change in serum 25(OH)D than women with higher skin reflectance(p=0.001).

The 14-year odds of optimal serum 25(OH)D (<75 nM) were 13% higher for every higher unit in skin reflectance [Odds Ratio 1.13, 95% Confidence Interval 1.08, 1.18]. ROC curve analyses revealed that the skin reflectance threshold that optimizes sensitivity and specificity for optimal serum 25(OH)D is a score of 55, with 31% of African American women and all of the Caucasian women having values above this value. The AUC statistic indicates that skin reflectance has a higher probability of correctly predicting optimal serum 25(OH)D than chance alone [AUC 0.66, 95% Confidence Interval 0.58-0.73], however, the single factor with the greatest predictive ability was vitamin D supplementation [AUC 0.75, 95%CI 0.70-0.80].

Skin color is an important contributor to circulating serum 25(OH)D in mid-life women over a 14-year period, with higher skin reflectance associated with higher 25(OH)D levels and higher odds of optimal 25(OH)D. Independent of race, the skin reflectance threshold for optimal vitamin D was identified. An unexpected finding was the strength of the association of vitamin D supplementation with optimal 25(OH)D and with the change in 25(OH)D over time.

Introduction

Emerging research indicates that vitamin D has a preventive impact on an expanding number of diseases and conditions including cardiovascular disease, periodontal disease, colorectal cancer, lower extremity dysfunction, and all-cause mortality, in addition to its previously-known relation with rickets in children and certain types of osteomalacia in adults.(1-4) Defining the pathobiological roles for vitamin D in relation to multiple health outcomes has generated renewed interest in characterizing factors that influence vitamin D production and vitamin D deficiency.

Vitamin D, a secosteroid measured as serum 25-hydroxycholecalciferol (25(OH)D), is produced in the skin or can be ingested through dietary intake.(5) In the skin, ultraviolet blue (UVB) exposure from sunlight converts subcutaneous 7-dehydrocholesterol (7-DHC) to previtamin D3 and thence to cholecalciferol. Cholecalciferol is the sole source of dietary vitamin D and the predominant source of vitamin D in nutritional supplements. Once produced or consumed, cholecalciferol is

transported to the liver where it is enzymatically converted by 25-hydroxylase to 25(OH)D.(6, 7)

Because the skin is a primary source of 25(OH)D, skin coloration is an important factor in the cutaneous production of 25(OH)D.(8) Melanin, the pigment of darker skin, is protective against the adverse effects of ultraviolet-blue (UVB) damage from prolonged sun exposure. However, the abatement of UVB absorption by melanin decreases the amount of 7-DHC converted to previtamin D, thereby ultimately reducing the amount of 25(OH)D produced by the skin.(9, 10) Furthermore, solar exposure to UVB is largely determined by seasonality and geographic location. Locations further from the equator provide less UVB exposure due to higher solar zenith angles (SZA) and the associated changes in season. Lower SZA, the angles between the sky directly overhead and the position of the sun, are found at latitudes closer to the equator and during summer months because of the tilt of the earth.(11) Because UVB is absorbed at wavelengths between 270-315 nm, higher SZA during the winter season decreases or precludes the UVB exposure required for 25(OH)D production. Compared to darker skin color, there is evidence linking lighter skin color and higher serum 25(OH)D for a given UVB dose.(12) Though there is evidence that the amount of UVB absorbed by the skin differs by melanin content, there is no evidence of a different rate of enzymatic conversion of 7-DHC to 25(OH)D.(13, 14)

It follows that lower serum 25(OH)D levels have been observed among individuals with darker skin color and among individuals living at higher latitudes, and that these effects are cumulative.(12, 15-17) Recent evidence suggest that optimal circulating 25(OH)D levels are values greater than or equal to 75 nM.(3, 18-20) In a population of Boston women aged 20-40 years, the mean value of plasma 25(OH)D ranged from 30.3 – 41.6 nM in African American women compared to 60.0 – 85.4 nM in Caucasian women.(21) In a similar study of reproductively-aged women evaluated in NHANES, African American women had a mean vitamin D value of 44.2 nM, with 42.4% experiencing levels below 37.5 nM, a previously suspected threshold for deficiency. In contrast, the mean vitamin D in Caucasian women was 82.5 nM with only 4.2% of women having levels below 37.5 nM.(22)

Other factors affecting circulating serum 25(OH)D levels may include older age, cigarette smoking, high body fat mass, and low dietary consumption.(8, 23) Decreases in gut motility, renal function and skin thickness with older age, the introduction of toxins which alter hepatic and renal function with smoking, and the affinity of 25(OH)D for sequestration in adipose tissue may explain these relationships.(4, 24-27) Among African Americans, a higher prevalence of smoking and obesity, combined with the higher melanin content of darker skin may lead to lower serum 25(OH)D.

It is notable, however, that while skin color is on average darker for African Americans than Caucasians, there is overlap in skin color between the two groups. Because of the correlation between skin color and vitamin D production in the skin, classifying people by race results in a distinction that while culturally appropriate, fails to account for differences in skin color that do not differ by race and may be biologically important. Examining skin melanin content, race, and other contributory factors will allow for a greater understanding of contributing factors to circulating 25(OH)D levels.

This report extends the current research on skin color and 25(OH)D using repeated serum 25(OH)D measures from the Michigan site of the Study of Women's Health Across the Nation. The report addresses two major questions 1) Does the 14-year longitudinal trajectory of serum 25(OH)D differ by a skin reflectance, a proxy measure of melanin content; and 2) Is there is a skin reflectance threshold, independent of self-reported race, that is predictive of optimal serum 25(OH)D defined as a value $\geq$75 nM?

Study Population

Data are from the Study of Women's Health Across the Nation (SWAN) Michigan site. SWAN, begun in 1996, is an ongoing multi-ethnic, multi-site longitudinal community-based study of health and the menopause transition in middle-aged women. The two-stage design used to generate the study population at the Michigan SWAN site has been described.(28) In the first stage, study personnel contacted adults in 24,283 residences located in 40 Census tracts of Ypsilanti and Inkster, MI to identify those households that included women aged 40-55 years. In those households with age-eligible women, health interviews were conducted (n=2621 women) to generate a list of women eligible for participation in the longitudinal study and to provide basic health information. Women were eligible for the longitudinal study (the second stage) if they were aged 42–

52 years, had an intact uterus and at least one ovary, were not using reproductive hormones, had a menstrual period in the 3 months prior to interview and self-identified as either African American or Caucasian. The Michigan SWAN site recruited 543 women, and by design, in a 60:40 ratio of African American to Caucasian women.

This report is based on assays for serum 25(OH)D data in specimens acquired during annual examinations in years 1-3, 5-10, and 14 and a single time quantitative measurement of skin color in year 14 (2010) at the Michigan SWAN site. Written informed consent was obtained annually from all participants, and approval for the study was obtained from the University of Michigan Institutional Review Board.

Study Measures

Skin Color Reflectance. Reflectance was measured using a narrow band PhotoVolt® 577-A digital reflectance meter. The instrument measures the amount of light reflected by Tristimulus filters in amber, blue, and green. During measurement, the light probe was positioned perpendicular to the skin without depressing the skin. Duplicate measures were taken for each color filter while women were seated and away from direct sunlight.(29) All reflectance measures are reported on the L* index scale using the Commission International d'Eclairage (CIE) L*a*b* system (1976). L* index measures the lightness-darkness axis and is highly correlated with the melanin index [R^2 = 0.93, p<0.001] in populations with a wide range of skin color values.(30)

Skin reflectance is measured on a 0-100 scale with 0 indicating light absorbance and no reflectance (darkness) while 100 indicating no light absorbance and complete reflectance (lightness). Natural skin reflectance, representing genetically inherited skin color, was measured on the inner upper arm, a non-UV exposed site. Facultative skin reflectance, a measure of natural skin color modified by UV exposure, was measured at the glabella, the area between the eyebrows at the center of the forehead.(15) The difference between natural skin reflectance and facultative skin reflectance is an approximation of sun exposure, reported as UV Tan reflectance. Larger UV Tan reflectance values indicate greater sun exposure and a lower reflectance measures for the sun-exposed forehead.

Skin reflectance was only measured in 2010. Because of this, natural skin reflectance, henceforth referenced as "skin reflectance", is treated as an invariant measure

and included as a part of longitudinal repeated-measures analyses for 1996-2010. In contrast, UV-Tan reflectance could vary with time and is only included in analyses for data collected concurrently in 2010.

Serum 25(OH)D. Blood was drawn annually and then separated within an hour of collection using a refrigerated centrifuge. Serum had minimal exposure to light and was stored at -80°C awaiting laboratory analyses. Serum 25(OH)D was measured singularly with an automated chemiluminescence immunoassay (Heartland Assays, Ames, IA). The assay has a 7.5 nM limit of detection, an 8% intra-assay coefficient of variation (CV), and an 11% inter-assay CV. Optimal vitamin D is defined as serum 25(OH)D values $\geq$ 75 nM.(3, 18-20)

In 2010, 405 (75%) women were available for contact and data collection and reflectance was measured in 365 (90%) of these participants. Measurements of the initially recruited cohort may have been unavailable because women were deceased (n=28), lost-to-follow-up (n=58), refused participation (n=32), were unable to complete the examination (n=38) or participated via telephone in lieu of a clinical visit (n=22). Cohort retention was similar by race. African American women still comprised 60% of the active participants in 2010, consistent with the 1996 baseline.

Serum 25(OH)D was measured at least once during the 14-year follow up period in 503 (93%) of the participants. Among women with at least one serum 25(OH)D measure, 211 (42%) had 9-10 annual 25(OH)D measures, 124 (25%) had 7-8 annual 25(OH)D measures, 73 (14%) had 5-6 annual 25(OH)D measures, and 95 (19%) had 1-4 annual serum 25(OH)D measures.

Covariate Measurement

Height and weight were measured at each examination and used to calculate Body Mass Index (BMI). BMI values of 30 kg/m^2 or greater are classified as obese. Body fat percentage was assessed with bioelectrical impedance in which the greater speed of electrical current conduction in muscle tissue and fluids is compared to the slower conduction in fat tissue.

An annual interview question indicating the presence or absence of difficulty in paying for necessities in the preceding 12 months (food, shelter and health care) was used as a time-varying indicator of financial hardship A dichotomous measure of current tobacco use (yes/no) was self –reported at each annual visit and treated as a time-varying covariate.

The use of vitamin D dietary supplements or multivitamin supplements containing vitamin D was assessed annually and treated as a time varying covariate, while the amount of dietary vitamin D intake was assessed at three examinations (baseline and follow-ups 5 and 9) using the National Cancer Institute food frequency questionnaire and provisional vitamin D data from the United States Department of Agriculture. Dietary information was combined with vitamin D values from supplements. A proxy variable for season of data collection was based on the average monthly UV index for southeast Michigan. Data collected between April 1 and September 30 was assigned to a high UV category based on monthly UV index average values of five or higher, whereas measures taken between October 1 and March 31 were grouped into a low UV category because the UV index average values were four or lower, with most months averaging zero. In data analysis, this measure was treated as a time-varying covariate with low UV as the referent group.

To estimate sun exposure in 2010, participants were asked to report the average number of hours spent in the sun during warm weather, the parts of the body typically exposed to sunlight during warm weather, and if they used sunscreen with a sun protective factor (SPF) of 15 or greater.

To account for the potential impact of cohort retention on study findings, study participation variable was created to compare deceased women or enrollees with 0-2 visits in the past five years to the active cohort of participants with 3-5 visits during the same time period. This dichotomous variable was included in longitudinal modeling with repeated measures.

Statistical Analysis

Analyses began with univariate and frequency computations for continuous and categorical variables, respectively; univariate statistics are reported with standard deviations (SD). Serum 25(OH)D was examined for normality to satisfy modeling

assumptions and log transformed to address the skewed distribution. Log transformed parameter estimates were back transformed in discussion to ease interpretation. An examination of correlation betweeen skin reflectance and UV Tan reflectance was performed; absent evidence of collinearity, both variables were included in cross-sectional models. Measures of association are reported with p-values or 95% confidence intervals (CI).

Cross Sectional Analytic Approach

Logistic regression modeling was used to evaluate the association between skin reflectance and optimal serum 25(OH)D, dichotomized at 75 nM in 2010. Covariates, including UV Tan reflectance, age, UV season, sunscreen use, body fat percentage and vitamin D supplement use, were included in the model. Receiver operator characteristic (ROC) curves were used to assess the adequacy of the skin reflectance in predicting optimal serum 25(OH)D by plotting sensitivity against one minus specificity. The summary statistic for the ROC curve is the area under the ROC curve (AUC) which was used to determine if measuring skin reflectance had a higher probability of identifying optimal serum 25(OH)D than would have occurred by chance (0.5); a perfect prediction would yield and AUC of 1.0.(31) The nonparametric approach was used to compare the AUC for ROC curves with different covariates.(32)

Longitudinal Repeated Measures Analytic Approach

Longitudinal linear mixed effects modeling was used to quantify the association between skin reflectance, assumed to be invariant over time, and the 14-year trajectory of 25(OH)D. Models included a linear time variable, representing the linear trajectory over the study period. The quadratic effect of time ($time^2$) was examined, but omitted from final models due to the absence of statistical association. For linear models, the independent variable coefficients are interpreted as the difference in baseline 25(OH)D according to the variable levels and the coefficients for interaction terms with time estimate the association between the variables and rate of 25(OH)D change. To account for correlated errors in closely spaced measures, we used the first-order autoregressive covariance structure.

Repeated measures logistic regression modeling was used to examine changes in the likelihood of optimal serum 25(OH)D(a categorical variable) over the 14 year follow up in relation to the skin reflectance as a continuous variable. The exchangeable covariance structure was used to account for correlated errors in the logistic regression model. For the logistic regression models, parameter estimates can be interpreted as the association between the variable and serum 25(OH)D at any time over the 14-year study.

Covariates (i.e. including skin reflectance, baseline age, UV season, body fat percentage and vitamin D supplement use) having demonstrated longitudinal associations with serum 25(OH)D were included in the final models. All statistical tests performed were two-sided and significance was defined at $\alpha=0.05$. Data management and analyses were completed using SAS v9.2.

Population Characteristics

The average ($\pm$ SD) age of cohort participants was 59 ($\pm$ 2.8) years. The mean skin reflectance was 57.0 ($\pm$ 10.3) on the inner upper arm. Facultative reflectance on the forehead was slightly darker 52.9 ($\pm$ 10.9). At the 1996 baseline, the mean serum 25(OH)D level ($\pm$ SD) was 28.4 ($\pm$ 18.4) nM and by 2010 the population mean had risen to 43.4 ($\pm$ 32.8) nM (p<0.0001). At baseline, 6% of the population had optimal vitamin D levels, defined as $\geq$ 75 nM, but by 2010 the percentage had risen to 26%.

In 2010, while half of women reported taking a vitamin D supplement, only 3/4 of the users reported the supplement dosage, which averaged 1291 ($\pm$ 1428) IU per day. Approximately 56% of the cohort conducted their annual visit during the high UV season and 36% reported regular use of sunscreen with an SPF rating of 15 or higher.

Current tobacco use was reported among 20% of study participants and 49% reported financial hardship. The mean BMI was 34.1 ($\pm$ 8.6) kg/m^2 and 63% of the population were classified as obese. The mean body fat percentage for this population was 44%. (Table 3.1)

There was overlap in skin reflectance measures for African Americans (range: 16.5-77.9) and Caucasians (range 58.0-75.9). (Figure 3.1). However, the mean ($\pm$ SD) skin reflectance for African Americans was lower [51.2 $\pm$ 8.9], whereas for Caucasians it was 65.6 ($\pm$ 6.7) (p<0.0001). African American women also had lower mean serum 25(OH)D values (38.3 $\pm$ 24.8 nM) than Caucasians (53.6 $\pm$ 37.4 nM), (p<0.0001).

African American women reported less use of vitamin D supplements (45% versus 63% p=0.0008) and sunscreen (28% versus 48% p=0.0002) than Caucasian women. There was no difference in BMI, body fat percentage, smoking status, or financial strain by self-reported race category.

Cross-Sectional Associations

In 2010, the odds of optimal serum 25(OH)D (75 nM) were 5% higher for every 1-unit higher skin reflectance value[1.05, 95% CI 1.02-1.08]. After adjustment for UV Tan reflectance, age, body fat percentage, vitamin D supplement use and sunscreen use, the odds of optimal 25(OH)D were 6% higher for every unit higher skin reflectance. (Table 3.2). UV Tan reflectance, age and UV season were not associated with 25(OH)D status. Vitamin D supplementation, lower body fat percentage, and reported use of sunscreen, were consonant with optimal 25(OH)D levels.

Figure 3.2 shows the ROC curve and AUC statistics for 1) skin reflectance, 2) vitamin D supplementation, 3) skin reflectance and vitamin D supplementation, and 4) the full model including skin reflectance, vitamin D supplementation, UV Tan reflectance, age, high UV season, body fat percentage, and sunscreen use. All models demonstrated a greater predictive probability than chance, demonstrated by the AUC statistic and confidence intervals that excluded 0.5. The two variables with the greatest independent predictive probability were skin reflectance [0.66, 95% CI 0.58-0.73] and vitamin D supplementation [0.75, 95%CI 0.70-0.80]. Together these variables comprised the majority of the predictive probability of the model [0.81, 95%CI 0.75-0.86]. The predictive probability for optimal 25(OH)D was greatest for the full model [0.84, 95% CI 0.79-0.89].

Adjusted for other covariates, the skin reflectance threshold that optimized sensitivity and specificity for the optimal vitamin D level was 55. At the skin reflectance threshold of 55, the sensitivity was 73% and specificity was 82%. The adjusted odds of having optimal serum 25(OH)D were 4 times higher among women with skin reflectance ≥ 55 [4.0, 95% CI 2.0-8.2] than women with skin reflectance measures below 55. Among the African American women, 31% had skin reflectance measures ≥ 55, whereas all of the Caucasian women had skin reflectance values ≥ 55. (Figure 3.1). Caucasian women

were 3 times as likely to have optimal 25(OH)D [2.8, 95% CI 1.6-5.2]. The odds ratio for the skin reflectance threshold at 55 and optimal 25(OH)D is higher than that for self-reported race and optimal 25(OH)D, however, the confidence intervals overlapped.

Longitudinal Repeated Measures Associations

Skin reflectance was associated with baseline serum 25(OH)D; for every one-unit higher skin reflectance, baseline serum 25(OH)D was 2% higher (p<0.0001). A one-unit lower skin reflectance was associated with a 0.1% greater annual increase in serum 25(OH)D (p=0.001).

Vitamin D intake was associated with 9% higher serum 25(OH)D at baseline and with a 2% greater increase in 25(OH)D over time. (Table 3.3). High UV season and lower body fat percentage were consistent with higher baseline serum 25(OH)D but were not consistent with the change in serum 25(OH)D over time. There was no baseline effect of age on serum 25(OH)D, but increasing age was related to increases in serum 25(OH)D.

In longitudinally examining natural skin reflectance in relationship to the repeated measures of the clinical threshold for optimal vitamin D (75 nM) during the 14 year study follow up, we find that the 14-year odds of optimal 25(OH)D increases by 13% for every unit increase in skin reflectance [OR 1.13, 95%CI 1.08-1.18]. (Table 3.4). Among women taking vitamin D supplements, the odds of optimal 25(OH)D were 19 times higher than those not reporting vitamin D supplementation [19.2, 95%CI 6.83-54.1]. Additionally, lower body fat and high UV season were related to optimal 25(OH)D status. Baseline age was not significantly associated with the 14-year odds of optimal 25(OH)D.

Discussion

Uniquely, this study examined the relationship between skin reflectance and longitudinal serum 25(OH)D. Higher skin reflectance was related to higher 25(OH)D values and higher overall odds of having optimal vitamin D levels. Over the 14-year study period, lower skin reflectance, however, is associated with a greater annual increase in 25(OH)D. This study also quantified the importance of skin reflectance by identifying the skin reflectance threshold for the current optimal serum 25(OH)D level. While this

threshold level in skin reflectance was somewhat consistent with self-reported racial category, the 31% discordance between skin reflectance and racial category suggested that 25(OH)D status is an important corollary of skin melanin content irrespective of self-identified racial category. Lastly, while the importance of skin reflectance was quantified, we also observed the importance of vitamin D supplementation on 25(OH)D status and the contribution of supplementation to the change in 25(OH)D levels over time.

Several previous studies have addressed the relationship between skin color and serum 25(OH)D using cross-sectional data. A Canadian study found that natural skin color (measured at the inner upper arm) and vitamin D intake were associated with serum 25(OHD).(15) A prospective trial of UVB irradiance also found a positive cross-sectional correlation between natural skin color value and 25(OH)D levels at baseline.(17) While most cross-sectional studies have confirmed that darker skin color (measured with reflectance or melanin index) is associated with lower serum 25(OH)D, the importance of skin color to this relationship has recently been challenged. A recent study's findings suggest that individuals with low baseline serum 25(OH)D and high baseline cholesterol have similar increases in serum 25(OH)D with the same UVB dosage irrespective of natural skin color.(33) These findings were based on a one week observation and the null finding for natural skin color and serum 25(OH)D may be due to short follow up time.

The longitudinal finding that higher skin reflectance was associated with higher 25(OH)D values and higher odds of optimal 25(OH)D over time extends the cross-sectional findings and identifies the long term effects of higher skin reflectance on optimal 25(OH)D levels. Notably, the change in serum 25(OH)D over the 14-year period was greater for women with lower skin reflectance. This may be driven by the increase in supplementation observed over the course of the study from 26% reporting supplementation at baseline to 52% in 2009-2010, a process which bypasses the rate-limiting UV dependent pathway;(9) alternatively, the greater increase among women with lower reflectance may support speculation that low baseline levels produce greater increases in 25(OH)D with UVB exposure.(33)

The geographic location of this research strongly influences its importance in examining the influence of skin reflectance on serum 25(OH)D as women age. The

Michigan SWAN site population resides at 42°N indicating a minimal SZA near 20° during summer months and a maximum SZA near 70° during winter months, sufficient to preclude UVB exposure for the duration of the extended winter.(11, 15) In this population, the absence of statistical associations with UV Tan reflectance (an approximation of sun exposure) may reflect the overall low UVB exposure and limited sun exposure observed among women at mid-life in our geographic region. Only 30% of women reported more than 2 hours of daily sun exposure and more than 90% reported covering most of their body surfaces during sun exposure. Furthermore, because UV Tan reflectance was only measured once during the study period, we cannot account for seasonal variation in exposed skin color or its change over time.

It is noteworthy that the single factor with the greatest predictive ability for optimal vitamin D was vitamin D supplementation. Research had suggested that the Food and Nutrition Board vitamin D recommendation was too low to show measurable differences in vitamin D, especially in relation to optimal circulating vitamin D levels.(9, 23) Although the majority of the population had suboptimal 25(OH)D throughout the study and supplement dosage was not available for all study visits, the significant difference in serum 25(OH)D associated with the self-report of supplementation gives credence to the viability of supplementation (even at low doses) in increasing serum 25(OH)D and helping to achieve optimal levels. Our findings suggest that in a population where dermal sun exposure is low or negligible, voluntary use of vitamin D supplementation is the most important predictor of optimal serum 25(OH)D for women of all skin reflectance values.

Compared to women with visits during the low UV season, high UV season was associated with higher serum 25(OH)D in the longitudinal analyses. Because SPF ratings of 15 or higher block 99% of the cutaneous absorption of UVA and UVB, we expected to see an inverse relationship between reported sunscreen use and serum 25(OH)D. (6) Unexpectedly, reported sunscreen use corresponded to higher serum 25(OH)D in our study population. However, the reported use of sunscreen was primarily observed among individuals reporting ample sun exposure. Though sunscreens provide protection from UVA and UVB, self-reported sunscreen and its SPF levels is not adequate to identify if UVB absorption is occluded and dermal production of 25(OH)D curtailed.

Using self-identified race as a proxy for skin color is only partly justified by our study data. Women self-classifying as African American comprised the entirety of those with darkest skin color values and Caucasians were disproportionately represented among those with the lightest skin color values. Though the odds ratio for optimal 25(OH)D and the skin color threshold was higher than the odds of optimal 25(OH)D and race, the 95% confidence intervals for the two estimates overlapped. Skin color (reflectance) and race were correlated, however, using race without consideration for skin color incorrectly classified 31% of the population as high risk for suboptimal serum 25(OH)D. The appropriate assessment of risk has important consequences for members of racial and ethnic groups with wider ranges of skin reflectance values. Assuming homogeneity of risk within racial categories fails to deconstruct the components of race that correspond with outcomes of interest. In the case of 25(OH)D, culture may influence diet and sun exposure habits, but should be considered in conjunction with biological skin color in determining the likelihood of optimal vitamin D status.

This study has strengths and limitations. Although cohort studies are vulnerable to the bias generated by loss-to-follow up, this study was largely successful in minimizing the potential bias with high retention among both African American and Caucasian women, retaining the 60:40 recruitment ratio. Because of the concern that lost participants may not be missing completely at random, we used data prior to censoring to account for the impact of differential loss on study findings and observed no difference in effect in our longitudinal models. Some variables of interest were not assessed annually. Of particular interest were measures of UV Tan reflectance and sun exposure, which were only assessed at year 14 in 2010. Because recent literature has questioned the role of skin color and sun exposure in the low serum 25(OH)D consistently observed in darker skinned populations, the absence of these measures precludes their consideration in our analyses. Though we obtained proxy measures for solar UVB exposure and amount of skin surface area exposed to sun, without a measure of UVB exposure, important questions remain about the rate of 25(OH)D production in darker populations and the overall importance of skin color to the overwhelming burden of low vitamin D throughout the world.

The study has important strengths. The 14-year study period provides unique data supporting the importance of skin reflectance to changes serum 25(OH)D over time and the likelihood of optimal 25(OH)D. Because this study includes women with a wide range of skin reflectance values, we were able to determine that race is a relevant proxy for skin color, but is subject to misclassification, resulting in overestimation of risk for suboptimal 25(OH)D in a racial group with a wide range of skin reflectance values. The location of this study helps establish the relevance of dietary and supplement vitamin D and the vitamin D from cutaneous production. In Michigan, where cutaneous 25(OH)D production is limited seasonally, the importance of skin color and supplementation must be considered concurrently. The population-based study sample increases confidence that the results can be applied to similarly-aged women in Michigan and locations with similar climates and sun exposure behaviors.

Overall, the study findings suggest that skin reflectance is an important contributor to circulating serum 25(OH)D in mid-life women over a 14-year period, with higher reflectance values increasing the likelihood of optimal levels. Notably, vitamin D supplementation emerged as an essential factor in achieving optimal 25(OH)D in this population of Michigan women. The results also suggest that race can be useful in identifying high-risk groups; however, optimally race classification be used in conjunction with the quantitative assessment of skin color. Future research should focus on elucidating factors associated with cutaneous 25(OH)D production including cholesterol levels, the nature and type of adiposity and skin color to determine their competing influence on circulating serum 25(OH)D and to develop appropriately tailored sun exposure and supplementation recommendations in public health messages.

Table 3.1. Michigan SWAN year 14 study population characteristics

Variable	N (%)	Mean ± SD
Skin Reflectance		57.0 ± 10.3
UV Tan Reflectance		4.2 ± 5.0
25(OH)D nM		43.4 ± 32.8
Vitamin D Intake (IU)		1291 ± 1478
% Vitamin D supplement use	189 (51.8%)	-
% Visits during High UV Season	221 (55.5%)	-
% Sunscreen use	127 (35.5%)	
Age (years)		59.0 ± 2.8
Percentage Body Fat (%)		44.0 ± 10.2
Body Mass Index (kg/m2)		34.1 ± 8.6
% African American	240 (60.3%)	-
% Current Smoker	74 (19.5%)	-
% Financial Hardship	181 (48.5%)	-

Skin reflectance approximates melanin content in the inner upper arm. UV Tan Reflectance, an estimate of sun exposure, is the difference between the reflectance measure in the inner upper arm and forehead. Values range from 0 (darkest) to 100 (lightest).

Table 3.2. Odds Ratio estimates for optimal serum 25(OH)D in year 14

Odds Ratio Estimates

Variable	Point Estimate	95% Wald Confidence Limits	
Skin Reflectance	**1.06**	**1.02**	**1.10**
UV Tan Reflectance	0.97	0.90	1.05
Age (years)	1.01	0.90	1.12
Percentage Body Fat (kg)	**0.95**	**0.91**	**0.98**
UV Season	0.75	0.40	1.41
Sunscreen Use	**1.94**	**1.04**	**3.60**
Vitamin D Supplement Use	**8.5**	**3.98**	**18.1**

Skin reflectance approximates melanin content in the inner upper arm. UV Tan Reflectance, an estimate of sun exposure, is the difference between the reflectance measure in the inner upper arm and forehead. Values range from 0 (darkest) to 100 (lightest).

"UV Season is a dichotomous categorical variable comparing High UV Season to the referent category Low UV Season.

Sunscreen Use and Vitamin D Supplement Use are dichotomous categorical variables which compare "yes" to the referent category "no"

Table 3.3. Longitudinal Linear Mixed Effects Parameter Estimates modeling the change in log transformed serum 25(OH)D during study years 1-14

	Population-Average Fixed Effects					
	Baseline Effect			Rate of 25(OH)D Change		
Independent Variables	β	SE	p	β	SE	p
Skin Reflectance	**0.02**	**0.003**	**<0.0001**	**-0.001**	**0.0002**	**<0.0001**
Baseline Age (years)	0.004	0.008	0.59	**0.002**	**0.0004**	**<0.0001**
Percentage Body Fat (kg)	**-0.008**	**0.002**	**0.001**	0.0003	0.0003	0.28
Vitamin D Supplement Use	**0.09**	**0.03**	**0.006**	**0.02**	**0.004**	**<0.0001**
UV Season	**0.18**	**0.04**	**<0.0001**	**-0.01**	**0.004**	**0.02**
Current Smoker	-0.10	0.06	0.09	-0.0004	0.006	0.95

Skin reflectance approximates melanin content in the inner upper arm.   Values range from 0 (darkest) to 100 (lightest). Skin reflectance was measured once during year 14 visit only.

UV season is a dichotomous categorical variable comparing High UV season with reference category Low UV season.

Table 3.4. Repeated measures logistic regression population-specific estimates modeling the longitudinal probability of optimal serum 25(OH)D during study years 1-14

Population-Average Odds Ratio Estimates		
Independent Variable	**Point Estimate**	**95% Wald Confidence Limit**
Skin Reflectance	**1.13**	**1.08** **1.18**
Baseline Age (years)	1.07	0.91 1.25
Percentage Body Fat (kg)	**0.89**	**0.85** **0.93**
Vitamin D Supplement Use	**19.2**	**6.83** **54.1**
UV Season	**3.51**	**1.41** **8.71**
Time (years)	**1.58**	**1.44** **1.74**

Skin reflectance approximates melanin content in the inner upper arm.   Values range from 0 (darkest) to 100 (lightest). Skin reflectance was measured once during year 14 visit only.

UV season is a dichotomous categorical variable comparing High UV season with reference category Low UV season.

Vitamin D Supplement Use is a dichotomous categorical variables which compares "yes" to the referent category "no"

Figure 3.1. Skin reflectance distribution by self-reported race
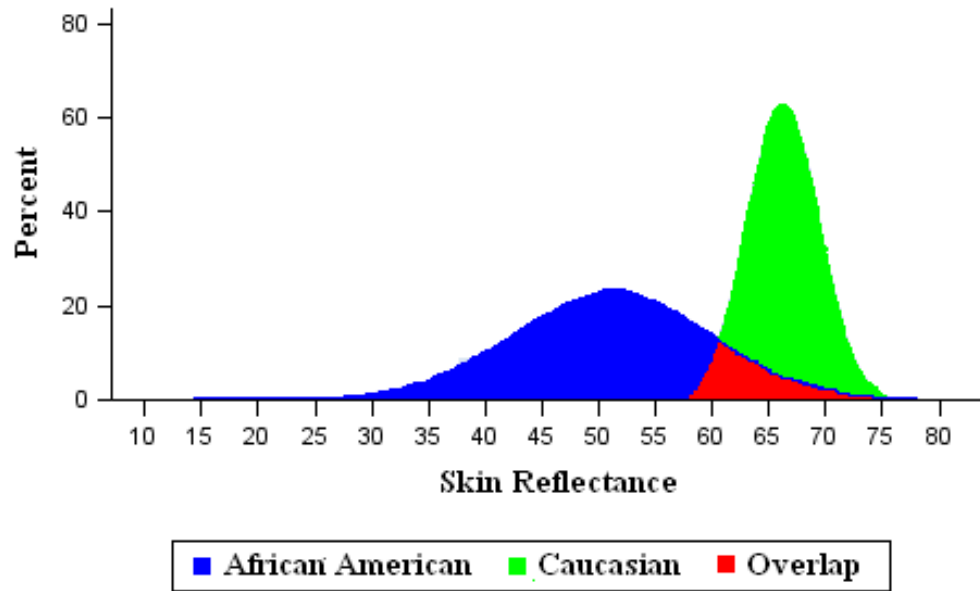
Figure 3.2. Receiver Operator Characteristic Curve Overlays for Optimal Serum 25(OH)D at year 14
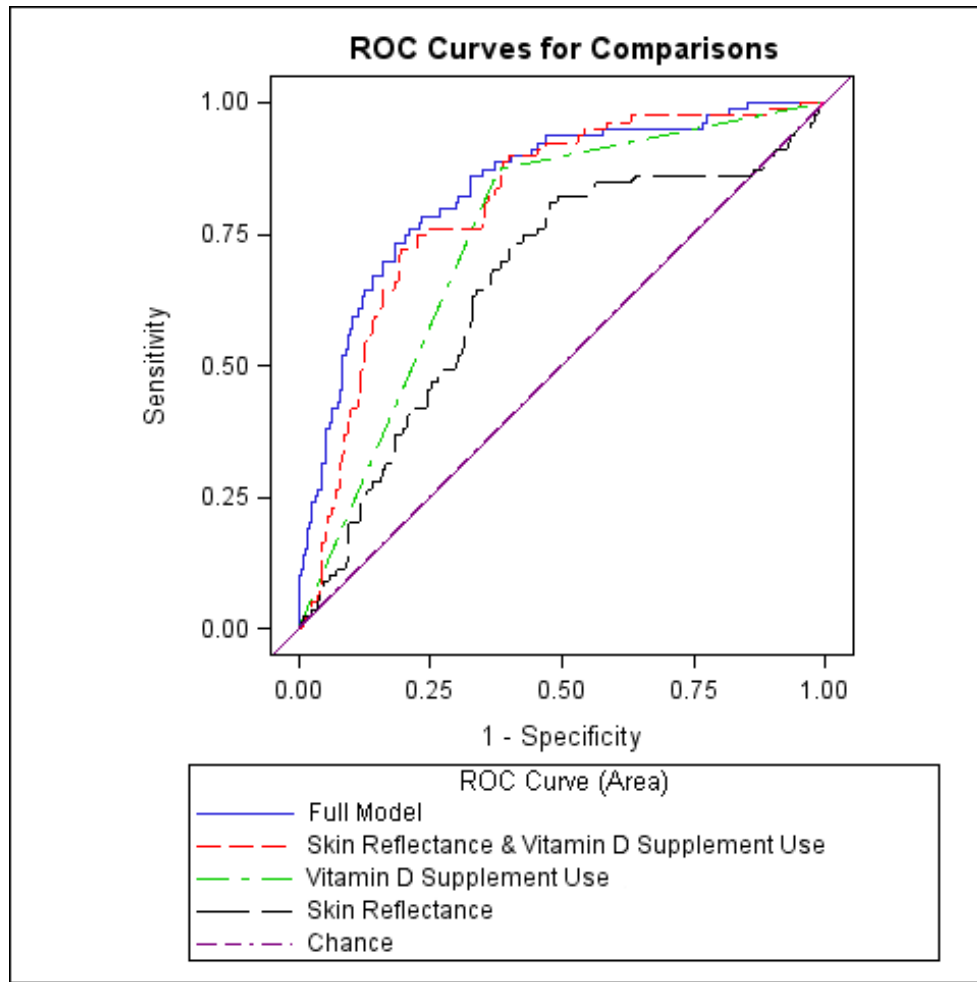
ROC Association Statistics

| ROC Model | Mann-Whitney | | | |
	AUC	SE	95% Wald Confidence Limits	
Full Model	0.84	0.03	0.79	0.89
Skin Reflectance & Vitamin D Supplement Use	0.81	0.03	0.75	0.86
Vitamin D Supplement Use	0.75	0.02	0.70	0.80
Skin Reflectance	0.66	0.04	0.58	0.73

Full Model includes skin reflectance, vitamin D supplement use, UV Tan reflectance, age, high UV season, body fat percentage, and sunscreen use.

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CHAPTER IV

Serum 25(OH)D mediates the relationship between Skin Color and Blood Pressure among Women in Michigan

Abstract

In an effort to understand risk factors for hypertension, serum 25(OH)D has been evaluated for potential influence in blood pressure regulation through the 25(OH)D receptors in the cardiovascular system and associated with control of renin expression, insulin resistance, and nervous system activation. It is hypothesized that the high burden of hypertensive disease among African Americans may be due in part to low serum 25(OH)D owed to darker skin color. Using data from mid-life women in Michigan, this study examined skin coloration in relation to systolic and diastolic blood pressure, considering whether these associations were explained or mediated in part by serum 25(OH)D levels.

Data were acquired from 405 women (60% African American and 40% Caucasian) aged 56-66 enrolled at the Michigan site of the Study of Women's Health Across the Nation (SWAN) in 2009-2010. Melanin content was approximated with measurement of skin reflectance, where values ranged from 0-100. Higher skin reflectance scores corresponded to lighter skin color and lower scores corresponded to darker skin color. Vitamin D was assayed as 25(OH)D in serum. Blood pressure was measured twice in the right arm using a mercury column sphygmomanometer and the two values averaged. Linear regression modeling was used to assess whether independent relationships exist between skin color, 25(OH)D and blood pressure, adjusting for covariates including age, financial hardship, smoking status, difficulty paying for necessities, anti-hypertensive medication use, sunscreen use, and vitamin D

supplementation. A bootstrap resampling method was used to estimate the indirect effect of skin color on blood pressure that was mediated by serum 25(OH)D.

Adjusted for confounders, significant independent relationships were observed between lower skin reflectance and lower serum 25(OH)D [0.01, p=0.002], lower serum 25(OH)D and higher systolic blood pressure [-0.04, p=0.004], and lower skin reflectance and higher systolic blood pressure [-0.003, p<0.0001]. Variation in skin reflectance accounted for less of the variation in systolic blood pressure after controlling for serum 25(OH)D. The bootstrapping-based mediation statistic [-0.0003, 95% CI -0.0010, -0.0001] indicated that 11% of the total effect of skin reflectance on systolic blood pressure is mediated through serum 25(OH)D. While skin reflectance was a significant correlate with diastolic blood pressure (DBP), there was no evidence of mediation by serum 25(OH)D with DBP.

The relationship between skin color and systolic blood pressure is mediated by serum 25(OH)D. Establishing the indirect effect of serum 25(OH)D on systolic blood pressure helps to target public health interventions aimed at ameliorating the risk of hypertensive disease and health disparities.

Introduction

Racial disparities in hypertension between African Americans and Caucasians have long been recognized and their elimination is a public health goal in the United States. (1) In the US population in 2009, the prevalence of hypertension was 43.5% in African American women whereas among Caucasian women (the population with the lowest US prevalence) the proportion was 28.5%. (2) Furthermore, in relation to Caucasians, African Americans have an earlier age at onset, greater severity, less success in achieving target control levels of hypertension with treatment, and higher rates of premature death from hypertension-related complications. (3) Age is also an important factor in hypertension, with the prevalence and disparities in hypertension being greatest in persons over the age of 55. In this age group, women comprise two-thirds of hypertension cases and the African American prevalence rate becomes two-fold higher than Caucasians. (4) In an effort to address the disparate burden of hypertensive disease, national programmatic goals and funding have emphasized the importance of research identifying contributory factors in hypertension.

Vitamin D is a factor of interest in blood pressure regulation. In cross-sectional and prospective epidemiologic studies, African Americans have lower serum 25(OH)D— a measure of the body's vitamin D stores—than Caucasians. (5, 6) Higher serum 25(OH)D may be central to the regulation of blood pressure through its control of renin expression, insulin resistance, and nervous system activation. (7-11) Skin coloration is an important factor in the production of 25(OH)D because the skin is a primary source of 25(OH)D. (12) In the skin, ultraviolet blue (UVB) exposure from sunlight converts subcutaneous 7-dehydrocholesterol (7-DHC) to 25(OH)D. Melanin, the pigment determining skin color, slows the absorption of UVB which decreases the amount of 7-DHC thereby reducing the production of 25(OH)D. (13, 14) It follows that compared to darker skin color, there is evidence linking lighter skin color and higher serum 25(OH)D. (15-18) The high burden of hypertensive disease among African Americans may be due in part to low serum 25(OH)D via darker skin color. (19-21)

Previous research has mainly focused on the relationship between skin color and serum 25(OH)D with very little examination of other health implications. Some studies examined skin color and blood pressure and found that darker skin color is related to higher blood pressure irrespective of self-reported racial classification. (22-29) These studies, however, have not examined the role of 25(OH)D as an important mediator in the skin color and blood pressure relationship. While it is necessary to elucidate the contribution of immutable risk factors such as skin color to hypertension, an important contribution is deconstructing immutable factors to find modifiable aspects that can promote healthy blood pressure regulation. Understanding the contribution of 25(OH)D to the relationship between skin color and blood pressure is a priority because circulating vitamin D levels are modifiable with dietary supplementation and sun exposure among women of all skin colors. Using data from mid-life women in Michigan, this study examined if skin color was associated with systolic and diastolic blood pressure, and if these associations were explained or mediated in part by serum 25(OH)D levels.

Study Population

Data are from the Study of Women's Health Across the Nation (SWAN) Michigan site. SWAN, begun in 1996, is an ongoing multi-ethnic, multi-site longitudinal community-based study of health and the menopause transition in middle-aged women.

The data for this study were generated at the Michigan SWAN site whose two-stage design used to generate the study population has been described. (30) Women were eligible for enrollment in the longitudinal cohort in 1996 if they were aged 42–52 years, had an intact uterus and at least one ovary, were not using reproductive hormones, had a menstrual period in the 3 months prior to interview and self-identified as either African American or Caucasian. The Michigan SWAN site recruited 543 women, and by design, in a 60:40 ratio of African American to Caucasian women.

This report is based on a quantitative measurement of skin color, assays for serum 25(OH)D, and blood pressure measurements acquired from annual examinations occurring between May 2009 and June 2010 at the Michigan SWAN site. In 2009-2010, 405 (75%) women enrolled at the 1996 baseline were available for contact and data collection. Measurements became unavailable because women were deceased (n=28), lost-to-follow-up (n=58), refused participation (n=32), were unable to complete the examination (n=38) or participated via telephone in lieu of a clinical visit (n=22). Cohort retention was similar by race. African American women still comprised 60% of the participants.

Written informed consent was obtained from all participants and approval for the study was obtained from the University of Michigan Institutional Review Board.

Study Measures

Skin Color Reflectance. Reflectance was measured using a narrow band PhotoVolt® 577-A digital reflectance meter. The instrument measures the amount of light reflected by Tristimulus filters in amber, blue, and green. During measurement, the light probe was positioned perpendicular to the skin without depressing the skin. Duplicate measures were taken for each color filter while women were seated and away from direct sunlight.(31) All reflectance measures are reported on the L* index scale using the Commission International d'Eclairage (CIE) L*a*b* system (1976). L* index measures the lightness-darkness axis and is highly correlated with the melanin index [R^2 = 0.93, p<0.001] in populations with a wide range of skin color values. (32)

Skin reflectance is measured on a 0-100 scale with a 0 score indicating total light absorbance with no reflectance (the darkest skin color) while a score of 100 indicates no light absorbance and complete reflectance (the lightest skin color). Natural skin

reflectance, representing genetically inherited skin color, was measured on the inner upper arm, without UV exposure. (15)

Serum 25(OH)D. Blood was drawn and then separated within an hour of collection using a refrigerated centrifuge. Serum was collected with minimal exposure to light and stored at -80°C awaiting laboratory analyses. Serum 25(OH)D was measured singularly with an automated chemiluminescence immunoassay (Heartland Assays, Ames, IA). The assay has a 7.5 nM limit of detection, an 8% intra-assay coefficient of variation (CV), and an 11% inter-assay CV. Optimal vitamin D is defined as serum 25(OH)D values ≥ 75 nM. (33-36)

Blood Pressure. Per protocol, blood pressure was measured twice in the right arm using a mercury column sphygmomanometer. Prior to measurement, the participant rested in a seated position for a minimum of five minutes with feet positioned on the floor and arm supported at heart level. The two measures were separated by a minimum of two minutes and the results averaged for reporting. Systolic blood pressure (SBP) was defined as the commencement of sound at Korotkoff phase 1; diastolic blood pressure (DBP) was defined as the cessation of sound at Korotkoff phase 5. The use of prescription anti-hypertensive medication was self-reported at the study visit and corroborated by interviewer observation of medication containers at examination.

Covariate Measurement

Height and weight were measured annually and used to calculate body mass index (BMI) as weight (kg)/height (m^2). BMI values of 30 kg/m^2 or greater are classified as representing obesity. Body fat percentage was assessed with bioelectrical impedance in which the greater transmission speed of electrical current in muscle tissue and fluids is compared to the slower conduction by fat tissue. Measures of fat mass have been demonstrated to be better correlates with serum 25(OH)D and blood pressure and will therefore be used in regression models; (37-39) the use of the BMI variable will be restricted to the description of the population.

The highest level of education completed was assessed at recruitment in 1996 and is reported as a dichotomous variable. Women reporting their highest level of completed education as college or post-graduate studies were grouped and compared to women

96

reporting less than high school, high school, or post-high school as their highest level of completed education. An interview question indicating the presence or absence of difficulty in paying for necessities in the preceding 12 months (food, shelter and health care) was used as an indicator of financial hardship. A dichotomous measure of current smoking was self –reported.

The use and dosage of vitamin D dietary supplements or multivitamin supplements containing vitamin D was assessed. Using the average monthly UV index for southeast Michigan, data collected between April 1 and September 30 was assigned to the high UV season based on monthly UV index average values of five or higher; measures taken between October 1 and March 31 were grouped into the low UV season because the UV index average values were four or lower, with most months averaging zero. To estimate sun exposure, participants were asked to report the average number of hours spent in the sun during warm weather, the parts of the body typically exposed to sunlight during warm weather, and if they used sunscreen with a sun protective factor (SPF) of 15 or greater.

Statistical Analyses

Data management and analyses were completed using SAS v9.2. Univariate and frequency computations were examined for continuous and categorical variables, respectively; univariate statistics are reported with means and standard deviations (SD) for continuous variables with counts and percentages for categorical variables. We examined variable distributions by race. Categorical data for the race categories were compared using the χ^2 test and continuous data were compared using the student's t-test. Blood pressure variables and serum 25(OH)D were examined for normality to satisfy linear regression model assumptions and were log transformed to address the right-skewed distribution. Log transformed parameter estimates were back transformed in the results and discussion to ease interpretation. When the response variable was log transformed, taking the anti-log of the estimate, subtracting 1 and multiplying by 100 yielded the percentage increase in the response variable associated with a one-unit increase in the explanatory variable. When both the response and explanatory variables were log transformed, the parameter estimate was interpreted as the percentage increase in the response variable associated with a 1% increase in the explanatory variable. (40)

All statistical tests performed were two-sided and significance was defined at α=0.05. Measures of association are reported with p-values or 95% confidence intervals (CI).

Linear regression was used to characterize the independent relationships between skin reflectance, serum 25(OH)D and blood pressure and to determine the degree of mediation by serum 25(OH)D in the relationship between skin reflectance and blood pressure. Mediation is a means of identifying an intermediate variable (25(OH)D) that is both caused by the explanatory variable (skin reflectance) and also affects the response variable (blood pressure) and helps explain how the skin reflectance affects blood pressure. The direct effect of skin reflectance is its immediate effect on blood pressure; the indirect effect of skin reflectance is the effect that operates through serum 25(OH)D; and the total effect is a combination of the indirect and direct effects. The Preacher and Hayes measure of indirect effects uses bootstrap resampling to calculate the quantity difference between the parameter estimates for the total effect and the direct effect in order to examine the underlying contribution of 25(OH)D in the association of skin reflectance and blood pressure. (41) Bootstrapping creates multiple subset samples of the data whose approximate sampling distributions are used to estimate the indirect effects. This approach to characterizing mediation avoids reliance on assumptions of normality in the distribution of the mediation statistic because it is based on an empirical estimation of the sampling distribution and thereby providing a more robust estimation of variance.

Figure 4.1 illustrates the Baron and Kenny relational criteria for evaluating mediation. (42) Serum 25(OH)D functioned as a mediator if, while controlling for confounders, the following criteria were met: 1) variation in skin reflectance values significantly accounted for variation in serum 25(OH)D (a path), 2) variation in serum 25(OH)D significantly accounted for variation in blood pressure (b path), 3) variation in skin reflectance significantly accounted for variation in blood pressure (c path), and 4) variation in skin reflectance accounted for less of the variation in blood pressure when controlling for serum 25(OH)D (the c path adjusted for the ab path, known as the c' path). The indirect effect of skin reflectance on blood pressure that was mediated through serum 25(OH)D was measured as the product of the a and b paths, which is equivalent to the difference between c and c' paths. This variable reflects a continuum,

with a difference of 0 demonstrating total mediation, that all of the effect of skin reflectance on blood pressure operates through 25(OH)D.

Selection of confounding variables for each model was based on biological significance; however, confounder selection for the models estimating the total (c path) and direct (c' path) effects of skin reflectance included all potential confounders. Potential confounders included age, body fat percentage, smoking status, financial hardship, anti-hypertensive medication use, sunscreen use, and vitamin D supplementation. To estimate variation explained by the model, the adjusted R^2 value is reported.

Results

Descriptive characteristics of the study population are shown in Table 4.1 according to self-reported race category. The average age of women was 59 years and body size measures were similar for African American and Caucasian women. The mean BMI was approximately 34 kg/m^2, the mean body fat percentage was approximately 44%, and 63% were classified as obese. Current tobacco use was reported by 23% of African American and 15% of Caucasian study participants. Completion of a college degree or higher was reported by 17% of African American women and 37% of Caucasian women. There was no statistically significant difference between African American and Caucasian women according to reported financial strain.

Blood pressure. Mean blood pressure measures differed by race category. SBP and DBP ($\pm$ SD) were higher among African American women [132 ($\pm$ 20) and 76 ($\pm$ 11) mm Hg] than Caucasian women [121 ($\pm$ 16) and 71 ($\pm$ 10) mm Hg]. Hypertensive treatment was also higher among African Americans (61%) than among Caucasians (42%).

Vitamin D. The mean serum 25(OH)D was 38.3 ($\pm$ 24.9) nM for African Americans and 53.6 ($\pm$ 37.5) nM for Caucasians; 18% of African Americans and 39% of Caucasians had optimal vitamin D levels, defined as a value of $\geq$ 75 nM. Though fewer African American women (45%) than Caucasian women (63%) reported taking a vitamin D supplement, only 3/4 of these users reported the supplement dosage, and this did not differ significantly between the race categories. Approximately 55% of women in both

race groups conducted their annual visit during the high UV season when 25(OH)D is produced from skin exposure to sunlight, and reported regular use of sunscreen with an SPF rating of 15 or higher was 28% among African Americans and 48% among Caucasians.

Lower mean skin reflectance was observed for African Americans (51.2 ± 8.9) as compared to Caucasians (66.2 ± 3.2) with the distribution of skin reflectance values by self-reported race category seen in Figure 4.2. Skin reflectance and race were correlated; women self-identifying as African American comprised the entirety of those with the darkest skin color values and Caucasians were disproportionately represented among those with the lightest skin color values. Despite this correlation, 31% of the African American population had skin reflectance values equal to or higher than the Caucasian women in the study.

Mediation relationships. Table 4.2 displays the results of the linear regression models evaluating the Baron and Kenny relational criteria for mediation. Differences in skin reflectance values significantly accounted for differences in serum 25(OH)D, demonstrating a positive relationship between the explanatory variable and the mediator (a path). For every one-unit higher reflectance value, serum 25(OH)D was 1% higher (p=0.002).

Likewise, as shown in Table 4.2, there was also evidence of a relationship between the mediator and the response variables (b path). Differences in serum 25(OH)D significantly accounted for differences in systolic blood pressure (SBP). For every 1% higher serum 25(OH)D, SBP was 0.04% lower (p=0.008). It follows that compared to the population mean serum 25(OH)D of 43.4 nM, a serum 25(OH)D value 100% higher (86.8 nM) was associated with 4% lower SBP. Notably, serum 25(OH)D was not significantly associated with diastolic blood pressure (DBP). There was also statistical evidence of a relationship between the explanatory variable and the response variable (c path). Differences in skin reflectance scores significantly accounted for differences in SBP and DBP. For every one-unit higher skin reflectance value, SBP was 0.3% lower (p<0.0001) and DBP was 0.2% lower (p=0.001). This corresponds to 17% lower SBP and 11% lower DBP when comparing the lightest skin reflectance value (73.9) to the darkest value (16.5).

Lastly, there was evidence that the relationship between the explanatory variable and one of the response variables decreased in the presence of the mediator (c' path). The association between skin reflectance and SBP was attenuated after controlling for serum 25(OH)D. For every one-unit higher skin reflectance value, SBP was 0.2% lower (p=0.008) when controlling for serum 25(OH)D. The parameter estimate for serum 25(OH)D was significant in this model, indicating that the relationship with systolic blood pressure persists in the presence of skin reflectance, indicated that 25(OH)D is acting as a mediator [-0.036, p=0.01]. The adjusted R^2 for this model indicates that the included variables account for 12.5% of the total variation in SBP (p<0.0001). The significant Preacher and Hayes bootstrapping mediation statistic -0.0003 (95% CI -0.0010, -0.0001) indicates that skin reflectance exhibits an indirect effect on SBP mediated through serum 25(OH)D; this effect is 11.1% of the total effect of serum 25(OH)D. The small magnitude of the bootstrap statistic considered with the significant parameter estimate of skin reflectance in the linear regression model suggests that that only part of the relationship between skin reflectance and systolic blood pressure is mediated through serum 25(OH)D.

Other statistically significant confounders included age, financial hardship, and use of hypertensive treatment. Being one year older was associated with 0.7% higher SBP (p=0.04), experiencing financial hardship was associated with 4% higher SBP (p=0.03), and use of hypertensive treatment was associated with a 6% higher SBP (p=0.002).

Though there was a relationship between skin reflectance and DBP, neither the bootstrapping statistic nor the comparison of the b path and c path regression models provides any evidence of mediation by serum 25(OH)D.

Discussion

This study examined the potential mediation of serum 25(OH)D between skin reflectance and systolic and diastolic blood pressure in a population of mid-life women in southeastern Michigan. Results demonstrated an association between skin reflectance and serum 25(OH)D and an association between serum 25(OH)D and systolic blood pressure after controlling for confounders. Serum 25(OH)D partially mediated the relationship between skin reflectance and systolic blood pressure, with 11% of the effect

of skin reflectance resulting from serum 25(OH)D. These outcomes support the conclusion that part of the relationship between skin color and systolic blood pressure acts through serum 25(OH)D. Though skin reflectance was associated with diastolic blood pressure, no relationship was observed between serum 25(OH)D and diastolic blood pressure, with and without adjustment for skin reflectance.

These study findings appear to be in concert with other findings that skin reflectance may act through two different mechanisms to affect blood pressure.(26) Skin color is an important part of one's cultural perception in many societies and plays a prominent role in social position and stress; additionally, differences in skin reflectance affect physiologic processes such as 25(OH)D production. Because of the overlap in skin reflectance values for African Americans and Caucasians observed in this study population and the importance of skin reflectance to systolic and diastolic blood pressure, our study findings suggest that there is value in examining skin reflectance independent of self-reported race when attempting to understand the mechanisms at play in racial and ethnic health disparities.

The Michigan location of this research strongly influences its importance in examining the relationship between skin reflectance, serum 25(OH)D and blood pressure in mid-life women. Hypertension is a contributing factor to cardiovascular morbidity and mortality, the burden of which is high in Michigan. According to the Centers for Disease Control and Prevention, between the years 2000-2006, the Michigan age-adjusted cardiovascular disease death rate per 100,000 women aged 35 years and older was 409 per 100,000 women, exceeding the national rate of 351 per 100,000 women. (43) The Michigan SWAN site population is comprised of African American and Caucasian women, and the stratum-specific death rates in Michigan for these groups (580 per 100,000 and 387 per 100,000) also exceeded the national rates of (479 per 100,000 and 341 per 100,000) respectively. In addition, this study population resides at 42°N, and during the extended winter, the tilt of the earth prevents the absorption of UVB wavelengths, resulting in no cutaneous production of 25(OH)D. (15, 44) This population also has limited sun exposure habits; only 30% of women reported more than 2 hours of daily sun exposure during warm weather in 2009-2010 and more than 90% of the women

reported covering most of their body surfaces during their sun exposure, precluding 25(OH)D production.

Our study findings extend preliminary findings from previous research in southeastern Michigan. The 1978 Detroit study of married blacks, aged 25-60 years, used a four point skin color rating system and reported that darker skin color was associated with higher blood pressure in men and women, though sampling effects in the lightest skin color category caused this relationship to be non-significant for women. (22) Skin color was not associated with blood pressure, possibly due to the minimal variation in skin color assessable by visual inspection among those with European ancestry. (45) The Detroit Study included a younger population, examined black and white populations separately, did not employ reflectance to measure skin color, and did not attempt to identify a mechanism for the observed association between skin color and blood pressure. However, this research is important because it was the first to highlight the potential importance of skin color to blood pressure among Michigan residents and also suggest the importance of physiologic components due to the interrelationship between pigment and hormone systems, which include 25(OH)D production.

Previous studies of skin color and blood pressure support our finding that skin reflectance is related to systolic and diastolic blood pressure. A Bolivian study of skin color and blood pressure found that skin reflectance and age were the most important predictors of variability in SBP and that body fat percentage had the least influence. (25) Investigators had hypothesized that the relative affluence of the darker skinned Bolivians compared to the lighter skinned Bolivians would not reflect the disparity in blood pressure observed between Blacks and Whites in the US. Although dark skin in the Bolivian population was not associated with negative social status, darker skinned persons had higher blood pressures and hypertension was six times as prevalent (7.9% versus 1.3%) than the lighter skinned Bolivians, implying that the effect of skin color on blood pressure may operate through socioeconomic position and unmeasured biological factors such as 25(OH)D.

Studies conducted in southeastern Puerto Rico, (26) reported no evidence of an association between darker skin reflectance and higher blood pressure. Instead, they found that darker "ascribed color", a cultural consensus describing skin coloration in

southeastern Puerto Rico, when moderated by socioeconomic position, was a strong predictor of higher blood pressure. The authors hypothesized that this interaction, resulting in greater importance of color as socioeconomic position increased, arises from more pernicious racism in middle and upper class contexts and results in darker individuals experiencing more frequent frustrating social interactions as their social position improves. While our measure of financial strain was a significant correlate with SBP, our findings differed in the importance of skin reflectance as a correlate with SBP, with skin reflectance emerging as an independently important correlate of SBP in the Michigan SWAN population, irrespective of measures of socioeconomic position. Notably, the Puerto Rican study population resides at 17°N in an equatorial clime with relatively ubiquitous UVB and ample sun exposure, which is in stark contrast to the environment in Michigan. The Puerto Rican population was also younger, aged 25-55 years, resulting in a lower prevalence of high blood pressure than the Michigan population aged 56-66.

In CARDIA, (27) higher skin reflectance was associated with lower systolic blood pressure. Lighter skinned African Americans experienced a decrease in SBP with increasing income, whereas darker-skinned African Americans experienced an increase in SBP with increasing income. For the SWAN study, financial strain was a significant parameter in the statistical models, however there was no statistical evidence of moderating effects of SEP on skin reflectance. We speculate that this difference reflects the similar economic strain in the African American and Caucasian women at the Michigan SWAN site.

A mediating effect of serum 25(OH)D on SBP was observed, which is important because elevated SBP drives the prevalence of hypertension. (46) It is postulated that lower serum 25(OH)D causes elevation of blood pressure through increases in renin expression, insulin resistance, and nervous system activation. (7-11) Establishing that 11% of the effect of skin reflectance on SBP operates through serum 25(OH)D allows us to target 25(OH)D as a small, but modifiable intermediate that can contribute to preventing unhealthy elevation of SBP and lowering the burden of hypertension.

There was no observed association between serum 25(OH)D and DBP, which is consistent with results of other studies that have examined this relationship. (19, 47)

Given the absence of a statistically significant direct relationship between 25(OH)D and DBP, it is not surprising that there was no evidence that serum 25(OH)D mediated the relationship between skin reflectance and DBP. Research suggests that SBP increases with age-related increases in peripheral resistance and/or arterial stiffness whereas these two factors contribute to DBP in opposite ways; thusly, similar age-related changes have differing consequences for SBP and DBP**.** (48) Thus, while serum 25(OH)D may not play a direct role in DBP, it may be correlated with peripheral resistance and arterial stiffness, explaining the observed association with SBP and the inconsistently observed association with DBP. Additional studies should examine and confirm these proposed mechanisms.

This study has numerous strengths and limitations. It is a population-based study of African American and Caucasian women at mid-life in Michigan. The high retention rate affords confidence that data from the study is representative of the base population from which it was drawn and the generalizability of results to similar populations.   This study includes quantitative measures of skin reflectance, serum 25(OH)D, blood pressure and important confounders allowing for the estimation of desired mediation effects. The cross-sectional nature of this data limits the estimation of temporal effects in observed associations and dose-response. Though this study only uses data from 2009-2010 only, measures from previous years allow us to more accurately identify and estimate measurement error. However, the study does not include diverse socioeconomic measures, perceptions of skin color and social status, or lifetime change in social position that have been hypothesized to be additional mediators in the relationship. (25-27, 49, 50) The low model R^2 (12.5%) suggest that missing or unmeasured parameters may have reduced the model fit. Notably, available measures of financial strain and education did not demonstrate significant effects (not shown).

Skin color has a biological and sociocultural impact on health. This study quantifies the effect of skin reflectance on systolic and diastolic blood pressure as well as the indirect effect that is mediated through serum 25(OH)D. The results suggest that interventions which include vitamin D supplementation or increased sun exposure may be useful in lowering the risk of elevated systolic blood pressure among all women, but should optimally be used in conjunction with skin reflectance to target women at greatest

risk for low serum 25(OH)D and high systolic blood pressure. Future research should focus on elucidating other factors associated with cutaneous skin reflectance and systolic and diastolic blood pressure to gain a greater understanding of potentially modifiable factors that are contributing to racial and ethnic health disparities.

Table 4.1. Michigan SWAN study population characteristics by self-reported race, year 2009-2010

Variable	African American		Caucasian		
	N(%)	Mean ± SD	N(%)	Mean ± SD	p-value
Age (years)	-	58.8 ± 2.9	-	59.3 ± 2.7	0.09
Percentage Body Fat (%)	-	44.3 ± 10.5	-	43.5 ± 9.7	0.44
Body Mass Index (kg/m2)	-	34.2 ± 8.5	-	33.9 ± 8.6	0.72
% Current Smoker	52 (22.6%)	-	22 (14.8%)	-	0.06
% Completing College	38 (16.5%)	-	57 (36.5%)	-	<0.0001
% Financial Hardship	118 (52.2%)	-	63 (42.9%)	-	0.08
Systolic Blood Pressure (mm Hg)	-	132 ± 20	-	121 ± 16	<0.0001
Diastolic Blood Pressure (mm Hg)	-	76 ± 11	-	71 ± 10	<0.0001
Hypertensive Treatment	141 (60.8%)	-	64 (42.4%)	-	0.0004
Serum 25(OH)D nM	-	38.3 ± 24.9	-	53.6 ± 37.5	<0.0001
% Optimal 25(OH)D (<75 nM)	39 (17.9)	-	54 (38.9)	-	<0.0001
% Vitamin D supplement use	101 (44.9%)	-	88 (62.9%)	-	0.0008
Vitamin D Intake (IU)	-	1190 ± 1422	-	1409 ± 1541	0.39
% Sunscreen use	61 (27.9%)		66 (47.5%)		0.0002
Skin Reflectance	-	51.2 ± 8.9	-	66.2 ± 3.2	<0.0001

Categorical data for the race categories were compared using the χ^2 test and continuous data were compared using the student's t-test.

Skin reflectance approximates melanin content in the inner upper arm. Values range from 0 (darkest) to 100 (lightest).

The "% completing college" includes women who reported completing college or higher levels of education.

Table 4.2. Associations between skin reflectance, serum 25(OH)D and blood pressure, controlling for confounders

		Response Variable					
		Serum 25(OH)D		Systolic Blood Pressure		Diastolic Blood Pressure	
Model	Explanatory Variable	β	p-value	β	p-value	β	p-value
a path	Skin Reflectance	0.01	0.002				
b path	Serum 25(OH)D			-0.035	0.004	-0.01	0.34
c path	Skin Reflectance			-0.003	<0.0001	-0.002	0.001
c' path	Skin Reflectance			-0.002	0.008	-0.002	0.03
	Serum 25(OH)D			-0.036	0.01	-0.005	0.69

Table 4.2 presents data from four separate models.

The a path model tests the relationship between skin reflectance and serum 25(OH)D while controlling for body fat percentage, sunscreen use, and vitamin D supplement use.

The b path model tests the relationship between 25(OH)D and blood pressure (systolic and diastolic) while controlling for hypertensive treatment, age, education, body fat percentage, financial hardship, UV season, and current smoking status.

The c path and c' path models tested the relationship between skin reflectance and blood pressure (systolic and diastolic) while excluding and including serum 25(OH)D. Both models controlled for hypertensive treatment, age, education, body fat percentage, sunscreen use, vitamin D supplement use, financial hardship, UV season, and current smoking status.

Serum 25(OH)D, Systolic Blood Pressure and Diastolic blood pressure were natural log transformed.

Skin reflectance approximates melanin content in the inner upper arm. Values range from 0 (darkest) to 100 (lightest).

Figure 4.1. Hypothesized relationships between skin reflectance, serum 25(OH)D, blood pressure and potential confounders
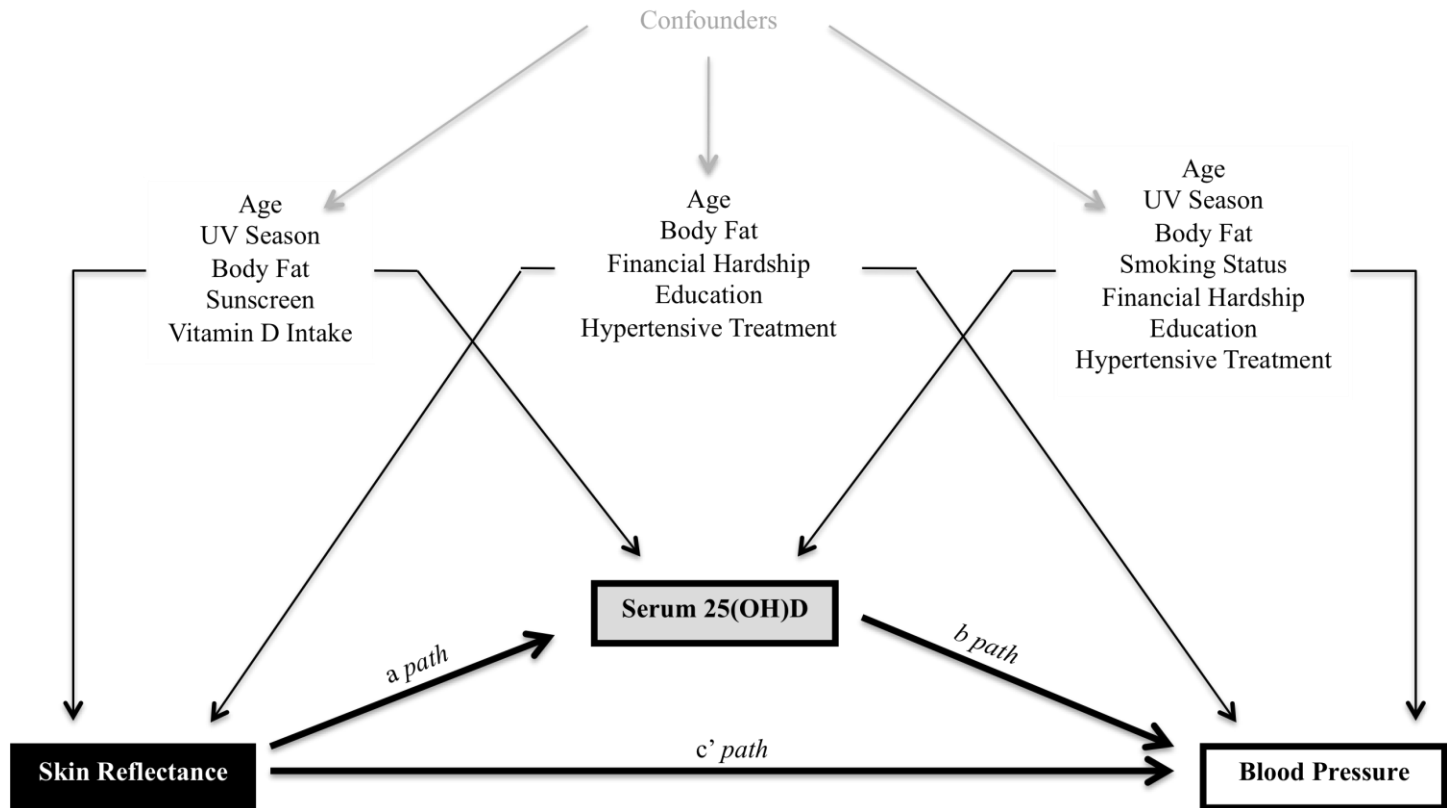
The c path provides the total effect of skin reflectance on blood pressure (systolic and diastolic) by estimating the association between skin reflectance and blood pressure while excluding serum 25(OH)D. The c' path provides the direct effect of skin reflectance on blood pressure by estimating the association between skin reflectance and blood pressure while including serum 25(OH)D. The difference between the total effect and the direct effect provides an estimate of the indirect effect of skin reflectance on blood pressure that operates through serum 25(OH)D.

Figure 4.2. Skin reflectance distribution by self-reported race
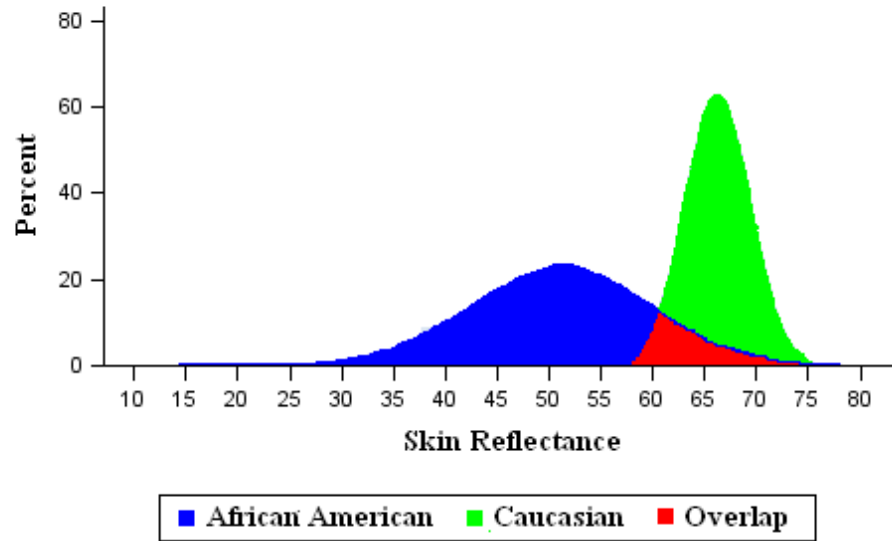
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CHAPTER V

Conclusions

Summary of Findings

This dissertation extends the research about health-related effects of serum 25(OH)D by examining its influence on blood pressure over time and quantifying its mediating effect on the relationship between skin color and blood pressure. This research, using data from a population-based sample of mid-life African American and Caucasian women in southeastern Michigan followed annually from 1996 to 2010, also permitted evaluation of the influence of skin color on serum 25(OHD levels over time in comparison to that of self-reported race.

Research directed toward understanding racial disparities in blood pressure between African Americans and Caucasians has examined genetic differences, environmental genetic polymorphisms (e.g. salt processing) and social dimensions including discrimination, neighborhood composition, and stress. (1-5) While findings from this prior research have contributed to an understanding of the multifactorial mechanisms for hypertension, this study examined those pathways related to skin color, an underlying factor determining self-reported race, that may physiologically operate through serum 25(OH)D.

Serum collection is invasive and assays of serum for 25(OH)D and measures of skin color with reflectance are expensive to collect. Therefore, most of the existing literature examining skin color, 25(OH)D and blood pressure is based on cross-sectional study designs and often employ self-reported race or a proxy measure for skin color, which are limited approximations for the capacity of skin and its coloration to contribution to circulating serum vitamin D levels (6-8) This study uniquely provides measures of skin reflectance, serum 25(OH)D and blood pressure, with the latter two measures encompassing a 14-year study period, to allow for greater examination of the

interrelationship between these variables. Investigating these relationships using longitudinal data allows for greater understanding of the long term changes in 25(OH)D levels and blood pressure, in addition to temporal changes in suboptimal 25(OH)D and hypertension risk with age. These findings help to inform the relevance of potential public health interventions using vitamin D to prevent the onset of conditions associated with high morbidity and premature mortality, including elevated blood pressure.

In Chapter II, we examined the longitudinal trajectory of 25(OH)D and blood pressure to determine if the ten-year trajectory of vitamin D differed in similarly aged African American and Caucasian women living with similar social and economic environments. Higher baseline serum 25(OH)D was associated with lower baseline systolic blood pressure among African American and Caucasian women; however, there were no relationships observed for baseline diastolic blood pressure. Annual change in serum 25(OH)D over the ten-year follow up period was not associated with systolic or diastolic blood pressure. Notably, while the baseline serum 25(OH)D values were lower in African American women compared to Caucasian women, the change in serum 25(OH)D was greater for African American women than for Caucasian women. These results suggest the importance of achieving recommended levels of serum 25(OH)D in early adulthood to modulate the likelihood of blood pressure increases with age and the need for targeted interventions to racial groups at differential risk for suboptimal 25(OH)D.

In Chapter III, we examined the relationship between skin reflectance and longitudinal serum 25(OH)D levels. Higher skin reflectance, observed with lighter skin coloration, was associated with higher 25(OH)D values at baseline. Higher skin reflectance, considered immutable, was associated with higher overall odds of having optimal vitamin D levels during the 14-year follow up. Skin reflectance was also related to the change in 25(OH)D levels over time. Over the study period, lower skin reflectance, associated with darker skin coloration, was associated with a greater annual increase in 25(OH)D. This was partly accounted for by the sharper increase in supplementation observed over the course of the study among women with lower reflectance.

We further quantified the contribution of degree of skin reflectance by identifying the skin reflectance threshold that best predicted optimal serum 25(OH)D levels. While

this threshold level in skin reflectance was somewhat consistent with the dichotomous self-reported racial category, the amount of discordance between skin reflectance and racial category (31%) suggested that skin melanin content (as approximated by skin reflectance measures) in relation to 25(OH)D status was an important element to health apart from self-identification of racial category. Importantly, we also observed the contribution of vitamin D supplementation on 25(OH)D status and the contribution of supplementation to the change in 25(OH)D levels over time. These results suggest that race can be useful in identifying high-risk groups; however, optimally race classification would be used in conjunction with the quantitative assessment of skin color to effectively identify at risk women.

In Chapter IV, we examined the potential mediating effect of serum 25(OH)D on the relationship between skin reflectance and systolic and diastolic blood pressure. Results from data analyses characterized the association between skin reflectance and serum 25(OH)D levels and an association between serum 25(OH)D levels and systolic blood pressure measures after controlling for confounders. Serum 25(OH)D levels partially mediated the relationship between skin reflectance and systolic blood pressure, with 11% of the effect of skin reflectance resulting from serum 25(OH)D. These findings support the conclusion that part of the relationship between skin color and systolic blood pressure acts through serum 25(OH)D. Though skin reflectance was associated with diastolic blood pressure, no relationship was observed between serum 25(OH)D and diastolic blood pressure, with and without adjustment for skin reflectance. The results suggest that serum 25(OH)D levels are an important modifiable component of the relationship between skin color and blood pressure.

We have extended the understanding of blood pressure in women at mid-life by contributing repeated measures in a biracial study population. The observed relationships between skin reflectance measures, serum 25(OH)D levels, and blood pressure were consistent in significance, magnitude and direction of the associations with those from earlier work that frequently failed to incorporate longitudinal measures and measures of skin coloration. (6, 8-22)

There are inconsistencies in this area of research, most frequently observed in the relationship between the compounds known as "vitamin D" and blood pressure. This

appears to be a reflection of differences in study measures, populations evaluated, and study design. (23-27)

In the following sections, I will address the strengths and limitations of this research. Following that, I discuss recommendations for future research direction in light of the study findings. Finally, I will conclude by discussing the application of these findings to clinical settings and public health interventions.

Strengths and Limitations

The objective measurement of skin color is expensive and cumbersome to acquire, thus studies frequently resort to applying proxy measures of race/ethnicity. Most 25(OH)D studies have used race/ethnicity as a proxy for skin color despite evidence that individuals can be of the same race/ethnicity and have markedly different skin colors. (28) This study directly measured skin color and evaluated this "finer" measure against the coarser estimate. Because skin color was measured as a continuous variable, the assessment of risk by skin color will not be restricted to racially defined boundaries. Further, this measure is objective and based on the underlying biology of cutaneous 25(OH)D synthesis and not culturally and socially defined classifications. Further, the data analysis included measures of social position and social stress (SEP) in addition to the biological skin coloration to allow for a more nuanced examination of factors frequently subsumed into the global measure of "race".

This study also examined skin color in a population with a range of skin colors. Many previous studies of the relationship between skin color and 25(OH)D had only a few participants in the darker skin color range; (8, 9, 29, 30) in contrast, many of the studies of skin color and blood pressure stratified by race or had fewer participants in the lighter skin color range. (6, 16-22) That this research could address a full range of skin reflectance measures is an important component of ensuring external validity to other similar populations. It may also contribute to the applicability for other ethnic groups with similar skin reflectance value ranges.

In much of the previous literature linking blood pressure to 25(OH)D, data collection occurs at one point in time. The cross-sectional NHANES study found an inverse association of 25(OH)D with blood pressure and this is important because this is the first report of this association in a large sample representative of the United States

population. (12) In a case-control study nested in the Nurses' Health Study, plasma 25(OH)D levels were inversely and independently associated with the risk of developing hypertension in young non-obese women. (13) An important contribution of this research to the existing literature is the analysis of population-based data acquired over ten years for women entering the menopausal transition at baseline. Thus, this study is unique in that it can address the longitudinal trajectory of these measures over time within an individual. This dataset provided the rare opportunity to investigate trends and determine the impact of changes in 25(OH)D levels on blood pressure measures to provide further evidence of any temporal association between study measures.

A primary strength of this study is conducting it in a population-based cohort of mid-age women. In so doing, this study examines the importance of 25(OH)D to blood pressure in a non-clinical population that is representative of similarly aged women in similar northern latitudes. The external validity of the study findings is supported by consistent findings from studies conducted in other populations. (13, 15, 25, 31) Whereas this study is a closed cohort, the retention and follow-up rates of the Michigan SWAN are particularly good for a study which is completing its fourteenth annual assessment with 75% follow-up overall and 81% follow-up among the still-living women.

Irrespective of the high retention rate, if there is differential participant loss is related to variables of interest related to poorer health, greater financial strain, skin coloration, or were disproportionately members of one race group, there would be increased concern that lost participants might not be missing completely at random and thereby violate assumptions for longitudinal modeling. Fortunately, there has not been a loss to follow-up related to race. We also used sensitivity analysis to address concerns about missing at random, by using data prior to censoring and after censoring to account for the impact of differential loss on study findings.

Although this is a longitudinal study with a number of annually acquired measures, not all variables of interest were assessed annually. Skin reflectance measurements (both natural and UV-tanned) were only measured at a single point in time. This is not a limitation for the use of natural skin color because it is a measure of biological skin color at a site unexposed to the sun which does not vary over time.

However, the use of UV- tanned skin color beyond cross-sectional analyses is limited. This restricts the information available about a measure of UV exposure over time, which is an important element of the relationship between skin color, 25(OH)D and blood pressure. Notably, there was surprising little difference in the measures of UV-tanned skin in these women at mid-life. Though we incorporated proxy measures for solar UVB exposure, including season of assessment, and amount of skin surface area exposed to sun (along with sunscreen use), we did not have a direct measure of UVB exposure.

Of additional interest were the measures of vitamin D dietary and supplement intake. These measures were particularly important in assessing the relationship between skin reflectance and 25(OH)D. While a strong association was observed, potentially, a more precise estimation of the contribution may have been possible with annually acquired measures. Because supplementation and dietary intake are modifiable factors, additional information on their efficacy in a population-based study is an important contribution to the scant and conflicting evidence on available on supplementation.

Additionally, the cross-sectional analysis of mediation is unable to account for the variable changes that occurred prior to study year 14 that contribute to the mediation of serum 25(OH)D in the relationship between skin reflectance and blood pressure.

Future Directions

Because this study population was recruited as one of seven sites for the Study of Women's Health Across the Nation (SWAN), an important continuation of this research would be to expand the population coverage and replicate these findings at the other sites. Among SWAN sites, study populations have similar age distributions, recruitment, follow-up period, study measures and frequency, collection and storage of specimens, staff training and study measures. However, important differences in geographic location, race, ethnicity, and socioeconomic status can be observed by conducting inter-site comparisons. Of particular interest are the geographic and ethnic differences. All seven SWAN sites include examination of Caucasian women; however, the Boston, Chicago and Pittsburgh sites also recruited African American participants, and the Los Angeles site enrolled Japanese and the Alameda and Contra Costa County sites enrolled Chinese women. The diversity in behavior, diet, and skin color that could be examined in this larger cohort is important to understanding the role of immutable and modifiable

factors related to hypertension. Because there would be overlap in skin reflectance values for all ethnic groups, it is particularly important to understand if the importance of skin color, irrespective of self-reported race, is maintained in a population with greater diversity. The geographic location also is important for understanding the relative contributions of skin color and supplementation to circulating 25(OH)D and its sequelae in different contexts. These locations range in latitudes from the south to north: Los Angeles 34°N, Alameda and Contra Costa 37°N , Pittsburgh 40°N, Chicago 41°N, Boston 42°N, and Michigan 42°N. The differences observed in average sun exposure, sunscreen use, and the fluctuation in monthly UV exposure would contribute to our understanding of the complex relationship between these factors.

Beyond extending the study to women of similar ages in other racial and ethnic groups, future studies should be powered to examine longitudinal trajectories of blood pressure (and other vascular outcomes) among younger adult women from various racial groups with a well-represented spectrum of serum 25(OH)D values at baseline. This would allow for a greater understanding of the contribution of 25(OH)D levels to the etiology of hypertensive disease throughout the lifespan. Confirming that higher 25(OH)D is associated with lower blood pressure and smaller increases in blood pressure over time will contribute to developing a consensus that 25(OH)D is important to the effort to maintain consistently normal blood pressure levels.

A study of UV irradiation suggests that individuals with low baseline serum 25(OH)D and high baseline cholesterol have similar increases in serum 25(OH)D with the same UVB dosage irrespective of natural skin color.(32) While these findings were based on a one week follow up period, our study findings demonstrated that darker women had lower 25(OH)D at baseline, but experienced a greater increase in 25(OH)D over the study period than their counterparts with higher skin reflectance (lighter) and higher baseline 25(OH)D levels. This observation appears to be partly driven by the sharper increase in supplementation observed over the course of the study among women with lower reflectance. However, the greater increase among women with lower reflectance may support the previous finding that low baseline levels produce greater increases in 25(OH)D with UVB exposure. Future research should focus on elucidating

factors associated with cutaneous 25(OH)D production including cholesterol levels and skin color to determine their competing influence on circulating serum 25(OH)D.

Most studies have not identified an association of serum vitamin D and diastolic blood pressure and this study did not provide evidence of an association between serum 25(OH)D and diastolic blood pressure. (15, 23) The literature suggests that systolic blood pressure increases with age-related increases in peripheral resistance and/or arterial stiffness whereas these two factors may have opposite contributions to DBP; thusly, similar age-related changes have differing consequences for systolic and diastolic blood pressure. (33) Interestingly, a significant association was observed between skin reflectance and diastolic blood pressure, despite the null finding for 25(OH)D. This suggests that the effect of 25(OH)D on vasculature may result in equal and opposite influences on diastolic blood pressure which negate observable differences. Future research should examine the relationship between serum 25(OH)D and peripheral resistance and arterial stiffness to expand and extend our understanding of the mechanisms underlying the serum 25(OH)D relationship with systolic blood pressure and to determine if competing effects explain many of the null findings for diastolic blood pressure.

Lastly, future studies should examine plausible etiologic pathways for the observed associations and consider implications for other conditions. Of particular import is the emerging role of obesity. With increasing rates of obesity in the United States, the role of inflammatory mediators such as IL-6 and IL-8 as well as metabolic regulators such as the adipocytokines, leptin and adiponectin, may play an increasingly important role in the vascular pathways for 25(OH)D production and those that link 25(OH)D to blood pressure. (34) The role of these factors and their relationship to vitamin D binding protein is an important continuation of this research. Additionally, given the association of 25(OH)D with insulin resistance, exploring the relationship between vitamin D binding protein and hemoglobin A1c as well diabetic sequelae such as vascular reactivity and peripheral neuropathy are important areas of future investigation.

Clinical Recommendations

More research is needed on the relationship between 25(OH)D and blood pressure, however, there is compelling evidence which suggests that higher serum

25(OH)D associated with beneficial health outcomes. This research demonstrated that the prevalence of suboptimal 25(OH)D is substantial and, as anticipated, darker women have a greater likelihood of having suboptimal 25(OH)D. Further, higher serum 25(OH)D was associated with lower systolic blood pressure. Given the potential importance of these findings in preventing or reducing cardiovascular disease morbidity and mortality, we pose the following recommendations with clinical and public health implications.

Increase rate of vitamin D supplementation. The primary finding of this dissertation research is that skin color is associated with 25(OH)D which is also associated with systolic blood pressure, and that part of the effect of skin color on blood pressure is mediated through 25(OH)D. This, combined with the finding that women who supplemented with vitamin D had higher serum 25(OH)D values, suggests the importance of vitamin D supplementation. While skin color is an immutable factor with important effects on health, 25(OH)D is a modifiable factor that can be increased to improve health outcomes. Increasing knowledge of the importance of vitamin D to health outcomes and the importance of maintaining consistently high 25(OH)D throughout the lifespan will help promote the practice of encouraging dietary and supplement intake as part of a healthy lifestyle.

In addition to its relevance for individuals with lower (darker) skin reflectance, this is also a public health message that can be a component of positive health practice among groups who practice substantial or complete coverage of the skin, thereby precluding the opportunity for dermal conversions of the naturally occurring 7-dehydroxycholesterol to vitamin D.

Increase supplementation dosage. Dietary sources of vitamin D are not ubiquitous, being limited to egg yolk, oils in fish, and selected organ meats such as liver. This suggests the need to consider vitamin D supplementation and its dose. Some milk, ready-to-eat cereals, and multivitamins are fortified to supplement dietary vitamin D intake and ensure adequate intake and have been demonstrated to increase serum 25(OH)D levels. (35-37) The 2011 Institute of Medicine (IOM) report increased the daily recommendations from 200-600 IU/d to a higher recommendation for all age groups; the current recommendation is 400 IU for infants up to 12 months, 600 IU for all

males over 12 months and females ages 1-70 years, and 800 IU/d for women aged 71 years or older. (38) These recommendations are substantially lower than the amount (approximately 10,000 IU) of vitamin D produced from short-term cutaneous exposure to sun, though intakes in this range have been demonstrated to increase circulating 25(OH)D by small amounts. (39) Importantly, however, research indicates that supplementation with vitamin D in amounts of 800, 1,000, 2,000 and 3,000 IU/d are too low to achieve optimal 25(OH)D status in most people. (40-42) Indeed, in a study of healthy postmenopausal black women supplemented with 800 IU/d for two years and 2000 IU/d for 1 year, 40% of the study population still had not achieved 75 nM at the end of the trial. (40) Our study demonstrated that women who reported vitamin D supplementation or had higher vitamin D intakes from diet and supplementation, had higher serum 25(OH)D and increasing values serum 25(OH)D over time. However, despite these higher values and increases over time, the majority of our study population remained in the suboptimal 25(OH)D category throughout the duration of the 10-year observation period.

The IOM report also challenged the 75 nM optimal 25(OH)D cutoff, citing that evidence for 50 nM was sufficient for bone health and that the relevance of 25(OH)D to other health outcomes is inconclusive. However, the report cited lack of data about optimal serum 25(OH)D levels and the risks and benefits of vitamin D intake in diverse ethnic groups, especially African Americans, who have higher bone density despite lower 25(OH)D levels. Despite this limitation, "Overall, the committee concludes that the majority of Americans and Canadians are receiving adequate amounts of both calcium and vitamin D." The findings of this dissertation research corroborate other US studies which offer conclusions in direct contrast with those of IOM. (43-48) Among African American Michigan SWAN participants, the mean serum 25(OH)D values at each annual visit were below the 50 nM threshold, and more than 45% of the Caucasian population failed to meet this lower threshold at each assessment over the 14 year study period. Although strong evidence suggests that 75 nM is the appropriate level of optimal 25(OH)D, even when using the lower 50 nM threshold, there is evidence that the majority of the US population is not receiving adequate vitamin D. (43, 45-48).

In an effort to achieve a scientific consensus to increase the recommended daily intake, it is important to publicly address the oft-referenced vitamin D toxicity. The IOM report suggests that The IOM states that the intake of vitamin D exceeding 4,000 IU/d, increases the risk of toxicity. (38) While the health community has repeatedly referenced vitamin D toxicity to justify low levels of vitamin D intake (38, 49), there is remarkably little evidence to corroborate a high risk of toxicity. Evidence to this effect is hinted in the limitations section of the IOM report which simultaneously supports the recommendation of low maximum daily intake dosages to ensure public health while conceding that there is limited data to support the recommendations. Evidence suggests that human toxicity probably arises after chronic daily consumption in excess of 40,000 IU/d. (50) This dosage would be the equivalent of increasing the 600 IU/d recommendation 67 fold, the dietary equivalent of consuming 400 8oz glasses of milk daily. A single documented case of pharmacologic vitamin D toxicity was reported in a man who had been unknowingly consuming 156,000–2,604,000 IU/d for two years due to a mislabeled package. (51) The combination of drastically lower 25(OH)D levels than the 75 nM recommendation in many populations, the observation that the current recommended daily intake has only a small influences on serum 25(OH)D, and the negligible risk of toxicity suggest the importance of promoting an increase in the dosage of vitamin D3 supplementation in all women to at least 3000-5000 IU/d. (Table 5.1) As surveillance for cardiovascular disease improves, there is an opportunity for monitoring the impact of this supplementation on cardiovascular health.

Consistent Brief Sun Exposure. Depending on skin color, humans make between 10,000 and 25,000 IU of vitamin D with a minimal erythemal dosage of UVB or exposure to the sun, usually occurring within 30-60 minutes. (52) To abate the risk of malignant melanoma which has been increasing annually, public health recommendations involve limiting sun exposure, increasing clothing coverage, and using sunscreen with SPF ratings of 15 or higher. These recommendations, however, substantially decrease the cutaneous production of 25(OH)D which are increasingly being demonstrated to be associated with many health outcomes apart from the traditional bone outcomes.

While light skinned individuals experience a malignant melanoma incidence rate of 50 per 100,000 per year, the incidence rate among dark skinned individuals is less than

1 per 100,000 per year. (53) Thus, the public health message of avoiding sun burn should be reiterated for all persons; however, avoidance of all sun exposure fails to balance the risk of malignant melanoma with consequences for other health conditions. Achieving an informed balance is particularly important for dark skinned persons with low risks of skin cancer and extremely high risks for sequelae of low 25(OH)D such as elevated blood pressure.

Our findings indicate that achieving optimal 25(OH)D is difficult in a population of mid-life women, though dietary supplementation and sun exposure help to increase circulating levels. These results suggest that sun exposure can be a healthy component of remediation and prevention of suboptimal 25(OH)D when undertaken with awareness and discretion. Based on our findings and previous literature, we therefore recommend 30-60 minutes of daily sun exposure, adjusted to be consistent with exposure to the minimal erythemal dose of UVB for an individual's skin color, geographical location and season. (Table 5.1)

Routine monitoring of 25(OH)D. Incorporating a routine assay of serum 25(OH)D into the battery of standard assays in preventive health maintenance would allow health care providers to recommend appropriate of vitamin D sources among individuals with lower 25(OH)D. This recommendation is in contrast to the 2011 IOM report, which only suggests routine monitoring of 25(OH)D in high risk populations. (38) Given the findings of this dissertation that high body fat, dark skin color and geographic locations distant from the equator play important roles in lower circulating 25(OH)D levels, more frequent monitoring of individuals in these higher-risk categories may be warranted to ensure optimal 25(OH)D status is achieved and maintained. While increasing the dosage and rate of supplementation in the entire population and targeting individuals with characteristics that put them at greater risk of suboptimal 25(OH)D is central to this public health effort, universal assessment is critical because low 25(OH)D occurs in all risk groups, is associated adverse outcomes, and should be individually monitored to ensure timely intervention.

Conclusions

Skin color and serum 25(OH)D has expanded in importance beyond rickets prevention and maintenance of bone health to include cardiovascular outcomes including

hypertension. This research expands current knowledge by examining longitudinal trajectories of serum 25(OH)D and blood pressure over time, characterizing their relationship to skin color. The overwhelming frequency of suboptimal circulating vitamin D levels, the finding that darker skin color increases the risk of suboptimal 25(OH)D, and that 25(OH)D mediates the relationship between skin color and systolic blood pressure has important relevance to the widening disparity in premature morbidity and mortality associated with hypertension affecting African Americans. Indeed, the research demonstrated that the association of measured skin reflection contributed to 11% of the association between 25(OH)D and blood pressure. The evolution and progression of research in this field will bring about greater awareness of the modifiable social and biological aspects of race that can be addressed to improve health outcomes. Having a greater understanding of these associations will ensure appropriate development of clinical practices and public health interventions targeted at promoting optimal health and preventing the onset of risk factors for cardiovascular disease.

Table 5.1. Comparison of Vitamin D intake recommendations from the 2011 Institute of Medicine Report and Dissertation Research in Michigan SWAN

Vitamin D Parameter	Institute of Medicine	Michigan SWAN Dissertation Research
Daily Intake	400-800 IU/d	3,000-5,000 IU/d
Sun Exposure	None. To minimize skin cancer risk	Daily minimal erythemal dose (215 J/m2)
Routine Monitoring	Not recommended in general population; high-risk populations only	General Population; emphasis on high-risk populations
Toxicity Threshold	>4,000 IU/d	>40,000 IU/d
Conclusions about Population Intake	Majority of US population (~97.5%) receive adequate intake	Michigan women have high prevalence of long-term suboptimal 25(OH)D and do not receive adequate intake

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APPENDIX

Collection of Skin Color and Vitamin D Data

Consent for Measurement of Skin Reflectance (Coloration) and Vitamin D
Study of Women's Health Across the Nation

Michigan Center

I, _____, hereby authorize Drs. MaryFran Sowers, Sioban Harlow, John Randolph, Janis Miller, and Roderick Little as well as Flojaune Griffin to include me in a special research study: The <u>S</u>tudy of <u>W</u>omen's Health <u>A</u>cross the <u>N</u>ation (SWAN) of vitamin D and health.

This study includes several components: 1) the amount of light reflected by your skin at your forehead and under your arm will be measured; 2) blood samples that you have provided in previous years will be tested for amount of vitamin D, and 3) there will be questions on the questionnaires, about how much time you are outside in the sun, how much of your body you expose to the sun when you are out and if you use a sunscreen or sun blocker.

This is a limited consent just for this study of skin coloration and vitamin D. You will be asked to sign another consent form, similar to the one that you have seen in earlier years, for the other measures during your visit.

Skin color will be measured in this study by exposing the skin on your forehead and under your arm to controlled source of light (as from a small flashlight) and measuring the amount of light that light that is reflected into a reflectance meter. This measurement will take approximately five minutes and involves no pain. You should tell the staff if you have an allergy to the application of alcohol (in a small pad) to clean your skin of its natural oils. Reflectance is higher in persons of lighter skin color and lower for

persons of darker skin color. Natural skin color will be measured in the inner upper arm. Your more tanned skin color will be measured on the forehead (and cleaned of makeup).

We will ask questions about how much time you are out-of-doors and what parts of your face, body, arms and legs you cover with either clothes (including hat) or sunscreen when you are out-of-doors. We will also ask you about foods or supplements that you might use which contain vitamin D.

If you consent, we will analyze your already collected and stored blood for vitamin D levels. You do not have to have extra blood drawn for this special study. These vitamin D levels will be analyzed in laboratories that specialize in this analysis in Iowa and Massachusetts.

As always, all information you provide will be computerized and will be identified by an identification number and **not** your name. A listing of participant names is kept only by the Principal Investigator, Dr. Sowers, and the Project Director and is in a locked file to insure confidentiality of information.

No data will be released to anyone, including your doctor or other healthcare provider, without your expressed permission. You will receive the results of your vitamin D analysis. If your results indicate a medical problem, you will be contacted by Dr. Sowers and the SWAN staff when the results are known.

Please remember that all information you provide is confidential. Your participation is entirely voluntary and can be withdrawn at any time. Your results will be confidential to the extent provided by local, state and federal law. No data will be released to anyone, including your doctor or other healthcare provider, without your written permission.

I understand that I may stop taking part in this project or refuse some of the tests at any time if I so choose. I understand that I have the right to ask questions at any time before, during, and after the project and that I should contact Dr. MaryFran Sowers at 734-936-3892 (109 Observatory, Ann Arbor, 48104) for answers. I understand that questions about the research protocol approval process or the rights of participants in research protocols can be sent to the Human Subject Protection Office, 540 East Liberty Street, Suite 202, Ann Arbor, MI 48104-2210 (phone 734-936-9033).

I have read this and understand what I will have to do and how much time is involved. I have been told how my records will be kept confidential. I freely consent to take part in this project.

_____

Printed Name of Participant

_____   _____

Signature of Participant   Date

_____   _____

Witness Signature   Date

Sunlight Exposure and Skin Reflectance Assessment Form

The Study of Women's Health Across the Nation (SWAN) of Vitamin D and Health

RESPONDENT ID:
| AFFIX ID LABEL HERE |

Date form Completed:___ ___ / ___ ___ / ___ ___

Respondent's DOB: ___ ___ / ___ ___ / ___ __

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1. When you are outdoors in warm weather, what parts of your body generally are <u>not covered</u> with clothing and are exposed to sunlight?

(Code 1 for all that apply, 2 for not applicable)

		Yes	**No**	**DK**
1.1	Head and Face	1	2	8
1.2	Arms and Hands	1	2	8
1.3	Lower Leg	1	2	8
1.4	Most of the Entire Leg	1	2	8
1.5	Back	1	2	8

2. In the past year during warm weather (include winter vacations) are you out of doors <u>and generally exposed to sunlight</u>?

1. Less than 1 hour per day
2. 1-2 hours per day
3. 3-5 Hours per day
4. More than 5 hours per day

3. Do you use a sunscreen with a rating of 15 or greater?

1. Yes
2. No
3. 8. Uncertain

4. Are you out-of-doors <u>and</u> exposed to sunlight _____ now in comparison to 10 years ago?

    1. More

    2. Less

    3. Same

    4. 8. Uncertain

5. Do you take a vitamin D supplement or a multivitamin supplement containing vitamin D?

    1. Yes

    2. No

    3. 8. Uncertain

        If YES →   5.1.   Do you take this supplement daily?

                              1. Yes

                              2. No

                              8. Uncertain

                        5.2.   What is the daily vitamin D dosage?

                              _____ IU   _____% RDI

                              8. Uncertain

To be completed by Clinic Staff

Forehead:

#1	Amber	__ __ . __%	Blue	__ __ . __%	Green	__ __ . __%
#2	Amber	__ __ . __%	Blue	__ __ . __%	Green	__ __ . __%

Inner Upper Arm:

#1	Amber	__ __ . __%	Blue	__ __ . __%	Green	__ __ . __%
#2	Amber	__ __ . __%	Blue	__ __ . __%	Green	__ __ . __%