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More than sun and skin:
Investigating the social and developmental determinants of vitamin D production

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ABSTRACT

Recent discoveries in vitamin D research indicate that vitamin D is a necessary component to several organs and tissues that extends beyond bone formation Furthermore, recent research indicates that vitamin D deficiency is rampant across the globe. Historically, anthropological research has focused on the adaptive significance of skin color to minimize the harmful effects of exposure to ultraviolet rays while maintaining vitamin D production. While foundational in biological anthropology, unanswered questions remain regarding the developmental and environmental factors that shape vitamin D production within populations, in a single generation. For example, within populations, gender, season of birth, time spent outdoors, urbanicity and socioeconomic factors all contribute to variation in patterns of sun exposure, and therefore levels of vitamin D. Therefore, the objective of this dissertation is to advance a biocultural anthropological understanding of vitamin D by exploring the more proximate environmental factors that explain vitamin D variation through the life course.

Data from the Philippines, a tropical country, was used to assess the impact of vitamin D among people in the Cebu Longitudinal Health and Nutrition Survey in the Philippines. Vitamin D assays were performed from whole blood samples from a sample of 349 young adults ages 20-22 years (66 males, 283 females) to determine circulating vitamin D levels. Socio-cultural environmental factors in both childhood and adulthood were analyzed to determine the impact of those variables on vitamin D levels in adulthood. Vitamin D status was then analyzed in conjunction with already existing DNA methylation data from the same participant samples. DNA methylation was evaluated as a predictor of the vitamin D receptor gene (*VDR*) and four

vitamin D metabolism enzyme genes (*CYP2R1, CYP24A1, CYP27A1, CYP27B1*). DNA methylation is a mechanism through which environments during development can regulate genome activity through the addition of methyl groups to CpG dinucleotides.

Combining ecosocial and epigenetic analyses, this project sought to understand what factors in early life and adulthood affect vitamin D variation for the entire sample and between females and males. This project also investigated whether DNA methylation provided a mechanism through which childhood environments can regulate genome activity resulting in vitamin D variation. DNA methylation of genes in the vitamin D metabolic process was found to not be predictive of circulating vitamin D levels. However, environmental factors in childhood and adulthood were found to be predictive of vitamin D with sex strongly predicting vitamin D levels.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

BMI – Body Mass Index

CLHNS – Cebu Longitudinal Health and Nutrition Study

DNAm – DNA methylation

DOHaD – Developmental origins of health and disease

FDA – Federal Drug Administration

IOM – Institute of Medicine

LC-MS/MS – Liquid chromatography with tandem mass spectrometry

NHANES – National Health and Nutrition Examination Survey

NIH – National Institutes of Health

SAT – Subcutaneous adipose tissue

SD – Standard deviation

UV – Ultraviolet

VDR – Vitamin D receptor

DEDICATION

To my mama, who always has the weather on her phone of wherever I am.

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CHAPTER 1: Vitamin D-New Perspectives

"Clarity of concepts, and attention to both gender relations and sex-linked biology, is critical for valid scientific research on population health."

-Nancy Krieger (Krieger 2003)

1.1 Introduction

On September 9, 2020 in a Live Instagram segment, Jennifer Garner interviewed Dr. Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases, where Dr. Fauci stated that as far as supplementation goes, he recommends both vitamin C and vitamin D to reduce susceptibility to Covid-19. In a matter of days, numerous articles were released stating that Dr. Fauci recommends vitamin D to boost immunity. While the research is still being conducted on whether vitamin D decreases the chances of Covid-19 susceptibility and whether subsequent vitamin D dosage after contracting covid-19 decreases symptom severity, interest in vitamin D research is on the rise.

More and more, popular media has touted vitamin D as the new miracle supplement, and more than a few books such as *The Vitamin D Miracle: How to Cure Common Health Problems and Have Optimal Health* (Vincent Miles, 2014), *The Vitamin D Solution: A 3-Step Strategy to Cure our Most Common Health Problems* (Michael F. Holick, 2010), *Vitamin D: The Sunshine Vitamin* (Zoltan P. Rona, 2011) espouse vitamin D deficiency as the root cause of numerous chronic diseases. To be clear, while vitamin D deficiency has been linked to some chronic and

infectious diseases, much more research is required before any of these claims can be made as definitive.

So why has vitamin D become one of the media's new cure alls? The answer lies in a resurgence of research in vitamin D. For years most of the research on vitamin D has focused on bone health -- rickets in children and osteoporosis in adults. Specifically, many researchers thought that vitamin D's only role was to assist in calcium absorption. However, there have always been doctors and researchers that have noticed that vitamin D obtained via sunlight may offer greater possibilities for health than bone health. As far back as 1895 vitamin D obtained through ultraviolet light exposure has been recommended as a treatment for tuberculosis (Zasloff, 2006).

Then, genetic researchers discovered the vitamin D receptor (VDR) in 1969 (Mark R. Haussler & Norman, 1969), and the subsequent discovery of the VDR in multiple tissues (Cantorna, 2011; M R Haussler, 1986; Norman, 2006; Wang, Zhu, & DeLuca, 2012) opened up completely new avenues of research regarding vitamin D. Suddenly, vitamin D was linked to immune cells, the intestines, the thyroid, the pancreas, the testis, and mammary glands amongst other tissues (Cantorna, 2011; M R Haussler, 1986; Norman, 2006; Wang et al., 2012).

In 2011, the Institute of Medicine (IOM), appointed a committee of 14 scientists to re-evaluate the 1994 dietary reference intakes for calcium and vitamin D as ample new research had been conducted to warrant an evaluation of vitamin D supplementation (Ross & Institute of Medicine (U. S.), 2011a). However, just as soon as the new recommendations were released there was disagreement about the dietary intake values (David B. Allison, Bhramar Mukherjee, John D. Kalbfleisch, & Suzanne P. Murphy, 2017; Maxmen, 2011; Vieth & Holick, 2018). The

controversy surrounding the new recommendations highlighted the gaps in the vitamin D literature (Brannon, Yetley, Bailey, & Picciano, 2008; Cashman & Kiely, 2011; Phinney, 2008). To be fair, the IOM made their recommendations based upon the research that was available to them at the time, but vitamin D research standards vary, and more research is required to fully understand what levels should be prescribed for females and males of various skin colors across the life course.

While formulating more accurate recommendations for vitamin D intakes are beyond the scope of this dissertation, as no dose-response research has been conducted, this dissertation adds to the vitamin D literature in several ways. For instance, this research discusses how environmental factors across the life course contribute to circulating vitamin D and is, to the best of the author's knowledge, the first to look at both childhood and adulthood factors for a single individual. This analysis finds that sex is a major predictor of vitamin D with females having a greater likelihood of vitamin D deficiency in this population. The environmental factors are stratified by sex to establish whether major gender differences contribute to the vitamin D disparities. Finally, epigenetics mechanisms are analyzed to determine whether vitamin D metabolism is affected by early life environments.

1.2 Dissertation Outline

This dissertation is organized into seven chapters. Chapter 2 provides an overview of the theoretical background pertaining to vitamin D and why gender may play a role in vitamin D deficiency. Chapter 3 provides an overview of the methods utilized within the dissertation. The background includes information about the study location, metropolitan Cebu in the Philippines,

and the history of the Cebu Longitudinal Health and Nutrition Study (CLHNS). Methods also includes a comprehensive summary of the variables included in each subsequent analysis in the proceeding chapters.

Chapter 4 through 6 are principal data analysis chapters. Chapter 4 analyzes the environmental factors in adulthood that predict vitamin D. Males were found on average to have higher vitamin D than females with a mean of 31.92 ng/ml compared to 24.86 ng/ml. Analyses are stratified by sex to explore whether gendered environmental experiences differ in relation to vitamin D levels in adulthood. No major differences in environmental predictors of vitamin D were found between females and males. However, sex, assets, and fish intake were found to be predictive of vitamin D in the total population.

Chapter 5 analyzes environmental factors in childhood that affect vitamin D in adulthood. In childhood, the strongest predictors of vitamin D were childhood urbanicity and childhood assets. Both were found to negatively predict vitamin D. Models were again stratified by sex to determine if gender differences exist in childhood environmental factors that contribute to vitamin D. Environmental factors were then combined in childhood and adulthood to create a lifetime model of factors that predict circulating serum vitamin D in adulthood.

In Chapter 6, methylation of genes in the vitamin D pathway is analyzed as a predictor of total vitamin D. Analyses were again stratified by sex to detect whether methylation is a mechanism by which differences are detected in vitamin D between females and males. Methylation patterns were not found to predict vitamin D levels nor did they explain the differences in vitamin D between females and males. Environmental predictors of vitamin D in

childhood and adulthood are also explored for their relationship to methylation of genes in the vitamin D metabolic pathway.

The last chapter (Chapter 7) summarizes the results of the dissertation and discusses the implications for future research from the analyzed vitamin D data. A discussion about the study limitations then follows. The broader implications for vitamin D research and public health are provided at the conclusion of chapter 7.

1.3 Contributions to the Literature-Study Significance

In this dissertation, I utilize a biocultural approach to understand how the socio-cultural experience of humans affects the body. The incorporation of gender into an analysis of vitamin D deficiency in an urbanizing society provides research on how the implications of gender changes with urbanizing societies. This research creates a more comprehensive picture of the factors that contribute to vitamin D levels beyond skin color and sun exposure. Furthermore, the integration of gender into the analysis allows reader to see how the cultural implications associated with environments and gendered spaces has implications for long-term health. Additionally, epigenetics is recognized as a mechanism in which the lived experience within an environment is incorporated into the body. By studying which socio-cultural factors contribute to methylation and circulating vitamin D, this research contributes to a growing body of research that seeks to determine whether vitamin D levels are determined by factors in childhood or young adulthood. In childhood, environmental factors tested are season of birth, birth weight, growth, breastfeeding duration, childhood urbanicity, and childhood assets. In adulthood, environmental variables researched are urbanicity, assets, fish consumption, exercise, and adiposity.

By analyzing sex differences between environmental determinants of vitamin D, this study delves deeper into the possible health risks associated with gendered lifestyle dynamics within a society. As defined by Nancy Krieger in an article titled *A Glossary for Social Epidemiology*, gender is a "social construct regarding culture-bound conventions, roles, and behaviors for, as well as relationships between and among, women and men and boy and girls" (Nancy Krieger, 2001). Sex is a "biological construct premised upon biological characteristics enabling sexual reproduction" (Nancy Krieger, 2001). The following are examples of sex-linked biology provided by Nancy Krieger: chromosomal sex, menstruation, secondary sex characteristics, sex-steroid-sensitive physiology of nonreproductive tissues, pregnancy, and menopause (N. Krieger, 2003). Societies undergoing urbanization may undergo simultaneous change in gender dynamics creating vast differences in gender constructs from childhood to adulthood. Gender is a cultural phenomenon that shifts expectations, which creates implications for health. Sex is one factor that affects biology and health, but the expectation and treatment of those sexes in society creates another factor that has biological ramifications when one considers how methylation allows the environment to have biological and health repercussions.

Testing gender in this analysis, sex differences in culturally determined environmental factors are analyzed for their effect on circulating vitamin D. If there are differences in environmental determinants of vitamin D between females and males, one can reasonably assume that gender is at work. However, there are some childhood factors, such as season of birth and birth weight that are more likely to be determined by sex characteristics that will also be tested for differences between the sexes.

Because there are so many communicable and non-communicable health implications, understanding the developmental and ecological determinants of circulating serum vitamin D is vital to understanding the long-term dynamics between vitamin D deficiency and disease. Because women seem to be more susceptible to vitamin D deficiency, a comparative analysis between males and females allows factors that affect methylation and overall circulating vitamin D levels to be analyzed. While, sex and cultural factors have long been associated with vitamin deficiency, the current research will allow cultural mediated environmental variables that may differ by gender to be analyzed as an independent factor predicting vitamin D levels.

CHAPTER 2: Background

Historically, research on vitamin D in anthropology has focused heavily on natural selection and the adaptive significance of both constitutive (basal) and facultative (tanning) skin pigmentation to generate vitamin D (Chaplin & Jablonski, 1998a; Frisancho, 1993; Nina G. Jablonski, 2004; Nina G. Jablonski & Chaplin, 2000; Juzeniene, Setlow, Porojnicu, Steindal, & Moan, 2009; Quillen, 2015; Yuen & Jablonski, 2010a). The hypothesis that lighter skin color evolved to optimize vitamin D production is vital to understanding the importance of vitamin D in cellular processes in the human body as a driver for evolution (Chaplin & Jablonski, 2009; Nina G. Jablonski & Chaplin, 2000; Yuen & Jablonski, 2010a). However, globalization has created a mismatch between skin color and environment. The accompanying rapid cultural change, stemming from globalization, influences vitamin D variation within a population within a single generation; however, little research has been conducted to understand which cultural factors and biological mechanisms interact to create this variation.

With growing concern for vitamin D deficiency, this chapter will delve into the benefits of vitamin D beyond the simple calcium absorption rhetoric that has been advertised in most of the scientific or popular nutrition literature to date. Special attention will be paid to the body's process of generating vitamin D from both an evolutionary viewpoint and a biological mechanistic stand. Factors that have been shown to affect vitamin D will be reviewed across the life course, and possible mechanisms for the embodying of cultural change and the effects those changes have on the body through vitamin D deficiency will be addressed. After reviewing past adaptation literature and environmental conditions that correspond to vitamin D deficiency, comparisons will be made with childhood and adult environment to determine how factors across

the life course differ in their determination of vitamin D quantification. Vitamin D in Cebu, Philippines will also be compared with the United States to discuss how vitamin D insufficiency differs and how vitamin D might be expected to change in the future. .

2.1 Vitamin D in the Anthropological Literature

Biological anthropologists study how environment and lifestyle shape biology. While the role of vitamin D in skin coloration is an adaptation story covering generations (Nina G. Jablonski, 2004; Nina G. Jablonski & Chaplin, 2000), there is increasing interest in how early environments shape human variation within a single generation (David James Purslove Barker, 1994; Mulligan, 2016). The anthropological literature on vitamin D has been discussed in both the adaptationist literature (Chaplin & Jablonski, 2009; Frisancho, 1993; Nina G. Jablonski, 2004; Relethford, 1997) and the nutrition literature (Garn, Sullivan, Decker, Larkin, & Hawthorne, 1992; Stini, 2003). Stini and Garn both described vitamin D as a vital component to bone structure (Garn et al., 1992; Stini, 1990, 2003). In *Human Adaptation and Accommodation*, Roberto Frisancho synthesized literature on human environmental stressors which included two chapters on human skin color as an adaptation to ultraviolet radiation and vitamin D (Frisancho, 1993). The adaptation literature was then heavily expanded upon by Nina Jablonski, whose lifetime work, largely, focuses on the adaption of human skin color globally (Chaplin & Jablonski, 1998b; Nina G. Jablonski, 2004; Nina G. Jablonski & Chaplin, 2010, 2018a). Further analysis on human evolution was added by John Relethford in the form of genetic adaptation (Relethford, 1997, 2000, 2002). As anthropologists, Stini, Garn, Frisancho, Jablonski, and

Relethford all underscored the importance of human skin color variation as adaptation to environmental influences not as biological categories separating human into subspecies or races.

Nutrition and Bone health

Stanley Garn and William Stini showed that both females and male experience bone loss at similar ages and over time, but bone loss in females is more impactful because females have smaller bones from which to lose bone mass (Garn et al., 1992; Stini, 1990, 2003). In his research, Garn showed that vitamin D did not impact bone loss during a two-decade study (Garn et al., 1992), perhaps indicating that bone health is only impacted before middle age. Utilizing the Arizona Bone Density Study, William Stini indicated that bone loss is a consequence of longer lifespans for human and for females is part of consequences of reproduction (Stini, 2003). It was in this article that Stini suggested that vitamin D, a hormone, may have differing effects based on sex.

Adaptation

Human variation in skin color is the product of natural selection wherein skin color is an adaptation to ultraviolet radiation exposure and the evolutionary response by the human species to accommodate the need for the biosynthesis of required solar dependent nutrients (Frisancho, 1993). Adaptation is based on the spectrum and range of ultraviolet radiation, which consists of thermonuclear rays in the 100-400 nm range further subdivided into three categories: UVA, UVB, and UVC rays (Kimlin, 2008). Earth's ozone layer prohibits UVC rays from entering the

atmosphere and reaching the surface leaving UVA and UVB rays as the remaining types of ultraviolet radiation humans have had to adapt to in their evolutionary lineage (**FIGURE 2.1**).

The prevailing anthropological hypothesis pertaining to vitamin D has up until now focused on skin color as a prototypical example of natural selection, where darker skin color is understood as an adaptation to minimize the harmful effects of exposure to ultraviolet rays while maintaining vitamin D production (Frisancho, 1993; Nina G. Jablonski & Chaplin, 2018a). Most of the variation in skin color is determined by melanin in the epidermis (Nina G. Jablonski & Chaplin, 2000, 2010), and dark skin, which is highly melanized, allowed for protection against UV radiation and the photolysis of folate (Branda & Eaton, 1978; N.G. Jablonski, 1999). Whereas vitamin D is generated from sun exposure, folate, a nutrient necessary for embryonic spinal and brain development (Hibbard, 1964; Lucock et al., 2017), is degraded by sun exposure. Hence, the necessary evolutionary need for dark or light skin, depending on ancestral past environmental exposure to UV light. UVA rays are responsible for the photolysis of folate (Branda & Eaton, 1978; Fukuwatari, Fujita, & Shibata, 2009; Nina G. Jablonski & Chaplin, 2010), while UVB rays determine vitamin D production (Nina G. Jablonski & Chaplin, 2010) resulting in a delicate balance between a biological need for greater melanocytes to avoid folate degradation and reduced melanocytes to produce vitamin D. This balance results in an individual's skin color.

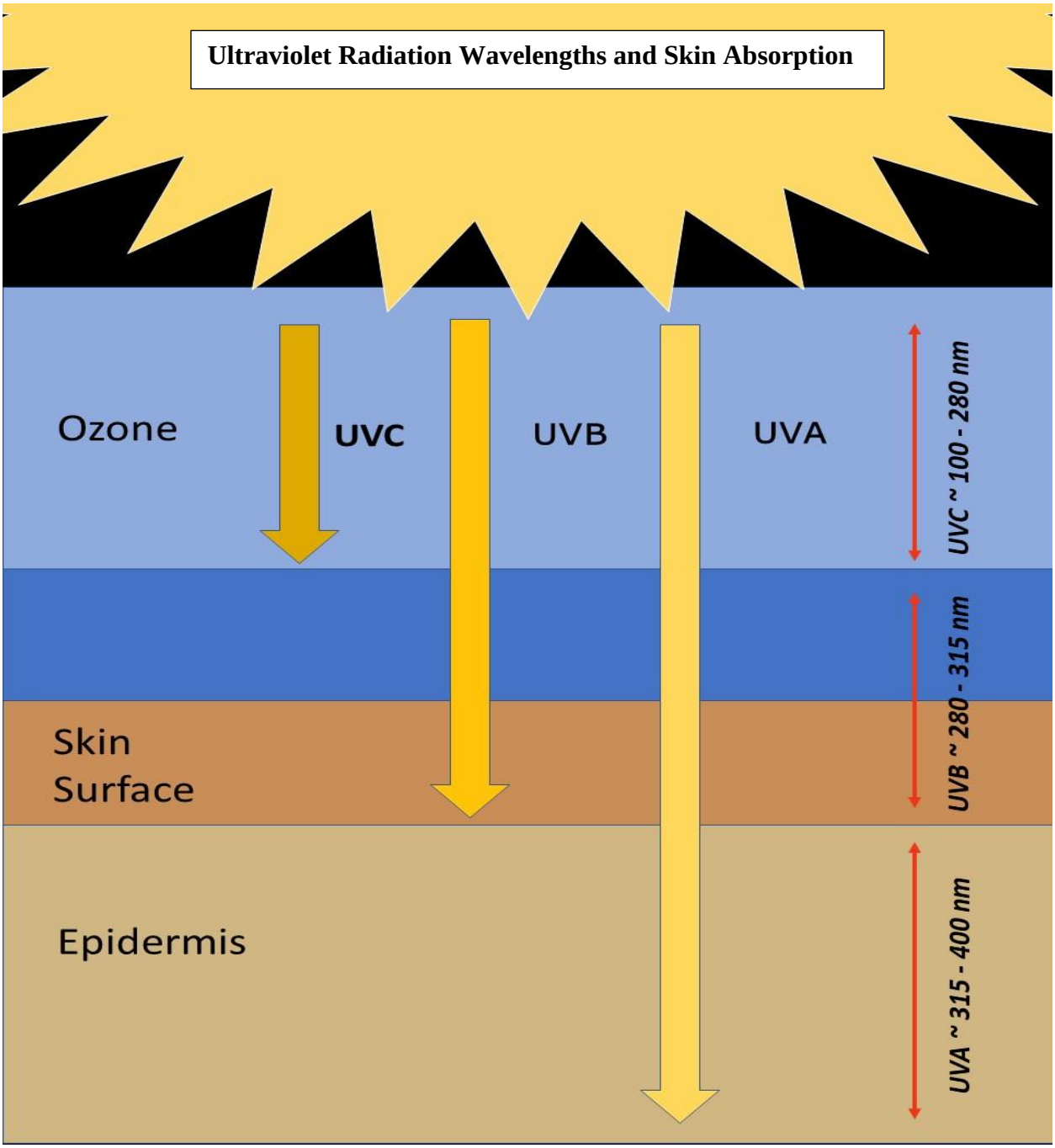


FIGURE 2.1. Ultraviolet radiation is produced by the sun in three types: UVA, UVB, and UVC. The wavelength of UVC is absorbed by the Earth's ozone layer leaving UVA and UVB to reach the Earth's surface. UVB rays penetrate a human's skin more superficially, while UVA rays penetrate more deeply into the epidermis. UVB rays produce a cascade of reactions leading the eventual production of vitamin D. Due to the tilt of the Earth, UVB rays are not available all year long in equatorial regions.

The earliest members of the hominid line likely had lightly pigmented or non-pigmented skin (fewer melanocytes) and dark colored hair (Nina G. Jablonski & Chaplin, 2000). However, for the *Homo sapiens* that currently live near the equator whose relatives have originated ancestrally in the equatorial region, we see highly melanized or visually darker skin with little body hair. Consequently, human skin color near the equator poses a scientific quandary as our closest living primate relatives have light skin under much fully hair that covers the entire body (Post, Szabó, & Keeling, 1975). The change seems to have occurred due to adaptation to UV rays. Likely part of thermoregulation for *Homo sapiens,* body hair loss occurred in our species. The hair loss and non-pigmented or lightly pigmented skin left *Homo sapiens* vulnerable to UVA rays. Due to our species' need for protection against folate degradation, darker skin was a driving force for greater melanin production and darker skin (Branda & Eaton, 1978; Nina G. Jablonski, 2004; Nina G. Jablonski & Chaplin, 2000, 2010). Consequentially, those *Homo sapiens* that ancestrally originated near the equator, where UVA and UVB rays are strongest, have darker skin than those that live further away from the equator. As humans migrated further from the equator, lighter skin re-emerged in indigenous peoples in Northern latitudes as a response to the need for vitamin D synthesis. One driver of adaptation towards greater vitamin D is in response to bone malformations in the pelvis which obstructs the birth canal in females leading to the inability to give birth vaginally (Yuen & Jablonski, 2010b).

The adaptive research on vitamin D has contributed to our understanding of skin color and sun exposure as determining factors in the production of vitamin D, (Nina G. Jablonski, 2004; Yuen & Jablonski, 2010a), and to our understanding of the origins and adaptive significance of variation in skin color across populations. While foundational in biological

anthropology, unanswered questions remain regarding the developmental and environmental factors that shape vitamin D production within populations, in a single generation. Increasingly, anthropologists are interested in how lived experiences within various environments becomes embodied to influence human variation (N. Krieger, 2005; Kuzawa & Sweet, 2009). While the adaptationist perspective on vitamin D and skin color is a multi-generational story driven by the biological influences of the sun on the body (Frisancho, 1993; Fukuwatari et al., 2009; Nina G. Jablonski & Chaplin, 2010; Lucock et al., 2017), more proximate exposures of vitamin D exist, driven by human cultural experience. Those experiences, such as increasing urbanization and socio-economic change driven by acculturation and globalization, can create vast differences in childhood and adult experiences. Additionally, there is increasing recognition of the importance of political economy to biological anthropology resulting in research that shows the health implications of rapid globalization and economic change (Goodman & Leatherman, 1998; Kelles & Adair, 2009; Kuzawa & Sweet, 2009; Thomas W McDade, 2001).

2.2 Vitamin D Comparison

A global comparison of vitamin D is difficult due to a lack of standardized protocols relating to the collection, analyzing, and reporting of vitamin D. For instance, over the years the National Center for Health Statistics that conducts the National Health and Nutrition Examination Survey (NHANES) has had to undertake rigorous quantitative analysis of their vitamin D testing as they have shifted from the utilization of the DiaSorin RIA kit (Stillwater MN) to a liquid chromatography tandem MS (LC-MS/MS) measurement procedure (Yetley et al., 2010). The change in laboratory analysis method was a result of a reformulation of the assay and noticeable fluctuations in quality controls. Even the 25(OH) vitamin D cutoffs for

deficiency can vary creating difficulty in comparing populations. While it is inappropriate to directly compare incidence or prevalence, trends in populations can and should be assessed. As previously mentioned, vitamin D deficiency has become a global concern. In the United States, NHANES data has indicated that serum vitamin D levels have decreased over time with mean serum 25(OH)D levels decreasing from 30 ng/ml in the 1988-1994 (NHANES III) survey to 24 ng/ml in the 2001-2004 survey (Ginde, Liu, & Camargo, 2009). Furthermore, in the NHANES II survey females had lower vitamin D levels than males at 24 ng/ml compared to 32 ng/ml for males, but the averages for these groups are equal at 24 ng/ml in the later survey. In the United States, females were at a greater risk for vitamin D deficiency during the 1988-1994 survey, but over time, the disparity between females and males decreasing within the total population (Ginde et al., 2009). There are also disparities within race/ethnic groups with non-Hispanic whites having less deficiency than Mexican Americans followed by non-Hispanic blacks (Ginde et al., 2009).

2.3 Globalization

Swift market integration resulting in large economic and political shifts, whether termed ‘Westernization’, ‘globalization’, or ‘acculturation’, creates a social experience wherein adult lives are often vastly different from that in which the individual grew up. These changes result in large social changes that dictate the environmental exposures of males and females. One biological avenue of research that is prone to these large political and economic changes may be vitamin D, often leading to drastic generational differences in vitamin D levels.

Research on global vitamin D status increasingly indicates that vitamin D deficiency is a health concern worldwide (Calvo, Whiting, & Barton, 2005; Hossein-nezhad & Holick, 2013;

Prentice, 2008). Along with the worldwide vitamin D deficiency pandemic, there is the re-merging concern over rickets, bone deformities in children, with the culprit being malabsorption of calcium due to poor vitamin D levels (Prentice, 2008; Rajakumar, Greenspan, Thomas, & Holick, 2007). Changes in behavior and understanding towards sun exposure may be contributing to the deficiency. For instance, market integration may lead to shifts from an agriculturally based economy to an industrial based economy, limiting sun exposure as the family unit spends less time outside as a consequence of requiring multiple family members outside in the fields to yield crops in the labor intensive farming economy.

Market shifts occurring during the globalization process often result in markets with more heavily processed foods which are high in carbohydrates, saturated fats, and sugars. These heavily processed foods often replace the less refined foods leading to diets with caloric surplus and increased adiposity (Kearney, 2010; Popkin, 2006). Higher adiposity correlates heavily with vitamin D deficiency in numerous populations (Cohen, 2010; Goryakin, Lobstein, James, & Suhrcke, 2015; Pereira-Santos, Costa, Assis, Santos, & Santos, 2015; Wakayo, Whiting, & Belachew, 2016; Wortsman, Matsuoka, Chen, Lu, & Holick, 2000a). Market shifts from primarily agriculturally based economies to industrial based economies also creates the potential for greater movement towards cities for economic gain. Whether through the creation of more pollution or the increase in multi-story buildings, the shift from rural to urban results in less exposure to sunlight and subsequently less vitamin D (Bailey, Manning, & Peiris, 2012; Kruger, Kruger, Wentzel-Viljoen, & Kruger, 2011).

In fact, urban environments have also been associated with vitamin D deficiency with populations living in urban environments tending to have less vitamin D than those living in

rural environments (EK Nichols et al., 2012). In industrialized countries and urban areas of developing nations, low socio-economic status is often correlated with vitamin D deficiency (Daly et al., 2012; Djennane et al., 2014; Voortman et al., 2015; Zgaga et al., 2011). In contrast in industrializing countries such India, vitamin D deficiency is associated with those working in higher income occupations compared to those in lower income brackets (Dharmshaktu et al., 2019). These results suggest that increasingly urbanizing environments result in increased vitamin D deficiency to those in lower socio-economic brackets because of occupation changes that results from economic shifts.

Overall, the changes that occur in economic and social arena lead to vast changes in vitamin D, sometimes within single generations. Research on vitamin D implicates these economic and social changes in vitamin D deficiency with implications for health outcomes. These avenues of research are predicated upon knowing how vitamin D impacts health.

2.4 Vitamin D and Health

Not considered a true vitamin, vitamin D is both a pro-hormone and a vitamin. A vitamin is a substance that must be acquired solely through diet. However, most vitamin D is generated in the body by the absorption of sunlight, specifically ultraviolet B radiation as few foods contain vitamin D. Vitamin D refers to both D_2 and D_3 types of vitamin D. Vitamin D_2, ergocalciferol, is plant or fungi derived vitamin D (Tripkovic et al., 2012), while D_3, cholecalciferol, is sourced from animals and is the type produced by the skin (DeLuca, 2004).

The production of vitamin D is initiated by one of two methods, either through the epidermis (via UV exposure) or through intestinal absorption (via food or vitamin consumption)

(Wagner, Taylor, & Hollis, 2008). When human skin is exposed to sunlight, 7-dehydrocholesterol is converted to vitamin D3 by ultraviolet B radiation in a heat dependent process (Holick, 2007; Serrano, Cañada, Moreno, & Gurrea, 2017). Both the dermis absorbed vitamin D3 and the ingested vitamin D2 bind to vitamin D-binding protein (Yousefzadeh, Shapses, & Wang, 2014). In a sequence of hydroxylation events vitamin D is converted to its active form via catalyzation by one of several types of enzymes in the cytochrome (**CYP**) P450 family (Christakos, Ajibade, Dhawan, Fechner, & Mady, 2012; Yasutake et al., 2010). After a series of hydroxylation event, biological functions are then performed through $1,25(OH)_2D$ binding to the vitamin D receptor (**VDR**) sites to regulate gene expression (Holick, 2007) (**FIGURE 2.2).**

While vitamin D is known for its role in calcium metabolism and bone health, research has determined that vitamin D receptors are pervasive throughout cells in the body, including intestinal epithelium, mammary epithelium, pancreas (beta islet cells), pituitary gland, skeleton (osteoblasts and chondrocytes), and immune system (monocytes, macrophages, and T-lymphocytes) (Bikle, 2009; DeLuca, 2004; Ooi, Chen, & Cantorna, 2012; Smolders et al., 2011). Upon the binding of 1,25(OH)2 to the vitamin D receptors, they up- or downregulate approximately 2000 different genes in the skeleton, skin, intestines, liver, thymus, spleen, lymph nodes, mammary glands, and testis, among other organs and tissue (Holick, 2007; Hossein-nezhad & Holick, 2012, 2013; Pike & Meyer, 2014; Ramagopalan et al., 2010; Wang et al., 2012). While the complete biological processes associated with vitamin D still require research, it can be assumed that the location of the vitamin D genes means that these tissues and organs are dependent on vitamin D to function.

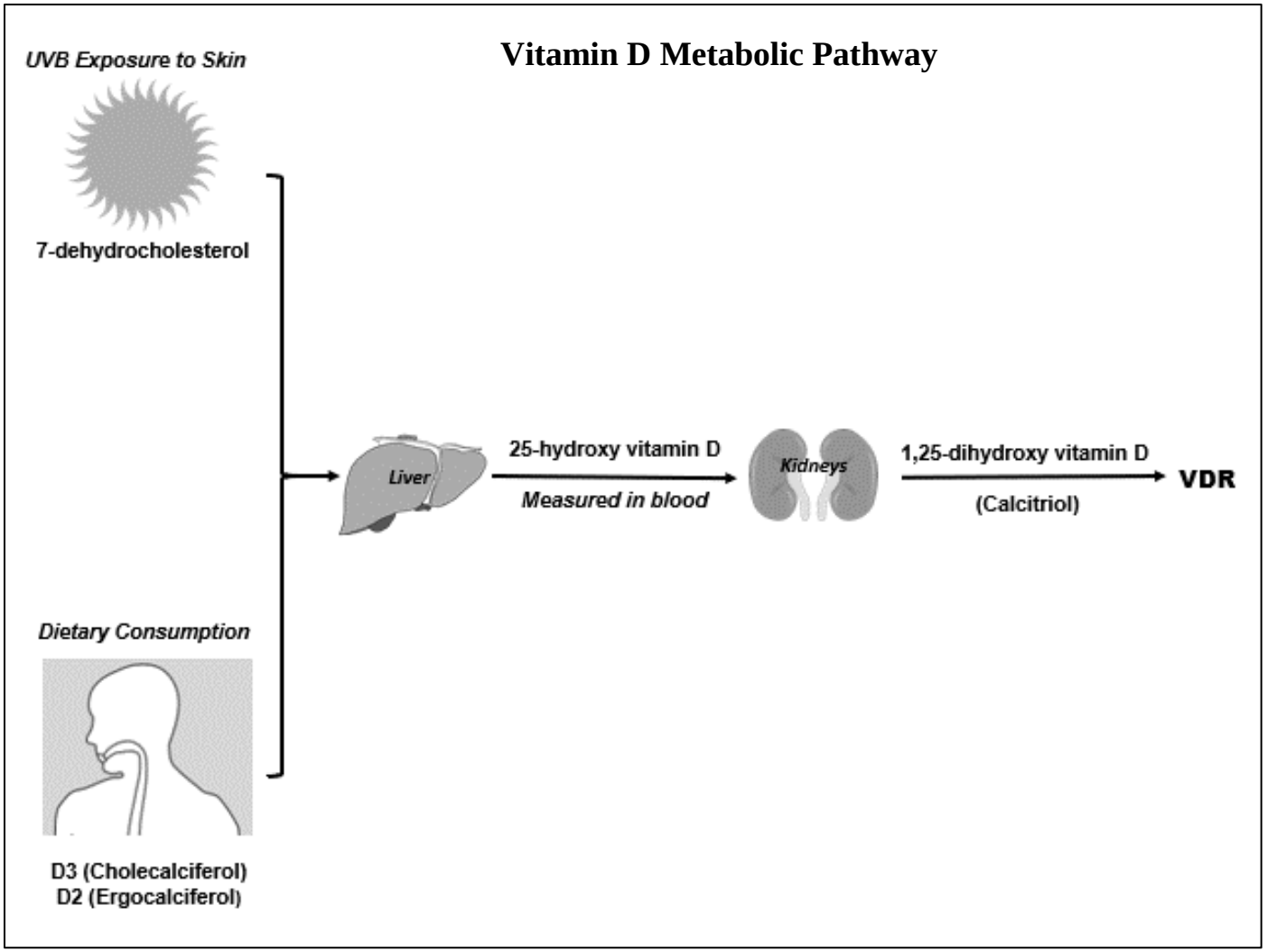


FIGURE 2.2 Vitamin D can be obtained via sun exposure and through food consumption. Both routes undergo hydroxylation events to produce 25-hydroxy vitamin D in the liver. After another hydroxylation event in the kidneys, 1, 25-dihydroxy vitamin D is formed, which is then regulated via the vitamin D receptor (VDR). VDR sites are expressed in various tissues and organs all over the body.

Vitamin D deficiency categorized as <20ng/mL (50 nmol/L) of 25OHD and levels between ≥20 and <30 ng/mL (51 and 74.9 nmol/L) considered insufficient levels. (Ross & Institute of Medicine (U. S.), 2011b). Deficiency is based on serum 25(OH)D values (Hossein-nezhad & Holick, 2013; Yousefzadeh et al., 2014). Though $1,25(OH)_2D$ is the biologically active form of vitamin D, research has shown that the precursor 25(OH)D is a better indicator of vitamin D status for a few reasons. For example, half-life for calcitriol is a few hours (K. S. Jones et al., 2015), while the half-life for 25(OH)D is 2 weeks or more (Datta et al., 2017; K. S. Jones et al., 2015). Furthermore, the close link between vitamin D and calcium absorption means low serum calcium, hypocalcemia, causes a cascade effect wherein parathyroid hormone converts 25(OH)D to $1,25(OH)_2D$ (Battault et al., 2013; Bikle, 2014; Christakos et al., 2012). The effects causes serum $1,25(OH)_2D$ to appear normal or even elevated when an individual is severely vitamin D deficient due to the upregulation by parathyroid hormone resulting in an inaccurate representation of vitamin D status if using calcitriol (Hossein-nezhad & Holick, 2013; Ross & Institute of Medicine (U. S.), 2011b).

2.5 Social Determinants of Vitamin D

Vitamin D deficiencies over long periods of time may lead to pathologies. With a considerable amount of vitamin D receptors in tissue and cell types throughout body, substantial research on mechanisms and pathways indicate that vitamin D is of clear import to the body throughout the life course, and that importance goes beyond bone formation. Furthermore, research indicates the body can be affected by low levels of vitamin D and have been linked to phenotypic changes in children and adults, leading to the more proximate diagnosis of vitamin D

deficiency, especially in women, and, perhaps, the more downstream diagnosis of disease states in adults. While research has yet to prove direct causal links, increased frequency of vitamin D deficiency has been associated with disease states such as autoimmune disease and cardiovascular disease (Arnson, Amital, & Shoenfeld, 2007; Camargo, 2011; Khadilkar, 2013; Talat, Perry, Parsonnet, Dawood, & Hussain, 2010; Tornhammar et al., 2014).

With so many diseases correlating with vitamin D, the determinants of vitamin D deficiency are important to understanding health outcomes in adulthood and beyond. Determinants of vitamin D are predicated upon our interactions with our environment. Our culture dictates how we interact with those environments. However, globalization can change cultures drastically in single generations leading to interactions with differing environmental exposures than those in which an individual grew up in. It is important to understand the circumstances in which vitamin D deficiency may arise.

Factors Affecting Vitamin D

As mentioned previously, urbanization is often associated with vitamin D deficiency. A myriad of other lifestyle factors that have also been implicated as risk factors for vitamin D deficiency such as obesity, diet, outdoor activities, and clothing choices. All these factors are attenuated by cultural norms and standards.

Like urbanization, obesity has been closely linked to vitamin D deficiency, and like vitamin D deficiency, obesity is a global epidemic. Studies have shown that micronutrients, of which vitamin D is one, are lower in people who are obese (García, Long, & Rosado, 2009; Kimmons, Blanck, Tohill, Zhang, & Khan, 2006). A metanalysis of 23 research articles indicated that those who were obese or overweight were more likely to be vitamin D deficient than those

within a normal body mass index regardless of age (Pereira-Santos et al., 2015). In fact, research on subcutaneous adipose tissue (SAT) and visceral body fat have shown that both are inversely related to vitamin D deficiency after adjusting for physical activity and vitamin D dietary intake (S. Cheng et al., 2010). Like the obesity epidemic, the vitamin D epidemic is rooted in the globalization process and is part of a much broader context of drastic change within populations. For instance, in a study on acculturation of non-Western immigrant groups in Nordic countries, results revealed that immigrant groups were both more likely to be overweight or obese and vitamin D deficient indicating that changes in environment may lead to changes in diet leading to poorer health outcomes.

Because most research on vitamin D deficiency has been case-control studies, the causal link has yet to be determined. That is, research has not yet discerned whether vitamin D deficiency contributes to obesity of whether obesity causes vitamin D deficiency. However, that are some indications that obesity may reduce availability of vitamin D because it is diluted within adipose tissue (Beckman, Earthman, Masodkar, & Sibley, 2012; Botella-Carretero et al., 2007; Wortsman et al., 2000a). Additionally, one examining the genetic polymorphisms in the vitamin D receptor (VDR) gene showed an inverse relationship between vitamin D metabolism and a genetic susceptibility to obesity which was affected by variants in the VDR (Reis, Hauache, & Velho, 2005). Taken together, it may be that individuals with certain VDR variants are genetically prone to metabolize vitamin D less efficiency leading to decreased levels of vitamin D which are then exacerbated by the uptake of circulating vitamin D by adipose tissue. Clearly, more research is required to discern the exact relationship between adipocyte receptors for vitamin D and circulating levels of vitamin D.

Studies have reached mixed conclusions on the degree to which diet contributes to circulating vitamin D. Much of the debate on diet is probably due to the variety of cultural, biological, and societal factors that control dietary intake of vitamin D. Vitamin D is present in a variety of fortified foods; however, the adoption of fortified foods is generally dependent on government legislation. For instance, in the United States milk, cheese, some calcium containing fruit juices, and breakfast cereals are all fortified (Calvo & Whiting, 2013). In contrast, Canada does not allow cheese or juice to be fortified but does have yogurt made from vitamin D fortified milk (Calvo & Whiting, 2013). Therefore, vitamin D oral consumption varies based upon the intake of milk, eggs, fish, and supplement intake, which an all vary by accessibility and food preference. Overall, most food items do not naturally contain high percentages of vitamin D except for fish (Macdonald, 2013), and research shows in Japan, a location where few most of the population is vitamin D sufficient, those who consumed larger amounts of fish (≥4 times per week) had significantly higher levels of vitamin D by an average of 10 nmol/L than those who ate fish sometimes (1-3 times per week) and a 15 nmol/L than those who ate no fish (Nakamura, Nashimoto, Hori, & Yamamoto, 2000).

Not surprisingly, within a given latitude, vitamin D is highly correlated with time spent outdoors. Time spent engaging in outdoor activities such as walking, cycling, physical exercise, and gardening are positively correlated with vitamin D levels (Zgaga et al., 2011). Conversely, among 6 year-old children in the Netherlands, vitamin D deficiency was more prevalent in children who watched more television, played outside less, and biked to school less (Voortman et al., 2015).

Clothing also attenuates the effects of time spent outside if clothing is covering the body: a study conducted in Jordan showed that women wearing a scarf/hijab were 1.60 times, and women wearing a niqab (full head covering with a slit for viewing), were 1.87 times more likely to be vitamin D deficient (EK Nichols et al., 2012). Furthermore, immigrants from nine different African and Middle Eastern countries, who were more likely to wear long-sleeved clothing in the summer, were at higher risk for vitamin D deficiency after moving to Sweden (L Granlund et al., 2016).

Sex Differences in Vitamin D Deficiency

Sex differences in vitamin D have been consistently reported across a range of populations, with women often having lower vitamin D across the life course (Basatemur, Horsfall, Marston, Rait, & Sutcliffe, 2017; Carnevale et al., 2001; Eikelenboom, Killestein, Kragt, Uitdehaag, & Polman, 2009; Karagüzel et al., 2014; H.-J. Yu, Kwon, Woo, & Park, 2016; S. Yu et al., 2015). Furthermore, women living in urban environments with at least secondary education were found to have 1.30 times greater vitamin D deficiency than women living in rural areas having the same education level, and those women with less than secondary education were found to have a 1.18 times greater prevalence of vitamin D deficiency (EK Nichols et al., 2012) indicating that rural to urban transitions may have some negative health effects in women.

Because of the negative correlation between vitamin D and autoimmune diseases, which affect women more than men (Eikelenboom et al., 2009; Gleicher & Barad, 2007; Knudsen, 2009; Rider, Foster, Evans, Suenaga, & Abdou, 1998; Whitacre, Reingold, & O'Looney, 1999), differences in vitamin D between the sexes are often assumed to be biological/genetic in origin

(Arabi, Mahfoud, Zahed, El-Onsi, & El-Hajj Fuleihan, 2010; Mithal et al., 2009; Zhao et al., 2017). However, it is possible that these differences represent gendered exposures to experiences and environments that are more proximately related to vitamin D production. For instance, in a study conducted in Nebraska that looked at children between the ages of 5 and 13, boys listed that they spent significantly more time in outdoor sports than girls at all ages, while girls stayed predominately indoors (Cherney & London, 2006). The phenomenon of girls spending more time indoors is not just seen in the United States. A cross-cultural analysis by Larson and Verma of time spent at work and at play for children and adolescents showed that girls consistently spent more time conducting indoor chores, while boys were more likely to spend time outdoors assisting in outdoor agricultural practices or in general yard work (Larson & Verma, 1999). The results indicate that gendered expectations of children may play a role in the decrease in vitamin D for girls in childhood.

Additionally, vitamin D deficiency in females was associated with increased cardiovascular disease risk and severity in adulthood as compared to males (J. S. Lim, Kim, Rhee, & Lim, 2012). There are more women than men living with cardiovascular disease (Mozaffarian et al., 2016), and the differences in vitamin D deficiency between males and females increases with age, with women becoming more deficient than men over time (J. S. Lim et al., 2012). These results may stem from gender becoming more salient as females and males age, leading to diverging exposures/environments that lead to differential health outcomes.

Sex differences in vitamin D deficiency exist and cultural norms regarding gender may impact those differences. These norms regarding gendered space and environmental exposure may occur during childhood. Because childhood or early life determinants of health are not

direct, anthropologists and other researchers are interested in how early life impacts later adult health outcomes. Epigenetics is one such mechanism.

2.6 Developmental Determinants of Vitamin D

Epigenetics is one mechanism through which early life events and experiences may become embedded in cellular memory. These processes can have lasting effects on gene function and phenotypic variation. Importantly, epigenetic mechanisms modify gene function without changing the underlying DNA sequence. DNA methylation (DNAm)—a key epigenetic mechanism widely studied in humans—involves the addition of methyl groups to CpG dinucleotides, with methylation of sites in gene promoter regions typically resulting in decreased transcription (Peter A. Jones & Daiya Takai, 2001), while methylation within gene bodies often resulting in increased gene expression (Lister & Lister, 2008).

While underlying biological mechanisms that mediate environmental effects on vitamin D metabolism within the body are unknown, methylation provides a plausible mechanism. Gender, season of birth, time spent outdoors, urbanicity, and socioeconomic factors are all plausible environmental determinants of vitamin D status that may become biologically embedded in the epigenome. By utilizing a longitudinal study in an environment with vast urbanization and socio-economic change, the factors in early life can be illuminated in how cultural environment effects the body at the molecular level.

Prior research has focused primarily on environmental factors measured concurrently with vitamin D. However, a large body of epidemiological research on the developmental origins of health and disease (D. J. P Barker, Osmond, Winter, Margetts, & Simmonds, 1989; DeBoer et

al., 2012; Heindel & Vandenberg, 2015; Thomas W. McDade et al., 2017), as well as anthropological research on developmental plasticity (Kuzawa, 2005), indicates environments early in life can have lasting effects on the development and function of a wide range of systems and tissues, with implications for disease in adulthood.

The implications for vitamin D have yet to be explored, but several lines of evidence suggest this is a promising direction for future research. For example, lower birth weight is associated with vitamin D deficiency in infancy (Kalanda, van Buuren, Verhoeff, & Brabin, 2005; Khalessi, Kalani, Araghi, & Farahani, 2015; Mannion, Gray-Donald, & Koski, 2006), and vitamin D supplementation early in childhood is associated with lower risk for Type 1 diabetes through adolescence and young adulthood (Hyppönen, Läärä, Reunanen, Järvelin, & Virtanen, 2001). Similarly, season of birth, specifically birth in winter, corresponds to an increased chance of developing cardiovascular and immune-regulated diseases later in adult life (Disanto et al., 2012; Sacheck et al., 2011; Tornhammar et al., 2014). Birth in winter is associated with an 11% increase in developing vitamin D deficiency in adulthood (Lippi, Bonelli, Buonocore, & Aloe, 2015; Merewood et al., 2010), and many studies show infants that breastfeed without vitamin supplementation are also at a higher risk to develop vitamin D insufficiency (Gordon et al., 2008; Hatun et al., 2005). To the extent that vitamin D influences the etiology of these diseases, it may be an important mediator of the long term effects of early environmental exposures (Abhimanyu & Coussens, 2017; Ananthakrishnan et al., 2012; Camargo, 2011; Lippi et al., 2015; Smolders, Damoiseaux, Menheere, & Hupperts, 2008)

2.7 Cebu

Despite recognition that vitamin D deficiency is a growing phenomenon worldwide, there is often the underlying idea that those in tropical regions have little to worry about in terms of vitamin D deficiency. Additionally, few studies have taken into consideration early life environmental exposures, adult exposures, methylation, and vitamin D levels. The Cebu Longitudinal Health and Nutrition Survey in the Philippines (CLHNS) is a longitudinal study (Adair et al., 2011) that will allow for close examination of childhood and early life factors that influence vitamin D in a tropical region. With UVB rays holding steady year-round, Cebu, Philippines allows socio-cultural factors to be analyzed in their contribution to circulating vitamin D levels.

The proposed research utilizes data from the CLHNS, which has followed the same individuals since their mothers were pregnant with them in 1983-84 (Adair et al., 2011). The longitudinal study began as a nutrition study on infant feeding practices. Random sampling selected 17 urban and 16 rural barangays to locate all pregnant women in these areas. Surveys were conducted immediately after birth, and then bimonthly for 24-months. Subsequent follow-up surveys took place in 1991–92, 1994–95, 1998–99, 2002 and 2005, 2007, and 2009 and have included collection of anthropometric data, diet, physical activity, illness, and neighborhood statistics among other variables. In 2005 blood samples were taken from 1,759 participants, and gene-methylation was conducted on a subsample of 500 individuals.

All data were collected and analyzed under conditions of informed consent with institutional review board approvals from the University of North Carolina at Chapel Hill, Northwestern University, and the University of British Columbia. All the aforementioned data

are available for use in the present proposal, and serum from 2005, the same year the DNA for the methylation data was acquired, are collected and stored at -70º C in the Laboratory for Human Biology Research at Northwestern.

2.8 Conclusion

We live in vastly different environments than our ancestors, and globalization is very quickly changing environments, sometimes in single generations. Clearly there are biological implications for the occurrence of quick changing environments and its repercussions for vitamin D. The next chapters will analyze socio-cultural factors that influence blood circulating vitamin D levels in childhood and adulthood. These factors will be compared in the same individuals to assess how urbanizing environments can change across the lifespan and how these environments influence vitamin D. Methylation will also be assessed to ascertain whether early life environments have lasting impact on the body's ability to produce vitamin D.

CHAPTER 3: Methods

The research utilizes data from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) (latitude10.3° N), an ongoing birth cohort study based in the Philippines. The CLHNS was originally conceptualized as an infant feeding study and has followed the same individuals since their mothers were pregnant with them in 1983 and 1984 (Adair et al., 2011). A wide range of environmental exposures have been collected in surveys over 30 years allowing for a rich source of information to address the how vitamin D is affected by environmental factors and whether methylation is a mediator between these environmental exposures and vitamin D production.

Utilizing a country near the equator allows sufficient ultraviolet-B light to produce vitamin D throughout the year in order to evaluate the social and developmental factors that influence vitamin D production without having to account for seasonal variation in UVB radiation (Kimlin, 2008). Furthermore, Cebu has undergone massive change in the form of rapid urbanization and economic change from the inception of the study in 1983 (Kelles & Adair, 2009). The change in Cebu across time allows for a range of rural-urban environments and socio-economic statuses both now and when the participants were born to explore how urbanization and socio-economic change effects vitamin D production.

3.1 Participants and Study Collection

Participants for the Cebu Longitudinal and Nutrition Study initially consisted of women (ages 14 to 47.1 years) who gave birth from between May 1, 1983 and April 30, 1984.

Participants were recruited from metropolitan Cebu, the second largest city in the country. A single stage cluster sampling procedure was used to randomly select 17 urban and 16 rural barangays, which are the equivalent of neighborhoods in urban areas or villages in rural areas. Households were surveyed to locate all pregnant women in these 33 barangays due to give birth between May 1983 and April 1984. Fewer than 4% refused resulting in 3,327 women being interviewed in individual and household level surveys. There was a total of 3080 single births, 26 multiple births, 42 has stillbirths or miscarriages, and 17 refused further participation after baseline. Furthermore, 136 women and their children out migrated. The survey initially followed mothers and the single infant births for the first two years of life. Surveys were conducted immediately after birth, and then bimonthly for twenty-four months to assess infant feeding practices, growth patterns, illness, and caregiving.

Subsequent follow-up surveys took place in 1991, 1994, 1998, 2002, 2005, 2007. The surveys have included collection of anthropometric data, diet, physical activity, illness, and neighborhood statistics among other variables from both mothers and their children. The average age for the index offspring during the subsequent surveys was 8.5, 11.5, 15.5, 18.7, 21.5, and 23.6 years. The 2009 follow-up survey was the first survey to only include the index children with the hope of also gathering data on the index children's children to create an inter-generational longitudinal study.

Attrition is primarily due to out migration, where study participants have migrated out of the study area. From the initial population that were asked to participate in the CLHNS, fewer than 4 percent refused. Of the 3327 women included at baseline, 3080 gave birth to live, single infants. In the 2007–2008 tracking survey, 63 percent of the 3080 mothers were located and

interviewed, and 59 percent of the 3080 singleton children were located and interviewed. Therefore, those that were interviewed in the subsequent surveys were less mobile than those in the original cohort.

Population

The 2005 survey for the children included 1,885 participants (20-22 years of age), Out of 1,885 participants, 1,759 individuals also provided a venipuncture blood sample. From this sample, three hundred ninety-five female participants from the subsample were chosen to assess their methylation based upon their participation in a 2009 pregnancy tracking study. An additional 99 male participants were also chosen for methylation analysis. Out of the initial 494 participants analyzed for methylation, three hundred eighty-two serum samples were available for analysis. Due to hemolysis of some of the serum samples, laboratory analysis produced three hundred seventy clean vitamin D quantification using the DiaSource 25(OH) Vitamin D Total enzyme-linked immunosorbent assay kit for serum. Vitamin D measures were then matched with full adult data for analysis resulting in a sample of 349. All data were collected and analyzed under conditions of informed consent with institutional review board approvals from the University of North Carolina at Chapel Hill, Northwestern University, and the University of British Columbia.

3.2 Dependent Variable

Vitamin D was assessed via enzyme-linked immunosorbent assay (ELISA). Several vitamin D kits were assessed but the DiaSource 25(OH) Vitamin D for serum was ultimately

decided upon because it is one of a few vitamin D ELISA kits that is approved by the Food and Drug Administration (FDA). The results from the ELISA also had a correlation of R=0.92 with liquid chromatography mass spectrometry (LC-MS), considered the best standard for determining vitamin D analysis. However, ELISA is a much more inexpensive alternative to LC-MS.

Laboratory Analysis of Vitamin D

Vitamin D was determined by using plasma samples from the CLHNS, currently stored at -70º C in the Human Biology Laboratory in the anthropology department at Northwestern University. Analyses focused on 383 participants that have been previously analyzed for genome-wide DNA methylation, using the same set of samples collected in 2005. These participants are representative of the CLHNS sample, with the exception that the methylation subsample is over-represented with women (N=395) (Thomas W. McDade et al., 2017). The DiaSource 25(OH) Vitamin D Total enzyme-linked immunosorbent assay kit for the *in vitro* quantitative measurement of 25-hydroxyvitamin D2 and D3 (25OH-D2 and 25OH-D3) can be used to quantify vitamin D in both serum and plasma. Total 25(OH) vitamin D is considered the best method for determining vitamin D (Adriana S. Dusso, Alex J. Brown, & Eduardo Slatopolsky, 2005; Neil A. Breslau, 1988; Saenger, Laha, Bremner, & Sadrzadeh, 2006).

Vitamin D kits were shipped from Belgium to Immuno-Biological Laboratories, Inc. (IBL-America) in Minneapolis, MN. After undergoing approval by the Federal Drug Administration (FDA), the kits were then overnight shipped to the Department of Anthropology's Human Biology Laboratory at Northwestern University. The kits were then

stored at 2-8℃ until use. All standards and controls were reconstituted by adding 1 milliliter of deionized water and gently mixed via vortex.

Strips with anti-25(OH) vitamin D_2 and D_3 (monoclonal antibodies) were selected and places on the 96-well microtiter plate. Fifty µL of calibrator, controls ,and samples were filled into the appropriate wells with an additional 150 µL of Incubation buffer administered into each well and incubated for 2 hours on a plate shaker at 400 rpm at room temperature. During the first incubation stage, the HRP conjugate solution was made by adding the appropriate amounts of conjugate buffer, concentrated conjugate, and concentrated HRP according to instructions.

After two hours, the plate is washed three times with 0.35 mL of wash solution, prepared that day, in each well. Them, 200 µL of HRP conjugate solution is pipetted into each well and incubated for 30 minutes at room temperature on a plate shaker at 400 rpm. Following three plate washes of 0.35 mL of wash solution, 100 µL of Chromogenic solution is pipetted into each well within 15 minutes of the last wash. The plate is incubated for 15 minutes at 400 rpm avoiding direct sunlight. Then 100 µL of Stop solution is administered into each well, and the plate absorbance is read at 450 nm.

Standard laboratory quality and control procedures were followed, which includes running samples in duplicate, inspecting calibration curves for fit and day-to-day variation, and measuring quality control samples with each assay. Hemolyzed plasma samples were not included in the final analysis.

3.3 Mediator

DNA methylation is an important epigenetic mechanism regulating gene function that is responsive to environmental exposures. Epigenetic mechanisms are the processes through which early life events and experiences may become embedded in cellular memory, which can then have lasting effects on gene function and phenotypic variation. Importantly, epigenetic mechanisms modify gene function without changing the underlying DNA sequence. DNA methylation (DNAm), a key epigenetic mechanism widely studied in humans, involves the addition of methyl groups to CpG dinucleotides, with methylation of sites in gene promoter regions typically resulting in decreased transcription (Peter A. Jones & Daiya Takai, 2001), while methylation within gene bodies often resulting in increased gene expression (Lister & Lister, 2008).

Underlying biological mechanisms that mediate environmental effects on vitamin D metabolism within the body are unknown, but methylation provides a plausible mechanism. Gender, season of birth, time spent outdoors, urbanicity, and socioeconomic factors are all plausible environmental determinants of vitamin D status that may become biologically embedded in the epigenome.

Methylation Protocol

Gene-methylation was assessed from venous blood samples from the 2005 survey. In 2005 blood samples were taken from 1,759 participants, and gene-methylation was conducted using the Illumina Human Methylation 450 Bead Chip. Genomic DNA was treated with sodium bisulfite, and converted DNA was applied to the Illumina Human Methylation 450 Bead Chip

using the manufacturer's standard conditions. Methylation analysis was conducted on subsample of 395 female participants, chosen based upon their participation in a 2009 pregnancy tracking study, and an additional 99 male participants. Quality control was performed via sex chromosome probes to confirm participant sex, which were then removed from further analysis. Unreliable probes with a detection p-*value* greater than 0.01, with fewer than three beads contributing to the signal, and those previously shown to bind to multiple genomic regions were also removed, leaving 434,728 probes. From these probes, the study analyzed vitamin D metabolism enzyme genes (*CYP2R1, CYP27B1, CYP24A1)* and the *vitamin D receptor gene (calcitriol)* resulting in 73 probes analyzed.

3.4 Independent Variables

Environment factors that are hypothesized to affect circulating vitamin D in adulthood were analyzed. To characterize the environment in young adulthood and infancy/childhood, measures of residential location (urban vs. rural), time spent outside, socioeconomic status, and diet were assessed from the 2005 survey and also from the averaged data from the surveys taken before puberty. Infant/childhood factors assessed were birthweight, season of birth, infant growth, and breastfeeding duration analyzed from survey data in 1984-1986.

Adult and Infancy/Childhood Environmental Factors

Adult environmental factors were measured from the 2005 survey data, the same year methylation analysis occurred. Here childhood is defined as the collective experience of an individual before puberty. Environmental factors in childhood were averaged across survey data

for any given variable from the 1983-1984, 1985-1986, 1991-1992, and 1994-1995 surveys for a collective measure of a variable.

Residential location

Location is measured on a previously validated scale of urbanicity that combines scores in population size and density, communications, educational facilities, transportation (vehicles and road composition), health services, and markets (Darren Lawrence Dahly & Adair, 2007) for both young adulthood and childhood. Communications includes phone service, mail, newspapers, the internet, cable TV, and cellular phones. Education facilities measures the presence of primary and secondary schools, colleges, and vocational schools. Transportation measures the density of paved roads and the availability of public transportation. Health services measure the presence of hospitals, medical clinics, maternal health clinics, family planning clinics, and community health centers. Markets measures Sari-Saris stores (small retail shops), and the presence of drug stores, grocery stores, and gas stations. Each component is measured on a scale from zero to ten with a final urbanicity score from zero to 70. The scaling method has been proven to capture more nuisance than the simple urban-rural dichotomy and is culturally relevant. Urbanicity is measured for both young adulthood in 2005 and infancy/childhood.

Time spent outdoors

Outdoor exposure is assessed by analyzing questionnaires from young adulthood. Time spent outdoors was approximated on an additive scale using the physical activity attribute in the 2005 survey.

Socio-economic status

Socio-economic status was measured as physical assets owned by the family. Household assets were used as a measure of socioeconomic status as assets provide a more stable measure of socioeconomic status in low-income settings due to inaccuracy in reports of household income (Vyas & Kumaranayake, 2006). A sum of household items was generated and averaged from survey data in 1983-1984, 1985-1986, 1991-1992, and 1994-1995 for a measure of household assets in childhood. Household assets were counted in 2005 for adulthood.

Diet

Diet was assessed by counting servings of fish during a 24-hour dietary recall for five different days. The number of servings were then summed. Few foods naturally contain high amounts of vitamin D (Cashman & Kiely, 2018; P. H. Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1994; O'Mahony, Stepien, Gibney, Nugent, & Brennan, 2011). While milk and other foods can contain vitamin D if fortified, there is no indication that vitamin D fortification of milk has been adopted in the Philippines (Republic of the Philippines Ministry of Health, 2000).

Body composition

Adiposity in the forms of body mass index and average skinfold were calculated from the 2005 survey. Skinfold measurements were taken from the triceps, suprailiac, and subscapular skinfold measurements. Body mass index (BMI) and skinfold average measures were assessed to determine which is a better predictor of vitamin, and one was used as a control variable since

vitamin D sequesters in fat diminishing circulating vitamin D (Alemzadeh, Kichler, Babar, & Calhoun, 2008; Cediel, Corvalan, Lopez de Romana, Mericq, & Uauy, 2016; Gilbert-Diamond et al., 2010; Sacheck et al., 2011).

Childhood Environmental Factors

To characterize the environment in infancy and childhood measures of birthweight, breastfeeding, growth in infancy, birth month, as well as the previously mentioned measures of residential location (urban vs. rural on a continuous scale), and socioeconomic status (measured as assets) in childhood were evaluated as predictors of vitamin D independent of current environmental factors identified in the 2005 early adulthood survey. Infant/childhood factors assessed were birthweight, season of birth, infant growth, and breastfeeding duration analyzed from the bimonthly data collected from the initial infant feeding survey. The data utilized was from the 1984-1986 survey.

Birthweight

Birthweight was measured in grams and serves as the first data point collected from the child. Weight was collected immediately in the home after birth using standard procedures (Lohman, Roche, & Martorell, 1988).

Season of birth

Birth month was evaluated and categorized by birth in the dry season or the rainy season. The dry season was defined as birth in February to April, and the rainy season was defined as

June to October. Diarrhea and respiratory infection in the first year of life was significantly higher for those born during the dry season, which suggests greater pathogen exposure for those born in the dry season (T. W. McDade, Rutherford, Adair, & Kuzawa, 2010). Because vitamin D is implicated in immune function (Bikle, 2009, 2011; Prietl, Treiber, Pieber, & Amrein, 2013), greater infection rate early in life may affect vitamin D during adulthood.

Breastfeeding

Breastfeeding was assessed using bimonthly feeding surveys during the first two years following birth. Two variables of breastfeeding, duration of exclusive breastfeeding and age at cessation were considered during for analysis. Exclusive breastfeeding is defined as the number of days of breastmilk consumption before the introduction of supplementary foods or liquids. The age at cessation is also measured in days. Ultimately, duration of exclusive breastfeeding was chosen for analysis.

Growth

Growth in infancy was measured by analyzing the bimonthly length (cm) and weight (kg) of each child during the first year of life. Anthropometric measures were collected at each bimonthly interview during the first two years following birth, and total weight gain and length achieved in the first year of life were used to analyze growth.

3.5 Analysis

For the current environment and infant/childhood environment analyses, vitamin D (ng/mL) was modeled as a continuous dependent variable, with secondary analyses using a

categorical approach (deficient, insufficient, and normal). For hypothesis testing descriptive and bivariate analyses were used to characterize each outcome and relationship to the independent variables of interest, followed by linear and logistic regressions. Stata (Version 15.0, College Station, TX) will be used for all analyses. Variables will be examined for outliers, which will be removed as appropriate. Regressions will be checked for normalization and heteroscedasticity, run with robust standard errors as needed, and α will be set at 0.05.

Each independent variable was evaluated independently with vitamin D status as the dependent variable after controlling for adiposity. All significant variables were then used to construct a comprehensive model of current adult environmental exposures that predict vitamin D status. Both linear regression (vitamin D as a continuous variable) and logistic regression (vitamin D as one of three categories) models were then tested. All variables were entered into multiple linear regression models controlling for sex.

Infancy/Childhood Environment Analyses

The analyses for environmental factors in infancy/childhood was similar to those conducted in adulthood. However, in addition to the residential location and socio-economic variables that were included in the analysis of adult factors that may influence vitamin D, the additional variables of birthweight, breastfeeding, growth in infancy, and birth month were analyzed as predictors of adult vitamin D.

Each environmental variable was evaluated independently with vitamin D status as the dependent variable. All significant variables were used to construct a comprehensive model of

infant and childhood environmental exposures that predict vitamin D status. Linear regression, vitamin D as a continuous variable, models were tested while controlling for sex.

3.6 Methylation Analysis

Multivariate analyses were performed using STATA 15 stratified by sex. Significant variables were included in multivariate modeling, controlling for sex and smoking since smoking can affect methylation patterns. Benjamini and Hochberg step-up procedure was used to correct for false discovery rate.

Methylation analysis followed procedure by Baron and Kenny procedures for testing mediation, as outlined by MacKinnon et al. (2002). The four steps are as follows:

1. Show that the primary variable of interest (X) is a significant predictor of the dependent variable (Y) in OLS regression.
2. Test whether X is a significant predictor of the proposed mediating variable (M).
3. Test whether M is a significant predictor of Y, while controlling for X.
4. Inspect coefficients from steps 1 and 3: If M completely mediates the association between X and Y, then the coefficient for X in step 3 should be reduced to zero.

First participant gender (X) was tested to see if it predicts vitamin D concentration (Y). Then, gender differences in socio-cultural variables (M) previously mentioned were tested. For variables M that differ by gender, mediation was tested for by considering gender and the subset of sociocultural variables in a single model predicting vitamin D. We hypothesized that that the coefficient for gender will be reduced in magnitude in the final model, which will be interpreted as evidence for mediation by socio-cultural factors.

For methylation analysis, the same procedure is used, but in this case the mediating variable (M) will be methylation status of vitamin D genes (M), which is evaluate as a mediator of the association between environmental variables (X) and circulating serum vitamin D (Y).

3.7 Study Sample Sizes

The sample sizes for each chapter are given below (**TABLE 3.1**). All independent environmental variables in chapter 4 were tested in multiple linear regression models to determine if they predicted vitamin D. Sex stratified regression models were also run to determine whether predictors determined vitamin D differently between females and males. All significant predictors were then run a single model controlling for sex.

Similar analyses, were conducted for chapter 4 but included season of birth, breastfeeding, growth, childhood assets, and childhood urbanicity as predictors of vitamin D. All predictive variables from childhood and adulthood were then included in a model. Stepwise regression was the implemented to create a final lifetime model of vitamin D. Both the childhood model and the final model were stratified by sex to determine if predictors varied between males and females over the life course. In the final chapter, methylation of genes in the vitamin D metabolic pathway are assessed to determine if they predict vitamin D levels. An exploratory analysis also correlated environmental variables with DNAm of the vitamin D genes.

TABLE 3.1 Sample Size for Each analysis by Environmental Variable in Each Chapter

Section	Variable	Female N	Male N	Total N
Chapter 4	Vitamin D	283	66	349
	Adult assets	283	66	349
	Adult urbanicity	283	66	349
	Fish consumption	283	66	349
	Physical activity	283	66	349
	Skinfold	279	66	345
	BMI	246	66	312
Chapter 5	Season of birth	283	66	349
	Breastfeeding (days)	278	66	344
	Growth	278	66	344
	Childhood Assets	265	66	331
	Childhood Urbanicity	265	66	331
Chapter 6	Methylation	283	66	349

3.8 Statistical Power

Post-hoc testing of statistical power using G*Power 3.1.9.7 indicated that the sample sizes are generally adequate to detect even small effects. For the adulthood regression, the statistical power approached 1 for the total sample size (N=345) to detect small effects (R^2=0.132) with 5 predictors in a two tailed multiple linear regression model. With the 6 predictors, the sample size was still more than adequate with a power calculated at 1.000 with a larger effect size (R^2=0.201). For the childhood multivariate regression model, the sample size decreased slightly (N=331) but power was 1.000 with 4 predictors.

For females, the sample size ranged from 265 to 283 depending on the predictor in question. Neither the female adult nor the childhood model was sufficiently powered to detect small effect sizes. For the adult model for females (N=279), the model was underpowered at 0.536 for a two-tailed regression model with 5 predictors and R^2=0.032. The implementation of stepwise regression for the female (N=283) model still indicated that the model was underpowered at 0.675 and R^2=0.022. For the childhood model, a two tailed multivariate regression model for females (N=265) with 3 predictors was also underpowered at 0.698. Unfortunately, the female sample is too small to detect the small effect sizes seen in the regression models.

The male sample size was the smallest (N=66). While the sample was large enough to detect moderate effects, small effects are more difficult. The statistical power was 0.795 for a two tailed multiple linear regression model for males with 5 predictors and an observed R^2=0.220 for the adult model. However, for the initial male childhood model with 2 predictors and an observed R^2=0.136, statistical power was low at 0.772. Because stepwise regression was

utilized, that same childhood model with only 1 predictor and an observed R^2=0.099 was sufficiently powered at 0.800.

CHAPTER 4: Adult Environment and Current Vitamin D

4.1 Introduction

While the research on vitamin D was once thought to be complete with the association to bone health and calcium absorption discovered, genetic evidence for the effect of vitamin D on multiple tissues and organs (Cantorna, 2011; M R Haussler, 1986; Wang et al., 2012) has opened completely new avenues of research regarding vitamin D levels through the commonly known pathways of sunlight exposure and diet. However, cultural and societal factors that influence these proximal pathways to circulating pathways are less well understood and should be explored to understand the broader picture surrounding vitamin D deficiency among populations worldwide.

Factors such as urbanization, socio-economic status, physical activity, occupation, and body mass index are all factors that influence vitamin D by either influencing sun and diet vitamin D intake or in the case of body mass index affecting the absorption process of vitamin D in the body. These factors are culturally determined and are ecological influences on a myriad of health outcomes. Here, these factors, are explored as influencers on circulating vitamin D in adults in the Philippines. Because sun exposure is a proximal factor in determining sun exposure, often more distal ecological contributors to vitamin D can be difficult to determine. Additionally, the urbanization process creates a transition from more agricultural, open environments to denser city life where buildings, pollution, and occupation changes may decrease sun exposure and change diets (Bailey et al., 2012; Fang et al., 2018; Kruger et al., 2011).

While vitamin D is a necessary nutrient for health for both children and adult, there are differing factors that influence vitamin D for differing life stages. Increasing growth is no longer a factor, but bone remodeling is still important. However, there are a number of chronic diseases (Ananthakrishnan et al., 2012; Borges, Martini, & Rogero, 2011; Camargo, 2011; S. Cheng et al., 2010; Mowry, 2011) that have been linked to vitamin D deficiency. Because the aforementioned diseases most often arise in adulthood, factors that affect vitamin D production in adulthood are vital for further study to understand the dynamics that lead to vitamin D deficiency. Furthermore, gender disparities in these factors are vital to understand why females are more susceptible to certain chronic diseases that are linked to deceased circulating vitamin D.

4.2 Background

Functions

It is well known that vitamin D in adulthood is vital for the prevention of bone deterioration brought upon by the remodeling process. However, vitamin D has also been implicated in many chronic diseases associated sedentary lifestyles (Hibler et al., 2016; Nina G. Jablonski & Chaplin, 2018b; Roomi, Farooq, Ullah, & Lone, 2015; Zgaga et al., 2011). Chronic diseases including cancer, cardiovascular disease, Type 1 diabetes, multiple sclerosis, and respiratory illness all have a proposed etiology that implicates vitamin D deficiency as a probable cause (Battault et al., 2013; Holick & Chen, 2008; Hossein-nezhad & Holick, 2013; Palacios & Gonzalez, 2014; Wacker & Holick, 2013). By contributing to the innate and adaptive immune system, vitamin D may contribute to the pathogenesis of disease (Bikle, 2011; Borges et al., 2011; Gois, Ferreira, Olenski, & Seguro, 2017).

Sources of Vitamin D

Generally, the main source of vitamin D is ultraviolet B rays from sunlight exposure, which in human evolutionary development would have been the primary method of vitamin D production. With new technologies, vitamin D sources have multiplied. For instance, sunbeds or tanning beds that emit UVB rays have been recommended by some researchers as a means to acquire vitamin D in some countries in Northern latitudes during the winter (Moan et al., 2009; Tangpricha et al., 2004). A large frequently cited fear of sun exposure and especially sun tanning is skin cancer. In fact, the American Cancer Society in a March 2019 article on their website titled Are you Getting Enough Vitamin D? states, "Do not skip using sunscreen or try other ways to get vitamin D from the sun", essentially stating that only food and supplement dietary resources should be utilized for vitamin D (Stacy Simon, 2019).

In fact, dermatology as a disciplines most often does not recommend natural sun exposure despite any benefits and certainly not tanning/sun bed exposure as a source of vitamin D production (H. W. Lim et al., 2005). While sun or tanning beds can cause skin cancer, researchers do state that populations should only get moderate, non-erythemal (non-sunburn causing) exposure when making the recommendation of sun beds (Moan et al., 2009; Tangpricha et al., 2004). Furthermore, one study found that sun beds exposure three times a week for a maximum of 12 minutes provided a greater increase in 25(OH)D than 1000 IU of vitamin D a day (de Gruijl & Pavel, 2012).

Diet

Oral vitamin D sources include both food related sources and oral supplements. Food sources of vitamin D can also be broken down into naturally occurring sources and vitamin D

fortified categories. For instance, naturally occurring vitamin sources include fish, especially fatty fish, egg yolks, and meat. Mushrooms can be a source of vitamin D if they have been subjected to UV light although they produce D_2 (ergocalciferol) as opposed to D_3 (cholecalciferol) (P. Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002; Stephensen et al., 2012). Animal products generate D_3 while D_2 is produced by invertebrates (O'Mahony et al., 2011; Schmid & Walther, 2013). While fish, eggs, and mushrooms can be rich sources of vitamin D, the vitamin D content vary widely due to a number of variables (P. Mattila et al., 2002; O'Mahony et al., 2011; Schmid & Walther, 2013).

Fish, generally, contain the most vitamin D per serving (O'Mahony et al., 2011). However, fish, depending on type, location caught, and wild or farm raised, can vary wildly on how much vitamin D they contain in that serving (O'Mahony et al., 2011). Wild mushrooms are more likely to contain larger amounts of vitamin D that the commercially grown button mushroom (P. H. Mattila et al., 1994; P. Mattila et al., 2002; Stephensen et al., 2012). Eggs also vary in their vitamin D content by season and feed provided to the animal (Schmid & Walther, 2013). Meat seems to vary widely on amount of D, which may be due to cut of meat, season, or possible vitamin D supplementation to the animal (Schmid & Walther, 2013). However, it is generally accepted that most cuts of meat will have low servings of vitamin D and is not a great primary source of vitamin D (O'Mahony et al., 2011; Schmid & Walther, 2013).

In an effort to prevent rickets in children, milk was fortified with vitamin D by irradiating milk, which promoted bone calcification better than untreated milk, before vitamin D was purified (Cowell, 1925). Later, fortification of milk was conducted by the addition of *viosterol*, a purified vitamin D product, which allowed for a greater array of products to be fortified with

vitamin D (Bishai & Nalubola, 2002; Hess, 1932). Vitamin D food fortification policy varies greatly by country in regards to types of food allowed and the amount per serving (Al Khalifah et al., 2020; Cashman & Kiely, 2018). For example, current policy allows the option to fortify dairy, dairy products, and cereals in the United States while policy dictates that Canadian manufacturers fortify milk, margarine, and plant-based milks (Calvo, Whiting, & Barton, 2004). Furthermore, vitamin D fortification of foods is not as widespread or prioritized as other forms of fortification such as iron, vitamin A, and iodine (World Health Organization, 2006). While food items vary in their vitamin D levels, there are also cultural values (vegetarianism), prohibitive costs (fish can be expensive), and dietary issues such as lactose intolerance that can prohibit individuals from getting enough vitamin D from dietary sources. Dietary supplements may be beneficial in these cases.

Ergocalciferol (D_2) is plant-based vitamin D which was used to formulate the first vitamin D supplements and has been used in commercial vitamins or multivitamins for decades (Houghton & Vieth, 2006). However, some doctors now prescribe cholecalciferol instead of ergocalciferol due to randomized clinical trials indicating that D_3 requires less to increase circulating 25(OH)D levels in the blood (Houghton & Vieth, 2006; Tripkovic et al., 2012). In the Philippines, there are no indications that foods are fortified with vitamin D. Fish consumption is a staple of the Filipino diet and will be assessed as a marker of vitamin D consumption.

Urbanization

The process of urbanization, whether through physical expansion or through migration from rural to urban centers is linked to both increased and decreased vitamin D (Bailey et al., 2012; Fang et al., 2018; Griffin et al., 2020). Because the transition from habitual sun exposure

to greater time spent indoors (Nina G. Jablonski & Chaplin, 2018b) would indicate that those living in urban environments receive less sun exposure, the general assumption has been that those in urban areas are more likely to be vitamin D deficient than those living in rural areas. However, research indicates that cultural factors affect sun exposure and the rural/urban dichotomy is not so straight forward with some scientists discovering that those living in rural areas are more likely to be vitamin D deficient that those living in urban areas (Fang et al., 2018; Griffin et al., 2020; Islam et al., 2002).

Clearly, the rural/urban dichotomy may not be nuanced enough to discuss the microcosmos in which communities live and interact. While there are many studies that compare groups of individuals within a single time and country, there are few studies that are able to compare vitamin D deficiency across larger shifts in cities across time. By utilizing an urbanicity score, the data used in the CLHNS can contribute a much more nuanced view pertaining to urbanicity over time and its relation to vitamin D deficiency.

SES

The urban/rural dichotomy as pertaining to vitamin D deficiency has shown to be much more complicated, perhaps in part, because of socio-economic status. Low socio-economic status is found time and time again as a risk factor for vitamin D deficiency (Djennane et al., 2014; Voortman et al., 2015; Zgaga et al., 2011). Lower socio-economic status often correlates to increased morbidity and mortality (Adler & Ostrove, 1999; Pappas, Queen, Hadden, & Fisher, 1993; Schrage et al., 2021), which may in part be due to a poorer quality diet. Owing that vitamin D deficiency arises with a change in environmental sun exposure, it follows that when sun exposure changes and there is an inability to compensate via foodstuffs vitamin D deficiency

is likely to occur. Those in lower socio-economic tiers are less likely to be able to compensate with the few dietary items containing vitamin D or afford vitamin D supplements when these items are highly priced. Of course, cultural norms affect both sun exposure and food availability, which means that it is not universal that those in lower socio-economic tiers would be the most deficient in the micronutrient, vitamin D.

Most research use country-level data or a two-tiered, low versus high, SES categorical variable to make comparisons in vitamin D research. However, those countries that are considered lower SES may actually produce a better measure of household adversity by using household assets instead of the traditional income report or education status most often generated by researchers (Vyas & Kumaranayake, 2006). Here we use household assets to better illustrate the relationship between SES and vitamin D deficiency over time. Therefore, household assets are a continuum instead of a category.

BMI vs. Skinfolds for adiposity

Because vitamin D is a fat-soluble vitamin, the vitamin sequesters in fat, and research has found that individuals that are obese were found to have greater amounts of vitamin D stored in adipose tissue (Carrelli et al., 2017) and had lower circulating vitamin D in serum (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000b). However, many studies determining vitamin D deficiency utilize BMI as a replacement for a more direct measure of body fat (Delle Monache et al., 2019; Lagunova, Porojnicu, Lindberg, Hexeberg, & Moan, 2009; Muscogiuri et al., 2019, 2019; Orces, 2019). In fact, a 2008 article about the key findings found at the National Institutes of Health conferences " Vitamin D and Health in the 21st Century: an Update specifically stated

that one of the limitations of existing research at the time of the conferences was the lack of controlling for body mass index (Brannon et al., 2008).

Later, in 2011, when the IOM met to discuss the revision of the dietary reference intakes, a research gap identified was to understand how factors such as "body weight and body composition affect the variability in serum 25OHD" (Ross & Institute of Medicine (U. S.), 2011a). While the issues may be a choice of semantics in referring to BMI as opposed to body fat specifically, the research implications are important, especially as research on vitamin D has had issues with comparison due to lack of uniformity in how research is conducted (Brannon et al., 2008). Therefore, it is imperative to correlate both in this sample to better distinguish if BMI is a reasonable estimator of body fat in this population. Most research that has controlled for BMI tends to be Western populations and BMI may not be a great indicator of body fat in the Philippines, an Asian population. After investigating both BMI and skinfold average, the best predictor of vitamin D will be used as a control in all analyses.

Physical Activity

As evidenced by the previously mentioned environmental factors, lifestyle changes often correlate with hypovitaminosis, and physical activity is no exception. Vitamin d deficiency is often found in those that partake in less physical activity (Hibler et al., 2016; Roomi et al., 2015). Physical activity is often associated with a healthier lifestyle, but in many lower income countries, heavy physical activity is the norm for those living in the lower echelons of society (Hallal et al., 2012; Yamauchi, Umezaki, & Ohtsuka, 2001). In industrialized countries, those individuals with higher income may be able to afford gym memberships that correspond to increased physical activity. In Cebu, physical activity will be assessed among individuals at

various income levels to better illuminate whether physical activity is a good predictor of serum vitamin D in a population undergoing urbanization.

Gender

Our experiences are affected by the lens of society. Those societal practices also dictate not only our gender, but the practices around gender. For instance, sun exposure is largely attenuated through our gendered practices regarding sun protection and societal thoughts on looking one's age (i.e., wrinkles). With sex or gender differences often being reported across populations (Abudawood et al., 2018; Muscogiuri et al., 2019; Verdoia et al., 2015), further research is warranted to determine whether lower vitamin D is a matter of sex or a matter of differences relating to gender and the cultural ideology placed upon gendered environments. If biological sex is the determining factor, one would expect little difference in environmental factors affecting vitamin D across the life course. If, however, gender is at play, one would expect environmental factors to vary, especially in adulthood.

In general, we do see clothing restrictions (i.e., wearing more concealing clothing) act as a barrier to vitamin d production with greater restrictions correlating with increased frequency of hypovitaminosis (Alagöl et al., 2000; Al-Musharaf et al., 2018). If a different set of expectations are placed upon one gender over another, then there exists a gendered environmental disparity and a cultural impact that goes beyond the biological differences of sex. In terms of clothing restrictions, gender does heavily influence vitamin d production (Alagöl et al., 2000). Here "sex" refers to biological, genetic markers, and "gender" refers to the cultural constructs placed upon certain groups that often but does not necessarily map directly onto "sex" groups. It is recognized that neither "sex" nor "gender" differentiate into discrete, binary groups. For the

purposes of "sex", genetic typing did confirm an XX or XY genotype for all participants. While analysis does use the female or male designation referring to XX or XY genotypes, analyses are determining whether these groups differ in environmental conditions affecting the groups. While the dichotomy is once again preserved in the analyses, the research aims to create a more comprehensive picture of the factors that influence vitamin D including how gender mediates these factors.

Participants

The 2005 survey for the child cohort included 1,885 participants (20-22 years of age). Out of 1,885 participants, 1,759 individuals also provided a venipuncture blood sample. From this sample, three hundred ninety-five female participants from the subsample were chosen to assess their methylation based upon their participation in a 2009 pregnancy tracking study. An additional 99 male participants were also chosen for methylation analysis. Out of the initial 494 participants analyzed for methylation, three hundred forty-nine were analyzed for vitamin D quantification using the DiaSource 25(OH) Vitamin D Total enzyme-linked immunosorbent assay kit for serum and had complete data for the analysis.

4.3 Methods

Tests of association were analyzed based upon predicted variables in adulthood that have previously been linked to vitamin D deficiency. To compare the correlation between BMI and adiposity, each was calculated as follows. BMI was calculated from weight in kilograms (kg) divided by height in meters (m) measured from the 2005 survey. BMI was calculated from the formula: $kg/(m^2)$. Skinfold thickness (mm) was calculated as the average of the triceps,

suprailiac, and subscapular skinfold measurements. Each skinfold was measured three times and averaged. The resulting measurements (in millimeters) for each skinfold were then added and divided by three for a measure of adiposity. Fish consumption was calculated by summing fish intake over a five-day dietary recall. Physical activity consisted of total times per month participants engaged in physical activity that is not part of normal work. Least squared regression models were used to determine the predictors of vitamin D, and to investigate potential mediators of gender differences in vitamin D.

4.4 Results

Results indicated that the mean vitamin D in the Cebu populations is 26.19 ng/ml. The mean for females is 24.86 ng/mL which is significantly lower than males which have a mean of 31.92 ng/ml. Average age for the population was 21 years of age. Surprisingly, BMI was statistically higher on average for males than females at 21.06 compared to 20.16. However, the comparison with skinfolds is vastly difference. Females carry more adipose tissue than males with an average skinfold of 20.16 mm compared to 11.81 mm. Males were more likely to have more assets than females, while females were more likely to live in more urban environments though neither asset nor urbanicity means were statistically significant from each other. Fish consumption was slightly higher for males though not significantly different than female fish consumption with an overall average of 2.25 servings over a five-day recall for the total population. Physical activity performed per month was less than one activity once a month for the total population with 0.55 activities. A significant difference was found between females and males with an average of 1.67 activities for males and 0.29 activities for females (**TABLE 4.1**).

TABLE 4.1: Adult Descriptive Statistics for Female and Male Participants. Mean (SD) are presented for continuous variables.

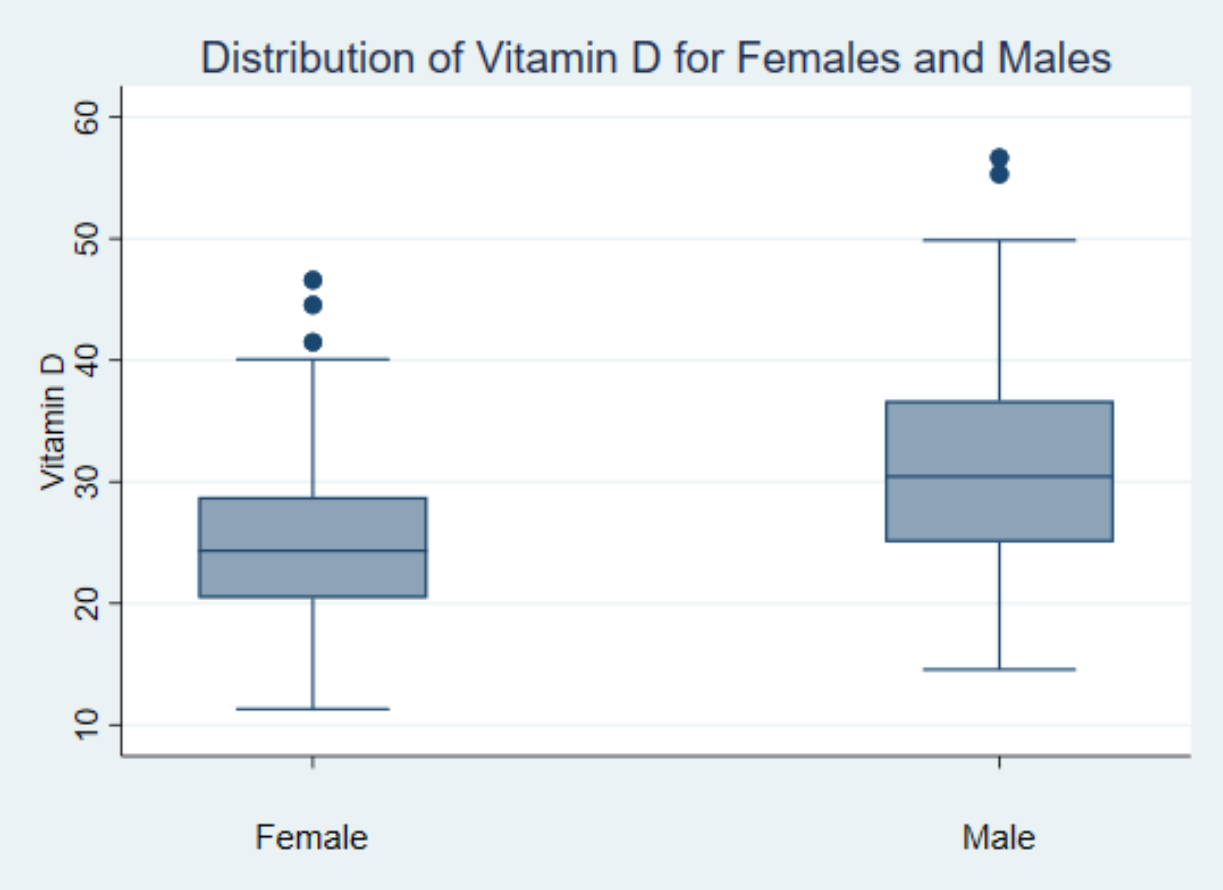
	Females (n=283)	**Males (n=66)**	**Total (n=349)**
age (years)	21.45 (0.30)	21.54 (0.34)	21.46 (0.31)
Vitamin D (ng/mL)*	24.86 (5.89)	31.92 (8.90)	26.19 (7.10)
BMI (kg/m^2)*	20.16 (2.92)	21.06 (2.56)	20.35 (2.87)
Skinfold average (mm)*	20.16 (6.45)	11.81 (4.82)	18.56 (6.69)
Assets	4.98 (1.89)	5.21 (2.00)	5.02 (1.91)
Urbanicity	41.48 (13.40)	39.17 (14.69)	41.04 (13.66)
fish consumption (servings in 5 days)	2.21 (0.84)	2.36 (0.91)	2.25 (0.86)
physical activity (activities/month)*	0.29 (0.89)	1.67 (2.30)	0.55 (1.37)

N=349 for total in each variable except for skinfold average in which N=345 (F=279 and M=66)

*$p<0.05$ for means that vary significantly between males and females

Boxplots were created to show the distribution on vitamin D for females and males (**FIGURE 4.1**). The range for females was from 11.311 to 46.591 ng/ml, while that for males was from 14.559 to 56.653 ng.ml.

FIGURE 4.1 Distribution of Vitamin D by Sex



Out of the total population, 18.33% were likely to be categorized as vitamin D deficient and 56.16% were likely to be categorized as insufficient by the standards set by the Endocrine society (Holick et al., 2011). Overall, females are more likely to be both deficient (21.91% vs. 3.03%) and insufficient (59.01% vs. 43.94%) with regards to their vitamin D status (**TABLE 4.2).**

TABLE 4.2: Sex Differences in Vitamin D Status (Deficiency, Insufficiency, Normal)

	Deficient (<20ng/ml)	Insufficient (≥20 to <30ng/ml)	Normal (≥30ng/ml)	Total *n*
Females	21.91%	59.01%	19.08%	283
Males	3.03%	43.94%	53.03%	66
Total	*18.34%*	*56.16%*	*25.50%*	*349*

A Pearson correlation matrix for both BMI and skinfold average was correlated with vitamin D. For BMI, the correlation matrix did not show significance at the $p<0.10$ level. Skinfold average was found to be significantly correlated with vitamin D (**TABLE 4.3**). As expected, skinfold average and body mass index were also highly correlated. Surprisingly, BMI had a positive trend with vitamin D rather than the expected inverse relationship that skinfold average has with vitamin D (**FIGURE 4.1**). Similar relationships were seen when comparing BMI for females but not for males. However, the correlation matrices indicate that neither relationship is significant for females nor males (**TABLE 4.4 and TABLE 4.5**). For skinfold average, the correlation indicates that as skinfold average increases vitamin D levels decrease. This trend was seen in the scatterplots for both females and males. However, sex stratified correlation matrixes do not indicate that these models are significant.

TABLE 4.3: Total Sample Correlations for Body Composition Variables and Vitamin D

	Vitamin D	BMI	Skinfold average
Vitamin D	1.00		
BMI	0.09	1.00	
Skinfold average	-0.28**	0.53**	1.00

*p<0.05

**p<0.01

TABLE 4.4: Female Correlations for Body Composition Variable and Vitamin D

	Vitamin D	BMI	Skinfold average
Vitamin D	1.00		
BMI	0.06	1.00	
Skinfold average	-0.09	0.70*	1.00

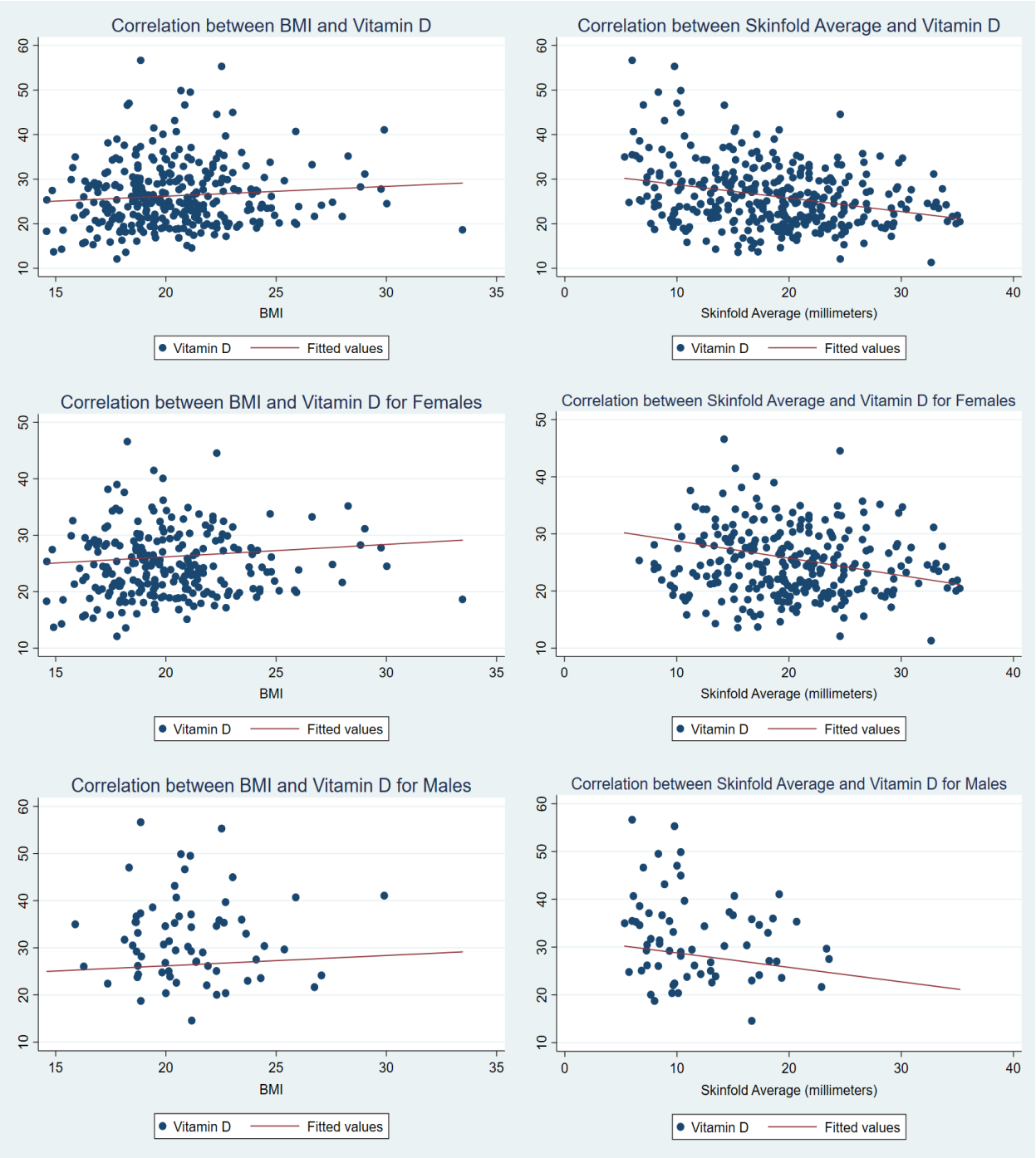
*p<0.05

TABLE 4.5: Male Correlations for Body Composition Variables and Vitamin D

	Vitamin D	BMI	Skinfold average
Vitamin D	1.00		
BMI	-0.01	1.00	
Skinfold average	-0.22	0.68*	1.00

*p<0.05

FIGURE 4.2: Correlations for Body Composition Variables and Vitamin D



A series of multiple linear regression were used to predict total 25(OH) vitamin D based upon the following independent variables: assets, urbanicity, fish consumption, and physical activity all while controlling for adiposity in each model. In the first model, adiposity was determined to be negatively associated with vitamin D (**TABLE 4.6, Model 1**). Subsequent models included the adiposity variable. After controlling for adiposity, assets, urbanicity, fish consumption, and physical activity were all significant predictors of vitamin D (**TABLE 4.6, Models 2-5**).

TABLE 4.6: Adult Environmental Predictors of Vitamin D Controlling for Adiposity for the Total Sample

	Model 1*	***Model 2****	***Model 3****	***Model 4 ****	***Model 5****
adiposity	-0.30±0.06 **	-0.29±0.06**	-0.28±0.06**	-0.28±0.06**	-0.27±0.06**
assets		-0.43±0.19*			
urbanicity			-0.05±0.03*		
Fish consumption				1.10±0.43*	
Physical activity					0.68±0.28*
Model adjusted R_2	0.078	0.089	0.086	0.092	0.092

β±S.E. are given for each model

*p<0.05

**p<0.01

Because of the significant sex difference in vitamin D, stratified regression models were run to consider whether predictors of vitamin D differed by sex. There were no statistically significant predictors of vitamin D for females (**TABLE 4.7**). For males, urbanicity and fish consumption were both significant predictors of vitamin D, while assets was trending but not statistically significant.

TABLE 4.7: Adult Regression Models of Environmental Predictors of Vitamin D Controlling for Adiposity for Females and Males

	Female	***p-value***	**Male***	***p-value***
adiposity	-0.08±0.06	0.231	0.26±0.28	0.362
assets	-0.27±0.20	0.162	-0.95±0.59 †	0.114
urbanicity	-0.02±0.03	0.488	-0.18±0.08 *	0.038
Fish consumption	0.58±0.43	0.172	2.95±1.1 7*	0.015
Physical activity	-0.26±0.42	0.526	0.59±0.44	0.188
adjusted R_2	0.014	0.117	0.179	0.004

β±S.E. are given for each model

*p<0.05

**p<0.01

†p<0.15

Stepwise regression was implemented for the above models. New regression models were run for each model with the cutoff for included variables of $p<0.15$. However, since no variables were near significance in the female regression model, a p-value of less than 0.20 was used instead (**TABLE 4.8**).

TABLE 4.8: Final Adult Regression Models for Environmental Predictors of Vitamin D for Females and Males

	Female	***p-value***	**Male***	***p-value***
assets	-0.28±0.19 †	0.118	-0.80±0.55	0.152
urbanicity	___	___	-0.13±0.07 †	0.080
Fish consumption	0.72±0.41 †	0.079	2.87±1.15 *	0.015
adjusted R_2	0.014	0.015	0.175	0.002

β±S.E. are given for each model

*p<0.05

**p<0.01

†p<0.15

Potential mediators of the sex difference in vitamin D were evaluated with a series of regression models including sex and the predictors considered above (**TABLE 4.9**). As expected, sex was a significant predictor of vitamin D, with lower concentrations for females (Model 1). Adiposity reduced the coefficient for sex by more than one whole unit indicating partial mediation between sex and adiposity in predicting vitamin D. Urbanicity and fish consumption also reduced the coefficient for sex but not at the same magnitude as adiposity. In the full model which includes all sex variables, sex remains significant. Assets and fish consumption are also significant in the full model. Most surprisingly, adiposity becomes non-significant in this model despite being significant in earlier analyses.

TABLE 4.9: Regression Models Controlling for Sex

	Model 1*	***Model 2****	***Model 3****	***Model 4 *****	***Model 5****	***Model 6****	***Model 7****
sex	7.05±0.90**	6.00±1.03**	7.18±0.89**	6.91±0.89**	6.86±0.89**	6.68±0.96**	6.04±1.06**
adiposity		-0.13±0.06*	-				-0.06±0.06
assets		-	-0.55±0.18**				-0.44±0.19*
urbanicity				-0.06±0.03*			-0.04±0.03
Fish consumption					1.30±0.40**		1.03±0.41*
Physical activity						0.27±0.28	0.28±0.27
Model adjusted R_2	0.149	0.160	0.169	0.161	0.171	0.149	0.193

β±S.E. are given for each model

*p<0.05

**p<0.01

4.5 CONCLUSIONS

In vitamin D research, scholars often control for adiposity by using the BMI. BMI is relatively easy to gather and requires less technical expertise than using calipers to gather skinfold thickness. However, results here show that BMI should not always be used as a control for adiposity in certain populations. In fact, BMI shows a positive correlation with vitamin D as opposed to the negative correlation vitamin D and skinfold thickness. Furthermore, in this population, BMI is higher in males than females, which is unexpected. This may be due to the fact that there is a relatively low sample size compared to females. Because females have greater adiposity as measured by skinfold thickness as opposed to BMI, the results could be an indicative of the fact that Asian populations often have higher body fat for a given BMI than their Caucasian counterparts (Deurenberg, Deurenberg-Yap, & Guricci, 2002; Haldar, Chia, & Henry, 2015). Because vitamin D research requires that a set of standards be created in order to more easily compare vitamin D research, it is important that a measure of adiposity be used that applies to all populations.

For females and males, the two groups differed in their mean vitamin D measurement, adiposity, and physical activity. Males were more likely to have higher 25(OH) vitamin D measurements and higher overall physical activity. Females, as expected, were more likely to have higher adiposity scores. Additionally, the female population in this sample have a greater chance of being categorized as deficient or insufficient (< 30 ng/ml) with regards to their vitamin D status as compared to males, and almost 75% of the population are not able to meet their vitamin D requirements.

Comparing the Cebu data to United States data is a bit difficult due to differing deficiency cut-off used. Ginde and colleagues use a deficiency cut-off of 10 ng/ml instead of 20

ng/ml (Ginde, Liu, & Camargo, 2009). There were zero individuals in the Cebu sample that had serum vitamin D less than 10 ng/ml. While there is no way to compare the Cebu sample in terms of deficiency, the percentages for Cebu and the United States samples in the insufficient sample can be compared.

Overall, the NHANES data indicated that 55% of the population had vitamin D levels less than 30 ng/ml during 1988-1994 and that percentage increased to 77% during the 2001-2004 survey (Ginde et al., 2009). This is a similar level to that seen in the Cebu sample as 74.5% of the sample were vitamin D insufficient as well. The NHANES sample stratifies age into four categories:12-19, 20-39, 40-59, and ≥ 60 years. As such, comparison of young adults of similar age in the Cebu sample falls close to two of these age categories (12-19 and 20-39). A comparison of general trends with the Cebu data indicated that only 19.08% of females are vitamin D sufficient in Cebu, putting females somewhere close to Mexican American females of similar age in the earlier NHANES III survey. For males, 53.03 % are vitamin D sufficient which places Cebu men somewhere between white males and Mexican American males from the earlier survey. If vitamin D trends continue with no intervention, we might expect that the percentage of those with sufficient vitamin D to decrease dramatically as seen in the later NHANES survey with less than 10% of females and somewhere between 10-30% of males getting sufficient vitamin D.

Because vitamin D differs dramatically by sex, it was imperative to analyze the sex stratified data to determine if there were any major factors that differed between groups despite the sample differences. The only significant predictor for males was fish, and no significant factors were found for the female group. Furthermore, the differences in fish consumption did

not differ by group. Nevertheless, the difference in fish consumption may represent a gender difference. The predictive nature of fish consumption in predicting vitamin D by males may be due to the fact that fishing is a male dominated occupation (Torell, Castro, Lazarte, & Bilecki, 2021). Comparisons of fishing communities in the Philippines indicates that females are vital to fisheries in their ability to process and sell fish as well as gather other aquatic items such as shells and seas cucumbers for sale (Siar, 2003; Torell et al., 2021). However, females experience an unequal balance of power in access to higher quality fishing and lack a decision making (Kleiber, Harris, & Vincent, 2018; Siar, 2003; Torell et al., 2021) which may translate to constrained social dynamics that limits their ability to consume fatty fish, that are full of vitamin D, and limits occupations in fisheries that expose individuals to the sun. It is also worth noting that the sample size for females and males are not large enough to detect small effect sizes, so it is possible that other environmental variables are just not detectable.

In models not controlling for sex, adiposity, measured by skinfolds, inversely predicted vitamin D as expected. In these models, assets and urbanicity scores inversely predict total 25(OH) vitamin D. When the predictors are stratified by sex, urbanicity and fish consumption were both significant predictors for vitamin D for males but not for females.

The urbanization score, which includes scores based upon population size and density, communications, educational facilities, transportation (vehicles and road composition), health services, and markets, indicates drastic changes in numerous facets of life. These urbanization process indicates that many individuals are spending time in a denser population that is accompanied by infrastructure that often includes multi-story buildings that black sunlight as

well as increases pollution that also serve to decrease vitamin D production. This may indicate that occupation shifts that accompany urbanicity may contribute to reduced vitamin D.

Fish consumption has historically been high in the Philippines as fishing is a major economic endeavor continuously ranking in the top 10 fish producing countries in the world (Bureau of Fisheries and Aquatic Resources, 2020; *Philippine Fisheries Profile, 2005*, 2006). For those that have are deficiency in vitamin D, fish consumption may be a sustainable recommendation for those in Cebu as fish is readily available in many forms such as fresh fish and dried fish, often eaten as snack.

In the total sample, the three predictors of vitamin D in adulthood are sex, assets, and fish consumption. Assets as a proxy for wealth indicates that those that are wealthier have a greater likelihood of having decreased vitamin D levels. Perhaps, these individuals are purchasing more processed food instead of the readily available fish. While it is often the case that vitamin D differs by sex, here we see a dramatic difference wherein more than 80% of females do not have sufficient vitamin D for biological functions compared to less than 50% of males. While more research should be conducted in the population, the vitamin D deficiency levels amongst females and males is a great concern. Future analysis that uses vitamin D to predict subclinical markers of disease might be beneficial in establishing connections between vitamin D and disease states.

CHAPTER 5: Childhood Environment and Vitamin D

5.1 Introduction

While the vitamin D measurement is quantified from a blood sample taken in adulthood, understanding which factors if any in childhood that affect that measurement is important for a few reasons. The first has its roots in life history theory. While life history theory is based on energetics and caloric intake, micronutrients may also play a limiting role in an organism's life investments. Second, as a continuation of the gendered perspective on health, factors that differ from females and males across the life course might point to the fact that cultural perspective on gender might have a lasting health impact in the form of vitamin D deficiency. Childhood environment can have a lasting impact on adult health and this dissertation affords us the opportunity to explore that possible impact with vitamin D. Furthermore, to the authors knowledge, this work is the first set of vitamin D analyses to test the hypothesize that early childhood environments may have an impact on vitamin D in adult life.

5.2 Background

Life History Theory

Life history theory posits that energy is the primary limiting factor to an organism's investments in maintenance, growth, and reproduction (Charnov & Schaffer, 1973; Stearns, Ackermann, Doebeli, & Kaiser, 2000). Organisms must balance these three types of investment over the life course to maximize reproductive capacity. For example, previous research has revealed energetic trade-offs between growth and immune function in humans (T. w. McDade,

Reyes-García, Tanner, Huanca, & Leonard, 2008). However, energy in the form of caloric intake may be an oversimplification, and vitamin D, a micronutrient, may represent an additional limiting factor that must be allocated between these two investments. From a life history perspective, vitamin D would contribute to growth in childhood and maintenance later in life, being used for both bone maintenance and possible immune function, leading to a possible tradeoff and ill health in adulthood.

In the past, vitamin D was seen as a simple compound required for only one system, the skeletal complex (DeLuca, 2008). While vitamin D is required for calcium absorption and overall bone growth (DeLuca, 2008), ongoing research suggests a much broader physiological role for vitamin D throughout the life course. Because early life allocation of resources may have an effect on adult health, this chapter explores whether early life factors have a lasting effect on vitamin D in adulthood.

Organisms use adapted strategies to allocate energetic resources between growth, maintenance, and reproduction, and life history theory is used to explain variation between populations in the three life investments (Charnov & Schaffer, 1973; Reiches et al., 2009). A major element of life history theory is that opportunity costs exist between growth, maintenance, and reproduction (Reiches et al., 2009). For example, energy allocated to one function, such as growth, can only be utilized for that function and may not be used for maintenance or reproduction. Furthermore, allocation to the main functions of growth, maintenance, and reproduction vary over time (Hawkes, O'Connell, Jones, Alvarez, & Charnov, 1998; Hill, 1993). As a child growth and maintenance are the main functions with reproduction not no taking up resources until puberty.

While energy allocation has long provided the foundation for life history theory, another limitation to the basic functions of growth, maintenance and reproduction is micronutrients. Micronutrients are required vitamins and minerals used by the body to carry out basic biological functions. In evolutionary history, these substances were probably closely linked with caloric intake. That is, if enough food was available, then the diet probably reflected adequate micronutrients to carry out biological functions. However, relatively recent evolutionary migration to cities has likely de-coupled these biological necessities. For the first time in evolutionary history a high caloric diet may not be coupled with the same relative amounts of micronutrients. High caloric density is associated with refined and processed foods, which provide high amounts of carbohydrates, sugar, and fat. As such, life history needs to expand to include these elements as a limiting factor for biological functioning. For instance, vitamin D is vital to both bone development/growth and immune function. While up-regulation of the immune system has shown to decrease growth in childhood (T. w. McDade et al., 2008; Reiches et al., 2009), it is possible that trade-off exist for vitamin D wherein the early usage may affect vitamin D levels in adulthood. Because vitamin D deficiency has been implicated in ill health, the early childhood allocation of vitamin D to certain functions, may lead to vitamin D deficiency and corresponding health outcomes.

It is beyond the scope of this paper to research direct trade-offs in vitamin D over time; however, environmental factors in childhood such as birth weight, infant growth, season of birth and breastfeeding duration that might have long term implications for vitamin D deficiency will be investigated. Furthermore, differing impacts of childhood environments may be indicative of

the fact that the social construct of gender, that often but not always maps onto biological sex, can create differing environments that can have health ramifications.

Sex, Gender, and Health

An estimated 5% of the world will develop an autoimmune disease. Of this 5%, approximately 80% of these cases will be women (Fairweather, Frisancho-Kiss, & Rose, 2008). Traditionally research on autoimmune disease focuses on the biological differences between sexes to understand autoimmune disease etiology. While autoimmune disease is often discussed in terms of biological differences in immune system function, rarely does current work explore socio-cultural influences that structure aspects of the environment for females. This perspective may also shed light on the larger gender differences in autoimmune disease risk.

In medical literature, sex and gender are often used interchangeably (Alex, Fjellman Wiklund, Lundman, Christianson, & Hammarström, 2012, p. 201; Nancy Krieger, 2003, p. 003) (Alex at al. 2012, Krieger 2003). However, many scholars argue that these terms should be considered separate categories and used more carefully in scientific literature (Nancy Krieger, 2003; Laner, 2000; Muehlenhard & Peterson, 2011). Sex denotes the biological characteristics such as chromosomes or hormones that pertain to reproduction, and sex can categories include female, male, and intersex (Krieger 2001). Gender refers to the cultural construction of appropriate behavior and roles between men/women and boys/girls (Krieger 2001). Gender can be thought of as defining what constitutes masculine and feminine traits within a society (Muehlenhard and Peterson 2011).

While there are clearly biological differences between the sexes that influence autoimmune disease etiology, there are also environmental conditions that interact with the biological predispositions to manifest in autoimmune disease. However, cultural influences may play a role in disease etiology by influencing the exposure certain groups have to the environment. There are distinct roles and behaviors for both males and females within their own cultural construct. These learned behaviors within a society are gender differences. While these terms should be used separately, this is by no means an argument that humans are either sexed or gendered. Humans are both sexed and gendered simultaneously (Krieger 2003). Therefore, it is time that gender is taken into consideration in disease etiology. Embodiment bridges the terms of sex and gender. Embodiment is useful to understand how the socio-cultural environment, specifically, gender relations in this work are internalized in the body and become part of the body and has health implications (Alex et al., 2012).

The terms used in this paper to discuss differences in sex are male and female, while the terms used to further describe gender are masculinity and femininity. While I do not seek to perpetuate the notion of only two sexes or two genders, discussing the full range of intersex or transgender is beyond the scope of this paper. Gender differences do not necessarily imply inequality. However, gender inequality occurs when one gender is granted more prestige, power, or influence to the detriment of the other gender. This inequality can lead to health disparities when the cultural norms lead to differential customs that lead to differences to healthcare access or predisposes an individual to certain behaviors that lead to differential health. Gender inequality can be said to create a more distinct divide between masculine and feminine behaviors

where masculine and feminine roles are clustered at polar opposites of the spectrum with little overlap between roles.

Differences in model between childhood and adulthood for males and females will be compared to determine if it is likely that gender differences are influencing vitamin D or if sex-linked differences are more likely. It is likely that as children grow, gender expectations between males and females grow resulting in differing health outcomes.

5.3 Methods

Tests of association were analyzed based upon predicted variables in childhood that have previously been linked to vitamin D deficiency. Childhood assets and urbanicity scores were averaged from parent asset and urbanicity score responses in surveys taken from the years 1983, 1986, 1991. The scores were then averaged to create a childhood index score. Least squared regression models were used to determine the predictors of vitamin D, and to investigate potential gender differences in predictors of vitamin D. Then, childhood and adult predictors of vitamin D from the total sample were combined in a single analysis to predict vitamin D. Thus, an early life model of prediction for vitamin D was created to determine what factors across the life course affect vitamin D outcomes in adulthood. Sex differences were investigated using least squares regression models controlling for sex.

5.4 Results

During childhood, participants exclusively breastfed for an average of 87.5 days or almost 3 months. Babies were born weighing approximately 3 kg. Independent samples t-tests

indicated that males had slightly more growth on average than females. In the first two years of life, male babies grew slightly more at an average of 30.82 cm while females grew an average of 29.03 cm for a difference of 1.79 cm. Slightly more than 20% of both males and females were born in the dry season. In childhood, the asset score averaged 2.84, while the urbanicity score was approximately 30 (**TABLE 5.1**).

TABLE 5.1: Descriptive Statistics for Female and Male Participants. Mean (s.d.) are presented for continuous variables.

	Females (n=278)	**Males (n=66)**	**Total (n=344)**
Duration of breastfeeding (days)	86.62 (59.31)	91.33(54.72)	87.52 (58.41)
Birthweight (kg)	3.01 (0.41)	3.07 (0.43)	3.02 (0.41)
Growth (cm/2 yr)*	29.03 (3.31)	30.82 (3.12)	29.39 (3.34)
Birth in dry season (%)	20.85%	24.24%	21.49%
Assets in childhood	2.84 (1.56)	2.83 (1.68)	2.84 (1.58)
Urbanicity in childhood	30.77 (12.92)	30.43 (13.77)	30.70 (13.07)

N=344 for total in each variable except for birth season in which N=349 (F=283 and M=66) and assets/urbanicity in which N=331 (F=265 and M=66)

*$p<0.05$ for means that vary significantly between males and females

A series of multiple linear regression were used to predict total 25(OH) vitamin D based upon the following independent variables: duration of exclusive breastfeeding, birthweight (kg), growth in the first two years of life (cm), birth in dry season, average household asset number in childhood, and average urbanicity score in childhood. In the first model, duration of exclusive breastfeeding was determined to be positively associated with vitamin D (**TABLE 5.2, Model**

1). Additionally, assets and urbanicity score in childhood were also found to be significant predictors of vitamin D (**TABLE 5.2, Models 5-6**). However, assets and urbanicity negatively predicted vitamin D. That is, those participants from wealthier and more urbanized households had lower vitamin D levels.

TABLE 5.2: Regression Models for Childhood Environmental Predictors of Vitamin D for the Total Sample

	Model 1	***Model 2***	***Model 3***	***Model 4***	***Model 5***	***Model 6***
Duration of exclusive breastfeeding (months)	0.01±0.01 *					
Birthweight (kg)		1.25±0.93				
Growth (cm/2 yr)			0.01±0.12			
Birth in dry season				-0.83±0.93		
Assets in childhood					-0.73±0.25**	
Urbanicity in childhood						-0.09±0.03**
Model adjusted R_2	0.010	0.002	-0.003	--0.001	0.023	0.023

β±S.E. are given for each model

*p<0.05

**p<0.01

All significant variables from TABLE 5.2 were run in a multiple linear regression to create a childhood model (**TABLE 5.3).** Only childhood assets and the urbanicity score were significant Because of the significant sex difference in vitamin D, stratified regression models were also run to consider whether predictors of vitamin D differed by sex. Both sex stratified models were significant, but no variables were found to be significant for the either model stratified model.

TABLE 5.3: Regression Models for Childhood Environmental Predictors of Vitamin D for Females, Males, and Total Sample

	Females*	***p-value***	***Males****	***p-value***	***Total*** **	***p-value***
Sex	____		_____	_____	6.89±0.89 **	0.00
Duration of exclusive breastfeeding (days)	0.01±0.01 †	0.09	-0.02±0.02	0.44	0.01±0.01	0.28
Assets in childhood	-0.31±0.24	0.20	-1.38±0.74 †	0.07	-0.52±0.24 *	0.03
Urbanicity in childhood	-0.04±0.03	0.23	-0.14±0.08 †	0.10	-0.06±0.03 *	0.03
Model adjusted R_2	0.022	0.03	0.085	0.02	0.18	0.00

β±S.E. are given for each model
*p<0.05
**p<0.01
†p<0.15

Stepwise regression was implemented for the above models. New regression models were run for each model with the cutoff for included variables of $p<0.15$ resulting in **TABLE 5.4**.

TABLE 5.4: Final Childhood Regression Models for the Environmental Predictors of Vitamin D for Females, Males, and Total Sample

	Females*	***p-value***	***Males****	***p-value***	***Total*** **	***p-value***
Sex	____	____	_____	_____	6.92±0.89 **	0.00
Duration of exclusive breastfeeding (days)	0.01±0.01*	0.03	____	____	____	____
Assets in childhood	____	____	-1.18±0.69 †	0.09	-0.56±0.24 *	0.02
Urbanicity in childhood	____	____	-0.14±0.08 †	0.11	-0.06±0.03 *	0.02
Model adjusted R_2	0.014	0.03	0.01	_____	0.18	0.00

β±S.E. are given for each model
*p<0.05
**p<0.01
†p<0.15

A multiple linear regression model was then performed with significant childhood and adulthood predictors of vitamin D to create a lifetime predictors of vitamin D model. Significant adult factors came from the previous analyses in chapter 4. Variables were tested based on a cutoff of $p<0.15$ from the adult and child analyses. For the total population, childhood predictors

included assets in childhood as well as the childhood urbanicity score, and adulthood factors from the total population included assets in adulthood and fish consumption. In the total population, sex was controlled for in the final model (**TABLE 5.5**). For females, no predictive variables were significant in the adult model, so a cut off of p<0.20 was used to determine which adult factors to include. For the male model, no variables were significant from childhood.

TABLE 5.5: Initial Regression Models for Lifetime Environmental Predictors of Vitamin D for Females, Males, and Total Sample

		Females	***Males****	***Total****
	Sex ***(female=0, male=1)***	_____	_____	6.90±0.88 **
Childhood Environmental Factors	**Duration of exclusive breastfeeding (*days*)**	0.01±0.01 †	_____	_____
	Childhood assets	_____	-0.80±0.68	-0.33±0.25
	Urbanicity in childhood	_____	-0.19±0.14	-0.05±0.03 †
Adult Environmental Factors	**Assets**	-0.30±0.19 †	____	-0.33±0.21 †
	Urbanicity	_____	0.02±0.13	____
	Fish consumption	0.55±0.42	3.21±1.13 **	1.02±0.42 *
	Model Adjusted R_2	0.014	0.187	0.200

β±S.E. are given for each model
*p<0.05
**p<0.01
†p<0.15

Stepwise regression was implemented for the above models. New regression models were run for each model with the cutoff for included variables of $p<0.15$ (**TABLE 5.6**). For the final lifetime mode, only breastfeeding was significant for females, and only fish consumption was significant for males.

TABLE 5.6: Final Regression Models for Lifetime Environmental Predictors of Vitamin D for Females, Males, and Total Sample

		Females *	***Males*****	***Total*****
	Sex ***(female=0, male=1)***	_____	_____	6.90±0.88 **
Childhood Environmental Factors	**Duration of exclusive breastfeeding (*days*)**	0.01±0.01 *	_____	_____
	Urbanicity in childhood	_	_____	-0.06±0.03 *
Adult Environmental Factors	**Assets**	-0.32±0.18 †	_____	-0.43±0.19*
	Fish consumption	_____	3.24±1.16**	1.07±0.42 *
	Model Adjusted R_2	0.022	0.095	0.199

β±S.E. are given for each model

*p<0.05

**p<0.01

5.5 CONCLUSIONS

In terms of investigating variables that are rooted in life history theory such as birth weight and growth, no evidence was found to indicate that either variable had lasting impacts on vitamin D long-term. The variables in childhood that predicted circulating vitamin D

were duration of exclusive breastfeeding, assets in childhood, and urbanicity in childhood. When stratifying each model by sex, only duration of breastfeeding is significant for females and breastfeeding accounts for less than 2% of the variability see within vitamin D for females. For males, only fish consumption is significant for the lifetime model. As mentioned in the previous chapter, fish consumption is an indication of a gendered social construct, namely fishing as a male occupation, that may have health benefits in men.

Breastfeeding as a predictor of vitamin D was surprising as it is often thought that breastfeeding can be a risk factor for vitamin D deficiency. Breastfeeding as a risk factor is due to the fact that vitamin D is not generated in breastmilk. Instead, vitamin D passes from mother to baby via breastmilk. Recently, there has been some worry that babies need to be supplemented with vitamin D as breastmilk may only contain minimal vitamin D. However, breastfeeding has been found to decrease childhood obesity risk (Metzger & McDade, 2010; Yan, Liu, Zhu, Huang, & Wang, 2014). While studies on obesity risk and breastfeeding are primarily conducted on children, the study by Metzger and McDade included children as old as 19 indicating that the protective effect of breastfeeding last at least as long as adolescence (Metzger & McDade, 2010). If the reduction in body fat does last long-term into early adulthood, breastfeeding may allow for greater circulating vitamin D in females from a reduction in adiposity.

The protective nature of breastfeeding in females may be an interaction of both gender and sex, wherein a social dynamic that dictates breastfeeding length has proven protective for one sex over another. Males are actually breastfed longer than females in this sample with males being breastfed 4.71 days longer than females although the difference is not statistically significant. However, the difference is significant for females but not males indicating that

females alone may benefit from the decrease in adiposity, a sex-linked trait driven by hormones to increase the fat deposition in females.

Furthermore, breastfeeding positively predicting vitamin D, may point to cultural practices around infant care as a reason breastfeeding is associated with higher vitamin D. It is possible that women in the Philippines who are more likely to breastfeed are practicing infant carrying at greater rates to provide nourishment for their babies. A byproduct of this carrying may be the increased chances for both mother and infant sun exposure allowing the infant to gain an increase vitamin D through their own solar production and through the passive transfer of vitamin D through feeding.

In the complete childhood model for the sample, childhood urbanicity score and childhood assets were found to negatively predict vitamin D. In a comparative analysis with China and the United States, higher household income in the Chinese elderly predicted decreased vitamin D but that was not the case in the United States (Wei, Zhu, & Ji, 2019). The authors surmised that rapid urbanization was responsible for this phenomenon and that those with greater assets are more likely to live in urban centers resulting in less sun exposure. In a related CLHNS analysis, Dahly and colleagues found that higher SES, as measured by assets, was positively associated with increased adiposity in males and after controlling for urbanicity, also associated with increased adiposity in females living in rural environments (Darren L. Dahly, Gordon-Larsen, Popkin, Kaufman, & Adair, 2010). Taken together, it seems that those with higher assets may live in urban centers that have a myriad of factors such as pollution that led to decreased vitamin D, and for those living within rural areas with higher SES, higher adiposity may lead to decreased circulating vitamin D as well.

When all the variables were combined from childhood and adulthood to create the lifetime vitamin D model, sex, childhood urbanicity, current adult assets, and fish were significant predictors of vitamin D. The persistence of urbanicity as predictor of vitamin D into adulthood may be indicative of the lasting effects of urbanization on health. While urbanizations might be thought of as beneficial to health outcomes, initial urbanization settlement is a difficult transition and results in long lasting health implications.

For instance, records from the 19th century in Britain indicate that urban dwelling resulted in increased mortality despite higher wages (Szreter & Mooney, 1998). Another example is the height decline seen in children during the industrial revolution in the United States (Komlos & A'Hearn, 2017). The transition to urban dwelling has lasting effects on health, and this research indicates that one of those lasting effects may be on vitamin D levels in children undergoing that transition.

It is glaringly apparent that sex makes a huge impact to the analysis, and it is still unclear whether the differences in vitamin D are sex-linked biological or cultural in nature. Perhaps, the most surprising aspect of the full model controlling for sex is that adiposity loses significance once sex is added to the model. Clearly, there is a difference in fat deposition for between females and males. Much of the influence of fat on vitamin D may be encompassed in controlling for sex.

Unfortunately, the lifetime models for females and males did not indicate differences across the life course. However, the sample sizes for females and males were too small to detect small effects, which may mean that there are other aspects of the environment that were tested but were undetectable with the current sample. Future analysis may benefit from a composite

score of gender equality or equity to attempt to create a better picture of how gender dynamics change over time and possible influence vitamin D levels.

In terms of gendered medicine, this lack of differences may indicate that there are no overarching gendered differences that just apply to either sex. Clearly, there are socio-cultural environmental conditions that influence vitamin D, but they affect the entire population undergoing urbanization. It may just be that for females the environmental factors are compounding to dictate their health more dramatically.

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CHAPTER 6: Methylation and Vitamin D

6.1 Introduction

Epigenetics is recognized as a mechanism in which the lived experience within an environment is incorporated into the body, a process which often described metaphorically as "getting under the skin" by anthropologists. The study of epigenetic mechanisms is central to research on developmental plasticity and the long-term effects of early life environments. Epigenetics is a mechanism by which heritable instructions result in genome function despite no change to the genetic code, and it is a plausible mechanism through which environments during development can regulate genome activity in adulthood. DNA methylation, the addition of methyl groups to cytosines in the DNA sequence, is one such epigenetic process that results in either upregulation or repression of gene transcription. The use of a biocultural anthropological approach allows for an exploration of how lived experience affects vitamin D production and health over the life course.

By studying which socio-cultural factors may contribute to methylation and circulating vitamin D, this chapter seeks to understand how life experiences become embodied to create biological consequences and variation among individuals. This chapter investigates the following: 1) whether methylation is a contributing factor to vitamin D variation and 2) determine whether social/environmental conditions measured in the previous chapter predict methylation of genes involved in vitamin D production . Overall, this chapter investigates how factors in childhood or young adulthood influence vitamin D human variation in a single generation without changing the underlying genetics of an individual, and whether there exist sex

differences in methylation that drive the circulating serum vitamin D variances seen between males and females.

6.2 Background

The developmental origins of health and disease (DoHaD) states that early life can have a lasting effect on long-term health (D. J. P. Barker, 2007). Epigenetics is a plausible biological mechanism in which the body can take-up instruction from early life environments and create a lasting reaction that leads to long-term health. Vitamin D has been linked to several diseases (Basson, 2014; Borges et al., 2011; Hyppönen et al., 2001; Smolders et al., 2008), which may indicate that early environments affecting vitamin D production may lead to the disease states by way of epigenetic processes.

Developmental Origins of Disease and Health

The failure of the human genome project to account for every health state through total genetic susceptibility led researchers to realize that other mechanisms were at work in the human body than just our genetic blueprint. Then, when David Barker published data linking fetal undernutrition to low birth weight and later cardiovascular disease (D. J. P Barker et al., 1989), researchers recognized that indicating that early life development can impact health in adulthood. With the inclusion of developmental plasticity (D. J. P. Barker, 2007), the Developmental Origins of Disease and Health (DOHaD) hypothesis expanded to include critical time periods in childhood as well as fetal development that influenced disease later in life.

The ‘Barker Hypothesis’ initially focused on fetal undernutrition and the lasting effect on heart health (D. J. P Barker et al., 1989). It stands to reason that restricted vitamin D, a vital nutritional component, early in life may also have a lasting effect on long-term health as an adult. While testing whether vitamin D deficiency in infancy and childhood is beyond the scope of this research, testing whether DNA methylation as an epigenetic process influences vitamin D is plausible. DNA methylation may be a signal to the body to produce vitamin D at certain rates because of early life environments.

Vitamin D Pathway

As mentioned previously, the production of vitamin D occurs through the epidermis (via UV exposure) or through intestinal absorption (via food or vitamin consumption) (Wagner et al., 2008). Upon human skin to sunlight, 7-dehydrocholesterol is converted to vitamin D3 by ultraviolet B radiation in a heat dependent process (Holick, 2007; Serrano et al., 2017). Both the dermis absorbed vitamin D3 and the ingested vitamin D2 binds to vitamin D-binding protein (Yousefzadeh et al., 2014). Then, in a sequence of hydroxylation events vitamin D is converted to its active form via catalyzation by one of several types of enzymes in the cytochrome (**CYP**) P450 family (Christakos et al., 2012; Yasutake et al., 2010).

After absorption, vitamin D is transferred to the liver via the bloodstream and is hydroxylated by **CYP2R1** and **CYP27A1** to produce the circulating 25-hydroxy vitamin D (25(OH) D) (Holick, 2007; Hossein-nezhad & Holick, 2013, p. 201; Yasutake et al., 2010). Then, the vitamin D derivative travels to the kidneys where it is hydroxylated again by 25(OH) D-1-α-hydroxylase (**CYP27B1**) to form 1,25-dihydroxy vitamin D ($1,25(OH)_2$ D), also known as

calcitriol (Hossein-nezhad & Holick, 2013; Smolders et al., 2008). Biological functions are then performed through 1,25$(OH)_2$D binding to vitamin D receptor (**VDR**) sites to regulate gene expression (Holick, 2007). (**FIGURE 6.1)**

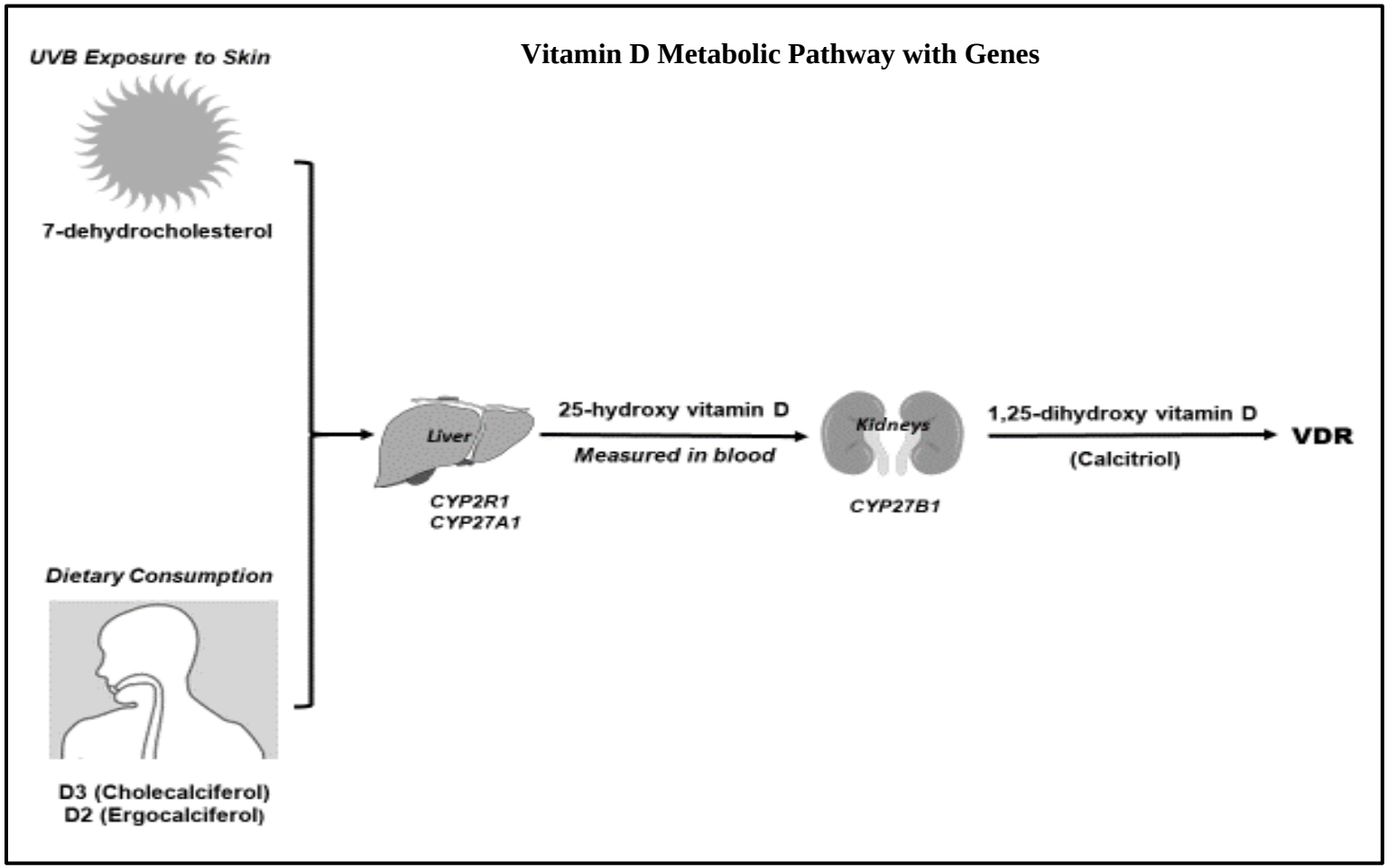

FIGURE 6.1. Vitamin D can be obtained via sun exposure and through food consumption. Both routes undergo hydroxylation events via Cytochrome P450 genes, CYP2R1 and CYP27A1, to produce 25-hydroxy vitamin D in the liver. After another hydroxylation event via CYP27B1 in the kidneys, 1, 25-dihydroxy vitamin D is formed, which is then regulated via the vitamin D receptor (VDR) genes. VDR sites are expressed in various tissues and organs all over the body**.**

Though $1,25(OH)_2$ D is the biologically active form of vitamin D, serum 25(OH)D is used to determine vitamin D status (Hossein-nezhad & Holick, 2013; Yousefzadeh et al., 2014) since serum $1,25(OH)_2$D can be normal or even elevated in secondary hyperparathyroidism, resulting in an inaccurate representation of vitamin D status (Hossein-nezhad & Holick, 2013). Vitamin D receptors are located throughout the body, and upon binding 1,25(OH)2, they up- or downregulate approximately 2000 different genes in the skeleton, skin, intestines, liver, thymus, spleen, lymph nodes, mammary glands, and testis, among other organs and tissue (Holick, 2007; Hossein-nezhad & Holick, 2012, 2013; Pike & Meyer, 2014; Ramagopalan et al., 2010; Wang et al., 2012).

Genes in Pathway

The vitamin D transduction process utilizes a series of cytochrome P450 enzymes that hydroxylates the inactive form of D_2 and D_3 (G. Jones, Prosser, & Kaufmann, 2014a). The process transforms vitamin D into a usable compound to be bound by the vitamin D receptor (G. Jones, Prosser, & Kaufmann, 2014b; Wikvall, 2001).

CYP2R1

CYP2R1 is also known as 25-hydroxylase . The gene is found on chromosome 11 and found to consist of five exons and four introns that are approximately 15kb long (J. B. Cheng, Levine, Bell, Mangelsdorf, & Russell, 2004).

CYP27A1

CYP27A1 is found on chromosome 2 (Cali, Hsieh, Francke, & Russell, 1991). The gene is also called sterol 27-hydroxylase, whereas the A1 designates subfamily A polypeptide 1 (MedlinePlus [Internet], 2021). The gene contains 9 exons and 8 introns and encompasses at least 18.6 kb of DNA (Leitersdorf et al., 1993).

CYP27B1

CYP27B1 is more commonly 25-hydroxyvitamin D3-1-alpha-hydroxylase. The gene is located on chromosome 12 and spans approximately 5 kb, comprising of nine exons and eight introns (Portale & Miller, 2000).

Vitamin D receptor (calcitriol)

The vitamin D receptor is also referred to as VDR and is formally named calcitriol. The VDR consists of 11 exons that span 75 kb (Miyamoto et al., 1997). The VDR allows the body to respond to the active form of vitamin D, the hormone 1α,25-dihydroxyvitamin D_3 (Malloy & Feldman, 2010), and these receptors are located in various tissues throughout the body. Because these sites are located in systems outside of the skeletal system, renewed interest in studying vitamin D has occurred.

6.3 Methods

Gene methylation was assessed from venous blood samples from the 2005 survey. In 2005 blood samples were taken from 1,759 participants, and methylation analysis was conducted

using the Illumina Human Methylation 450 Bead Chip. Genomic DNA was treated with sodium bisulfite, and converted DNA was applied to the Illumina Human Methylation 450 Bead Chip using the manufacturer's standard conditions. Methylation analysis was conducted on a subsample of 395 female participants, chosen based upon their participation in a 2009 pregnancy tracking study, and 100 male participants. Quality control was performed via sex chromosome probes to confirm participant sex, which were then removed from further analysis. Unreliable probes with a detection p-*value* greater than 0.01, with fewer than three beads contributing to the signal, and those previously shown to bind to multiple genomic regions were also removed, leaving 434,728 probes. Raw data from Illumina Genome studio are loaded into the lumi R package, which converts array intensity values to both beta values (proportional methylation values from 0 to 1) and M values (Du et al., 2010). The M-value is the $\log_2$ ratio of the intensities of the methylated probes versus the unmethylated probe, and a negative value means that more CpG sites are unmethylated than methylated, while a positive values indicated greater methylation (Du et al., 2010).

From these probes, the study analyzed vitamin D metabolism enzyme genes (*CYP2R1, CYP27B1, CYP24A1)* and the *vitamin D receptor gene (calcitriol)* resulting in 73 probes analyzed. Those missing data were excluded, and individual variability within these probes was filtered to exclude probes for which variability in beta-values between the 10^{th} and 90th percentiles was greater than 5%. There were 11 probes left to analyze. Bivariate analyses were performed on these 11 probes.

6.4 Results

Methylation level for each of the eleven sites was averaged (TABLE 6.1). These sites represent the genes CYP27A1, CYP27B1, CYP24A1, and the vitamin D receptor in the vitamin D metabolism pathway.

TABLE 6.1: Methylation M-Value at each vitamin D site

Probe	Symbol	Gene region	Methylation M-Value (Average and s.d.)
cg01872077	CYP27A1	TSS200	-3.53±0.27
cg26104932	CYP27A1	Body	-0.00±0.20
cg01182309	CYP27B1	Body	-2.98±0.34
cg02547054	VDR	5'UTR	1.37±0.19
cg04905829	CYP27B1	5'UTR	0.82±0.15
cg05190176	VDR	5'UTR	0.08±0.13
cg07060721	CYP27B1	Body	-1.95±0.19
cg10592901	VDR	Body	-0.09±0.23
cg18413900	CYP27B1	TSS200	0.99±0.13
cg00287413	CYP24A1	Body	2.67±0.26
cg18956481	CYP24A1	5'UTR	-2.59±0.23

Vitamin D values and methylation at each gene along the vitamin D pathway site were run in a Pearson correlation matrix (**TABLE 6.2**). Correlation values for serum vitamin D and methylation values with $P<0.1$ are marked as significant. Each probe cg- designation corresponds to either CYP27A1, CYP27B1, CYP24A1, or the VDR gene associated with a step in the vitamin D production process.

TABLE 6.2: Correlation Table for Each Genes and Vitamin D

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) Vitamin D	1.000											
(2) cg01872077	-0.072	1.000										
(3) cg26104932	-0.106*	-0.069	1.000									
(4) cg01182309	-0.042	-0.043	-0.002	1.000								
(5) cg02547054	-0.054	0.025	0.033	0.061	1.000							
(6) cg04905829	0.063	0.025	0.322*	-0.062	0.001	1.000						
(7) cg05190176	0.098†	0.025	0.062	-0.074	0.158*	0.136*	1.000					
(8) cg07060721	-0.092†	-0.056	0.247*	-0.016	0.086	0.140*	0.097	1.000				
(9) cg10592901	0.023	-0.011	0.076	-0.040	-0.022	0.036	-0.042	0.051	1.000			
(10) cg18413900	0.023	-0.104	0.123*	-0.004	0.122*	0.237*	0.236*	0.152*	0.070	1.000		
(11) cg00287413	-0.060	-0.087	0.023	-0.004	0.023	-0.087	-0.001	0.105	0.048	0.018	1.000	
(12) cg18956481	-0.026	-0.016	0.033	-0.004	0.029	0.133*	-0.031	0.036	-0.113*	0.090	-0.023	1.000

** $p<0.05$, † $p<0.1$*

For each probe, female and male methylation average was compared (**TABLE 6.3**). Statistically significant differences were found between cg26104932, cg05190176, and cg07060721. Females had greater on average methylation at sites cg26104932 and cg07060721.

TABLE 6.3: Female/Male Methylation Comparison by Site

Probe	**Symbol**	**Female (n=283)**	**Male (n=66)**
cg01872077	CYP27A1	-2.65(0.27)	-2.66 (0.26)
cg26104932 *	CYP27A1	0.03 (0.19)	-0.15 (0.02)
cg01182309	CYP27B1	-2.96 (0.34)	-3.03 (0.33)
cg02547054	VDR	1.38 (0.18)	1.37 (0.20)
cg04905829	CYP27B1	0.81 (0.15)	0.83 (0.15)
cg05190176*	VDR	0.07 (0.13)	0.15 (0.13)
cg07060721*	CYP27B1	-1.93 (0.19)	-2.03 (0.02)
cg10592901	VDR	-0.09 (0.23)	-0.08 (0.23)
cg18413900	CYP27B1	0.99 (0.14)	1.00 (0.12)
cg00287413	CYP24A1	2.67 (0.02)	2.65 (0.04)
cg18956481	CYP24A1	-2.60 (0.23)	-2.59 (0.24)

* statistically different means at $p<0.05$

For each probe that was statistically different on average between females and males, a linear regression model was run with and without controlling for sex to determine if methylation predicted vitamin D. With the exclusion of cg26104932 (CYP27A1), no methylation site was not found to be predictive of circulating serum vitamin D independent of sex. Furthermore, when controlling for sex, cg26104932 (CYP27A1) was no longer significant.

TABLE 6.4: Regression of Methylation Sites with and without Controlling for Sex

	Symbol	Model 1*	Model 2**	Model 3†	Model 4**	Model 5†	Model 6
Sex *(female=0, male=1)*	___	___	7.28±0.96**	___	7.02±0.92**	___	7.00±0.92**
cg26104932	***CYP27A1***	-3.72±1.87*	1.26±1.85	___	___	___	___
cg05190176	***VDR***	___	___	5.21±2.85	0.41±2.72	___	___
cg07060721	***CYP27B1***	___	___	___	___	-3.38±1.96	-0.48±1.86
R^2 adjusted	___	0.008	0.148	0.007	0.146	0.009	0.147

β±S.E. are given for each model
*p<0.05
**p<0.01
†p<0.10

While DNA methylation was not found to predict vitamin D while controlling for sex, further investigation into whether any environmental factors in childhood or adulthood is correlated with DNA methylation was also investigated. A Pearson correlation table for each environmental factor in childhood and adulthood was run for each CpG site differentiated by sex (**TABLES 6.5 and 6.6**). Correlation between DNAM and environmental factors in childhood and adulthood differed by sex and are worth future investigation. For females, birth in the dry season, growth, childhood assets, and childhood urbanicity all had 1 or more methylated genes correlated with each factor. For males, birth in the dry season, duration of breastfeeding and childhood urbanicity all had one or more methylated genes associated with each factor. Adulthood environmental factors that correlated with one or more methylated CpG sites for women included assets, urbanicity, fish, and outdoor activity, while males only had fish and outdoor activity associated with CpG sites.

TABLE 6.5: Correlation Table for Each Gene with Adult and Childhood Environmental Variables for Females

DNAm Probe Designation	Childhood Environment						Adult Environment				
	Birth in dry season	*Birth Weight*	*Growth*	*Duration of BF*	*Child Assets*	*Child Urbanicity*	*Assets*	*Urbanicity*	*Fish*	*Adiposity*	*Outdoor Activity*
cg01872077	0.001	0.040	-0.030	0.001	**-0.192***	-0.101	-0.042	**-0.112†**	0.042	0.003	0.007
cg26104932	-0.016	0.040	-0.030	0.001	**-0.212***	-0.101	-0.042	**-0.112†**	0.042	0.003	0.007
cg01182309	-0.028	0.044	-0.014	0.052	0.034	-0.007	**0.110†**	0.061	0.010	-0.028	0.012
cg02547054	0.025	0.023	-0.077	-0.048	**0.122***	0.012	0.096	-0.031	0.024	-0.088	**0.140***
cg04905829	0.056	0.004	-0.049	-0.003	-0.110†	-0.095	-0.099†	-0.071	0.042	0.009	0.017
cg05190176	0.023	0.009	-0.030	-0.056	-0.049	-0.014	0.002	0.028	**-0.148***	0.025	0.029
cg07060721	-0.094	0.042	**0.118†**	0.021	-0.034	0.066	0.007	-0.034	-0.047	0.0170	-0.022
cg10592901	0.079	0.075	0.015	-0.063	0.062	0.057	-0.004	0.023	-0.034	-0.043	0.053
cg18413900	0.096	0.040	-0.039	0.012	-0.047	**-0.137***	-0.080	**-0.202***	0.049	-0.098	0.046
cg00287413	**0.131***	0.052	0.071	0.008	-0.028	-0.026	0.042	-0.011	-0.003	0.099†	0.038
cg18956481	-0.011	-0.073	0.008	-0.015	-0.069	-0.035	0.017	0.011	0.078	-0.066	0.063

** p<0.05*
† p<0.1

TABLE 6.6: Correlation Table for Each Gene with Adult and Childhood Environmental Variables for Males

DNAm Probe Designation	Childhood Environment					Adult Environment					
	Birth in dry season	*Birth Weight*	*Growth*	*Duration of BF*	*Child Assets*	*Child Urbanicity*	*Assets*	*Urbanicity*	*Fish*	*Adiposity*	*Outdoor Activity*
cg01872077	0.036	0.045	0.050	0.033	0.025	0.107	0.099	0.072	0.153	0.016	-0.183
cg26104932	0.138	0.115	-0.070	-0.108	-0.034	-0.086	0.022	-0.056	**0.244***	-0.007	**0.254***
cg01182309	-0.013	-0.025	-0.187	-0.100	-0.198	0.102	-0.179	0.032	-0.133	-0.052	0.042
cg02547054	0.041	0.038	0.133	-0.188	0.194	0.014	0.049	0.110	-0.159	0.070	0.024
cg04905829	**-0.233†**	0.157	-0.063	-0.078	-0.094	-0.033	-0.065	-0.079	0.128	-0.064	0.026
cg05190176	-0.131	-0.076	0.028	-0.049	0.036	0.013	0.128	0.056	-0.049	-0.183	0.119
cg07060721	0.057	0.118	0.046	**-0.208†**	-0.058	0.087	-0.047	0.075	**-0.226†**	0.099	**0.251***
cg10592901	0.080	-0.062	-0.060	0.137	-0.064	-0.016	-0.082	0.109	0.096	-0.164	0.197
cg18413900	-0.032	0.058	-0.134	**0.220†**	-0.160	0.137	-0.078	0.135	0.081	-0.149	**0.268***
cg00287413	0.135	-0.136	-0.138	-0.007	0.173	-0.090	0.005	-0.092	-0.031	-0.119	0.127
cg18956481	0.143	0.132	0.137	0.171	**-0.212†**	0.073	-0.047	-0.041	0.104	-0.083	-0.056

** p<0.05*
† p<0.1

6.5 Conclusions

DNA methylation of genes in the vitamin D pathway were not found to be predictive of circulating serum vitamin D levels. DNA methylation is one epigenetic mechanism in which the body may assess the physical and social environment and create a lasting message in a body to predict the future. For vitamin D, methylation was not shown to leave a lasting mark on total 25(OH) vitamin D despite childhood urbanicity having an impact on vitamin D in adulthood. Because the production of vitamin D is vital to function throughout the life course, methylation, and the up- and down-regulation of certain genes in the pathway may not be beneficial long-term to human health.

Because childhood urbanicity is negatively associated with vitamin D in adulthood, it is still possible that there is some epigenetic mechanism at play. However, I think that is more likely that like urbanization during the industrial revolution that transition from rural to urban is a process that affects a multitude of environmental factors such as pollution, infrastructure, and diet. It is more likely that those factors affected by the urbanization process is what leads to decreased vitamin D in adulthood, and those downstream repercussions of urbanization create lasting environmental changes that persist into adulthood.

While no significant effect of methylation was found on vitamin D, the effect of methylation on gene variants were not tested. It is also possible that methylation interacts with gene variation to reduce serum vitamin D. Gene variants in the vitamin D pathway have shown to lead to reduced circulating vitamin D (Bakos et al., 2020; Jamka et al., 2018; Pillai et al., 2011). Future analysis may benefit from an analysis that includes gene variants as well as methylation at these sites for each variant to determine if the variants and methylation interact to

reduce circulating vitamin D in this population. However, comparisons between females and males would most likely not be possible because multiple variants would be tested splitting the male group by variants. An analysis of methylation of gene variants would require the total sample to detect differences across the variants.

Overall, it seems that childhood may not dictate vitamin D in adulthood. The impact of this on overall health seems promising because results indicate that doctors and individuals can continue to influence a person's vitamin D status into adulthood. However, the data does not add to the body of literature linking vitamin D to various diseases. Is the link with vitamin D just coincidental? Perhaps, vitamin D deficiency is a result of those with an illness getting less sun exposure due to staying indoors for more optimal comfort. And further still, an illness may utilize greater vitamin D in immune function resulting in vitamin D deficiency. Further research is warranted to better understand if greater immune activation results in a greater usage of vitamin D.

While methylation did not affect total serum vitamin D, environmental factors may still affect methylation patterns in the vitamin D pathway. TABLES 6.5 and 6.6 indicate that there are differences in environmental factors that correlate with methylation patterns, and these factors vary by sex across the lifespan. Some DNA methylation differences seem to reflect the differences between females and males seen in earlier environmental factors such as breastfeeding and fish consumption. Perhaps, some of the environmental factors lead to differential methylation, but only further investigation will illuminate this relationship.

CHAPTER 7: Conclusions

7.1 Summary

Overall, vitamin D in this population differed between females and males differed in their mean vitamin D measurement with males were more likely to have higher 25(OH) vitamin D measurements than females. Additionally, the female population in this sample is more likely to be categorized as deficient (< 20ng.mL) or insufficient (< 30 ng/mL) with regards to their vitamin D status as compared to males, and almost 75% of the population are not able to meet their vitamin D requirements.

Furthermore, females were more likely to have greater adiposity and less physical activity than their male counterparts. Here, research indicates that the measure of adiposity requires the more nuanced and rigorous measurement of collecting skinfold thickness. In the NIH 2008 conference on vitamin D gaps (Brannon et al., 2008), researchers emphasized the need to control for adiposity by using BMI. However, the research results on body composition and vitamin D indicate that BMI is not the best control for adiposity in this population. Skinfold thickness is a much better measure of adiposity. Without the more rigorous measurement of skinfold thickness, BMI would portray an unrealistic of the relationship between adiposity and vitamin D.

Because vitamin D can differ by sex, it is vital to vitamin D research that sex stratification is conducted and reported. For adulthood environment, no major differences were found between the female and male groups. The only significant predictor for males in adulthood was fish consumption, and no significant factors were found for female.

However, evidence for the total population did elicit some interesting results. For instance, the three biggest factors in determining vitamin D in adulthood are sex, assets, and fish consumption with the strongest predictor being sex. Assets inversely predicted total 25(OH) vitamin D, while those who consumed the most fish per week were more likely to have higher vitamin D levels. assets as a proxy for wealth indicates that those that are wealthier have a greater likelihood of having decreased vitamin D levels. Taken together, these results indicate that individuals who are wealthier many be purchasing more processed food instead of the readily available fish rich in vitamin D.

For the childhood environment, the negative results are in many ways just as interesting as the positive results. For instance, because growth utilizes vitamin D, greater growth might correlate with reduced vitamin D production instead no evidence was found to indicate that early growth had lasting impacts on vitamin D over the long-term. However, the results might be indicative that those that do grow more in the first two years of life might actually have an abundance of vitamin to grow.

The variables in childhood that predicted circulating vitamin D were duration of exclusive breastfeeding, assets in childhood, and urbanicity in childhood. Because breastfeeding has recently become a cause for concern due to breastfeeding not producing vitamin D (Wagner et al., 2008), instead passing vitamin D from mother to child, breastfeeding as a positive predictor of vitamin D was enlightening. However, in the complete childhood model only the childhood urbanicity score and childhood assets predicted vitamin D. Childhood urbanicity and childhood assets both negatively predict vitamin D.

The predictors of vitamin D in childhood and adulthood were then combined to create a lifetime predictive model of circulating vitamin D. The lifetime predictors of vitamin D were sex, childhood urbanicity, fish consumption, and adult assets. When stratifying each model by sex, only duration of breastfeeding is significant for females for the lifetime model. For males, only fish consumption is significant for the lifetime model. While vitamin D differs dramatically by sex, no lifetime explanation was found based upon the tested environmental factors from childhood and adulthood.

On interesting aspects of these regressions is that once sex is introduced into the adult equation, adiposity no longer becomes significant in the model in predicting vitamin D. In fact, sex is a strong predictor of vitamin D in this population, with females more likely to be deficient in vitamin D. Clearly, there is a difference between those that identify as females and males; however, no dominant predictors were found to explain the disparity between the two groups. DNA methylation of genes in the vitamin D pathway were not found to be predictive of circulating serum vitamin D. DNA methylation is one epigenetic mechanism in which the body may assess the physical and social environment and create a lasting message in a body to predict the future. For vitamin D, methylation was not shown to leave a lasting mark on total 25(OH) vitamin D. Because the creation of vitamin D is vital to function throughout the life course, methylation, and the up- and down-regulation of certain genes in the pathway may not be beneficial long-term to human health.

Overall, lasting effects of childhood on vitamin D via methylation was not established. No methylation of genes tested in the vitamin D pathway predicted vitamin D. These results may

indicate that vitamin D levels are malleable across the lifespan. However, these results do not lead us to a mechanism linking decreased vitamin D to long-term health.

While methylation did not affect total serum vitamin D, environmental factors may still affect methylation patterns in the vitamin D pathway. There are some differences in environmental factors that correlate with methylation patterns, and these factors vary by sex across the lifespan. Future investigation into these environmental factors will be conducted to if these factors predict methylation at CpG sites in the vitamin D pathway.

7.2 Study Limitations

Perhaps, the biggest limitation is that circulating total 25(OH) vitamin D is derived from a single sample at a single point in time. Vitamin D is not a stagnant measure. A better measure of the lasting effects of childhood and adult environments would have been to have include several measure of vitamin D over time. Clearly, that is much more rigorous undertaking to both study participants and researchers. Furthermore, a comparison between childhood and adult measure of vitamin D would have provided a much more comprehensive look at how impacts in childhood with a baseline vitamin D comparison affects adult vitamin D measures.

Another limitation is an ongoing issue with vitamin D research writ large. That is the issue of comparison across studies. I chose the DiaSource 25(OH) Vitamin D Total enzyme-linked immunosorbent assay kit for serum because it is one of a few vitamin D ELISA kits that is approved by the Food and Drug Administration (FDA). However, assay vary in their correlation with liquid chromatography mass spectrometry (LC-MS), which is considered the best standard for determining vitamin D analysis, because the process of synthesizing vitamin D produces

several vitamin D metabolites that also circulate within the body (Brandi, 2010; Cashman, 2012; Hibler et al., 2016). The vitamin D metabolites will often bind to the assay plate resulting in inaccurate readings for total 25(OH) vitamin D. The result is that it is difficult to compare vitamin D across populations and time periods.

Another limitation is the unequal group sizes studied. Because the original collection aimed to study women of reproductive age, the sample sizes for females and males differ greatly with the female sample being greater than four times the male sample. Comparisons between groups of such different sample sizes makes extrapolation to the population quite difficult.

7.3 Broader Impacts

As in prior studies with this dataset, the results will be shared with collaborators in the Philippines and assist in their efforts to inform local policy as they deem appropriate. Additionally, as is customary of the CLHNS data, the vitamin D information will eventually be open access to those that would like to use the data for further research.

Biological Anthropology Methods to Inform Intervention

By integrating biological anthropology methods, this research begins to delve deeper into the possible health risks associated with gendered upbringing. The dynamics of gender may undergo change just as societies undergoing urbanization creates different dynamics from societies in childhood. Gender is a cultural phenomenon that also shifts which creates implications for health. Sex is one factor that affects biology and health, but the expectation and treatment of those sexes in society creates another factor that can have biological ramifications,

especially when one considers how methylation allows the environment to have biological and health repercussions. While the testing of environmental differences did not immediately illuminate how gender affects vitamin D differences, this dissertation begins to provide a template for how work on gender in health can be carried out especially as it pertains to vitamin D.

Furthermore, the results in this dissertation clearly indicate that sex is the strongest predictor of vitamin D in this population. Because vitamin D is a growing phenomenon, it is imperative that more research be undertaken to understand the sex disparities. Vitamin D deficiency may be a growing issue that is occurring in the Philippines, and more research should be conducted to gather evidence that over time of the shifts in culture that may be leading to vitamin D deficiency. One hopeful recommendation is that the traditional, large quantities of fish consumption in the Philippines seems to be protective against vitamin D deficiency. Therefore, to prevent vitamin D deficiency, healthcare workers may be able to recommend the culturally relevant and readily available option of fish consumption in its many forms to combat vitamin D deficiency in this population. Thus, healthcare workers can provide a sustainable recommendation for those in Cebu instead of immediately recommending vitamin D supplementation.

Vitamin D and Health

Because there are so many communicable and non-communicable health implications associated with vitamin D deficiency, understanding the developmental and ecological determinants of circulating serum vitamin D is vital to understanding the long-term dynamics between vitamin D deficiency and disease. Furthermore, women seem to be more susceptible to

vitamin D deficiency, so a comparative analysis between males and females allows factors that affect methylation and overall circulating vitamin D levels to analyzed. While no clear indicators were detected for the vitamin D differences between females and males, more research should clearly state that the appropriate statistical comparisons have been made. Often in vitamin D research, readers are left wondering if a difference was not detected or whether the researcher simply did not bother to test for the difference.

Furthermore, childhood urbanicity, adult assets, fish consumption, and sex did predict vitamin D levels for the sample. Though further replication would need to be conducted in the Philippines, a simple questionnaire with these four questions might one day indicate whether an individual should be tested for vitamin D deficiency. Another aspect that is beneficial to health is the sampling of young adults. Rarely is the age range studied for vitamin D deficiency and the factors that impact that deficiency.

7.4 Future Research Directions

Because the topic of vitamin D has been so closely linked to our bones, much of the research regarding vitamin D has been at a standstill. Still, there are many scientists that have long discussed the greater nuances and theory regarding vitamin D. Overall, there is much research that is required to understand the unknowns of vitamin D. One first step to better understanding how vitamin D might lead to a myriad of communicable and non-communicable diseases is link vitamin D directly with immune function. A next step is to utilize the extensive data from the CLHNS to analyze whether vitamin D predicts immune markers in the study population. With the connections and links to infectious disease (Esposito & Lelii, 2015; Gois et

al., 2017; Kearns, Alvarez, Seidel, Tangpricha, & Tangpricha, 2015; Talat et al., 2010), research on vitamin D levels and T-cell production during an infectious disease would establish a mechanism by which vitamin D can contribute to immune activation. These results would go a long way in providing the beginning evidence toward understanding how vitamin D works in other systems beyond the skeletal system.

Other research that can be undertaken is, of course, the further analysis of child and adult environmental factors that are correlated with DNA methylation at genes in the vitamin D pathway. Analyzing whether the environmental factors affect methylation might at least explain some of the variability between females and males at some of the CpG sites. Furthermore, an analysis of the methylation differences between gene variants of the vitamin D pathway may be a better method of detecting whether methylation plays a role in the differences between female and male vitamin D levels.

Longer-term research goals would be to investigate how vitamin D affects growth patterns in children. Do children require stores of vitamin D to undergo a growth spurt? Are there trade-offs in childhood between growth and immune function in regard to circulating vitamin D? Beyond rickets, little research has been conducted on the more subtle effects of vitamin D on growth in childhood. For those populations undergoing famine or even periods of intermediate color restriction, there may be tradeoffs between growth and immune function as vitamin D is theorized to contribute to both. Populations undergoing urbanization and industrialization may be particularly vulnerable to the hypothesized trade-offs between growth and immune function as cultural and economic shifts drive populations to decreased UVB exposure.

Additionally, the links between vitamin D and COVID-19 are beginning to be investigated. That is, do individuals with higher vitamin D levels have reduced chances of contracting COVID-19, and if those individuals with higher vitamin D do contract COVID, are their symptoms less severe? Furthermore, research on the susceptibility of other infectious diseases and vitamin D is also an area of great interest. After all, prescribing a vitamin D supplement is a lot easier than treating an outbreak of a high contagious disease. Researching links between vitamin D and infectious disease would once again begin to illustrate the possible links between vitamin D and immune function.

A set of clear standardized practices need to be created to allow researchers and public health officials to make comparisons on vitamin D levels across populations over time. Unfortunately, the standardized practices may have to include vitamin D quantification with mass spectrometry. Mass spectrometry is more expensive than assay such as the one that I used to quantify vitamin D in these analyses, but mass spectrometry allows the vitamin D metabolites that are produced in the body to be properly quantified resulting in a much more accurate quantification of circulating vitamin D. Other standards are set cut-offs for deficiency and insufficiency. If no value can be agreed upon, researchers may need to then produce data with multiple deficiency cutoffs to allow for comparison across samples.

7.5 Concluding Remarks

Vitamin D research has in many ways been a neglected topic. This research begins to study the long-term effects of vitamin D on adult health, but more research needs to be conducted to gain clearer answers on the effects of vitamin D beyond the skeletal system. In

order to gain clearer answers, collaborative work will need to be conducted with physicians, endocrinologists, public health experts, and social scientists to understand how the social and physical environment interacts with the vitamin D metabolic pathway and the impact on overall health.

REFERENCES

Abhimanyu, A., & Coussens, A. K. (2017). The role of UV radiation and vitamin D in the seasonality and outcomes of infectious disease. *Photochem. Photobiol. Sci.* https://doi.org/10.1039/C6PP00355A

Abudawood, M., Tabassum, H., Ansar, S., Almosa, K., Sobki, S., Ali, M. N., & Aljohi, A. (2018). Assessment of gender-related differences in vitamin D levels and cardiovascular risk factors in Saudi patients with type 2 diabetes mellitus. *Saudi Journal of Biological Sciences*, *25*(1), 31–36. https://doi.org/10.1016/j.sjbs.2017.04.001

Adair, L. S., Popkin, B. M., Akin, J. S., Guilkey, D. K., Gultiano, S., Borja, J., … Hindin, M. J. (2011). Cohort Profile: The Cebu Longitudinal Health and Nutrition Survey. *International Journal of Epidemiology*, *40*(3), 619–625. https://doi.org/10.1093/ije/dyq085

Adler, N., & Ostrove, J. (1999). Socioeconomic Status and Health: What We Know and What We Don't. *Annals of the New York Academy of Sciences*, *896*, 3–15. https://doi.org/10.1111/j.1749-6632.1999.tb08101.x

Adriana S. Dusso, Alex J. Brown, & Eduardo Slatopolsky. (2005). Vitamin D. *American Journal of Physiology Renal Physiology*, *289*(1), F8–F28.

Al Khalifah, R., Alsheikh, R., Alnasser, Y., Alsheikh, R., Alhelali, N., Naji, A., & Al Backer, N. (2020). The impact of vitamin D food fortification and health outcomes in children: A systematic review and meta-regression. *Systematic Reviews*, *9*(1), 144. https://doi.org/10.1186/s13643-020-01360-3

Alagöl, F., Shihadeh, Y., Boztepe, H., Tanakol, R., Yarman, S., Azizlerli, H., & Sandalci, Ö. (2000). Sunlight exposure and vitamin D deficiency in Turkish women. *Journal of Endocrinological Investigation*, *23*(3), 173–177. https://doi.org/10.1007/BF03343702

Alemzadeh, R., Kichler, J., Babar, G., & Calhoun, M. (2008). Hypovitaminosis D in obese children and adolescents: Relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism*, *57*(2), 183–191. https://doi.org/10.1016/j.metabol.2007.08.023

Alex, L., Fjellman Wiklund, A., Lundman, B., Christianson, M., & Hammarström, A. (2012). Beyond a Dichotomous View of the Concepts of 'Sex' and 'Gender' Focus Group Discussions among Gender Researchers at a Medical Faculty. *PLoS ONE*, *7*(11), e50275. https://doi.org/10.1371/journal.pone.0050275

Al-Musharaf, S., Fouda, M. A., Turkestani, I. Z., Al-Ajlan, A., Sabico, S., Alnaami, A. M., … Saravanan, P. (2018). Vitamin D Deficiency Prevalence and Predictors in Early Pregnancy among Arab Women. *Nutrients*, *10*(4), 489. https://doi.org/10.3390/nu10040489

Ananthakrishnan, A. N., Khalili, H., Higuchi, L. M., Bao, Y., Korzenik, J. R., Giovannucci, E. L., … Chan, A. T. (2012). Higher Predicted Vitamin D Status Is Associated With Reduced Risk of Crohn's Disease. *Gastroenterology*, *142*(3), 482–489. https://doi.org/10.1053/j.gastro.2011.11.040

Arabi, A., Mahfoud, Z., Zahed, L., El-Onsi, L., & El-Hajj Fuleihan, G. (2010). Effect of age, gender and calciotropic hormones on the relationship between vitamin D receptor gene polymorphisms and bone mineral density. *European Journal of Clinical Nutrition*, *64*(4), 383–391. https://doi.org/10.1038/ejcn.2010.5

Arnson, Y., Amital, H., & Shoenfeld, Y. (2007). Vitamin D and autoimmunity: New aetiological and therapeutic considerations. *Annals of the Rheumatic Diseases*, *66*(9), 1137–1142. https://doi.org/10.1136/ard.2007.069831

Bailey, B. A., Manning, T., & Peiris, A. N. (2012). The Impact of Living in Rural and Urban Areas: Vitamin D and Medical Costs in Veterans. *The Journal of Rural Health*, *28*(4), 356–363. https://doi.org/10.1111/j.1748-0361.2012.00407.x

Bakos, B., Szili, B., Szabó, B., Horváth, P., Kirschner, G., Kósa, J. P., … Takács, I. (2020). Genetic variants of VDR and CYP2R1 affect BMI independently of serum vitamin D concentrations. *BMC Medical Genetics*, *21*(1), 129. https://doi.org/10.1186/s12881-020-01065-3

Barker, D. J. P. (2007). The origins of the developmental origins theory. *Journal of Internal Medicine*, *261*(5), 412–417. https://doi.org/10.1111/j.1365-2796.2007.01809.x

Barker, D. J. P, Osmond, C., Winter, P. D., Margetts, B., & Simmonds, S. J. (1989). Weight in infancy and death from Ischaemic heart disease. *The Lancet*, *334*(8663), 577–580. https://doi.org/10.1016/S0140-6736(89)90710-1

Barker, David James Purslove. (1994). *Mothers, babies and disease in later life*. BMJ Publishing Group.

Basatemur, E., Horsfall, L., Marston, L., Rait, G., & Sutcliffe, A. (2017). Trends in the Diagnosis of Vitamin D Deficiency. *Pediatrics*, *139*(3), e20162748. https://doi.org/10.1542/peds.2016-2748

Basson, A. (2014). Vitamin D and Crohn's Disease in the Adult Patient A Review. *Journal of Parenteral and Enteral Nutrition*, *38*(4), 438–458. https://doi.org/10.1177/0148607113506013

Battault, S., Whiting, S. J., Peltier, S. L., Sadrin, S., Gerber, G., & Maixent, J. M. (2013). Vitamin D metabolism, functions and needs: From science to health claims. *European Journal of Nutrition*, *52*(2), 429–441. https://doi.org/10.1007/s00394-012-0430-5

Beckman, L. M., Earthman, C. P., Masodkar, K., & Sibley, S. D. (2012). The link between obesity and low circulating 25-hydroxyvitamin D concentrations: Considerations and implications. *International Journal of Obesity*, *36*(3), 387+. Academic OneFile. Retrieved from Academic OneFile.

Bikle, D. D. (2009). Vitamin D and immune function: Understanding common pathways. *Current Osteoporosis Reports*, *7*(2), 58.

Bikle, D. D. (2011). Chapter one—Vitamin D Regulation of Immune Function. In G. Litwack (Ed.), *Vitamins & Hormones* (pp. 1–21). Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123869609000010

Bikle, D. D. (2014). Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. *Chemistry & Biology*, *21*(3), 319–329. https://doi.org/10.1016/j.chembiol.2013.12.016

Bishai, D., & Nalubola, R. (2002). The History of Food Fortification in the United States: Its Relevance for Current Fortification Efforts in Developing Countries. *Economic Development and Cultural Change*, *51*(1), 37–53. https://doi.org/10.1086/345361

Borges, M. C., Martini, L. A., & Rogero, M. M. (2011). Current perspectives on vitamin D, immune system, and chronic diseases. *Nutrition*, *27*(4), 399–404. https://doi.org/10.1016/j.nut.2010.07.022

Botella-Carretero, J. I., Alvarez-Blasco, F., Villafruela, J. J., Balsa, J. A., Vázquez, C., & Escobar-Morreale, H. F. (2007). Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. *Clinical Nutrition*, *26*(5), 573–580. https://doi.org/10.1016/j.clnu.2007.05.009

Branda, R. F., & Eaton, J. W. (1978). Skin color and nutrient photolysis: An evolutionary hypothesis. *Science*, *201*(4356), 625–626. https://doi.org/10.1126/science.675247

Brandi, M. L. (2010). Indications on the use of vitamin D and vitamin D metabolites in clinical phenotypes. *Clinical Cases in Mineral and Bone Metabolism*, *7*(3), 243–250.

Brannon, P. M., Yetley, E. A., Bailey, R. L., & Picciano, M. F. (2008). Overview of the conference "Vitamin D and Health in the 21st Century: An Update." *The American Journal of Clinical Nutrition*, *88*(2), 483S-490S. https://doi.org/10.1093/ajcn/88.2.483S

Bureau of Fisheries and Aquatic Resources. (2020). *Philippine Fisheries Profile 2019*. Quezon City.

Cali, J. J., Hsieh, C. L., Francke, U., & Russell, D. W. (1991). Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *Journal of Biological Chemistry*, *266*(12), 7779–7783. https://doi.org/10.1016/S0021-9258(20)89518-0

Calvo, M. S., & Whiting, S. J. (2013). Survey of current vitamin D food fortification practices in the United States and Canada. *The Journal of Steroid Biochemistry and Molecular Biology*, *136*, 211–213. https://doi.org/10.1016/j.jsbmb.2012.09.034

Calvo, M. S., Whiting, S. J., & Barton, C. N. (2004). Vitamin D fortification in the United States and Canada: Current status and data needs. *The American Journal of Clinical Nutrition*, *80*(6), 1710S-1716S. https://doi.org/10.1093/ajcn/80.6.1710S

Calvo, M. S., Whiting, S. J., & Barton, C. N. (2005). Vitamin D Intake: A Global Perspective of Current Status. *The Journal of Nutrition*, *135*(2), 310–316. https://doi.org/10.1093/jn/135.2.310

Camargo, C. A. (2011). Vitamin D and Cardiovascular Disease. *Journal of the American College of Cardiology*, *58*(14), 1442–1444. https://doi.org/10.1016/j.jacc.2011.06.037

Cantorna, M. T. (2011). Why do T cells express the vitamin D receptor?: Vitamin D and T cells. *Annals of the New York Academy of Sciences*, *1217*(1), 77–82. https://doi.org/10.1111/j.1749-6632.2010.05823.x

Carnevale, V., Modoni, S., Pileri, M., Di Giorgio, A., Chiodini, I., Minisola, S., … Scillitani, A. (2001). Longitudinal evaluation of vitamin D status in healthy subjects from southern Italy: Seasonal and gender differences. *Osteoporosis International*, *12*(12), 1026–1030.

Carrelli, A., Bucovsky, M., Horst, R., Cremers, S., Zhang, C., Bessler, M., … Stein, E. M. (2017). Vitamin D Storage in Adipose Tissue of Obese and Normal Weight Women. *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research*, *32*(2), 237–242. https://doi.org/10.1002/jbmr.2979

Cashman, K. D. (2012). The role of vitamers and dietary-based metabolites of vitamin D in prevention of vitamin D deficiency. *Food & Nutrition Research*, *56*. https://doi.org/10.3402/fnr.v56i0.5383

Cashman, K. D., & Kiely, M. (2011). Towards prevention of vitamin D deficiency and beyond: Knowledge gaps and research needs in vitamin D nutrition and public health. *The British Journal of Nutrition; Cambridge*, *106*(11), 1617–1627. http://dx.doi.org.turing.library.northwestern.edu/10.1017/S0007114511004995

Cashman, K. D., & Kiely, M. (2018). Vitamin D and Food Fortification. In *Vitamin D, Volume II: Health, Disease and Therapeutics* (4th ed., pp. 109–127). Philadelphia, PA. Retrieved from http://www.clinicalkey.com/#!/content/book/3-s2.0-B9780128099636000638?scrollTo=%23hl0002035

Cediel, G., Corvalan, C., Lopez de Romana, D., Mericq, V., & Uauy, R. (2016). Prepubertal Adiposity, Vitamin D Status, and Insulin Resistance. *PEDIATRICS*, *138*(1), e20160076–e20160076. https://doi.org/10.1542/peds.2016-0076

Chaplin, G., & Jablonski, N. G. (1998a). Hemispheric difference in human skin color. *American Journal of Physical Anthropology*, *107*(2), 221–223. https://doi.org/10.1002/(SICI)1096-8644(199810)107:2<221::AID-AJPA8>3.0.CO;2-X

Chaplin, G., & Jablonski, N. G. (1998b). Hemispheric difference in human skin color. *American Journal of Physical Anthropology*, *107*(2), 221–223. https://doi.org/10.1002/(SICI)1096-8644(199810)107:2<221::AID-AJPA8>3.0.CO;2-X

Chaplin, G., & Jablonski, N. G. (2009). Vitamin D and the evolution of human depigmentation. *American Journal of Physical Anthropology*, *139*(4), 451–461. https://doi.org/10.1002/ajpa.21079

Charnov, E. L., & Schaffer, W. M. (1973). Life-History Consequences of Natural Selection: Cole's Result Revisited. *The American Naturalist*, *107*(958), 791–793.

Cheng, J. B., Levine, M. A., Bell, N. H., Mangelsdorf, D. J., & Russell, D. W. (2004). Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(20), 7711–7715. https://doi.org/10.1073/pnas.0402490101

Cheng, S., Massaro, J. M., Fox, C. S., Larson, M. G., Keyes, M. J., McCabe, E. L., … Wang, T. J. (2010). Adiposity, Cardiometabolic Risk, and Vitamin D Status: The Framingham Heart Study. *Diabetes*, *59*(1), 242–248. https://doi.org/10.2337/db09-1011

Cherney, I. D., & London, K. (2006). Gender-linked differences in the toys, television shows, computer games, and outdoor activities of 5-to 13-year-old children. *Sex Roles*, *54*(9–10), 717–726.

Christakos, S., Ajibade, D. V., Dhawan, P., Fechner, A. J., & Mady, L. J. (2012). Vitamin D: Metabolism. *Rheumatic Disease Clinics of North America*, *38*(1), 1–11. https://doi.org/10.1016/j.rdc.2012.03.003

Cohen, P. (2010). The Hypogonadal–obesity–vitamin D-insufficiency/deficiency cycle in men. *Medical Hypotheses*, *75*(5), 473. https://doi.org/10.1016/j.mehy.2010.04.027

Cowell, S. J. (1925). Irradiation Of Milk And The Healing Of Rickets. *The British Medical Journal*, *1*(3352), 594–595. JSTOR. Retrieved from JSTOR.

Dahly, Darren L., Gordon-Larsen, P., Popkin, B. M., Kaufman, J. S., & Adair, L. S. (2010). Associations between Multiple Indicators of Socioeconomic Status and Obesity in Young Adult Filipinos Vary by Gender, Urbanicity, and Indicator Used. *The Journal of Nutrition*, *140*(2), 366–370. https://doi.org/10.3945/jn.109.114207

Dahly, Darren Lawrence, & Adair, L. S. (2007). Quantifying the urban environment: A scale measure of urbanicity outperforms the urban-rural dichotomy. *Social Science & Medicine (1982)*, *64*(7), 1407–1419. https://doi.org/10.1016/j.socscimed.2006.11.019

Daly, R. M., Gagnon, C., Lu, Z. X., Magliano, D. J., Dunstan, D. W., Sikaris, K. A., … Shaw, J. E. (2012). Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: A national, population-based study. *Clinical Endocrinology*, *77*(1), 26–35. https://doi.org/10.1111/j.1365-2265.2011.04320.x

Datta, P., Philipsen, P. A., Olsen, P., Bogh, M. K., Johansen, P., Schmedes, A. V., … Wulf, H. C. (2017). The half-life of 25(OH)D after UVB exposure depends on gender and vitamin D receptor polymorphism but mainly on the start level. *Photochemical & Photobiological Sciences*, *16*(6), 985–995. https://doi.org/10.1039/C6PP00258G

David B. Allison, Bhramar Mukherjee, John D. Kalbfleisch, & Suzanne P. Murphy. (2017, May 15). *Purported mathematical errors in the 2011 IOM report, Dietary Reference Intakes: Calcium and Vitamin D* [Letter to Marcia K. McNutt]. Retrieved from https://www.nap.edu/resource/13050/Vit%20D%20panel%20report%20final.pdf

de Gruijl, F. R., & Pavel, S. (2012). The effects of a mid-winter 8-week course of sub-sunburn sunbed exposures on tanning, vitamin D status and colds. *Photochemical & Photobiological Sciences*, *11*(12), 1848. https://doi.org/10.1039/c2pp25179e

DeBoer, M. D., Lima, A. A., Oría, R. B., Scharf, R. J., Moore, S. R., Luna, M. A., & Guerrant, R. L. (2012). Early childhood growth failure and the developmental origins of adult disease: Do enteric infections and malnutrition increase risk for the metabolic syndrome? *Nutrition Reviews*, *70*(11), 642–653. https://doi.org/10.1111/j.1753-4887.2012.00543.x

Delle Monache, S., Di Fulvio, P., Iannetti, E., Valerii, L., Capone, L., Nespoli, M. G., … Angelucci, A. (2019). Body mass index represents a good predictor of vitamin D status in women independently from age. *Clinical Nutrition*, *38*(2), 829–834. https://doi.org/10.1016/j.clnu.2018.02.024

DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition*, *80*(6), 1689S-1696S. https://doi.org/10.1093/ajcn/80.6.1689S

DeLuca, H. F. (2008). Evolution of our understanding of vitamin D. *Nutrition Reviews*, *66*, S73–S87. https://doi.org/10.1111/j.1753-4887.2008.00105.x

Deurenberg, P., Deurenberg-Yap, M., & Guricci, S. (2002). Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. *Obesity Reviews*, *3*(3), 141–146. https://doi.org/10.1046/j.1467-789X.2002.00065.x

Dharmshaktu, P., Saha, S., Kar, P., Sreenivas, V., Ramakrishnan, L., & Goswami, R. (2019). Absence of vitamin D deficiency among common outdoor workers in Delhi. *Clinical Endocrinology*, *91*(2), 356–362. https://doi.org/10.1111/cen.14012

Disanto, G., Chaplin, G., Morahan, J. M., Giovannoni, G., Hyppönen, E., Ebers, G. C., & Ramagopalan, S. V. (2012). Month of birth, vitamin D and risk of immune-mediated

disease: A case control study. *BMC Medicine*, *10*(1). https://doi.org/10.1186/1741-7015-10-69

Djennane, M., Lebbah, S., Roux, C., Djoudi, H., Cavalier, E., & Souberbielle, J.-C. (2014). Vitamin D status of schoolchildren in Northern Algeria, seasonal variations and determinants of vitamin D deficiency. *Osteoporosis International*, *25*(5), 1493–1502. https://doi.org/10.1007/s00198-014-2623-7

Du, P., Zhang, X., Huang, C.-C., Jafari, N., Kibbe, W. A., Hou, L., & Lin, S. M. (2010). Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics*, *11*(1), 587. https://doi.org/10.1186/1471-2105-11-587

Eikelenboom, M. J., Killestein, J., Kragt, J. J., Uitdehaag, B. M. J., & Polman, C. H. (2009). Gender differences in multiple sclerosis: Cytokines and vitamin D. *Journal of the Neurological Sciences*, *286*(1–2), 40–42. https://doi.org/10.1016/j.jns.2009.06.025

EK Nichols, IMD Khatib, N J Aburto, K M Sullivan, KS Scanlon, JP Wirth, & MK Serdula. (2012). Vitamin D status and determinants of deficiency among non-pregnant Jordanian women of reproductive age. *European Journal of Clinical Nutrition*, *66*(6), 751–756.

Esposito, S., & Lelii, M. (2015). Vitamin D and respiratory tract infections in childhood. *BMC Infectious Diseases*, *15*(1). https://doi.org/10.1186/s12879-015-1196-1

Fairweather, D., Frisancho-Kiss, S., & Rose, N. R. (2008). Sex Differences in Autoimmune Disease from a Pathological Perspective. *The American Journal of Pathology*, *173*(3), 600–609. https://doi.org/10.2353/ajpath.2008.071008

Fang, F., Wei, H., Wang, K., Tan, L., Zhang, W., Ding, L., … Zhu, M. (2018). High prevalence of vitamin D deficiency and influencing factors among urban and rural residents in Tianjin, China. *Archives of Osteoporosis*, *13*(1). https://doi.org/10.1007/s11657-018-0479-8

Frisancho, A. R. (1993). *Human adaptation and accommodation*. Ann Arbor: University of Michigan Press.

Fukuwatari, T., Fujita, M., & Shibata, K. (2009). Effects of UVA Irradiation on the Concentration of Folate in Human Blood. *Bioscience, Biotechnology, and Biochemistry*, *73*(2), 322–327. https://doi.org/10.1271/bbb.80530

García, O. P., Long, K. Z., & Rosado, J. L. (2009). Impact of micronutrient deficiencies on obesity. *Nutrition Reviews*, *67*(10), 559–572. https://doi.org/10.1111/j.1753-4887.2009.00228.x

Garn, S. M., Sullivan, T. V., Decker, S. A., Larkin, F. A., & Hawthorne, V. M. (1992). Continuing bone expansion and increasing bone loss over a two-decade period in men and women from a total community sample. *American Journal of Human Biology*, *4*(1), 57–67. https://doi.org/10.1002/ajhb.1310040109

Gilbert-Diamond, D., Baylin, A., Mora-Plazas, M., Marin, C., Arsenault, J. E., Hughes, M. D., … Villamor, E. (2010). Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: A prospective study. *American Journal of Clinical Nutrition*, *92*(6), 1446–1451. https://doi.org/10.3945/ajcn.2010.29746

Ginde, A. A., Liu, M. C., & Camargo, C. A. (2009). Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Archives of Internal Medicine, 169*(6), 626–632.

Gleicher, N., & Barad, D. H. (2007). Gender as risk factor for autoimmune diseases. *Journal of Autoimmunity, 28*(1), 1–6. https://doi.org/10.1016/j.jaut.2006.12.004

Gois, P. H. F., Ferreira, D., Olenski, S., & Seguro, A. C. (2017). Vitamin D and Infectious Diseases: Simple Bystander or Contributing Factor? *Nutrients*, *9*(7), 651. https://doi.org/10.3390/nu9070651

Goodman, A. H., & Leatherman, T. L. (1998). *Building a new biocultural synthesis: Political-economic perspectives on human biology*. Ann Arbor: University of Michigan Press.

Gordon, C. M., Feldman, H. A., Sinclair, L., Williams, A. L., Kleinman, P. K., Perez-Rossello, J., & Cox, J. E. (2008). Prevalence of Vitamin D Deficiency Among Healthy Infants and Toddlers. *Archives of Pediatrics & Adolescent Medicine*, *162*(6), 505–512. https://doi.org/10.1001/archpedi.162.6.505

Goryakin, Y., Lobstein, T., James, W. P. T., & Suhrcke, M. (2015). The impact of economic, political and social globalization on overweight and obesity in the 56 low and middle income countries. *Social Science & Medicine, 133*, 67–76. https://doi.org/10.1016/j.socscimed.2015.03.030

Griffin, T. P., Wall, D., Blake, L., G Griffin, D., Robinson, S., Bell, M., … O'Shea, P. M. (2020). Higher risk of vitamin D insufficiency/deficiency for rural than urban dwellers. *The Journal of Steroid Biochemistry and Molecular Biology*, *197*, 105547. https://doi.org/10.1016/j.jsbmb.2019.105547

Haldar, S., Chia, S. C., & Henry, C. J. (2015). Chapter Four—Body Composition in Asians and Caucasians: Comparative Analyses and Influences on Cardiometabolic Outcomes. In J. Henry (Ed.), *Advances in Food and Nutrition Research* (Vol. 75, pp. 97–154). Academic Press. https://doi.org/10.1016/bs.afnr.2015.07.001

Hallal, P. C., Andersen, L. B., Bull, F. C., Guthold, R., Haskell, W., & Ekelund, U. (2012). Global physical activity levels: Surveillance progress, pitfalls, and prospects. *The Lancet*, *380*(9838), 247–257. https://doi.org/10.1016/S0140-6736(12)60646-1

Hatun, S., Ozkan, B., Orbak, Z., Doneray, H., Cizmecioglu, F., Toprak, D., & Calikoglu, A. S. (2005). Vitamin D Deficiency in Early Infancy. *The Journal of Nutrition*, *135*(2), 279–282. https://doi.org/10.1093/jn/135.2.279

Haussler, M R. (1986). Vitamin D Receptors: Nature and Function. *Annual Review of Nutrition*, *6*(1), 527–562. https://doi.org/10.1146/annurev.nu.06.070186.002523

Haussler, Mark R., & Norman, A. W. (1969). CHROMOSOMAL RECEPTOR FOR A VITAMIN D METABOLITE*. *Proceedings of the National Academy of Sciences of the United States of America*, *62*(1), 155–162.

Hawkes, K., O'Connell, J. F., Jones, N. G. B., Alvarez, H., & Charnov, E. L. (1998). Grandmothering, menopause, and the evolution of human life histories. *Proceedings of the National Academy of Sciences*, *95*(3), 1336–1339. https://doi.org/10.1073/pnas.95.3.1336

Heindel, J. J., & Vandenberg, L. N. (2015). Developmental Origins of Health and Disease: A Paradigm for Understanding Disease Etiology and Prevention. *Current Opinion in Pediatrics*, *27*(2), 248–253. https://doi.org/10.1097/MOP.0000000000000191

Hess, A. F. (1932). The Rôle of Activated Milk in the Anti-Rickets Campaign. *American Journal of Public Health and the Nations Health*, *22*(12), 1215–1219.

Hibbard, B. M. (1964). THE ROLE OF FOLIC ACID IN PREGNANCY; WITH PARTICULAR REFERENCE TO ANAEMIA, ABRUPTION AND ABORTION. *The Journal of Obstetrics and Gynaecology of the British Commonwealth*, *71*, 529–542.

Hibler, E. A., Sardo Molmenti, C. L., Dai, Q., Kohler, L. N., Warren Anderson, S., Jurutka, P. W., & Jacobs, E. T. (2016). Physical activity, sedentary behavior, and vitamin D metabolites. *Bone*, *83*, 248–255. https://doi.org/10.1016/j.bone.2015.11.016

Hill, K. (1993). Life history theory and evolutionary anthropology. *Evolutionary Anthropology: Issues, News, and Reviews*, *2*(3), 78–88. https://doi.org/10.1002/evan.1360020303

Holick, M. F. (2007). Vitamin D Deficiency. *New England Journal of Medicine*, *357*(3), 266–281. https://doi.org/10.1056/NEJMra070553

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., … Weaver, C. M. (2011). Evaluation, Treatment, and Prevention of Vitamin D Deficiency: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, *96*(7), 1911–1930. https://doi.org/10.1210/jc.2011-0385

Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. *The American Journal of Clinical Nutrition*, *87*(4), 1080S-1086S.

Hossein-nezhad, A., & Holick, M. F. (2012). Optimize dietary intake of vitamin D: An epigenetic perspective. *Current Opinion in Clinical Nutrition and Metabolic Care*, *15*(6), 567–579. https://doi.org/10.1097/MCO.0b013e3283594978

Hossein-nezhad, A., & Holick, M. F. (2013). Vitamin D for Health: A Global Perspective. *Mayo Clinic Proceedings*, *88*(7), 720–755. https://doi.org/10.1016/j.mayocp.2013.05.011

Houghton, L. A., & Vieth, R. (2006). The case against ergocalciferol (vitamin D2) as a vitamin supplement. *The American Journal of Clinical Nutrition*, *84*(4), 694–697. https://doi.org/10.1093/ajcn/84.4.694

Hyppönen, E., Läärä, E., Reunanen, A., Järvelin, M.-R., & Virtanen, S. M. (2001). Intake of vitamin D and risk of type 1 diabetes: A birth-cohort study. *The Lancet*, *358*(9292), 1500–1503. https://doi.org/10.1016/S0140-6736(01)06580-1

Islam, M. z., Lamberg-Allardt, C., Kärkkäinen, M., Outila, T., Salamatullah, Q., & Shamim, A. a. (2002). Vitamin D deficiency: A concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region. *European Journal of Clinical Nutrition*, *56*(1), 51.

Jablonski, N.G. (1999). A possible link between neural tube defects and ultraviolet light exposure. *Medical Hypotheses*, *52*(6), 581–582. https://doi.org/10.1054/mehy.1997.0697

Jablonski, Nina G. (2004). The Evolution of Human Skin and Skin Color. *Annual Review of Anthropology*, *33*(1), 585–623. https://doi.org/10.1146/annurev.anthro.33.070203.143955

Jablonski, Nina G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution*, *39*(1), 57–106. https://doi.org/10.1006/jhev.2000.0403

Jablonski, Nina G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(Suppl 2), 8962–8968. https://doi.org/10.1073/pnas.0914628107

Jablonski, Nina G., & Chaplin, G. (2018a). The roles of vitamin D and cutaneous vitamin D production in human evolution and health. *International Journal of Paleopathology*. https://doi.org/10.1016/j.ijpp.2018.01.005

Jablonski, Nina G., & Chaplin, G. (2018b). The roles of vitamin D and cutaneous vitamin D production in human evolution and health. *International Journal of Paleopathology*. https://doi.org/10.1016/j.ijpp.2018.01.005

Jamka, M., Arslanow, A., Bohner, A., Krawczyk, M., Weber, S. N., Grünhage, F., … Stokes, C. S. (2018). Effects of Gene Variants Controlling Vitamin D Metabolism and Serum Levels on Hepatic Steatosis. *Digestion*, *97*(4), 298–308. https://doi.org/10.1159/000485180

Jones, G., Prosser, D. E., & Kaufmann, M. (2014a). Cytochrome P450-mediated metabolism of vitamin D. *Journal of Lipid Research*, *55*(1), 13–31. https://doi.org/10.1194/jlr.R031534

Jones, G., Prosser, D. E., & Kaufmann, M. (2014b). Cytochrome P450-mediated metabolism of vitamin D. *Journal of Lipid Research*, *55*(1), 13–31. https://doi.org/10.1194/jlr.R031534

Jones, K. S., Assar, S., Vanderschueren, D., Bouillon, R., Prentice, A., & Schoenmakers, I. (2015). Predictors of 25(OH)D half-life and plasma 25(OH)D concentration in The Gambia and the UK. *Osteoporosis International*, *26*(3), 1137–1146. https://doi.org/10.1007/s00198-014-2905-0

Juzeniene, A., Setlow, R., Porojnicu, A., Steindal, A. H., & Moan, J. (2009). Development of different human skin colors: A review highlighting photobiological and photobiophysical aspects. *Journal of Photochemistry and Photobiology B: Biology*, *96*(2), 93–100. https://doi.org/10.1016/j.jphotobiol.2009.04.009

Kalanda, B. F., van Buuren, S., Verhoeff, F. H., & Brabin, B. J. (2005). Catch-up growth in Malawian babies, a longitudinal study of normal and low birthweight babies born in a malarious endemic area. *Early Human Development, 81*(10), 841–850. https://doi.org/10.1016/j.earlhumdev.2005.06.006

Karagüzel, G., Dilber, B., Çan, G., Ökten, A., Değer, O., & Holick, M. F. (2014). Seasonal Vitamin D Status of Healthy Schoolchildren and Predictors of Low Vitamin D Status. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(5), 654–660. https://doi.org/10.1097/MPG.0000000000000274

Kearney, J. (2010). Food consumption trends and drivers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2793–2807. https://doi.org/10.1098/rstb.2010.0149

Kearns, M. D., Alvarez, J. A., Seidel, N., Tangpricha, V., & Tangpricha, V. (2015). Impact of Vitamin D on Infectious Disease. *The American Journal of the Medical Sciences*, *349*(3), 245–262. https://doi.org/10.1097/MAJ.0000000000000360

Kelles, A., & Adair, L. (2009). Offspring consume a more obesogenic diet than mothers in response to changing socioeconomic status and urbanization in Cebu, Philippines. *International Journal of Behavioral Nutrition and Physical Activity*, *6*(1), 47. https://doi.org/10.1186/1479-5868-6-47

Khadilkar, S. S. (2013). The Emerging Role of Vitamin D3 in Women's Health. *Journal of Obstetrics and Gynaecology of India*, *63*(3), 147–150. https://doi.org/10.1007/s13224-013-0420-4

Khalessi, N., Kalani, M., Araghi, M., & Farahani, Z. (2015). The Relationship between Maternal Vitamin D Deficiency and Low Birth Weight Neonates. *Journal of Family & Reproductive Health*, *9*(3), 113–117.

Kimlin, M. G. (2008). Geographic location and vitamin D synthesis. *Molecular Aspects of Medicine*, *29*(6), 453–461. https://doi.org/10.1016/j.mam.2008.08.005

Kimmons, J. E., Blanck, H. M., Tohill, B. C., Zhang, J., & Khan, L. K. (2006). Associations Between Body Mass Index and the Prevalence of Low Micronutrient Levels Among US Adults. *Medscape General Medicine*, *8*(4), 59.

Kleiber, D., Harris, L., & Vincent, A. C. J. (2018). Gender and marine protected areas: A case study of Danajon Bank, Philippines. *Maritime Studies*, *17*(2), 163–175. http://dx.doi.org.turing.library.northwestern.edu/10.1007/s40152-018-0107-7

Knudsen, G. P. (2009). Gender bias in autoimmune diseases: X chromosome inactivation in women with multiple sclerosis. *Journal of the Neurological Sciences*, *286*(1–2), 43–46. https://doi.org/10.1016/j.jns.2009.04.022

Komlos, J., & A'Hearn, B. (2017). Hidden negative aspects of industrialization at the onset of modern economic growth in the U.S. *Structural Change and Economic Dynamics*, *41*, 43–52. https://doi.org/10.1016/j.strueco.2017.03.001

Krieger, N. (2003). Genders, sexes, and health: What are the connections--and why does it matter? *International Journal of Epidemiology*, *32*(4), 652–657. https://doi.org/10.1093/ije/dyg156

Krieger, N. (2005). Embodiment: A conceptual glossary for epidemiology. *Journal of Epidemiology & Community Health*, *59*(5), 350–355. https://doi.org/10.1136/jech.2004.024562

Krieger, Nancy. (2001). A glossary for social epidemiology. *Journal of Epidemiology and Community Health*, *55*(10), 693–700.

Krieger, Nancy. (2003). Genders, sexes, and health: What are the connections—and why does it matter? *International Journal of Epidemiology*, *32*(4), 652–657. https://doi.org/10.1093/ije/dyg156

Kruger, M. C., Kruger, I. M., Wentzel-Viljoen, E., & Kruger, A. (2011). Urbanization of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition Research*, *31*(10), 748–758. https://doi.org/10.1016/j.nutres.2011.09.012

Kuzawa, C. W. (2005). Fetal origins of developmental plasticity: Are fetal cues reliable predictors of future nutritional environments? *American Journal of Human Biology*, *17*(1), 5–21. https://doi.org/10.1002/ajhb.20091

Kuzawa, C. W., & Sweet, E. (2009). Epigenetics and the embodiment of race: Developmental origins of US racial disparities in cardiovascular health. *American Journal of Human Biology*, *21*(1), 2–15. https://doi.org/10.1002/ajhb.20822

L Granlund, A Ramnemark, C Andersson, M Lindkvist, E Fharm, & M Norberg. (2016). Prevalence of vitamin D deficiency and its association with nutrition, travelling and clothing habits in an immigrant population in Northern Sweden. *European Journal of Clinical Nutrition*, *70*(3), 373–379.

Lagunova, Z., Porojnicu, A., Lindberg, F., Hexeberg, S., & Moan, J. (2009). The dependency of vitamin D status on body mass index, gender, age and season. *Obesity and Metabolism*, *6*(4), 52. https://doi.org/10.14341/2071-8713-4886

Laner, M. R. (2000). "Sex" versus "Gender": A Renewed Plea. *Sociological Inquiry*, *70*(4), 462–474. https://doi.org/10.1111/j.1475-682X.2000.tb00920.x

Larson, R. W., & Verma, S. (1999). How children and adolescents spend time across the world: Work, play, and developmental opportunities. *Psychological Bulletin*, *125*(6), 701–736. (1999-01567-005). https://doi.org/10.1037/0033-2909.125.6.701

Leitersdorf, E., Reshef, A., Meiner, V., Levitzki, R., Schwartz, S. P., Dann, E. J., … Berginer, V. M. (1993). Frameshift and splice-junction mutations in the sterol 27-hydroxylase gene cause cerebrotendinous xanthomatosis in Jews or Moroccan origin. *The Journal of Clinical Investigation*, *91*(6), 2488–2496. https://doi.org/10.1172/JCI116484

Lim, H. W., Gilchrest, B. A., Cooper, K. D., Bischoff-Ferrari, H. A., Rigel, D. S., Cyr, W. H., … Stone, S. P. (2005). Sunlight, tanning booths, and vitamin D. *Journal of the American Academy of Dermatology*, *52*(5), 868–876. https://doi.org/10.1016/j.jaad.2005.03.015

Lim, J. S., Kim, K. M., Rhee, Y., & Lim, S.-K. (2012). Gender-Dependent Skeletal Effects of Vitamin D Deficiency in a Younger Generation. *The Journal of Clinical Endocrinology & Metabolism*, *97*(6), 1995–2004. https://doi.org/10.1210/jc.2011-3098

Lippi, G., Bonelli, P., Buonocore, R., & Aloe, R. (2015). Birth season and vitamin D concentration in adulthood. *Annals of Translational Medicine*, *3*(16). https://doi.org/10.3978/j.issn.2305-5839.2015.09.30

Lister, L., & Lister, R. (2008). Highly Integrated Single-Base Resolution Maps of the Epigenome in Arabidopsis. *Cell*, *133*(3), 523–536.

Lohman, T. G., Roche, A. F., & Martorell, R. (1988). *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books.

Lucock, M., Beckett, E., Martin, C., Jones, P., Furst, J., Yates, Z., … Veysey, M. (2017). UV-associated decline in systemic folate: Implications for human nutrigenetics, health, and evolutionary processes. *American Journal of Human Biology*, *29*(2). https://doi.org/10.1002/ajhb.22929

Macdonald, H. M. (2013). Contributions of Sunlight and Diet to Vitamin D Status. *Calcified Tissue International*, *92*(2), 163–176. https://doi.org/10.1007/s00223-012-9634-1

MacKinnon, D., Lockwood, C., Hoffman, J., West, S., & Sheets, V. (2002). MacKinnon DP, Lockwood CM, Hoffman JM, West SG, Sheets V. A comparison of methods to test mediation and other intervening variable effects. *Psychological Methods*, *7*, 83–104. https://doi.org/10.1037/1082-989X.7.1.83

Malloy, P. J., & Feldman, D. (2010). Genetic Disorders and Defects in Vitamin D Action. *Endocrinology and Metabolism Clinics of North America*, *39*(2), 333–346. https://doi.org/10.1016/j.ecl.2010.02.004

Mannion, C. A., Gray-Donald, K., & Koski, K. G. (2006). Association of low intake of milk and vitamin D during pregnancy with decreased birth weight. *CMAJ : Canadian Medical Association Journal*, *174*(9), 1273–1277. https://doi.org/10.1503/cmaj.1041388

Mattila, P. H., Piironen, V. I., Uusi-Rauva, E. J., & Koivistoinen, P. E. (1994). Vitamin D Contents in Edible Mushrooms. *Journal of Agricultural and Food Chemistry*, *42*(11), 2449–2453. https://doi.org/10.1021/jf00047a016

Mattila, P., Lampi, A.-M., Ronkainen, R., Toivo, J., & Piironen, V. (2002). Sterol and vitamin D2 contents in some wild and cultivated mushrooms. *Food Chemistry*, *76*(3), 293–298. https://doi.org/10.1016/S0308-8146(01)00275-8

Maxmen, A. (2011). Nutrition advice: The vitamin D-lemma. *Nature*, *475*(7354), 23–25. https://doi.org/10.1038/475023a

McDade, T. w., Reyes-García, V., Tanner, S., Huanca, T., & Leonard, W. r. (2008). Maintenance versus growth: Investigating the costs of immune activation among children in lowland Bolivia. *American Journal of Physical Anthropology*, *136*(4), 478–484. https://doi.org/10.1002/ajpa.20831

McDade, T. W., Rutherford, J., Adair, L., & Kuzawa, C. W. (2010). Early origins of inflammation: Microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 1129–1137. https://doi.org/10.1098/rspb.2009.1795

McDade, Thomas W. (2001). Lifestyle incongruity, social integration, and immune function in Samoan adolescents. *Social Science & Medicine*, *53*(10), 1351–1362. https://doi.org/10.1016/S0277-9536(00)00414-7

McDade, Thomas W., Ryan, C., Jones, M. J., MacIsaac, J. L., Morin, A. M., Meyer, J. M., … Kuzawa, C. W. (2017). Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *Proceedings of the*

National Academy of Sciences of the United States of America, *114*(29), 7611–7616. https://doi.org/10.1073/pnas.1620661114

MedlinePlus [Internet]. (2021). Cyp27a1.pdf. In *CYP27A1*. Bethesda (MD): National Library of Medicine (US). Retrieved from https://medlineplus.gov/genetics/gene/cyp27a1/#references

Merewood, A., Mehta, S. D., Grossman, X., Chen, T. C., Mathieu, J. S., Holick, M. F., & Bauchner, H. (2010). Widespread Vitamin D Deficiency in Urban Massachusetts Newborns and Their Mothers. *PEDIATRICS*, *125*(4), 640–647. https://doi.org/10.1542/peds.2009-2158

Metzger, M. W., & McDade, T. W. (2010). Breastfeeding as obesity prevention in the United States: A sibling difference model. *American Journal of Human Biology*, *22*(3), 291–296. https://doi.org/10.1002/ajhb.20982

Michael F. Holick. (2010). *The Vitamin D Solution: A 3-Step Strategy to Cure Our Most Common Health Problems*. United States: Penguin Publishing Group. Retrieved from https://books.google.com/books?id=P4xbcP19H0AC

Mithal, A., Wahl, D. A., Bonjour, J.-P., Burckhardt, P., Dawson-Hughes, B., Eisman, J. A., … others. (2009). Global vitamin D status and determinants of hypovitaminosis D. *Osteoporosis International*, *20*(11), 1807–1820.

Miyamoto, K., Kesterson, R. A., Yamamoto, H., Taketani, Y., Nishiwaki, E., Tatsumi, S., … Pike, J. W. (1997). *Department of Clinical Nutrition (K.-i.M., H.Y., Y.T., E.N., S.T., Y.I., K.M., E.T.) Tokushima University Tokushima 770, Japan Department of Molecular and*

Cellular Physiology (R.A.K., J.W.P.) University of Cincinnati Cincinnati, Ohio 45267. *11*(8), 15.

Moan, J., Lagunova, Z., Cicarma, E., Aksnes, L., Dahlback, A., Grant, W. B., & Porojnicu, A. C. (2009). Sunbeds as Vitamin D Sources. *Photochemistry and Photobiology*, *85*(6), 1474–1479. https://doi.org/10.1111/j.1751-1097.2009.00607.x

Mowry, E. M. (2011). Vitamin D: Evidence for its role as a prognostic factor in multiple sclerosis. *Journal of the Neurological Sciences*, *311*(1–2), 19–22. https://doi.org/10.1016/j.jns.2011.06.035

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., … Turner, M. B. (2016). Heart Disease and Stroke Statistics—2016 Update: A Report From the American Heart Association. *Circulation*, *133*(4), e38–e360. https://doi.org/10.1161/CIR.0000000000000350

Muehlenhard, C. L., & Peterson, Z. D. (2011). Distinguishing Between Sex and Gender: History, Current Conceptualizations, and Implications. *Sex Roles*, *64*(11–12), 791–803. https://doi.org/10.1007/s11199-011-9932-5

Mulligan, C. J. (2016). Early Environments, Stress, and the Epigenetics of Human Health. *Annual Review of Anthropology*, *45*(1), 233–249. https://doi.org/10.1146/annurev-anthro-102215-095954

Muscogiuri, G., Barrea, L., Somma, C. D., Laudisio, D., Salzano, C., Pugliese, G., … Savastano, S. (2019). Sex Differences of Vitamin D Status across BMI Classes: An Observational Prospective Cohort Study. *Nutrients*, *11*(12), 3034. https://doi.org/10.3390/nu11123034

Nakamura, K., Nashimoto, M., Hori, Y., & Yamamoto, M. (2000). Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. *The American Journal of Clinical Nutrition, 71*(5), 1161–1165. https://doi.org/10.1093/ajcn/71.5.1161

Neil A. Breslau. (1988). Normal and Abnormal Regulation of 1,25-(OH)2D Synthesis. *The American Journal of the Medical Sciences, 296*(6), 417–425.

Norman, A. W. (2006). Vitamin D Receptor: New Assignments for an Already Busy Receptor. *Endocrinology, 147*(12), 5542–5548. https://doi.org/10.1210/en.2006-0946

O'Mahony, L., Stepien, M., Gibney, M. J., Nugent, A. P., & Brennan, L. (2011). The Potential Role of Vitamin D Enhanced Foods in Improving Vitamin D Status. *Nutrients*, *3*(12), 1023–1041. https://doi.org/10.3390/nu3121023

Ooi, J. H., Chen, J., & Cantorna, M. T. (2012). Vitamin D regulation of immune function in the gut: Why do T cells have vitamin D receptors? *Molecular Aspects of Medicine, 33*(1), 77–82. https://doi.org/10.1016/j.mam.2011.10.014

Orces, C. (2019). The Association between Body Mass Index and Vitamin D Supplement Use among Adults in the United States. *Cureus, 11*(9), e5721–e5721. PubMed (31720189). https://doi.org/10.7759/cureus.5721

Palacios, C., & Gonzalez, L. (2014). Is vitamin D deficiency a major global public health problem? *The Journal of Steroid Biochemistry and Molecular Biology*, *144*, 138–145. https://doi.org/10.1016/j.jsbmb.2013.11.003

Pappas, G., Queen, S., Hadden, W., & Fisher, G. (1993). The Increasing Disparity in Mortality between Socioeconomic Groups in the United States, 1960 and 1986. *New England Journal of Medicine*, *329*(2), 103–109. https://doi.org/10.1056/NEJM199307083290207

Pereira-Santos, M., Costa, P. R. F., Assis, A. M. O., Santos, C. a. S. T., & Santos, D. B. (2015). Obesity and vitamin D deficiency: A systematic review and meta-analysis. *Obesity Reviews*, *16*(4), 341–349. https://doi.org/10.1111/obr.12239

Peter A. Jones & Daiya Takai. (2001). The Role of DNA Methylation in Mammalian Epigenetics. *Science*, *293*(5532), 1069–1070.

Philippine Fisheries Profile, 2005. (2006). Quezon City.

Phinney, K. W. (2008). Development of a standard reference material for vitamin D in serum. *The American Journal of Clinical Nutrition*, *88*(2), 511S-512S. https://doi.org/10.1093/ajcn/88.2.511S

Pike, J. W., & Meyer, M. B. (2014). Fundamentals of vitamin D hormone-regulated gene expression. *The Journal of Steroid Biochemistry and Molecular Biology*, *144*, 5–11. https://doi.org/10.1016/j.jsbmb.2013.11.004

Pillai, D. K., Iqbal, S. F., Benton, A. S., Lerner, J., Wiles, A., Foerster, M., … Freishtat, R. J. (2011). Associations between genetic variants in vitamin D metabolism and asthma characteristics in young African Americans: A pilot study. *Journal of Investigative Medicine : The Official Publication of the American Federation for Clinical Research*, *59*(6), 938–946. https://doi.org/10.231/JIM.0b013e318220df41

Popkin, B. M. (2006). Technology, transport, globalization and the nutrition transition food policy. *Food Policy*, *31*(6), 554–569. https://doi.org/10.1016/j.foodpol.2006.02.008

Portale, A. A., & Miller, W. L. (2000). Human 25-hydroxyvitamin D-1α-hydroxylase: Cloning, mutations, and gene expression. *Pediatric Nephrology*, *14*(7), 620–625. https://doi.org/10.1007/PL00009639

Post, P. W., Szabó, G., & Keeling, M. E. (1975). A quantitative and morphological study of the pigmentary system of the chimpanzee with the light and electron microscope. *American Journal of Physical Anthropology*, *43*(3), 435–443. https://doi.org/10.1002/ajpa.1330430325

Prentice, A. (2008). Vitamin D deficiency: A global perspective. *Nutrition Reviews*, *66*, S153–S164. https://doi.org/10.1111/j.1753-4887.2008.00100.x

Prietl, B., Treiber, G., Pieber, T., & Amrein, K. (2013). Vitamin D and Immune Function. *Nutrients*, *5*(7), 2502–2521. https://doi.org/10.3390/nu5072502

Quillen, E. E. (2015). The Evolution of Tanning Needs Its Day in the Sun. *Human Biology*, *87*(4), 352–360.

Rajakumar, K., Greenspan, S. L., Thomas, S. B., & Holick, M. F. (2007). SOLAR Ultraviolet Radiation AND Vitamin D. *American Journal of Public Health*, *97*(10), 1746–1754. https://doi.org/10.2105/AJPH.2006.091736

Ramagopalan, S. V., Heger, A., Berlanga, A. J., Maugeri, N. J., Lincoln, M. R., Burrell, A., … Knight, J. C. (2010). A ChIP-seq defined genome-wide map of vitamin D receptor binding: Associations with disease and evolution. *Genome Research*, *20*(10), 1352–1360. https://doi.org/10.1101/gr.107920.110

Reiches, M. W., Ellison, P. T., Lipson, S. F., Sharrock, K. C., Gardiner, E., & Duncan, L. G. (2009). Pooled energy budget and human life history. *American Journal of Human Biology*, *21*(4), 421–429. https://doi.org/10.1002/ajhb.20906

Reis, A., Hauache, O., & Velho, G. (2005). Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence. *Diabetes & Metabolism*, *31*(4), 318–325. https://doi.org/10.1016/S1262-3636(07)70200-8

Relethford, J. H. (1997). Hemispheric difference in human skin color. *American Journal of Physical Anthropology*, *104*(4), 449–457. https://doi.org/10.1002/(SICI)1096-8644(199712)104:4<449::AID-AJPA2>3.0.CO;2-N

Relethford, J. H. (2000). Human skin color diversity is highest in sub-Saharan African populations. *Human Biology*, *72*(5), 773–780.

Relethford, J. H. (2002). Apportionment of global human genetic diversity based on craniometrics and skin color. *American Journal of Physical Anthropology*, *118*(4), 393–398. https://doi.org/10.1002/ajpa.10079

Republic of the Philippines Ministry of Health. Republic Act 8976: An Act Establishing the Philippine Food Fortification Program And For Other Purposes. , Republic Act No. 8976 § (2000).

Rider, V., Foster, R. T., Evans, M., Suenaga, R., & Abdou, N. I. (1998). Gender Differences in Autoimmune Diseases: Estrogen Increases Calcineurin Expression in Systemic Lupus Erythematosus. *Clinical Immunology and Immunopathology*, *89*(2), 171–180. https://doi.org/10.1006/clin.1998.4604

Roomi, M. A., Farooq, A., Ullah, E., & Lone, K. P. (2015). Hypovitaminosis D and its association with lifestyle factors. *Pakistan Journal of Medical Sciences*, *31*(5), 1236–1240. https://doi.org/10.12669/pjms.315.7196

Ross, A. C., & Institute of Medicine (U. S.) (Eds.). (2011a). *Dietary reference intakes: Calcium, vitamin D*. Washington, DC: National Academies Press.

Ross, A. C., & Institute of Medicine (U. S.) (Eds.). (2011b). *Dietary reference intakes: Calcium, vitamin D*. Washington, DC: National Academies Press.

Sacheck, J., Goodman, E., Chui, K., Chomitz, V., Must, A., & Economos, C. (2011). Vitamin D Deficiency, Adiposity, and Cardiometabolic Risk in Urban Schoolchildren. *The Journal of Pediatrics*, *159*(6), 945–950. https://doi.org/10.1016/j.jpeds.2011.06.001

Saenger, A. K., Laha, T. J., Bremner, D. E., & Sadrzadeh, S. M. H. (2006). Quantification of Serum 25-Hydroxyvitamin D2 and D3Using HPLC–Tandem Mass Spectrometry and Examination of Reference Intervals for Diagnosis of Vitamin D Deficiency. *American Journal of Clinical Pathology*, *125*(6), 914–920. https://doi.org/10.1309/J32UF7GTQPWN25AP

Schmid, A., & Walther, B. (2013). Natural Vitamin D Content in Animal Products. *Advances in Nutrition*, *4*(4), 453–462. https://doi.org/10.3945/an.113.003780

Schrage, B., Lund, L. H., Benson, L., Stolfo, D., Ohlsson, A., Westerling, R., … Savarese, G. (2021). Lower socioeconomic status predicts higher mortality and morbidity in patients with heart failure. *Heart*, *107*(3), 229–236. https://doi.org/10.1136/heartjnl-2020-317216

Serrano, M.-A., Cañada, J., Moreno, J. C., & Gurrea, G. (2017). Solar ultraviolet doses and vitamin D in a northern mid-latitude. *Science of The Total Environment*, *574*, 744–750. https://doi.org/10.1016/j.scitotenv.2016.09.102

Siar, S. V. (2003). Knowledge, Gender, and Resources in Small-Scale Fishing: The Case of Honda Bay, Palawan, Philippines. *Environmental Management*, *31*(5), 569–580. https://doi.org/10.1007/s00267-002-2872-7

Smolders, J., Damoiseaux, J., Menheere, P., & Hupperts, R. (2008). Vitamin D as an immune modulator in multiple sclerosis, a review. *Journal of Neuroimmunology*, *194*(1–2), 7–17. https://doi.org/10.1016/j.jneuroim.2007.11.014

Smolders, J., Thewissen, M., Theunissen, R., Peelen, E., Knippenberg, S., Menheere, P., … Damoiseaux, J. (2011). Vitamin D-related gene expression profiles in immune cells of patients with relapsing remitting multiple sclerosis. *Journal of Neuroimmunology*, *235*(1–2), 91–97. https://doi.org/10.1016/j.jneuroim.2011.03.012

Stacy Simon. (2019, March 5). Are You Getting Enough Vitamin D? Retrieved March 22, 2020, from American Cancer Society website: https://www.cancer.org/latest-news/are-you-getting-enough-vitamin-d.html

Stearns, S. C., Ackermann, M., Doebeli, M., & Kaiser, M. (2000). Experimental evolution of aging, growth, and reproduction in fruitflies. *Proceedings of the National Academy of Sciences*, *97*(7), 3309–3313. https://doi.org/10.1073/pnas.97.7.3309

Stephensen, C. B., Zerofsky, M., Burnett, D. J., Lin, Y., Hammock, B. D., Hall, L. M., & McHugh, T. (2012). Ergocalciferol from Mushrooms or Supplements Consumed with a Standard Meal Increases 25-Hydroxyergocalciferol but Decreases 25-

Hydroxycholecalciferol in the Serum of Healthy Adults. *The Journal of Nutrition*, *142*(7), 1246–1252. https://doi.org/10.3945/jn.112.159764

Stini, W. A. (1990). "Osteoporosis": Etiologies, prevention, and treatment. *American Journal of Physical Anthropology*, *33*(S11), 151–194. https://doi.org/10.1002/ajpa.1330330508

Stini, W. A. (2003). Sex differences in bone loss—An evolutionary perspective on a clinical problem. *Collegium Antropologicum*, *27*(1), 23–46. PubMed (12974131). Retrieved from PubMed. (12974131)

Szreter, S., & Mooney, G. (1998). Urbanization, Mortality, and the Standard of Living Debate: New Estimates of the Expectation of Life at Birth in Nineteenth-Century British Cities. *The Economic History Review*, *51*(1), 84–112.

Talat, N., Perry, S., Parsonnet, J., Dawood, G., & Hussain, R. (2010). Vitamin D Deficiency and Tuberculosis Progression. *Emerging Infectious Diseases*, *16*(5), 853–855. https://doi.org/10.3201/eid1605.091693

Tangpricha, V., Turner, A., Spina, C., Decastro, S., Chen, T. C., & Holick, M. F. (2004). Tanning is associated with optimal vitamin D status (serum 25-hydroxyvitamin D concentration) and higher bone mineral density. *The American Journal of Clinical Nutrition*, *80*(6), 1645–1649. https://doi.org/10.1093/ajcn/80.6.1645

Torell, E., Castro, J., Lazarte, A., & Bilecki, D. (2021). Analysis of Gender Roles in Philippine Fishing Communities. *Journal of International Development*, *33*(1), 233–255. https://doi.org/10.1002/jid.3520

Tornhammar, P., Ueda, P., Hult, M., Simila, H., Eyles, D., & Norman, M. (2014). Season of birth, neonatal vitamin D status, and cardiovascular disease risk at 35 y of age: A cohort

study from Sweden. *The American Journal of Clinical Nutrition*, *99*(3), 472–478. https://doi.org/10.3945/ajcn.113.072520

Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., … Lanham-New, S. (2012). Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, *95*(6), 1357–1364. https://doi.org/10.3945/ajcn.111.031070

Verdoia, M., Schaffer, A., Barbieri, L., Di Giovine, G., Marino, P., Suryapranata, H., & De Luca, G. (2015). Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease. *Nutrition, Metabolism and Cardiovascular Diseases*, *25*(5), 464–470. https://doi.org/10.1016/j.numecd.2015.01.009

Vieth, R., & Holick, M. F. (2018). Chapter 57B - The IOM—Endocrine Society Controversy on Recommended Vitamin D Targets: In Support of the Endocrine Society Position. In D. Feldman (Ed.), *Vitamin D (Fourth Edition)* (pp. 1091–1107). Academic Press. https://doi.org/10.1016/B978-0-12-809965-0.00059-8

Vincent Miles. (2014). *The Vitamin D Miracle: How to Cure Common Health Problems and Have Optimal Health Free Book Offer Included*. CreateSpace Independent Publishing Platform. Retrieved from https://books.google.com/books?id=LMmhoAEACAAJ

Voortman, T., van den Hooven, E. H., Heijboer, A. C., Hofman, A., Jaddoe, V. W., & Franco, O. H. (2015). Vitamin D Deficiency in School-Age Children Is Associated with Sociodemographic and Lifestyle Factors. *The Journal of Nutrition*, *145*(4), 791–798. https://doi.org/10.3945/jn.114.208280

Vyas, S., & Kumaranayake, L. (2006). Constructing socio-economic status indices: How to use principal components analysis. *Health Policy and Planning*, *21*(6), 459–468. https://doi.org/10.1093/heapol/czl029

Wacker, M., & Holick, M. F. (2013). Vitamin D — Effects on Skeletal and Extraskeletal Health and the Need for Supplementation. *Nutrients*, *5*(1), 111–148. https://doi.org/10.3390/nu5010111

Wagner, C. L., Taylor, S. N., & Hollis, B. W. (2008). Does Vitamin D Make the World Go 'Round'? *Breastfeeding Medicine*, *3*(4), 239–250. https://doi.org/10.1089/bfm.2008.9984

Wakayo, T., Whiting, S. J., & Belachew, T. (2016). Vitamin D Deficiency is Associated with Overweight and/or Obesity among Schoolchildren in Central Ethiopia: A Cross-Sectional Study. *Nutrients*, *8*(4). https://doi.org/10.3390/nu8040190

Wang, Y., Zhu, J., & DeLuca, H. F. (2012). Where is the vitamin D receptor? *Archives of Biochemistry and Biophysics*, *523*(1), 123–133. https://doi.org/10.1016/j.abb.2012.04.001

Wei, J., Zhu, A., & Ji, J. S. (2019). A Comparison Study of Vitamin D Deficiency among Older Adults in China and the United States. *Scientific Reports*, *9*(1), 19713. https://doi.org/10.1038/s41598-019-56297-y

Whitacre, C. C., Reingold, S. C., & O'Looney, P. A. (1999). A Gender Gap in Autoimmunity. *Science*, *283*(5406), 1277–1278.

Wikvall, K. (2001). Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (Review). *International Journal of Molecular Medicine*, *7*(2), 201–209. https://doi.org/10.3892/ijmm.7.2.201

World Health Organization. (2006). *Guidelines on food fortification with micronutrients* (Lindsay Allen, Bruno de Benoist, Omar Dary, & Richard Hurrell, Eds.). Geneva: World Health Organization. Retrieved from http://search.proquest.com/docview/200185097?rfr_id=info%3Axri%2Fsid%3Aprimo

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000a). Decreased bioavailability of vitamin D in obesity. *The American Journal of Clinical Nutrition*, *72*(3), 690–693.

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000b). Decreased bioavailability of vitamin D in obesity. *The American Journal of Clinical Nutrition*, *72*(3), 690–693.

Yamauchi, T., Umezaki, M., & Ohtsuka, R. (2001). Physical activity and subsistence pattern of the Huli, a Papua New Guinea Highland population. *American Journal of Physical Anthropology*, *114*(3), 258–268. https://doi.org/10.1002/1096-8644(200103)114:3<258::AID-AJPA1024>3.0.CO;2-Y

Yan, J., Liu, L., Zhu, Y., Huang, G., & Wang, P. P. (2014). The association between breastfeeding and childhood obesity: A meta-analysis. *BMC Public Health*, *14*. https://doi.org/10.1186/1471-2458-14-1267

Yasutake, Y., Fujii, Y., Nishioka, T., Cheon, W.-K., Arisawa, A., & Tamura, T. (2010). Structural Evidence for Enhancement of Sequential Vitamin D3 Hydroxylation Activities by Directed Evolution of Cytochrome P450 Vitamin D3 Hydroxylase. *The Journal of Biological Chemistry*, *285*(41), 31193–31201. https://doi.org/10.1074/jbc.M110.147009

Yetley, E. A., Pfeiffer, C. M., Schleicher, R. L., Phinney, K. W., Lacher, D. A., Christakos, S., … Picciano, M. F. (2010). NHANES Monitoring of Serum 25-Hydroxyvitamin D: A Roundtable Summary123. *The Journal of Nutrition*, *140*(11), 2030S-2045S. https://doi.org/10.3945/jn.110.121483

Yousefzadeh, P., Shapses, S. A., & Wang, X. (2014). Vitamin D Binding Protein Impact on 25-Hydroxyvitamin D Levels under Different Physiologic and Pathologic Conditions [Research article]. https://doi.org/10.1155/2014/981581

Yu, H.-J., Kwon, M.-J., Woo, H.-Y., & Park, H. (2016). Analysis of 25-Hydroxyvitamin D Status According to Age, Gender, and Seasonal Variation. *Journal of Clinical Laboratory Analysis*, *30*(6), 905–911. https://doi.org/10.1002/jcla.21955

Yu, S., Fang, H., Han, J., Cheng, X., Xia, L., Li, S., … Qiu, L. (2015). The High Prevalence of Hypovitaminosis D in China. *Medicine*, *94*(8). https://doi.org/10.1097/MD.0000000000000585

Yuen, A. W. C., & Jablonski, N. G. (2010a). Vitamin D: In the evolution of human skin colour. *Medical Hypotheses*, *74*(1), 39–44. https://doi.org/10.1016/j.mehy.2009.08.007

Yuen, A. W. C., & Jablonski, N. G. (2010b). Vitamin D: In the evolution of human skin colour. *Medical Hypotheses*, *74*(1), 39–44. https://doi.org/10.1016/j.mehy.2009.08.007

Zasloff, M. (2006). Fighting infections with vitamin D. *Nature Medicine*, *12*(4), 388–391.

Zgaga, L., Theodoratou, E., Farrington, S. M., Agakov, F., Tenesa, A., Walker, M., … Campbell, H. (2011). Diet, Environmental Factors, and Lifestyle Underlie the High Prevalence of Vitamin D Deficiency in Healthy Adults in Scotland, and Supplementation Reduces the

Proportion That Are Severely Deficient. *The Journal of Nutrition, 141*(8), 1535–1542. https://doi.org/10.3945/jn.111.140012

Zhao, D., Ouyang, P., de Boer, I. H., Lutsey, P. L., Farag, Y. M. K., Guallar, E., … Michos, E. D. (2017). Serum vitamin D and sex hormones levels in men and women: The Multi-Ethnic Study of Atherosclerosis (MESA). *Maturitas, 96*, 95–102. https://doi.org/10.1016/j.maturitas.2016.11.017

Zoltan P. Rona. (2011). *Vitamin D: The Sunshine Vitamin*. Book Publishing Company. Retrieved from https://books.google.com/books?id=Po66BgAAQBAJ