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Vitamin D and Age-Related Macular Degeneration

Mandy Hemphill
Walden University

Walden University

College of Health Sciences

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Miranda Hemphill

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Review Committee
Dr. Hadi Danawi, Committee Chairperson, Public Health Faculty
Dr. Aimee Ferraro, Committee Member, Public Health Faculty
Dr. James Rohrer, University Reviewer, Public Health Faculty

Chief Academic Officer
Eric Riedel, Ph.D.

Walden University
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Abstract

Vitamin D and Age-Related Macular Degeneration

by

Mandy Hemphill

BS, Southern Methodist University, 1996

Dissertation Submitted in Partial Fulfillment of the

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Abstract

Age-related macular degeneration (AMD) is the leading cause of vision loss in individuals aged 50 years and older and is estimated to affect as many as 11 million individuals in the United States. The purpose of this study was to examine the association between vitamin D and AMD disease progression. The life course epidemiology framework model was used to explore how vitamin D level as a risk factor may have an association to AMD disease through time. Data in the 2005–2008 National Health and Nutrition Examination Survey (NHANES) database were collected on vitamin D levels and identified stages of AMD level based on graded fundus eye exams from an available sample size of 5,604 participants. A quantitative cross-sectional study approach was used to address this gap in knowledge. A bivariate analysis was used to examine each independent variable (age, race/ethnicity, smoking status, and diabetes) to the dependent variable AMD from the 2005–2008 NHANES dataset. A multivariate logistic regression analysis was performed with AMD including each independent variable found to be significant. The findings from this study failed to suggest an association between vitamin D levels to AMD, with or without the covariates included in the model. There was not an association found between vitamin D level and presence of AMD. An association was found between age, smoking, and race to presence of AMD in each of the bivariate models. The findings from this study could be used for positive social change by encouraging medical and public health agencies to target screening programs at high-risk age, smoking, and race groups. There remains to be conflicting data in the literature. This study adds to the body of literature suggesting that higher levels of vitamin D are not necessarily beneficial as they pertains to AMD.

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Chapter 1: Introduction to the Study

Introduction

As the term implies, *age-related macular degeneration* (*AMD*) refers to macular degeneration that occurs with age without any other precipitating conclusive associative risk factors in individuals aged 50 years and older (National Eye Institute [NEI], 2015). AMD is an ophthalmic condition leading to gradual loss of vision that may not cause noticeable symptoms in the early stages of the disease. However, through time, blurring of vision, and loss of sharpness in vision can occur. AMD can result in a wide range of vision-related symptoms causing impairment that include blurriness, decreased central vision, distortion of images and lines, decreased intensity or brightness of colors, reduced night vision, glare or scotoma (blind spot) (NEI, 2015).

Should modifiable factors that are related to AMD development or disease progression be identified, then there is a potential to have a positive effect on predicted growing numbers. In Chapter 1, I discuss a review of the existing literature on the topic of the association of vitamin D levels to AMD and why this association needs to be explored further. In addition, the research questions and hypotheses that are examined are stated along with the theoretical framework that was used for the study, discussion of the nature of the study, assumptions, limitations, terms used in the study are defined, and, last, how this study will advance knowledge of the AMD disease.

Background

Several studies have evaluated vitamin D levels and its association with AMD. Parekh et al. (2007) provided information suggesting that vitamin D might have a protective effect against AMD. However, Cougnard-Gregoire et al. (2015) found contradicting results in a population-based sample of French older subjects from a community dwelling that did not find a specific role for vitamin D in AMD. One notable difference between these studies was that the former included 7,752 individuals in their study population compared to 697 subjects in the latter (Parekh, 2007; Cougnard-Gregoire, 2015).

Millen and colleagues (2015) conducted a study that found that vitamin D deficiency was associated to AMD, with the highest risk in individuals that also contained 2 risk alleles for complement factor H (CFH) and complement factor I (CFI) genotypes. Variants in these genes have been shown to be associated with increased AMD risk – for both early- and late-stage disease (Edwards et al., 2005). The recent study conducted by Millen and colleagues (2015) suggested there was a joint effect with vitamin D level and genotypes of known high genetic risk to AMD.

Itty and colleagues (2014) provided information that compared 25-hydroxyvitamin D (25OHD) levels in subjects with different forms of AMD demonstrating lower levels of 25OHD in neovascular AMD (NVAMD), an advanced form, suggesting vitamin D could be a modifiable risk factor. Kim, Han, and Jee (2014) conducted a study that concluded that high serum levels of vitamin D were inversely associated to the advanced form of AMD in men, but not in women. This study differs

from the one by Parekh and colleagues (2007) in that the latter found serum vitamin D levels to demonstrate a relationship or association with early AMD, but not in the advanced form of AMD.

Several studies have evaluated the association between vitamin D levels and AMD. Of the few studies that have been done, conflicting results have suggested that it is difficult to draw definitive conclusions based on what is currently known. The association between vitamin D to AMD still remains unclear and the small number of studies conducted suggests that this specific association warrants further study.

AMD is a disease of the retina, or back of the eye, that, if left untreated, can lead to blindness. AMD is classified by four stages: no AMD, early AMD, intermediate AMD, or late AMD (Van Lookeren Campagne, LeCouter, Yaspan, & Ye, 2014). There are two types of late stage AMD: NVAMD (often referred to as wet AMD) and geographic atrophy (GA; often referred to as dry AMD) (Van Lookeren Campagne et al., 2014; Ferris et al., 2013).

Few recognized phenotypes exist for AMD. AMD fundus photos are imaging pictures that are taken of the retina (the back part of the eye) in a doctor's office to identify and diagnose the disease (Bird et al., 1995). Classification of the photos using different grading systems can result in patients that demonstrate a phenotype that falls in a category that is specific to the disease (NEI, n.d.). AMD patients will often demonstrate similar fundus photo characteristics that can be categorized as having the same phenotype (NEI, n.d.).

Vitamin D (25OHD) is a fat-soluble vitamin that can be found in some foods and is broken down by ultraviolet light (National Institutes of Health Office of Dietary Supplements, 2015). Vitamin D has been shown to play multiple roles in human health including its anti-inflammatory effects and that it is also an immune-modulator (Golan, Shalev, Treister, Chodick, & Loewenstein, 2011; Manolagas, Provvedini, Murry, Tsoukas, & Deftos, 1986). Calcitriol is the active hormonal form of vitamin D and has been demonstrated to affect endothelial cells (Albert et al., 2007). Proteins for the vitamin D receptor and the enzyme that converts the major circulating metabolites of vitamin D to its active hormone, calcitriol, are expressed in the retina (Zampatti et al., 2014). Albert and colleagues (2007) utilized a mouse model to demonstrate that calcitriol was a potent inhibitor of retinal neovascularization. Their data suggested that calcitriol might provide benefits in patients with neovascular eye diseases (Albert et al., 2007).

Vitamin D deficiency may increase the risk for developing AMD (Itty et al., 2014; Parekh et al., 2007). Few studies have examined the association of vitamin D levels and AMD. Parekh and colleagues (2007) conducted a study using the National Health and Nutrition Examination Survey (NHANES) data from 1988 through 1994 and found levels of vitamin D to be inversely associated with early AMD, but not advanced AMD, suggesting the vitamin may provide a protective role for the disease. Since then, several studies have shown an association of AMD with vitamin D (Millen et al., 2015; Itty et al., 2014; Kim et al., 2014;). However, several studies have not found an association (Cougnard-Gregoire et al., 2015; Park et al., 2014). Study design and data collection methods could be weaknesses that prevented the detection of an association. For

example, data collection methods and samples were taken primarily from a Korean population in the Park and colleagues (2014) study.

Statement of the Problem

Current global estimates indicate that 30–50 million people have AMD (Birch & Liang, 2007). Those numbers are projected to rise to 288 million by the year 2040 (Wong, 2014). AMD is the leading cause of severe and irreversible vision loss in the developed world in people older than 50 years and continues to grow as a public health problem (Wong, 2014). It is critical to continue to attempt to understand factors that affect the development of the disease or potentially advance disease progression. Conflicting results from the limited number of studies on the association of vitamin D and AMD suggest a gap in the literature and justification that further investigation is warranted. Additional research on the association of vitamin D and AMD may provide more information that will assist in awareness and understanding of factors related to the disease. Identifying factors that could be easily modified has the potential to positively affect the growing public health burden.

Purpose of the Study

The purpose of this study was to examine the association between vitamin D and AMD. I used a quantitative cross-sectional approach to address this gap in knowledge. I also used a logistic regression statistical methods analysis to examine data in the NHANES database that was collected by examiners on vitamin D and identified stages of AMD level graded from fundus eye exams. The study compared vitamin D levels to

stages of AMD disease across participants attempting to explore whether an association existed.

The independent variable in the study was level of vitamin D. The dependent variable was stage of AMD. The covariates were diabetes, age, race, and smoking status.

Research Questions and Hypotheses

The following research questions and hypotheses have been derived from a review of existing literature on the topic of vitamin D level and its association with AMD.

RQ1: What is the association between vitamin D level (high versus low) and the development of AMD?

$H_0$1: There is no association between vitamin D level (high versus low) and the development of AMD.

H_a1: There is an association between vitamin D level and the development of AMD.

RQ2: What is the association between diabetes (yes/no) and development of AMD?

$H_0$2: There is no association between diabetes (yes/no) and the development of AMD.

H_a2: There is an association between diabetes (yes/no) and the development of AMD.

RQ3: What is the association between age and development of AMD?

$H_0$3: There is no association between age and the development of AMD.

H_a3: There is an association between age and the development of AMD.

RQ4: What is the association between race and the development of AMD?

$H_0 4$: There is no association between race and the development of AMD.

$H_a 4$: There is an association between race and the development of AMD.

RQ5: What is the association between smoking (yes/no) and the development of AMD?

$H_0 5$: There is no association between smoking (yes/no) and the development of AMD.

$H_a 5$: There is an association between smoking (yes/no) and the development of AMD.

RQ6: What is the association between vitamin D level and the development of AMD, after controlling for covariates?

$H_0 6$: There is no association between vitamin D level and the development of AMD after controlling for covariates.

$H_a 6$: There is an association between vitamin D level and the development of AMD after controlling for covariates.

I analyzed the first five questions using binary logistic regression with a binary outcome AMD (yes = 1 and no = 0). I analyzed the final (sixth) question using a multivariate model. Logistic regression modeling was used to develop the most parsimonious model with significant risk factors associated with the development of AMD.

Theoretical Framework for the Study

The theoretical base for this study was the life course epidemiology model. According to Parekh and Zizza (2013), the life course theory when applied in epidemiology can provide a framework that will examine how nutrition along with other risk factors can have a long-term effect on chronic disease development. This theory was utilized to explore how certain risk factors have an association to the disease in question through time or the in the course of an individual's life (Parekh & Zizza, 2013). This theory was utilized to better understand biological, environmental, and psychosocial pathways and how they collectively affect the natural history of an individual's health (Parekh & Zizza, 2013). This approach was utilized to examine the association of vitamin D and the role it potentially played in development of AMD, which is a disease that develops through time. I considered only biological and environmental factors in this study. I provide a more detailed explanation in Chapter 2. In Table 1, I present an overview of the constructs utilized for this research.

Table 1

Overview of Constructs

Variable	Biological pathway	Environmental pathway
Vitamin D level		X
Diabetes	X	
Age	X	
Race	X	
Smoking status	X	X

Nature of the Study

Although with conflicting results, several studies have been conducted to better understand the association between level of vitamin D and AMD disease development and stage of disease. Therefore, the rationale for this study was to evaluate large numbers of subjects with the hope to strengthen the results of the data while adding to the existing literature. The selection of utilizing the existing NHANES database and running a secondary analysis provided the opportunity to have these large numbers.

The key study variables included vitamin D level; AMD status; and presence of diabetes, age, race, and smoking status. In this study, I sought to better understand the association between vitamin D level with the development of AMD, while considering the covariates of age, race, diabetes, and smoking status.

In my cross-sectional study, I utilized a quantitative methods approach to implement a logistic regression analysis using an existing dataset. The 2005–2008 NHANES dataset contains serum concentration levels of vitamin D as well as retinal fundus photographs graded and categorized by AMD severity scale. This 2005–2008 dataset from NHANES was utilized to analyze the association of vitamin D levels to AMD severity from an available sample size of 5,604 individuals (Centers for Disease Control and Prevention [CDC], 2015).

Definitions

Age-related macular degeneration (*AMD*): A progressive degenerative disease of the macula – an area of the retina responsible for visual acuity and color vision (Van Lookeren Campagne et al., 2014).

Early age-related macular degeneration : Generally referred to as dry AMD – is characterized by medium drusen >63 μm and ≤125 μm with no AMD pigmentary abnormalities, and generally causes only minimal visual acuity impairment (Van Lookeren Campagne et al., 2014).

Diabetes: A disease in which an individual's blood glucose, or blood sugar levels are too high – typically a fasting plasma glucose higher than 7.0 mmol/l (NIH, 2016).

Fundus imaging eye exams: The process where 2-D or 3-D retinal semitransparent tissues are projected using reflected light onto an imaging plane (Abramoff, Garvin, & Sonka, 2010).

Intermediate age-related macular degeneration : Also generally referred to as dry AMD – is characterized by large druze >125 μm and/or any AMD pigmentary abnormalities, and generally causes only minimal visual acuity impairment (Van Lookeren Campagne et al., 2014).

Late age-related macular degeneration : Also referred to as advanced AMD, this condition has two forms: (a) NVAMD (wet/exudative AMD) and (b) GA (Van Lookeren Campagne et al., 2014).

Vitamin D: A fat-soluble vitamin naturally occurring in few foods, added to others and available as a dietary supplement (NIH, 2014).

Assumptions

I utilized data collected from NHANES. The NHANES database is publicly available and I assumed that the fundus photos taken have been read and graded correctly. I also assumed that the correct sampling and testing methods were utilized for quantitative analysis of vitamin D level. In addition, I assumed that individuals that participated did so willingly, thereby lessening the chance of bias. These assumptions were necessary to the validity of the study being conducted.

Scope and Delimitations

The 2005–2008 NHANES database used an oversampling of persons aged 60 years and older (CDC, 2015). This particular aspect of the data was beneficial for the current study as I sought to better understand the association between variables in a disease that occurs in the aging population. The nature of the NHANES database sampling accounted for why it made sense to use this population to run an analysis looking at better understanding a disease in an aging population. In addition, having the independent and dependent variables both measured in a manner that minimized recall bias contributed to the strength of the data outcomes and conclusions.

A drawback was that all of the individuals in this population dataset were from the United States. It was a large sampling in the NHANES database and was generalizable to the overall U.S. population. However, utilizing data from only one country creates a challenge in saying the conclusions are generalizable to populations in other countries and delimits implications to U.S. populations.

Limitations

A limitation of the study was the potential for individual participant bias for the portions of data that were self-reported. Covariates smoking and diabetes were both self-reported by participants. Smokers may contribute bias by providing self-reported answers they believed were more socially desirable. In addition, medical records indicating diabetes diagnosis could have been more accurate than participant self-reports. As stated previously, AMD is a disease of those older than 55 years. Memory recall may be potentially worse in this age population contributing to recall bias. Other covariates included age and race; these are both straightforward and should not contribute to bias that influences outcomes. Fortunately, the manner of data collection for both the independent variable (vitamin D level) and dependent variable (stage of AMD) minimized recall bias. Examiners in a lab tested vitamin D level and masked readers did the reading of retinal images (NHANES, 2015). However, the lab method for vitamin D measurement changed (discussed further in Chapter 3) from the 2005–2006 and 2007–2008 data collection years and presents a potential limitation through measurement bias. Additional measures were not needed to address limitations of the study.

Significance

In the United States, an estimated 1.75 million adults older than 40 years have some form of advanced AMD (Cheung & Eaton, 2013). By 2020, that number is expected to climb to approximately 3 million (Wong et al., 2014; Brantley et al., 2012). AMD is still the leading cause of blindness in the Western world (Brantley et al., 2012). Globally, AMD is the third leading cause of visual impairment with an estimated

blindness prevalence of 8.7% (WHO, 2014). Several risk factors have been associated with AMD including age, race, smoking, a number of genetic risk factors, and diet (Chew, Clemons, Milton, & Sperduto, 2007; Klein, Peto, Bird, & Vannewkirk, 2004). The greatest risk for developing AMD is attributed to age, as data suggested that greater than 15% of white women older than 80 years have advanced forms of AMD (Klein et al., 2004). AMD has a significant and growing effect on public health and poses significant economic and social burdens. By demonstrating that there is an association between vitamin D level and development of AMD, this research may effect positive social change at a global level. If an association is found and can successfully be established, AMD disease progression may be addressed with vitamin D supplementation. If identifying this association can lead to a supplement that lessens disease progression and AMD patients have better outcomes, then an additional social implication includes the potential to a reduced burden on the cost of health care.

Summary

AMD is a complicated disease with a growing burden on public health. Several potential modifiable risk factors warrant further exploration. There is limited research on the association of serum vitamin D levels with AMD disease progression. This study may add to the literature on this topic and address this gap in knowledge. If an association between the two can be more clearly defined, then the study results may have a meaningful effect on AMD disease progression and mitigating the effects on the growing future public health burden. If vision can be spared, then patients may have better outcomes and lead more normal lives.

In Chapter 2, I review the existing literature on the topic of vitamin D association to AMD. I also discuss how the existing research serves as a foundation for this study and a need to learn more. In Chapter 2, I provide a discussion of the literature with a focus on the findings as well as challenges. I conclude Chapter 2 with how outcomes and implications from past research have the potential to guide further research and address existing gaps.

Chapter 2: Literature Review

Introduction

AMD is a disease of the back of the eye, which can result in blindness if left untreated. AMD is classified by four stages: no AMD, early AMD, intermediate AMD, or late AMD, with two types of late stage AMD: neovascular (or wet) AMD and GA (or dry) AMD (Van Lookeren Campagne et al., 2014; Ferris et al., 2013). It is projected that the current estimate of 30–50 million individuals living with AMD will grow to 288 million by 2040 (Singer, 2014; Wong, 2014). With this growing prevalence and affect on the public health system, it will be beneficial to better understand underlying factors that contribute to AMD disease development that could potentially be modified. The risk for developing AMD may be increased in the presence of vitamin D deficiency (Itty et al., 2014; Parekh et al., 2007). A limited number of studies have been done that look at the association of vitamin D levels and AMD. Vitamin D deficiency is a modifiable risk factor that is still not fully understood as it pertains to AMD and warrants further exploration. Several studies have been completed that I explored in detail. These studies have been largely inconclusive, with conflicting results among them. Some conclude by suggesting there is a possible association, whereas others indicate that an association between vitamin D deficiency and AMD development does not exist.

In Chapter 2, I discuss the literature review strategy. In addition, I describe the theoretical foundation with a rationale for why I chose it and I examine a review of the current literature related to key variables and concepts. Last, I conclude Chapter 2 with a summary of major themes in the literature and how this study fills the identified gap.

Literature Search Strategy

The search strategy included review of publications that reported on AMD, vitamin D, and vitamin D deficiency by searching the following databases: PubMed, Academic Search Premier, Science Direct, Google Scholar, and the Walden library. I searched these databases for relevant papers that were published on this topic until May 2016, which included the following search terms in various combinations: *age-related macular degeneration*, *vitamin D*, *vitamin D deficiency*, *serum vitamin D*, *risk*, *AMD status*, or *cross-sectional study*. This strategy assisted in identifying studies that examined the association of vitamin D to AMD in different types of studies. It is a relatively new topic to assess the link between vitamin D level and development of AMD. Therefore, the search was not limited in terms of how far back I searched for studies, but the relevant literature included was 10 published papers from 2007 to 2016. The two papers from 2007 were relevant and included because one was the only other NHANES database review on this topic and the other evaluated the role of calcitriol/vitamin D on retinal neovascularization in animals precipitating the necessity to understand the effect in humans (Albert et al., 2007; Parekh et al., 2007). There remains a gap in the literature on this topic of vitamin D association to AMD despite the list of studies discussed here.

Theoretical Foundation

I introduced the theoretical base for this study in Chapter 1, the life course epidemiology model. The life course theory studies biological, environmental, and psychosocial pathways that play a role in an individual's life span as it relates to development of chronic disease (Parekh & Zizza, 2013). However, my work focused on

the biological and environmental pathways only. The life course approach is a methodology that provides a framework to analyze how risk factors can affect disease development in an individual's life through time. The life course theory was introduced in the 1960s by connecting several general principles including social change, social structure, and individual action to guiding the foundation of life course research (Giele & Elder, 1998).

This life course theory helps explain disease patterns across populations through time and attempts to better understand if and how exposures effect chronic disease development. The life course theory may employ either qualitative or quantitative approaches. Quantitative approaches include the following: life event history analysis, longitudinal studies, as well as cohort and cross-sectional designs. The current research utilized a cross-sectional design for data analysis.

Ben-Shlomo and Kuh (2002) discussed propositions, hypotheses, and challenges of the life course approach to chronic disease epidemiology back when increasing interest in using this framework began. The life course theory employs an approach that examined long-term affect of physical and social exposures throughout gestation, childhood, adolescence, young adulthood, and later life attempting to explore how the exposures had an effect on chronic disease risk (Ben-Shlomo & Kuh, 2002). There have been chronic disease cohort studies conducted in the past that implemented the life course theory by collecting baseline measures, then following patients and analyzed changes in exposure data based on the follow up metrics collected. A challenge of this type of analysis that has been observed was that despite baseline measures (including birth

weight and socioeconomic status) being accounted for, there existed a temporal relationship between variables that was not considered (Ben-Shlomo & Kuh, 2002).

Considering there are multiple factors or exposures that play a role in disease and the significance of understanding the timing of the exposures, it should come as no surprise that disease risk is affected by the exposures in multiple ways (Ben-Shlomo & Kuh, 2002). The ways the exposures are interrelated should be acknowledged and considered. For the purposes of this study, a cross-sectional approach was utilized to look at a snapshot of multiple factors and how they each played a role in development of disease. Further research can be conducted to look more closely at the temporal relationship between the variables.

Ben-Shlomo and Kuh (2002) postulated that it is important to understand the natural history of a biological system, the temporal relationship between exposures and how across different periods through the life course that the exposures can influence biological development differently. For example, an exposure earlier in life may not necessarily have an appreciable effect on an individual's disease development rate or health decline in the same way that it might if the same exposure happened later (Ben-Shlomo & Kuh, 2002). Ku and Ben-Shlomo (2004) share an example of how an early life exposure can reduce lung function potential, but by comparison the mid to later life exposure accelerates the age-related decline even further. This observation makes a case for the following assumption: there is a critical period that an exposure can have a lasting or lifelong effect on a biological system or affect the development of disease (Kuh & Ben-Shlomo, 2004). It also seems counter intuitive as one might think the earlier

exposure would send a person on a longer path of decline that started sooner resulting in a worse outcome through time. If vitamin D level does affect development of AMD disease or disease progression, it should be considered at what point is it important to manage vitamin D levels through supplementation that has the greatest chance to reduce the risk of disease. This could be explored later, but a critical period may exist that vitamin D level as an exposure for disease poses the greatest risk. If so, it is possible that the critical period relates to the temporal relationship it plays with the other variables.

The life course epidemiology model makes sense for this research because it provides a framework for understanding how behavioral changes can affect disease prevention and may interrupt a disease cycle (Parekh & Zizza, 2013). The current research attempts to understand if modifying vitamin D levels in deficient individuals can interrupt the AMD disease cycle by slowing or preventing disease progression and address this gap in the literature.

Literature Review Related to Key Variables

AMD Risk Factors

The Age-Related Eye Disease Study (AREDS) Research Group ran a large clinical trial sponsored by the NEI from 1992 through 1998 that enrolled 4,757 participants with varying degrees of AMD severity in an attempt to learn more about and better understand the clinical course, risk factors, and prognosis of AMD and age-related cataracts (AREDS, 2005). The primary results at the end of the study in 2001 concluded that the AREDS vitamin supplement that was studied was successful in slowing or preventing progression of advanced AMD (AREDS, 2005). The AREDS study has

resulted in many subsequent papers and analysis to further understand AMD and confirmed or established various associated risk factors. Among them were the risk factors of age, race, smoking status, and diabetes that were included in this study as covariates.

Age. AMD is a progressive disease that can ultimately lead to GA or NVAMD as individual's age. AMD prevalence has been demonstrated to be age related in multiple studies. The AREDS study found increasing age (60 years and older, up to 80 years old) to be a risk factor for NVAMD and GA (AREDS, 2000). Chew and colleagues (2014) confirmed age as a risk factor in the subsequent AREDS2 study. The age risk factor continues to increase as the individual becomes older (Friedman et al., 2004; AREDS, 2000). According to the Chew and colleagues (2014) AREDS report, increasing age causes risk progression to the advanced form of AMD to go up. A pooled analysis from three large population-based studies, the Beaver Dam Study, the Rotterdam Study, and the Blue Mountains Eye Study that included 14,752 participants demonstrated age to be a risk factor with a clear association across AMD subtypes and all studies (Smith et al., 2001).

Race. When analyzing race as a risk factor, multiple large population based-studies have indicated being Caucasian presents the greatest risk (Friedman et al., 2004). Combining the Beaver Dam Eye Study, the Blue Mountains Eye Study, the Rotterdam Study, and the AREDS study including 1000's of participants, all have found being white versus other races presented the greatest risk in developing AMD (Chew et al., 2014; Friedman et al., 2004; AREDS, 2000). The Eye Diseases Prevalence Research Group

reported that more than 1 in 10 Caucasians aged 80 years and older has the advanced form or neovascular form of AMD (Friedman et al, 2004). Hispanic persons were found to have the lowest rate of AMD when compared to white and black counterparts (Friedman et al., 2004). The current study attempted to explore the association of race to AMD even further.

Smoking. Smoking has been extensively studied and fairly well established as a risk factor to AMD. The AREDS study found smoking to be a risk factor associated to the advanced neovascular form of AMD (nAMD) (AREDS, 2000). Chew and colleagues (2014) also found an association between smoking and AMD disease and found smoking to be the greatest risk in the oldest group of individuals (age 75 to 80 years old) that were in the most severe AMD category at baseline. The Blue Mountains Eye Study that included 3,654 participants, found smoking at the time of baseline exam was associated with an increased risk of both incident GA as well as the late form of AMD (Wang et al., 2007; Tomany et al., 2004). Furthermore, it has been found in multiple studies that current smokers were at higher risk than both past and non-smokers of developing incident AMD (Chew et al., 2014; Tomany et al., 2004). Tomany and colleagues (2004) reported that current smokers are 6.19 times more likely and past smokers were 5.52 times more likely to develop NVAMD than their non-smoking counterparts.

A ten-year follow-up of the Blue Mountains Eye Study that included 2,454 participants continued to demonstrate smoking association to AMD (Wang et al., 2007). Smith and colleagues (2001) reported that smoking was the principal known preventable exposure associated with any form of AMD based on pooled data from three large studies

including 14,752 individuals. This pooled analysis of the Beaver Dam Study, Rotterdam Study, and Blue Mountains study found tobacco smoking to be the only factor, apart from age, to demonstrate a clear association to both types of AMD (the neovascular form as well as GA) across all studies (Smith et al., 2001). At the time this research was conducted, smoking status presented the most easily modifiable risk factor that can affect AMD disease incidence and progression.

Diabetes. Diabetes is a major public health issue in the United States and continues to increase in prevalence. As prevalence increases, so does the demand on health care facilities through increased amount of disability, loss of productivity, and in some cases premature mortality (Rahman, Rahman, Ismail, & Rashid, 2007). Microvascular and macrovascular comorbidities are common in patients with diabetes (Rahman et al., 2007). Diabetes has been studied as a potential risk factor to association for development of AMD, a microvascular disease. The AREDS study (2005) found a weak association of diabetes as a risk factor to AMD based on medical history that reported the individual was under treatment for diabetes. In addition, the Blue Mountains Eye Study found having diabetes to be associated with the development of incident GA (Tomany et al., 2004). Diabetes as a risk factor for AMD still has limited data to support it. Exploring this risk factor further in the current study had the greatest potential of the covariates to add to the existing gap in the literature on exploring AMD risk factors. A better understanding of the association of diabetes to AMD broadens the scope of further understanding implications of growing incidence of both diabetes and AMD diseases.

Vitamin D. Using the aforementioned literature research strategy, ten research papers that include similar constructs of interest and the cross-sectional methodology are discussed in further detail. Of the ten papers, six studies (spanning from 2007 through 2015) utilized cross-sectional methodology similar to the scope of this study. The other four studies included: a study conducted by Albert and colleagues (2007) done in animals, a case series study done by Seddon et al. (2011), a prospective combined systems analysis by Morrison and colleagues (2011) including a family-based cohort as well as multiple case control cohorts, and a retrospective longitudinal analysis study done by Day and colleagues (2012).

Of all the research that has been done on this topic so far, most of the various researchers have utilized the cross-sectional study design approach, with the exception of the longitudinal cohort study done by Day et al. (2012), case series by Seddon et al. (2011), and prospective case control study by Morrison et al. (2011). The cross-sectional approach allows the researcher to view the association between a variable and a disease at a single point in time. The researchers that utilized this approach to attempt to better understand the association between vitamin D level and AMD disease are discussed in further detail.

Strengths of the cross-sectional study approach on this specific area of study include: these studies are relatively quick and easy to conduct; the data on selected variables is only collected once; multiple exposures and outcomes can be measured; the ability to measure prevalence for all variables being studied; and the importance of understanding the prevalence for the disease being studied in a specific population. The

many listed strengths of the cross-sectional approach contributed to the justification and rationale for use of the cross-sectional study design in the current study. The cross-sectional study design in this particular study allowed for data on the selected independent variable of vitamin D level and covariates of age, race, diabetes, and smoking status to all be studied from the measurements that were collected for the NHANES database.

A strength of the retrospective longitudinal cohort study conducted by Day and colleagues (2012) was that the study design looked at incident cases of AMD, which by nature of design required vitamin D deficiency to have occurred before AMD. This can be viewed as a weakness in cross-sectional study designs that assess prevalence and does not allow assessment of whether deficient vitamin D level or AMD disease occurred first (Day et al., 2012).

Weaknesses of the cross-sectional approach in this specific area of study included the following: difficulty in determining, which came first – the outcome or the exposure variable; it may be difficult to interpret the associations identified; or inability to measure incidence. Weaknesses in the retrospective cohort study conducted by Day and colleagues (2012) included: Medicare claims files were utilized for data and this data does not contain laboratory findings.

Weaknesses of the prospective case control cohorts conducted by Morrison and colleagues (2011) discussed here included recall bias and sample size. Telephone interviews were conducted in this particular study collecting various information. One key variable where recall bias may have influenced outcome data was on the amount of

sun exposure reported (Morrison et al., 2011). This data affected how vitamin D as a variable played a role in disease. In addition, the sample size of the advance AMD cases was very small and only included 10 individuals (Morrison et al., 2011).

Of the pieces of literature mentioned above, there were conflicting findings as it pertained to association of vitamin D to AMD. Based on these conflicting findings and limited number of studies conducted thus far, it would be plausible to consider the existing cross-sectional study attempting to further validate the existing findings. This study adds to the literature on the topic of association of vitamin D to AMD. The key variables for this study included vitamin D level, AMD status, and the presence of diabetes, age, race, and smoking status. With this study, I sought to better understand the association between vitamin D level with the development of AMD, while considering the covariates of age, race, diabetes, and smoking status.

Albert and colleagues (2007) set the stage for attempting to better understand the role vitamin D played in a variety of eye diseases that contained a neovascular component by examining calcitriol (1,25-hydroxyvitamin D3) in an animal model. A mouse oxygen-induced ischemic retinopathy (OIR) model was utilized to evaluate the role of calcitriol (Albert et al., 2007). Results from this mouse model in the study by Albert et al. (2007) demonstrated that calcitriol-treated animals showed a significant decrease in retinal neovascularization compared to control. These results suggested a link of vitamin D level to neovascular driven retinal disease that warranted exploration to begin in human studies. This particular study contributed to a gap of the association between vitamin D to AMD that needed further exploration.

Cross-sectional studies

Parekh and colleagues (2007) conducted the first study to attempt to better understand the association between vitamin D and AMD. This first study utilized a cross-sectional approach to evaluate the third NHANES from 1988 through 1994 (Parekh et al., 2007). The study was attempting to find additional risk factors and considered vitamin D because sunlight exposure had been suggested as a potential risk factor (Klein et al., 2004; Taylor et al., 1992). Parekh and colleagues (2007) explored the association of food and supplement sources of vitamin D to prevalence of AMD. The final analysis from the NHANES III utilized a logistic regression to study data from 7752 individuals and found vitamin D to be inversely associated to early AMD, but did not find the same association to advanced AMD (Parekh et al., 2007). Parekh and colleagues (2007) study was limited by having a small number of only 10 individuals in the advanced AMD group. However, the paper set the stage for justifying further research to confirm these findings. As a result, six subsequent cross-sectional studies were conducted as follow up in attempt to better understand the suggested association.

Millen et al. (2011) and Golan et al. (2011) published the subsequent cross-sectional studies on this topic and published conflicting results within the same year. Millen and colleagues (2011) were the second study that demonstrated a relationship by confirming an association of vitamin D status to development of AMD in Caucasian postmenopausal women from the Carotenoids in Age-related Eye Disease Study (CAREDS) population. AMD classification in this study was based on retinal fundus photographs taken of the 1,313 sample size of individuals from the years of 2001 through

2004 (Millen et al., 2011). This cross-sectional study model utilized a logistic regression statistical analysis method to evaluate the association of serum vitamin D concentrations from multiple sources (dietary, supplement, and sunlight) to prevalence of AMD in a study population of 1,313 women (Millen et al., 2011).

Millen and colleagues (2011) did not find a protective effect from reported direct sunlight hours. However, analyses of this postmenopausal sample of women confirmed the protective effects of vitamin D status to prevalence of AMD, similar to the results from Parekh and colleagues (2007). A limitation of this study was that the conclusions could only be extrapolated to Caucasian postmenopausal women. In addition, there was a potential for recall bias in this study as sunlight exposure since the age of 18 years of age was self-reported by participants (Millen et al., 2011).

Golan and colleagues (2011) utilized a cross sectional study approach to evaluate the connection between vitamin D levels and AMD in a study population including members of the Maccabi Healthcare Services (MHS) aged 60 years or above. The study population included 9,176 individuals – 1,045 with AMD and 8,124 non-AMD subjects (Golan et al., 2011). Golan et al. (2011) expected to find that AMD patients would have lower levels of vitamin D compared to their non-AMD counterparts, but instead they found higher levels of vitamin D were not associated with lower prevalence of AMD. Potential limitations of this study included: food and supplement intake of individuals was not accounted for; nor was the amount of sun exposure (Golan et al., 2011). A strength of this study was the relatively large number of AMD individuals included in the

study, which made it unlikely that the negative results were a result of a type II error (Golan et al., 2011).

Two large cross-sectional epidemiology studies investigating the association of vitamin D with AMD were conducted in 2014; one by Kim et al. (2014) and another by Itty and colleagues (2014). The three large epidemiologic studies prior generated conflicting results (Parekh et al., 2007; Millen et al., 2011; Golan et al., 2011). The study by Itty et al. (2014) concluded with similar findings to Parekh and colleagues (2007) and Millen et al. (2011). Whereas Kim et al. (2014) concluded with conflicting results suggesting there was an inverse association between high levels vitamin D and late AMD in men, but not in women.

Kim et al. (2014) evaluated a large sample of 17,045 individuals from the Korean National Health and Nutrition Examination Survey (KHANES) from 2008 to 2012. This study defined AMD in two categories, early and late. Early AMD was defined by presence and type of drusen, whereas late AMD was defined by the presence of wet (nAMD) or dry AMD (GA) (Kim et al., 2014). Simple and multiple logistic regression statistical methods were utilized in this study and results found the prevalence of late AMD was inversely associated with high vitamin levels in men but not in women (Kim et al., 2014). There was not a correlation found for early AMD in either men or women (Kim et al., 2014).

A strength of the study by Kim et al. (2014) was the large sample size. However, there were several limitations that included: inability to adjust for seasonal variation in vitamin D level, difficulty inferring causality based on study design type, capturing of

data on individuals that had their wet AMD treated with antivascular growth factors, and last the lack of detailed data on sunlight exposure (Kim et al., 2014). Sunlight exposure may be variable throughout the year and was not accounted for in this study, but it is questionable if the Asian population demonstrated seasonal variation in vitamin D status (Kim et al., 2014).

Itty and colleagues (2014) sought to explore the association of vitamin D deficiency to NVAMD and nonneovascular AMD (NNVAMD) compared to matched controls from a Duke University Medical Center population (aged 55 years and older) from July 1997 through November 2011. A total of 462 participants whose vitamin D levels had been measured were included in the study: 146 with NVAMD, 216 with NNVAMD, and 100 matched controls (Itty et al., 2014). This study done by Itty et al. (2014) found higher rates of vitamin D deficiency in the NVAMD group compared to those in the NNVAMD group and matched controls. There were limitations to this study, including the lack of temporal connection that goes along with a retrospective review. These findings were consistent with some of the previous studies mentioned and justify further review.

Cougnard-Gregoire and colleagues (2015) conducted the most recent cross-sectional population based study to explore the association between vitamin D deficiency and AMD. The study included 697 individuals from an elderly population (aged 72.7 $\pm$ 4.4 years) in Bordeaux France (Cougnard-Gregoire et al., 2015). Methods included collection of plasma vitamin D concentrations collected from 1999–2001 compared to retinal photographs with images classified as early or late AMD (Cougnard-Gregoire et

al., 2015). Early AMD was defined by drusen whereas late AMD was defined by NVAMD or GA (Cougnard-Gregoire et al., 2015). The study found prevalence of vitamin D deficiency to be very high at 83.2%, yet no associations of vitamin D status were found with any stage of AMD (Cougnard-Gregoire et al., 2015).

A limitation of the study by Cougnard-Gregoire and colleagues (2015) was the small number. However, there were several strengths including: objective measurement of vitamin D, blood samples taken at different times of the year, and confounders such as BMI, sociodemographic status, use of certain medications, factors related to vascular diseases, relevant genetic polymorphisms (Cougnard-Gregoire et al., 2015). The results reported in the Cougnard-Gregoire et al. (2015) study that did not support a role of vitamin D to AMD were similar to what Day et al. (2012) found as well as Golan et al. (2011). The existing evidence of an association between vitamin D level and AMD status were inconsistent and warranted further study.

Longitudinal Cohort Study

Day and colleagues (2012) conducted a retrospective longitudinal cohort study in Medicare beneficiaries examining a possible association between vitamin D deficiency and subsequent AMD diagnosis (of either the neovascular or non-neovascular form). Medicare 5% claims files from 2004 through 2006 were utilized to identify a patient population of 6,966 individuals with vitamin D deficiency (Day et al., 2012). Individuals with an AMD diagnosis that preceded the vitamin D deficiency diagnosis were excluded (Day et al., 2012). Results from this study did not find an association of vitamin D deficiency to diagnosis of either form (neovascular or non-neovascular) of AMD to be

statistically significant (Day et al., 2012). Although the findings of Day et al. (2012) of no association of vitamin D levels to AMD were similar to those of Golan and colleagues (2011), they were contrary to what several previous studies had shown.

A strength of the study by Day and colleagues (2012) was the design looked at incident cases and therefore differed from the other studies mentioned because in this study it was known that vitamin D deficiency occurred first (Day et al., 2012). This was the first study to examine incident cases rather than prevalence of AMD disease (Day et al., 2012). A weakness of this study was lack of laboratory results from the type of claims data utilized (Day et al, 2012).

Case Series Study

Seddon and colleagues (2011) conducted a case series study to evaluate the affect of behavioral and nutritional factors (including vitamin D dietary intake) on AMD in Caucasian male monozygotic twins. Fundus photographs that were graded by Wisconsin Grading System were utilized to evaluate stage of disease (Seddon et al., 2011). The Wilcoxon signed-rank test and linear regression statistical models were utilized to assess the associations between the various nutritional and behavioral characteristics (including vitamin D level) and stage of AMD disease (Seddon et al., 2011). The results of this study found dietary intake of vitamin D to be inversely associated with AMD, drusen size, and drusen area (Seddon et al., 2011). Although they explored different population types, the results of this case series by Seddon et al. (2011) along with those of the cross-sectional study conducted in the same year by Millen and colleagues (2011) both

supplemented the original findings by Parekh and colleagues (2007) that suggested there was an association between vitamin D level and AMD disease status.

Prospective Cohort Studies

Morrison and colleagues (2011) utilized a family based cohort study of 50 sibling pairs to examine vitamin D levels compared to AMD. Data on vitamin supplementation was collected via questionnaire and UV exposure was based on location of where individuals lived for the majority of their lives (Morrison et al., 2011). Limitations of this type of data collection included recall bias as it pertains to vitamin D supplementation and quantification of sunlight exposure is difficult to assess at best. Results from this study suggested a trend toward lower levels of vitamin D in the siblings with NVAMD compared to higher concentrations of vitamin D in the unaffected counterparts (Morrison et al., 2014). However, the difference was not statistically significant. This study done by Morrison et al. (2014) was the first to detect a genetic association between vitamin status and AMD and is discussed further in Chapter 5.

The limited number of studies discussed above on the topic of vitamin D association to AMD generated inconsistent findings and suggested further exploration was needed. There remains a gap in the literature on this topic and more research on the association of vitamin D status to AMD needs to be done.

Summary and Conclusions

The themes in the literature on this topic to date suggest data is inconclusive. Most of the studies that have been done exploring the topic of the association of vitamin D to AMD status were cross-sectional design studies. What is known is that vitamin D

levels have been shown to be protective in a variety of different conditions (Morrison et al., 2011). What is not known is how exactly vitamin D plays a role in prevalence of AMD disease. The limited number of studies on this topic in recent years has found conflicting results and suggests that more research needs to be done. The present study explored in a large sample size the association of vitamin D to AMD status and adds to the existing literature. Using the NHANES database, the association between vitamin D level and AMD progression was investigated here.

In Chapter 3, I discuss the research design and rationale for this study. I review the methodology, including: the population, sampling, recruitment, participation, data collection, and instrumentation. I conclude Chapter 3 with threats to validity and explore possible ethical concerns or issues.

Chapter 3: Research Method

Introduction

AMD is a progressive disease that can ultimately lead to one of two forms of the disease: GA or NVAMD. The most common type of AMD is the nonneovascular form (dry) or GA. The neovascular, or wet, form of AMD is the leading cause of blindness in older adults affecting an estimated 1.6 million adults older than age 50 years in the United States (Melnikova, 2005). Approximately 200,000 new cases of the neovascular form of AMD are diagnosed in the United States each year (Melnikova, 2005). Limited data supports the suggestion of a possible association between vitamin D status and AMD. The purpose of this study was to evaluate the association between vitamin D and AMD further.

Currently, it is not clear whether there is an association between vitamin D deficiency and AMD. There is limited data on this topic with conflicting results. The findings from this study may support the importance of monitoring and maintaining healthy vitamin D levels. This particular cross-sectional study design using NHANES data from 2005–2008 used a large population based data sample and tested the five hypotheses listed in Chapter 1. The first of these hypotheses attempted to understand the association between vitamin D level (high versus low) and the development of AMD. The subsequent four hypotheses explored the association of diabetes, age, race, and smoking status to AMD.

In Chapter 3, I discuss the research method. In addition, I describe the instrumentation along with threats to validity and ethical procedures. I conclude Chapter 3 with a summary of the study design and methodology.

Research Design and Rationale

In this cross-sectional analysis, I utilized the NHANES data from 2005–2008 to test the association between independent variable vitamin D and dependent variable AMD as well as covariates age, race, smoking status, and diabetes. This research design was similar to some of the other research studies on this topic. The cross-sectional research design provides several advantages for this type of study including: cross-sectional studies are relatively quick and easy to conduct; the data on selected variables is only collected once; multiple exposures and outcomes can be measured; the ability to measure prevalence for all variables being studied; and the importance of understanding the prevalence for the disease being studied in a specific population (Giele & Elder, 1998). The many listed strengths of the cross-sectional approach contribute to the justification and rationale for use of the cross-sectional study design in the current study. In this particular study, the cross-sectional study design allowed for data on the selected independent variable of vitamin D level and covariates of age, race, diabetes, and smoking status to all be studied from the measurements that were collected in the 2005–2006 and 2007–2008 NHANES database.

In my study, I examined a specific point in time to assess the relationship between the independent variable vitamin D and the outcome variable AMD, as well as the associations to each of the covariates age, race, diabetes, and smoking status. This

snapshot in time represented the current status of the disease AMD and the tested factors in a large nationally representative sample of the population. The results from this analysis are generalizable to the greater population as to whether associations were present between the tested variables. Data from this study were extracted from the NHANES database according to the variables listed in Tables 3 through 6. The total sample sizes for NHANES 2005–2006 and 2007-2008 were 9,950 and 9,762, respectively (CDC, 2015b). AMD is a disease that is most likely to occur after the age of 60 years, but the disease can occur at a younger age. Therefore, I restricted the age cutoff for the 5,604 sample size to adults aged 40 years and older because AMD is a disease of the aging population and prevalence increases with age. In this cross-sectional study, I utilized the NHANES database seeking to understand the association between vitamin D and AMD including covariates age, race, diabetes, and smoking status addresses an important gap in the literature.

NHANES Dataset

NHANES is a program that began in the early 1960s designed to assess and study the health of children and adults in the United States (CDC, 2015a). NHANES is a program of the National Center for Health Statistics (NCHS), which is a part of the Centers for Disease Control and Prevention (CDC).

The NHANES population is a nationally representative sample across the United States taken of about 5,000 individuals each year (CDC, 2015a). NHANES over-samples individuals aged 60 years and older, African Americans, and Hispanics in order to produce statistics that most closely match the U.S population (CDC, 2015a). The

interview of this population included socioeconomic, demographic, dietary, and health-related questions (CDC, 2015a). The examination portion was carried out by highly trained medical professionals and included medical, dental, physiological measurements, and laboratory tests (CDC, 2015a).

Data Collection

NHANES survey data was collected from conducting interviews, physical examinations, and drawing samples for laboratory testing (CDC, 2013). Interviews took place in respondents' homes and health measurements were performed in mobile centers that were equipped to travel throughout the country (CDC, 2015a). The interviews collected person-level demographic, health, and nutrition information, in addition to information about the household (CDC, 2013). The examination included collecting physical measurements, a dental examination, and the collection of blood and urine samples for laboratory testing (CDC, 2013).

The study team (many of whom were bilingual in English and Spanish) that conducted these assessments and collected the information and data included a physician, dietary and health interviewers, and medical and health technicians (CDC, 2015a). Advanced computer systems made it possible for interviewers to use notebook computers and electronic pens to collect information on site. The information was then transmitted to the NHANES database through devices that included digital scales and stadiometers (CDC, 2015a). To ensure privacy, respondents were able to enter sensitive information using touch-sensitive computer screens (CDC, 2015a).

To encourage participation, the local media was allowed to share information about the survey. In addition, transportation was provided to and from the mobile center if needed by the participants (CDC, 2015a). The data collected from respondents was available to the NCHS staff within 24 hours, expediting the ability to share results with the public. NHANES data is provided to the public at no charge.

Sampling and Sampling Procedures

The type of sampling methodology changed from NHANES 2005–2006 and 2007–2008 years. All Hispanics (not just Mexican-Americans) were oversampled in the 2007–2008 dataset. The sampling frame for NHANES included all ages of the noninstitutionalized civilian population of the United States (CDC, 2013). Predesignated domains of sex-age groups for black persons, Mexican-American persons, and income-sex-age groups for other persons were utilized for oversampling in 2005-2006 (CDC, 2013). Predesignated domains of sex-age groups for non-Hispanic black persons, Hispanic persons, and income-sex-age groups for other persons were utilized for oversampling in 2007–2008 (CDC, 2013).

The sample size from 2005–2006 and 2007–2008 included 10,348 and 10,149 individuals respectively. There were 7,081 individuals in the 2005–2008 combined dataset that were 40 years and older (Klein et al., 2011). Of those, there was a final sample total of 5,604 individuals from NHANES 2005–2008 of which there were gradable fundus photographs that have been included for the AMD analysis purposes of this current study. This final sample of 5,604 participants included 3,017 non-Hispanic

white individuals, 401 other Hispanic individuals, 1,139 non-Hispanic black individuals, 864 Mexican American individuals, and 183 individuals of other races/ethnicities.

G*Power 3.1 statistical software was utilized to run a power analysis and compute the required sample size for this study. A priori is the ideal type of power analysis and is done before a study takes place (Mayr, Erdfleder, Buchner, & Faul, 2011). An a priori power analysis was conducted using a two-tailed *z* test for linear regression with an alpha of 0.05 for statistical significance, a power of 90%, and an odds ratio of 1.2, which powered it to detect a weak association. According to Monson (1990) an odds ratio below 1.2 is not powered to indicate an association. The probability utilized for this power analysis was 0.065 because the prevalence or probability of having any AMD in the United States based on the 2005–2008 NHANES survey data was 6.5% (Klein et al., 2011). Based on the power analysis conducted using G*Power statistical software, the minimum sample needed to detect a difference was 5,156. The entire sample size available of 5,604 was utilized. Evaluation of the association of vitamin D to AMD using this sample contributed to understanding if there was an association and adds to the literature on this topic.

Study Variables

Dependent Variable: AMD

In the 2005–2006 and 2007–2008 datasets, AMD was determined by information on the 3-level severity classification (no ARM, early ARM, or late ARM) of AMD from the worse eye (CDC, 2015b). Digital images using a Canon CR6-45NM Opthalmic Digital Imaging System and Canon EOS 10D digital camera were captured from all

participants aged 40 years and older (CDC, 2005). Fundus photo images were read twice by an experienced reader using a modification of the Wisconsin Age-Related Maculopathy Grading Sytem (CDC, 2005). Early ARM was defined by presence or absence of drusen and/or pigmentray abnormalities (CDC, 2015b). Late ARM was defined by exudative ARM signs and/or GA (CDC, 2015b). In order to run a binary logistic regression statistical analysis, ARM codes for 1 = early ARM and 2 = late ARM were combined and any AMD was defined as early and late AMD. For the purposes of this study, the eye with the worst severity of lesion was utilized.

Independent Variable: Vitamin D

In the 2005–2006 and 2007–2008 datasets, vitamin D level was determined from analysis of a serum sample that was collected from participants at a mobile examination center (MEC) (CDC, 2015b). The staff members were observed for equipment operation, specimen collection and preparation, and testing procedures (CDC, 2015b). In the 2005–2006 dataset, the diasorin 25OHD assay method was utilized to determine vitamin D levels from the individual's serum (CDC, 2015b). In the 2007–2008 dataset, the vitamin D lab method changed and utilized an ultra-high performance liquid chromatography-tandem mass spectrometric method to calculate a total 25OHD that was the sum of measured 25-hydroxyvitmain D2 (25OHD2) and 25-hydroxyvitamin D3 (25OHD3) (CDC, 2015b).

Covariates

Covariates for this study included age, race, smoking status, and diabetes; participants age 40 years and older were included, self-reported race, self-reported

smoking status based on how they answered if they had ever smoked a cigarette or smoked at least 100 cigarettes in life, and if they had ever been told by a physician that they have diabetes.

Variables: Coding and Questions

This section outlines the NHANES dataset questions and coding that was utilized. I collected the data from the NHANES 2005–2006 and 2007–2008 datasets.

Demographic data. Demographic covariates age and race were part of the NHANES datafile 'Demographic Variables and Sample Weights' Doc file DEMO_E.xpt that were collected at the time of screening interview for both data years 2005–2006 and 2007–2008. Age was recorded in years at the time of screening interview. In the 2005–2006 dataset, responses of age 85 years and older were all coded as 85. In the 2007–2008 dataset, responses of age 80 years and older were all coded as 80. Race/ethnicity were coded from responses given to the survey questions. These are presented in Table 2.

Table 2

Baseline Characteristics: 2005–2006 and 2007–2008

Variable name	Description and coding	Variable type	Study code
RIDAGEYR	Age at screening Adjudicated - recode	Categorical	0 to 79, 80 (2007–2008)
RIDAGEYR	Age at screening Adjudicated - recode	Categorical	0 to 84, 85 (2005–2006)
RIDRETH1	Race/ethnicity recode	Categorical	Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race – Including Multi-Racial

Laboratory Data. The independent vitamin D variable data was obtained from the NHANES datafile VID_D_Doc (2005–2006) and VID_E_Doc (2007–2008). The vitamin D lab method from 2005–2006 measured total 25-hydroxyvitamin D. The vitamin D method changed for 2007–2008 and the total 25OHD was the sum of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. These are presented in Table 3.

Table 3

Vitamin D

Variable name	Description and coding	Variable type	Study code
LBDVIDMS	Vitamin D (nmol/L)	Binary	High versus low
LBXVIDMS	250HD2+250HD3	Binary	High versus low

Questionnaire Data. Covariate smoking was self-reported by participants as a part of NHANES Doc file SMQ_D_Doc (2005–2006) and SMQ_E_Doc (2007–2008). The data collected for the variable utilized in the current study was based on if the participant was a current smoker or past smoker at the time of questionnaire. Covariate diabetes was a part of Doc file DIQ_D_Doc (2005–2006) and DIQ_E_Doc (2007–2008). The diabetes status was self-reported by participants as to whether they had ever been told by a physician that they had diabetes. The question was answered before the physical examination. I present these in Table 4.

Table 4

Covariate: Smoking and Diabetes

Variable name	Description and coding	Variable type	Study code
SMQ040	Do you now smoke cigarettes	Binary	Yes (Every day, Some days) or No (Not at all)
SMQ020	Smoked at least 100 cigarettes in life	Binary	Yes, No
DIQ010	Doctor told you have diabetes (both males and females 1 year – 150 years). Will limit to age 40–100 years	Binary	Yes, No

Examination Data. The AMD severity determination variable provided information on the 3-level severity classification of AMD regarding the worse eye. Early ARM was defined by presence or absence of drusen and/or pigmentary abnormalities. Late ARM was defined by signs of exudative ARM and/or GA. For *any* ARM, codes for 1 = early ARM and 2 = late ARM were combined. These are presented in Table 5.

Table 5

AMD Severity Determination: 2005-2006 and 2007-2008

Variable name	Description and coding	Variable type	Study code
OPDUARM	ARM, 3 severity levels, worse eye	Categorical	No ARM, early, late, missing

The NHANES dataset categorized the AMD variables as early, late, and none. Fundus photos taken from participants that have been read by experienced Wisconsin grading center photographer were utilized to determine AMD status. Similar methods have been utilized in other epidemiological studies (the AREDS study for example) to diagnose disease with fundus photography that was more closely similar to clinical exam. The existing NHANES database utilized for this study relied on fundus photos to determine AMD status. This was an appropriate way and more accurate way to assess AMD status than patient recall. This eliminated the possibility of including participants that had not yet been diagnosed and were not aware that they had early stage AMD disease.

Methodology

Instrumentation

The data collection instrument I utilized for this dissertation was the NHANES existing secondary dataset. NHANES data comprised three levels of data from a representative sample of the U.S. noninstitutionalized civilian population: a household screener, an interview, and a physical examination (CDC, 2013). When combining 2-year survey cycles it is important to combine the cycles using the appropriate weights (CDC, 2013b). Therefore, I utilized the 4-year examination weights generated from the MEC examination for comparisons. I discussed the data analysis of how the four consecutive years 2005–2008 were weighted in the data collection sampling section.

NHANES data is available for public use and therefore permission to use the data was not necessary. The Walden University Institutional Review Board (IRB) approved this study before being conducted (IRB approval number 10–28–16–0229912). The validity and reliability of the NHANES database was evaluated in a comparative analysis across three national health surveys that also included the Behavioral Risk Factor Surveillance System (BRFSS) and the National Health Interview Survey (NHIS) (Li et al, 2012). According to Li and colleagues (2012), the results of their study supported the external validity and reliability of all three national health surveys (including NHANES) and were consistent with previous findings.

Data Analysis Plan

I utilized the IBM SPSS Statistics software package to run the statistical analysis for this study (IBM Marketplace, n.d.). I utilized a bivariate logistic regression to explore the association of vitamin D to AMD. The association of AMD to age, race/ethnicity, smoking status, and diabetes were explored individually and those found to be significant were included in further analysis using a multivariate logistic regression. Calculations included descriptive statistics on the tested association between vitamin D and AMD including the covariates.

Hypothesis 1: There is an association between vitamin D level (high versus low) and the development of AMD. This variable data was created from blood draw samples that were taken from participants. I ran a binary logistic regression analysis between vitamin D level and AMD to test this hypothesis. For this statistical analysis, AMD codes

for 1 = early AMD and 2 = late AMD were combined to make this a binary logistic regression analysis.

Hypothesis 2: There is an association between diabetes and the development of AMD. This variable data was created from participant responses to the question if they have ever had a "doctor told you have diabetes". Responses included were yes, no, borderline, refused, and don't know. I ran a binary logistic regression analysis between diabetes and AMD to test this hypothesis.

Hypothesis 3: There is an association between age and the development of AMD. The participant in response to the survey question reported age at time of screening. The age cutoff for the sample was restricted to adults aged 40 years and older because AMD is a disease of the aging population and prevalence increases with age. I ran a binary logistic regression analysis between age and AMD to test this hypothesis.

Hypothesis 4: There is an association between race and the development of AMD. I ran a binary logistic regression analysis between race and AMD to test this hypothesis. This variable data was created from participant self-reported race responses to the survey questions on race and Hispanic origin. Responses included were Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other race – including multi-racial.

Hypothesis 5: There is an association between smoking and the development of AMD. The variable data was created from participant's responses to the survey questions 'do you now smoke cigarettes' and have you 'smoked at least 100 cigarettes in life'. The following responses to select from in response to 'do you now smoke cigarettes' were

provided - every day, some days, not at all, don't know, or refuse to answer. The following responses to select from in response to have you 'smoked at least 100 cigarettes in life' were provided – yes, no, or refuse to answer. I ran a binary logistic regression analysis between smoking and AMD to test this hypothesis. For this statistical analysis, answers to the smoking questions were re-coded for 0 = Non-Smoker, 1 = Past Smoker, and 2 = Current Smoker. I utilized these answers for analysis in this binary logistic regression to test the association between current smoking, past smoking, and no smoking to likelihood of having AMD.

Hypothesis 6: There is an association between vitamin D level and the development of AMD after controlling for covariates. I ran a multivariate logistic regression analysis between all covariates and AMD to examine whether the combined variables predicted AMD and test this hypothesis.

Threats to Validity

There are a number of strengths to this study, including that the study utilized a nationally represented large population based sample size including multiple race/ethnicities. It is also important to consider limitations to internal and external validity of this study using the NHANES dataset. The institutionalized population was not included in the sample. Fundus photographs were not able to be collected for all participants for one reason or another. This should be taken into consideration when making generalizations to the entire U.S. population. I assume that the vitamin D instrument utilized to measure serum levels was conducted correctly and has not affected interpretation of results. Recall bias in this aging population has the potential to affect the

validity of results. Self-reported data as it pertains to smoking status as well as diabetes diagnosis needs to be considered. All of these factors should be taken into account when making assessments based on the results of this study.

Ethical Considerations

The NHANES data is free to be utilized by the public. Individuals that participated in the NHANES survey received compensation and a report of medical findings (CDC, 2015a). All of the survey information collected was de-identified and kept strictly confidential (CDC, 2015a). Participant's privacy was protected by public laws (CDC, 2015a). There were no ethical concerns related to participant recruitment, materials or methods utilized to draw samples, or collection of data for the 2005–2006 and 2007–2008 NHANES dataset utilized for this dissertation.

Summary

I applied the cross-sectional approach to this quantitative study analyzing the association between vitamin D and AMD using the NHANES data from 2005–2006 and 2007–2008. I included additional research areas of the confounding variables of age, race, smoking status, and diabetes for analysis. In Chapter 4, I present results of the analysis I utilized to test the six hypotheses for this study. In it, I discuss data collection issues and the results of the study.

Chapter 4: Results

Introduction

The purpose of this study was to examine the association between vitamin D and AMD. AMD is a destructive eye disorder. Loss of vision translates into an effect on the emotional aspects of life, puts individuals at risk for falls, and prevents them from interacting with their families. Many dimensions of this study make it important. The research questions and corresponding hypotheses were the following:

RQ1: What is the association between vitamin D level (high versus low) and the development of AMD?

$H_0$1: There is no association between vitamin D level (high versus low) and the development of AMD.

H_a 1: There is an association between vitamin D level and the development of AMD.

RQ2: What is the association between diabetes (yes/no) and development of AMD?

$H_0$2: There is no association between diabetes (yes/no) and the development of AMD.

H_a 2: There is an association between diabetes (yes/no) and the development of AMD.

RQ3: What is the association between age and development of AMD?

$H_0$3: There is no association between age and the development of AMD.

H_a 3: There is an association between age and the development of AMD.

RQ4: What is the association between race and the development of AMD?

$H_0$4: There is no association between race and the development of AMD.

H_a 4: There is an association between race and the development of AMD.

RQ5: What is the association between smoking (yes/no) and the development of AMD?

$H_0$5: There is no association between smoking (yes/no) and the development of AMD.

H_a 5: There is an association between smoking (yes/no) and the development of AMD.

RQ6: What is the association between vitamin D level and the development of AMD, after controlling for covariates?

$H_0$6: There is no association between vitamin D level and the development of AMD after controlling for covariates.

H_a 6: There is an association between vitamin D level and the development of AMD after controlling for covariates.

In Chapter 4, I discuss the data collection and I present the descriptive statistics for independent, dependent, and potentially confounding variables. I provide the results of my analysis of the six research questions. Chapter 4 concludes with a summary and transition to Chapter 5.

Data Collection

This study utilized data collected from the 2005–2008 NHANES database. The NHANES program began in the 1960s and is a series of surveys designed to assess the

health and nutrition of adults and children in the United States. It is a nationally representative sample across the United States taken of about 5,000 individuals each year (CDC, 2013). NHANES survey data was collected from conducting interviews, physical examinations, and drawing samples for laboratory testing (CDC, 2013).

I downloaded and coded the NHANES dataset per the aforementioned categories for analysis with SPSS software. The data was weighted with the NHANES sample strata and cluster weights as well as full sample 2-year interview (WTINT2YR) and MEC exam (WTMEC2YR) weights according to the NHANES least common denominator method (CDC, 2015c; CDC, 2015d). The sample weights were utilized to aid in obtaining an unbiased estimate of the population because the sample participants were chosen with unequal probabilities. The strata and cluster statements name the variables that form strata and identified clusters in the cluster sample NHANES design.

Results

A sample of 10,348 and 10,149 individuals were interviewed in the NHANES years 2005–2006 and 2007–2008 samples, respectively. From the total, 2,413 and 3,191 individuals in 2005–2006 and 2007–2008 respectively had gradable fundus photos (CDC, 2015c). This allowed me to use a combined total sample of 5,604 individuals with gradable fundus photos categorized into ARM status for this analysis. Within this 5,604 partial study sample, there were reported results from all 5,604 individuals on age, diabetes status, and race/ethnicity. There were 5,036 individuals in the study sample with reported values of vitamin D and 5,602 individuals with reported smoking status. Table 6 provides a further breakdown of the descriptive statistics of each variable by category. In

table 6, I present the frequencies and percentages for the categorical variables of the study sample, NHANES sample, and percentages by the weighted population sample.

Table 6

Respondent Sociodemographic characteristics: Study Sample, NHANES Sample, and Weighted Sample to Represent Population

	Study Sample n = 5604*		NHANES Sample N = 20,497		Weighted Sample
	Frequency	%	Frequency	%	%
ARM					
Yes	443	7.9	NA	NA	7.9
No	5163	92.1	NA	NA	92.1
Vitamin D					
1-9.99	177	3.2	534	2.6	2.0
10-19.99	1590	28.4	4748	23.2	25.0
20-29.99	2049	36.6	6414	31.3	43.0
30+	1220	21.8	3550	17.3	30.0
Diabetes					
Yes	831	15.2	1298	6.3	10.6
No	4773	85.2	18,190	88.7	89.4
Age					
40-59	2829	50.5	3357	16.4	64.4
60+	2775	49.5	3724	18.2	35.6
Race/Ethnicity					
Non-Hispanic White	3017	53.8	8043	39.2	77.1
Other Hispanic	401	7.2	1550	7.6	3.2
Mexican American	864	15.4	5004	24.4	5.4
Non-Hispanic Black	1139	20.3	4921	24.0	9.6
Other race	183	3.3	979	4.8	4.7
Smoking status					
Current smoker	1154	20.6	2408	11.7	20.3
Past smoker	1731	30.9	2741	13.4	31.2
Non-smoker	2717	48.5	5752	28.1	48.5

*Number of individuals with gradable fundus photography

Results for Research Question 1

To answer research question 1, "What is the association between vitamin D level (high versus low) and the development of AMD?", I utilized binary logistic regression using complex samples for vitamin D level and AMD that were extracted from the NHANES database. The study population was n = 5,036. The NHANES dataset reported vitamin D levels on only 5,036 individuals from the 5,604 study sample. Vitamin D level data was missing or unreported for 568 individuals. Table 7 contains the results from the binary logistic regression.

Table 7

Individual Logistic Regression Results for Variables Vitamin D: Association with Dichotomous Age-related Macular Degeneration

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Vitamin D (ng/ml)	.279			
1-9.99 vs. 20-29.99		.409	.209	.801
10-19.99 vs. 20-29.99		.858	.631	1.168
≥30 vs. 20-29.99		1.001	.758	1.323

The p value of the results of the analysis of the association between vitamin D level and AMD were found to be .279. The results in the highest levels of vitamin D (30+ ng/ml) had an odds ratio of 1.001 (95% CI: .758 to 1.323) when compared to 20-29.99 ng/ml. The odds ratio for individuals with vitamin D level 10-19.99 ng/ml was .858 (95% CI: .631 to 1.168) when compared to individuals with 20-29.99 ng/ml. The odds ratio in

the lowest level of vitamin D (1-9.99 ng/ml) was .409 (95% CI: .209 to .801) when compared to 20-29.99 ng/ml. These results suggested that overall; vitamin D level was not associated with AMD. I did not find a vitamin D level association to AMD to be statistically significant in response to research question 1. However, the only comparison where the confidence interval does not cross 1 was in the lowest level (1-9.99 ng/ml) suggesting that there may be a protective association in the lowest level of vitamin D category when compared to individuals with 20-29.99 ng/ml in this sample. Vitamin D was the main focus of this analysis; therefore I included it in the multivariate logistic regression model for research question 6. I failed to reject the null hypothesis for research question 1 (there is no association between vitamin D level (high versus low) and the development of AMD) based on the results found for the binary analysis in this study.

Results for Research Question 2

To answer research question 2, "What is the association between diabetes (yes/no) and development of AMD?", I utilized binary logistic regression using complex samples for diabetes status and AMD that were extracted from the NHANES database. The study population was 5,604. The NHANES dataset reported diabetes status on the entire study sample of 5,604 with graded fundus photography. Table 8 contains the results from the binary logistic regression.

Table 8

Individual Binary Logistic Regression Results for Variable Diabetes: Association with Dichotomous Age-related Macular Degeneration

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Diabetes				
Yes vs. no	.272	1.258	.952	1.662

The *p* value associated with this result ($p = .272$) indicated there was not a statistically significant association between diabetes status and AMD. The odds ratio result was 1.258, with a 95% CI of .952 to 1.662, including 1, which means that there was not a statistical significance indicating that people with diabetes in this sample were not at higher risk for AMD than those without diabetes. Therefore, I left diabetes out of the multivariate logistic regression analysis run for research question 6. Based on the results found on the association between diabetes and AMD to answer this question, I failed to reject the null hypothesis for research question 2.

Results for Research Question 3

To answer research question 3, "What is the association between age and development of AMD?", I utilized binary logistic regression using complex samples for reported age and AMD that were extracted from the NHANES database. The study population was $N = 5,604$. The NHANES dataset reported age on the entire study sample of 5,604 with graded fundus photography. Table 9 contains the results from the binary logistic regression.

Table 9

Individual Binary Logistic Regression Results for Variable Age: Association with Dichotomous Age-related Macular Degeneration

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Age				
≥ 60 vs. 40-59	≤.001	5.455	3.773	7.887

The *p* value result of <.001 indicated a statistically significant association between age and AMD. The odds ratio result of 5.455 (95% CI: 3.773 to 7.887) suggested individuals in the higher age category (aged 60 years and older) were 5.455 times more likely to have AMD than their younger counterparts aged 40-59 years. Therefore, I included age in the multivariate logistic regression analysis. I rejected the null hypothesis for research question 3 (there is no association between age and the development of AMD) based on the results found for this study.

Results for Research Question 4

To answer research question 4, "What is the association between race and the development of AMD?", I utilized binary logistic regression using complex samples for reported race and AMD that were extracted from the NHANES database. The study population was *N* = 5,604. The NHANES dataset reported race on the entire study sample of 5,604 with graded fundus photography. Table 10 contains the results from the binary logistic regression.

Table 10

Individual Binary Logistic Regression Results for Variable Race/Ethnicity: Association with Dichotomous Age-related Macular Degeneration

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Race/Ethnicity	≤.001			
Other Hispanic vs. Non-Hispanic White		.576	.341	.975
Mexican American vs. Non-Hispanic White		.670	.472	.950
Non-Hispanic Black vs. Non-Hispanic White		.333	.227	.489
Other race vs. Non-Hispanic White		.608	.321	1.152

The *p* value result of <.001 indicated a statistically significant association between race/ethnicity and AMD. The odds ratios presented in Table 10 utilized non-Hispanic white as the referent. All results suggested that non-Hispanic whites were at higher risk of AMD than the other groups. The odds ratio result of .576 (95% CI: .341 to .975) for individuals of other Hispanic race/ethnicity suggested non-Hispanic whites were 1.73 times more likely to have AMD. The odds ratio result of .670 (95% CI: .472 to .950) for individuals of Mexican American race/ethnicity suggested that their non-Hispanic white counterparts were 1.49 times more likely to have AMD. The odds ratio result of .333 (95% CI: .227 to .489) for individuals of non-Hispanic black race/ethnicity also showed that their non-Hispanic white counterparts were 3 times more likely to have AMD. The odds ratio result of .608 (95% CI: .321 to 1.152) for individuals of other race

race/ethnicity suggested non-Hispanic whites were 1.64 times more likely to have AMD. I converted these odds ratios to suggest the increased risk for non-Hispanic whites when compared to each of the other groups. I found race to be statistically significantly associated with AMD and therefore I included race in the multivariate logistic regression analysis. I rejected the null hypothesis for research question 4 (there is no association between race and the development of AMD) based on the results found using this study sample.

Results for Research Question 5

To answer research question 5, "What is the association between smoking (current/past/non) and the development of AMD?", I utilized binary logistic regression using complex samples for smoking status and AMD that were extracted from the NHANES database. The study population was n = 5,602. The NHANES dataset reported smoking status on 5,602 individuals from the 5,604 participant study sample. Smoking status data was missing or unreported for only 2 individuals. Table 11 contains the results from the binary logistic regression.

Table 11

Individual Binary Logistic Regression Results for Variable Smoking Status: Association with Dichotomous Age-related Macular Degeneration

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Smoking status	≤.001			
Past vs. Non-smoker		1.584	1.229	2.048
Current vs. Non-smoker		.809	.569	1.151

The *p* value result of <.001 indicated a statistically significant association between smoking status and AMD. The odds ratio result for past smokers was 1.586 (95% CI: 1.229 to 2.048) when compared to non-smokers. These results indicate that past smokers were 1.58 times more likely to have AMD than non-smokers. The odds ratio result for current smokers was .809 (95% CI: .569 to 1.151) when compared to non-smokers suggesting that current smokers are 1.23 times less likely to have AMD than non-smokers. However, the confidence interval does cross 1, indicating that there was no difference between current smokers and non-smokers when it comes to having AMD. I found smoking status to be statistically significant; therefore, I included smoking status in the multivariate logistic regression analysis. I rejected the null hypothesis for research question 5 (there is no association between smoking status (yes/no) and the development of AMD) based on the results found for this study.

Results for Research Question 6

To answer research question 6, "What is the association between vitamin D level and the development of AMD, after controlling for covariates?", I utilized a multivariate logistic regression using complex samples for vitamin D level and AMD status after including covariates age, race, and smoking status. I found all of the included covariates to show statistical significance in each of their individual binary logistic regression analyses. Based on the bivariate logistic results, I did not include diabetes in the model. I included the entire study population in the multivariate analysis for a total of $N = 5{,}604$. Results for this multivariate logistic regression are included in Table 12.

Table 12

Multivariate Logistic Regression Results for the Association of Vitamin D Level with Dichotomous Age-related Macular Degeneration after Controlling for Age, Race/Ethnicity, and Smoking Status

Characteristic	*p*	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Vitamin D (ng/ml)	.553			
1-9.99 vs. 20-29.99	.454	.570	.255	1.271
10-19.99 vs. 20-29.99	.145	.969	.731	1.284
≥30 vs. 20-29.99	.156	.924	.683	1.249
Age ≥ 60 vs. 40-59	<.001	5.004	3.382	7.405
Race/Ethnicity	.011			
Other Hispanic vs. Non-Hispanic White	.314	.738	.412	1.323
Mexican American vs. Non-Hispanic White	.198	.863	.591	1.262
Non-Hispanic Black vs. Non-Hispanic White	.237	.430	.274	.675
Other race vs. Non-Hispanic White	.456	.647	.289	1.446
Smoking status	.293			
Past vs. Non-smoker	.144	1.247	.943	1.649
Current vs. Non-smoker	.209	1.07	.725	1.603

The *p* value result varied for each independent variable comparison to AMD suggesting statistical significance in the multivariate model. For vitamin D, I found the *p* value to be .553, not statistically significant. The results in the highest levels of vitamin D (30+ ng/ml) had an odds ratio of .924 (95% CI: .683 to 1.249) when compared to 20-

29.99 ng/ml. The odds ratio in the lowest level of vitamin D (1-9.99 ng/ml) was .570 (95% CI: .255 to 1.271) when compared to 20-29.99 ng/ml. The odds ratio for individuals with 10-19.99 ng/ml of vitamin D was .969 (95% CI: .731 to 1.284) when compared to individuals with 20-29.99 ng/ml of vitamin D. The confidence interval crosses 1 in all three comparisons, indicating there was no difference between any of the groups.

I found the *p* value for age to be <.001 and statistically significant in the multivariate model. For age, older individuals had an odds ratio of 5.004 (95% CI: 3.382 to 7.405), indicating individuals aged 60 years and older were 5 times more likely to have AMD than their younger counterparts. I found age to be associated with AMD in both the bivariate and multivariate models. This odds ratio result indicated little to no modifying effect occurred when I added the other variables.

The *p* value result of .011 for association between race/ethnicity indicated statistical significance. I found the odds ratio results for race/ethnicity to be .738 (95% CI: .412 to 1.323) for 'other Hispanic', .863 (95% CI: .591 to 1.262) for 'Mexican American', .430 (95% CI: .274 to .675) for 'non-Hispanic black', and .647 (95% CI: .289 to 1.446) for 'other race'. These results indicated that (similar to the bivariate model) non-Hispanic whites were more likely to have AMD than individuals in each race category when included in the multivariate model.

For smoking, I found the *p* value to be .293 and was not statistically significant in the multivariate model. For past smokers, the odds ratio result was 1.247 (95% CI: .943 to 1.649) when compared to non-smokers indicating that past smokers are 1.247 times more likely to have AMD, but it was not statistically significant. The odds ratio result for

current smokers was 1.078 (95% CI: .725 to 1.603) when compared to non-smokers. I failed to reject the null hypothesis for research question 6 (there is no association between vitamin D level and the development of AMD after controlling for covariates) based on the results found for this study. I compared the six models, the five univariate logistic regressions, and the multivariate logistic regression using Pseudo R^2 and present them in Table 13.

Table 13

Cox and Snell Pseudo R^2, Nagelkerke Pseudo R^2, McFadden Pseudo R^2 Values by Model

Model	Cox and Snell	Nagelkerke	McFadden
Univariate			
Vitamin D	.001	.002	.002
Diabetes	.000	.001	.001
Age	.040	.104	.084
Race/Ethnicity	.005	.013	.010
Smoking status	.004	.011	.009
Multivariate	.043	.110	.089

Cox and Snell R^2, Nagelkerke R^2, and McFadden R^2 values (sometimes referred to as Pseudo R^2 values) are methods of calculating explained variation and I utilized these to compare models. I included these in the analysis to understand which model fits the best in relation to the other models. The Cox and Snell, Nagelkerke, and McFadden pseudo R^2 values for vitamin D ranged from .001 to .002, for diabetes ranged from .000 to .001, for age ranged from .040 to .104, for race ranged from .005 to .013, and for smoking status ranged from .004 to .011. In the multivariate model, the Cox and Snell, Nagelkerke, and McFadden pseudo R^2 values ranged from .043 to .110. This means that all variables

combined explained 4.3% to 11% of the variance in the model and the majority of the variance was explained by age. In addition, this indicated that the multivariate model was an improved model compared to the bivariate models. As I expected, the multivariate model demonstrated that it was the best model by having the highest pseudo R^2 values. These results demonstrate that the multivariate model provided the best fit, though it was not predictive of AMD.

Summary

Based on the results found from the analysis in this study, I failed to reject the null hypotheses from the individual research questions 1 (there is no association between vitamin D level (high versus low) and the development of AMD) and research question 2 (there is no association between diabetes (yes/no) and the development of AMD). I rejected the null hypotheses for research questions 3, 4, and 5. I concluded that there were statistically significant associations between age, race, and smoking status in individual comparisons to AMD status. I failed to reject the null hypothesis for research question 6 (there is no association between vitamin D level and the development of AMD after controlling for covariates) because results indicated that there was not a statistically significant association ($p = .553$) between vitamin D level and presence of AMD in participants when controlling for covariates age, race, and smoking status.

In Chapter 5, I discuss and interpret the results described in Chapter 4. I interpret these results in the context of existing theories based on the current literature on the topic of vitamin D association to AMD. In addition, I review the strengths and limitations of the current study. In Chapter 5, I summarize the findings of the study, propose

recommendations for further research on the topic based on the findings, and last review the potential affect this research study will have on social change.

Chapter 5: Discussion

Introduction

In this cross-sectional study, I utilized the NHANES existing database to evaluate the association between vitamin D and AMD, while also examining the association between covariates diabetes, age, smoking status, and race independent of AMD. I found covariates age, race, and smoking status to be statistically significant and I included them in a multivariate model testing association between vitamin D and AMD. In Chapter 5, I provide interpretations of the data findings, review limitations of the study, discuss recommendations based on the findings, provide implications for social change, and, last, provide conclusions to the study.

Interpretation of the Findings

Overall, the results from this study failed to suggest an association between vitamin D levels to AMD is present with or without the covariates included in the model. There was not an association found between vitamin D level and presence of AMD. I found an association between age, smoking, and race to presence of AMD in each of the bivariate models. Age and race still had an association in the multivariate model. However, the smoking association was no longer present in the multivariate model. Running the pseudo R^2 tests provided a better understanding of how much variation in the dependent variable can be explained by the model. The 4.3% to 11% variance results of the pseudo R^2 test demonstrated that age was the main variable that explained the variance. The other variables contributed a minor percentage (1% or less) to the model. When running the multivariate model, the assumption was that the multivariate model

would demonstrate higher pseudo R^2 numbers than each of the individual models. This was confirmed in the results found for this analysis. These results suggested that once the additional variables of age, smoking status, and race were all added to the model, it created a better model.

Vitamin D and AMD

In this study, I did not find vitamin D to be statistically significantly associated to presence of AMD, even after controlling for covariates. There were not enough patients to do an analysis including late AMD. Therefore, I combined early and late AMD as any AMD for the analysis. In this cross-sectional study of 5,604 individuals aged 40 years and older, only 7.9% of the population had any form of AMD and only 31.6% of the population had low levels of vitamin D. Vitamin D levels were broken down into the following four categories for the analysis: 1–9.99 ng/ml, 10–19.99 ng/ml, 20–29.99 ng/ml, and 30+ ng/ml. The reference category for this analysis was 20–29.99 ng/ml, based on the normal range for vitamin D level and this category had the largest number of individuals in the study sample (n = 2,049).

A statistically significant association between vitamin D level and AMD was not found in either the bivariate or multivariate models. In the multivariate model, vitamin D was found to have a p value result of .553 (not statistically significant). The results in the highest levels of vitamin D (30+ ng/ml) had an odds ratio of .924 (95% CI: .683 to 1.249) when compared to 20–29.99 ng/ml. The odds ratio in the lowest level of vitamin D (1–9.99 ng/ml) was .570 (95% CI: .255 to 1.271) when compared to 20–29.99 ng/ml. The odds ratio for individuals with 10–19.99 ng/ml of vitamin D was .969 (95% CI: .731 to

1.284) when compared to individuals with 20–29.99 ng/ml of vitamin D. The confidence interval crosses 1 in all three comparisons, suggesting there is no difference between any of the groups.

In the bivariate analysis, the odds ratio in the lowest level of vitamin D (1-9.99 ng/ml) was .409 (95% CI: .209 to .801) when compared to 20-29.99 ng/ml. The odds ratio for individuals with vitamin D level 10-19.99 ng/ml was .858 (95% CI: .631 to 1.168) when compared to individuals with 20-29.99 ng/ml. The highest levels of vitamin D (30+ ng/ml) had the highest odds ratio of 1.001 (95% CI: .758 to 1.323) when compared to 20-29.99 ng/ml. The odds ratio went up as vitamin D level went up. However, these results suggested that overall vitamin D level was not predictive of AMD. The only comparison where the confidence interval did not cross 1 was in the lowest level (1-9.99 ng/ml) compared to 20-29.99 ng/ml. Vitamin D serum concentrations may fluctuate throughout the year or by season and this was not taken into account in the current study.

The results from the current study provided similar findings (of no association of vitamin D status to AMD) to what the most recent similar cross sectional study by Cougnard-Gregoire and colleagues (2015) found. The latter study included 697 individuals from an elderly population (aged 72.7 $\pm$ 4.4 years) in Bordeaux France (Cougnard-Gregoire et al., 2015), considerably less than the current study sample of 5,604. Cougnard-Gregoire and colleagues (2015) classified AMD as early or late instead of presence or no presence of AMD. The current study did not contain a high prevalence of vitamin D deficiency like the study by Cougnard-Gregoire and colleagues (2015) that

observed 83.2%. It is possible that because only 31.6% of the population had low levels of vitamin D (<19.99ng/ml) and 3.2% of those had extremely low levels (<9.9 ng/ml), that it played a role in whether a signal was observed.

Results from the current study conflict with the results from the cross-sectional study conducted by Itty and colleagues (2014) which found a statistically significant association when comparing vitamin D levels in individuals with NVAMD (late AMD) to those with NNVAMD (early AMD) and controls. Late AMD was very rare in the current NHANES study and therefore the early and late AMD individuals needed to be combined. It is possible that had the numbers been larger that an association may have been observed and there were not enough participants to show an affect.

The current study utilized data from NHANES that did not provide information of whether blood samples were taken during different times of the year. NHANES data also does not provide information of location/latitude of participants or source of vitamin D. A breakdown of latitude of individuals that participated in the study would have provided a better understanding of potential for gaining vitamin D source from sunlight. The study sample size of 5,604 was powered to detect a weak association with a power of 90% and an odds ratio of 1.2. Therefore, it was not likely that the negative study findings were due to a type II error.

Diabetes and AMD

In the current study, I did not find diabetes to be statistically significantly associated to presence of AMD as a single predictor. The *p* value result was .272 with an odds ratio of 1.258 (95% CI: .952 to 1.662) indicating there was not a statistically

significant association between diabetes status and AMD. In the current study, I found that people with self-reported diabetes in this sample were not at higher risk for AMD than those without diabetes. Therefore, I left diabetes out of the multivariate model.

Diabetes disease in the eye involves microvascular complications and has been studied as a potential risk factor to association for development of AMD in the AREDS study and Blue Mountains Eye Study (Tomany et al., 2004; AREDS 2005). The AREDS study (2005) found a weak association of diabetes as a risk factor to AMD based on medical history that reported the individual was under treatment for diabetes. The Blue Mountains Eye Study found having diabetes to be associated with the development of incident GA, a different form of AMD than was studied here (Tomany et al., 2004). Diabetes as a risk factor for AMD has limited data behind it and studying this covariate offered the greatest potential to add the existing literature on this topic. However, the results from the current study did not offer a finding to support a weak association or an association at all. There is still an opportunity to examine this association further in other ways. Perhaps using medical records with physician diabetes diagnosis information would be another approach to evaluate this association further.

Age and AMD

In this study, I found age to be statistically significantly associated to the presence of AMD in both the bivariate and multivariate models. The *p* value result for age was <.001 in both the bivariate and multivariate models. The odds ratio result was 5.455 (95% CI: 3.773 to 7.887) in the bivariate model and 5.004 (95% CI: 3.382 to 7.405) indicating there was a statistically significant association between age and AMD, where individuals

60 years of age and older were 5 times more likely to have AMD than individuals aged 40-59 years of age in both models. The age *p* value was similar in both models whereas the *p* value for other variables vitamin D and smoking status changed. It is likely that age had a modifying effect on these other variables. I discuss in further detail in those sections.

The findings reported from the current study regarding age were similar and consistent with results in the existing literature from previous studies testing this association. The current study findings results were not surprising, as age has been well established as a predictor for AMD in multiple large population based studies such as the AREDS study, the Beaver Dam Eye study, the Blue Mountains Eye study, and the Rotterdam study. All of these studies included age as a risk factor in 1,000's of individuals and found increasing age to be a strong risk factor to development of AMD (Chew et al., 2014; Friedman et al., 2004; AREDS, 2000). As such, the term *age* is now included in the AMD disease name.

Race and AMD

In this study, I found race/ethnicity to be statistically significantly associated to the presence of AMD in both the bivariate and multivariate models. The *p* value result for race/ethnicity was $\leq$.001 in the bivariate model and .011 in the multivariate model. The odds ratio and confidence interval results for each race and ethnicity category compared to non-Hispanic whites suggested a statistically significant association between each race category and AMD. Non-Hispanic whites were 1.49–3 times more likely to have the

presence of AMD than other Hispanics, Mexican Americans, non-Hispanic blacks, and other race categories.

The findings of the current study confirmed what multiple other large population based studies have found suggesting non-Hispanic whites are at the greatest risk for developing AMD. The Beaver Dam Eye study, the Blue Mountains Eye study, the Rotterdam study, and the AREDS study have studied race as a risk factor in 1,000's of individuals and found whites to be more likely to develop AMD (Chew et al., 2014; Friedman et al., 2004; AREDS, 2000). The Eye Diseases and Prevalence Research Group have reported Hispanics to have the lowest rate of AMD when compared to white and black counterparts (Friedman et al., 2004). In contrast, the current study found non-Hispanic blacks to have the lowest likelihood of having AMD to all other race categories. In the current study 'Mexican American' (15.4 % of the study population) and 'other Hispanic' (7.2% of the study population) were labeled separately. It is important to consider how it might have affected the results as they compare to 'non-Hispanic blacks' (20.3% of the study population) if they had been combined together. In the multivariate model, the results were similar with a statistically significant finding suggesting that non-Hispanic whites were at the greatest risk for AMD. The multivariate results did not appear to be modified by the other covariates.

Smoking and AMD

In this study, I found smoking to be statistically significantly associated to the presence of AMD in the bivariate model, but not the multivariate model. In the bivariate model, the odds ratio was 1.586 (95% CI: 1.229 to 2.048) for past smokers when

compared to non-smokers. The odds ratio was .809 (95% CI: .569 to 1.151) for current smokers when compared to non-smokers. These results suggested that past smokers were 1.58 times more likely to have AMD than non-smokers and current smokers are 1.23 times less likely to have AMD than non-smokers. The confidence interval for the past smokers comparison did not include 1 suggesting that smoking was predictive of AMD. The bivariate results confirmed previous findings from the AREDS study suggesting smoking is a risk factor for AMD. However, findings in this study indicating past smokers were at higher risk for having AMD than current smokers differed from the findings of Chew and colleagues (2014) and Tomany and colleagues (2004) of the inverse where current smokers were at higher risk than past smokers of having AMD.

In the multivariate model, the *p* value for smoking comparison to AMD went up to .283 (losing statistical significance) indicating the other variables in the model had a modifying effect on smoking. Age was most likely the variable that had the most effect on the multivariate model as I found age to be the strongest predictor of AMD. The pseudo R^2 tests showed the multivariate model as the strongest with a range from .043 to .110. Age had the next highest values with a range from .040 to .104 and was found to have the highest pseudo R^2 values of all of the individual models. These findings indicate that age was the most predictive of AMD and provided the strongest model behind the multivariate model potentially modifying the other variables.

The life course epidemiology model was utilized as the theoretical framework for this study. I found a history of past smoking to be associated with development of AMD. How smoking through the course of an individual's life affects AMD disease

development was represented in this comparison. The smoking variable demonstrated how the life course theory plays a role in studying disease development. Age as a modifying factor played a role in how the smoking variable had a potential to change in the multivariate model further demonstrating how other factors have modifying effects on one another.

Limitations of the Study

Limitations of the study include: not understanding the source of vitamin D (food, supplements, sun exposure), self-reports of data, small number of participants that have AMD compared to non-AMD, small percentage of participants with low vitamin D and even smaller percentage with extremely low (deficient) vitamin D, and differing vitamin D level measurements between 2-year datasets. Understanding the source of vitamin D would allow for better understanding of the current versus past state of vitamin D status. For example, if vitamin D levels were attributed from use of supplements, then perhaps vitamin D level would have recently changed thereby making it difficult to ascertain an association to the affect of presence or absence of disease.

Individual participant bias for the self-reported data variables included smoking and diabetes. Smokers may have provided answers they felt were more socially acceptable and therefore contributed to bias in the study. In addition, when answering specifics about smoking habits, memory recall may not have been accurate also contributing to bias. A more accurate data collection method for diabetes status would have been to collect medical records for participants in the study rather than to collect data by asking participants to self-report. This is another potential limitation of the study

because it contributed to potential bias. AMD by definition is a disease of older individuals and increases potential recall bias due to worse memory recall in the aging population.

Race as a covariate is straightforward and should not have contributed to bias that influenced outcomes. The method of data collection for both the independent variable (vitamin D level) and dependent variable (stage of AMD) were collected in a manner that minimized recall bias. Those methods included vitamin D testing done in a lab and reading of retinal images that were performed by masked readers (NHANES, 2015). However, the lab method for vitamin D measurement changed from the 2005–2006 and 2007–2008 data collection years (as discussed in more detail in Chapter 3), which presented a potential limitation through measurement bias. In addition, source of vitamin D from food, supplements, or amount of sun exposure was not estimated, which may have influenced the results.

Recommendations

A recommendation based on the findings from this study is to attempt to better understand the diabetes to AMD association. Future researchers should consider using physician medical records with diagnosis information as an alternative approach to evaluate this association further. Of all of the variables studied here, diabetes is the least studied in terms of association to AMD. Age, smoking status, and ethnicity are well established at this point. The body of literature on vitamin D association to AMD is still growing, but it appears that the evidence remains unclear. The data from this study and the literature have provided conflicting results. Additional research on this topic should

continue with considerable thought on how to strengthen studies with large AMD populations. This study did not evaluate the association broken down by early and late AMD and further research with large enough numbers to evaluate both will be important. The number of NVAMD (late AMD) individuals has been small in the previous studies and provides an opportunity for further exploration. Perhaps finding a population to study with large numbers of individuals in higher age categories that are more likely to have advanced disease would be beneficial.

Implications for Social Change

The findings from this study could be used for positive social change by encouraging medical and public health agencies to target screening programs at high-risk age, smoking, and race groups. There remains to be conflicting data in the literature. This study adds to the body of literature suggesting that higher levels of vitamin D are not necessarily beneficial. There has been an increase in the community recommending more focus on and testing of vitamin D levels. The associated emphasis has been on maintaining increased vitamin D levels for health benefits. Perhaps it is not the case when evaluating AMD risk and vitamin D is not as closely associated with AMD as the thinking was trending toward. However, this study does confirm previous findings of age, race, and smoking status association to AMD. Health care professionals can use this information to target screening for AMD among at-risk groups. Perhaps AMD screenings among older, white individuals would be beneficial. Since an association was also not found between diabetes and AMD, perhaps more research needs to be conducted on this topic as the numbers of individuals with diabetes continues to grow.

Conclusion

This study attempts to better understand the association between vitamin D and AMD in a large weighted sample from the publicly available NHANES dataset that is representative of the U.S. population. The study fails to find an association between vitamin D and AMD. Covariates that are included in the analysis are diabetes, age, race, and smoking status. Age is strongly predictive of AMD and has an affect on the multivariate model.

The findings from this study conducted from a large population sample representative of the U.S. population adds to the literature on the topic of vitamin D association to AMD. The results of this study do not support an inverse association of vitamin D to AMD and fails to support a specific role for vitamin D in AMD. It is still unknown whether vitamin D supplements, a diet rich in vitamin D, or safe exposure to the sun would play a role in development of AMD.

References

Abràmoff, M. D., Garvin, M. K., & Sonka, M. (2010). Retinal imaging and image analysis. *IEEE Transactions on Medical Imaging*, *3*, 169-208. doi:10.1109/RBME.2010.2084567

Age-Related Eye Disease Study Research Group. (2005). Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study (AREDS): AREDS report no. 19. *Ophthalmology*, *112*(4), 533-539. doi:10.1016/j.ophtha.2004.10.047

Age-Related Eye Disease Study Research Group. (2000). Risk factors associated with age-related macular degeneration: A case-control study in the age-related eye disease study: Age-related eye disease study report number 3. Ophthalmology, 107(12), 2224-2232. doi:10.1016/S0161-6420(00)00409-7

Albert, D. M., Scheef, E. A., Wang, S., Mehraein, F., Darjatmoko, S. R., Sorenson, C. M., & Sheibani, N. (2007). Calcitriol is a potent inhibitor of retinal neovascularization. *Investigative Ophthalmology & Visual Science*,*48*(5), 2327-2334. doi:10.1167/iovs.06-1210

Ben-Shlomo, Y., & Kuh, D. (2002). A life course approach to chronic disease epidemiology: Conceptual models, empirical challenges and interdisciplinary perspectives. *International journal of epidemiology*, *31*(2), 285-293. doi:10.1093/ije/31.2.285

Birch, D. G., & Liang, F. Q. (2007). Age-related macular degeneration: A target for

nanotechnology derived medicines. *International Journal of Nanomedicine*, *2*(1), 65-77. doi:10.2147/nano.2007.2.1.65

Bird, A., Bressler, N., Bressler, S., Chisholm, I., Coscas, G., Davis, M., . . . Klein, R. (1995). An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Survey of Ophthalmology, 39*(5), 367-374. doi:10.1016/s0039-6257(05)80092-x

Brantley Jr, M. A., Osborn, M. P., Sanders, B. J., Rezaei, K. A., Lu, P., Li, C., . . . Sternberg Jr, P. (2012). Plasma biomarkers of oxidative stress and genetic variants in age-related macular degeneration. *American Journal of Ophthalmology, 153*(3), 460-467. doi:10.1016/j.ajo.2011.08.033

Buitendijk, G. H., Rochtchina, E., Myers, C., Van Duijn, C. M., Lee, K. E., Klein, B. E., . . . & Uitterlinden, A. G. (2013). Prediction of age-related macular degeneration in the general population: The Three Continent AMD Consortium. *Ophthalmology*, *120*(12), 2644-2655. doi:10.1016/j.ophtha.2013.07.053

Centers for Disease Control and Prevention (CDC). (2015a). About the National Health and Nutrition Examination Survey. Retrieved from http://www.cdc.gov/nchs/nhanes/about_nhanes.htm

Centers for Disease Control and Prevention (CDC). (2015b). National Health and Nutrition Examination Survey. Retrieved from http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm

Centers for Disease Control and Prevention (CDC). (2015c). National Health and Nutrition Examination Survey. 2005–2006 Data Documentation, Codebook, and Frequencies. Retrieved from https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/OPXRET_D.htm

Centers for Disease Control and Prevention (CDC). (2015d). National Health and Nutrition Examination Survey. 2007–2008 Data Documentation, Codebook, and Frequencies. Retrieved from https://wwwn.cdc.gov/Nchs/Nhanes/2007-2008/DEMO_E.htm

Centers for Disease Control and Prevention (CDC). (2013). National Health and Nutrition Examination Survey: Sample Design. Retrieved from http://www.cdc.gov/nchs/data/series/sr_02/sr02_160.pdf

Centers for Disease Control and Prevention (CDC). (2013b). National Health and Nutrition Examination Survey: Vital and Health Statistics. The National Health and Nutrition Examination Survey: Analytic Guidelines, 1999–2010. Retrieved from http://www.cdc.gov/nchs/data/series/sr_02/sr02_161.pdf

Centers for Disease Control and Prevention (CDC). (2012). National Health and Nutrition Examination Survey: Vital and Health Statistics. The National Health and Nutrition Examination Survey: Sample Design, 1999–2006. Retrieved from http://www.cdc.gov/nchs/data/series/sr_02/sr02_155.pdf

Centers for Disease Control and Prevention (CDC). (2005). NHANES Digital Grading Protocol. Retrieved from

http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/NHANES_ophthamology_digital_grading_protocol.pdf

Cheung, L. K., & Eaton, A. (2013). Age-related macular degeneration. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 33*(8), 838-855. doi:10.1002/phar.1264

Chew, E. Y., Clemons, T. E., Agrón, E., Sperduto, R. D., SanGiovanni, J. P., Davis, M. D., & Ferris, F. L. (2014). Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report no. 36. *JAMA Ophthalmology*, *132*(3), 272-277. doi:10.1001/jamaophthalmol.2013.6636

Chew, E. Y., Clemons, T. E., Milton, R. C., & Sperduto, R. D. (2007). The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study. *Arch Ophthalmol*, *125*(9), 1225-1232. doi:10.1001/archopht.125.9.1225

Coleman, H. R., Chan, C. C., Ferris, F. L., & Chew, E. Y. (2008). Age-related macular degeneration. *The Lancet*, *372*(9652), 1835-1845. doi:10.1016/s0140-6736(08)61759-6

Cougnard-Gregoire, A., Merle, B. M., Korobelnik, J. F., Rougier, M. B., Delyfer, M. N., Feart, C., Delcourt, C. (2015). Vitamin D Deficiency in Community-Dwelling Elderly Is Not Associated with Age-Related Macular Degeneration. J Nutr, 145(8), 1865-1872. doi:10.3945/jn.115.214387

Day, S., Acquah, K., Platt, A., Lee, P. P., Mruthyunjaya, P., & Sloan, F. A. (2012). Association of vitamin D deficiency and age-related macular degeneration in

medicare beneficiaries. *Archives of ophthalmology*, *130*(8), 1070-1071. doi:10.1001/archophthalmol.2012.439

Edwards, A. O., Ritter, R., Abel, K. J., Manning, A., Panhuysen, C., & Farrer, L. A. (2005). Complement factor H polymorphism and age-related macular degeneration. *Science*, *308*(5720), 421-424. doi:10.1126/science.1110189

Ferris, F. L., Wilkinson, C. P., Bird, A., Chakravarthy, U., Chew, E., Csaky, K., ... & Beckman Initiative for Macular Research Classification Committee. (2013). Clinical classification of age-related macular degeneration. *Ophthalmology*, *120*(4), 844-851. doi:10.1016/j.ophtha.2012.10.036

Friedman, D. S., O'Colmain, B. J., Munoz, B., Tomany, S. C., McCarty, C., De Jong, P. T., ... & Kempen, J. (2004). Prevalence of age-related macular degeneration in the United States. Arch ophthalmol, 122(4), 564-572. doi:10.1001/archopht.122.4.564

Giele, J. Z., & Elder, G. H. (Eds.). (1998). *Methods of life course research: Qualitative and quantitative approaches*. Sage Publications. doi:10.4135/9781483348919

Golan, S., Shalev, V., Treister, G., Chodick, G., & Loewenstein, A. (2011). Reconsidering the connection between vitamin D levels and age-related macular degeneration. *Eye*, *25*(9), 1122-1129. doi:10.1038/eye.2011.174

IBM Marketplace (n.d.). IBM SPSS Statistics – What it can do for your business. Retrieved from https://www.ibm.com/us-en/marketplace/statistical-analysis-and-reporting

Itty, S., Day, S., Lyles, K. W., Stinnett, S. S., Vajzovic, L. M., & Mruthyunjaya, P. (2014). Vitamin D deficiency in neovascular versus nonneovascular age-related

macular degeneration. *Retina*, *34*(9), 1779-1786. doi:10.1097/iae.0000000000000178

Jager, R. D., Mieler, W. F., & Miller, J. W. (2008). Age-related macular degeneration. *New England Journal of Medicine*, *358*(24), 2606-2617. doi:10.1056/nejmra0801537

Kim, E. C., Han, K., & Jee, D. (2014). Inverse relationship between high blood 25-hydroxyvitamin D and late stage of age-related macular degeneration in a representative Korean population. *Invest Ophthalmol Vis Sci*, 55(8), 4823-4831. doi:10.1167/iovs.14-14763

Klein, R., Chou, C. F., Klein, B. E., Zhang, X., Meuer, S. M., & Saaddine, J. B. (2011). Prevalence of age-related macular degeneration in the US population. *Archives of Ophthalmology*, 129(1), 75-80. doi:10.1001/archophthalmol.2010.318

Klein, R., Peto, T., Bird, A., & Vannewkirk, M. R. (2004). The epidemiology of age-related macular degeneration. *American Journal of Ophthalmology*,*137*(3), 486-495. doi:10.1016/j.ajo.2003.11.069

Kuh, D., & Shlomo, Y. B. (2004). *A life course approach to chronic disease epidemiology* (No. 2). Oxford University Press. doi:10.1093/acprof:oso/9780198578154.001.0001

Li, C., Balluz, L. S., Ford, E. S., Okoro, C. A., Zhao, G., & Pierannunzi, C. (2012). A comparison of prevalence estimates for selected health indicators and chronic diseases or conditions from the Behavioral Risk Factor Surveillance System, the National Health Interview Survey, and the National Health and Nutrition

Examination Survey, 2007–2008. *Preventive Medicine*,*54*(6), 381-387. doi:10.1016/j.ypmed.2012.04.003

Manolagas, S. C., Provvedini, D. M., Murry, E. J., Tsoukas, C. D., & Deftos, L. J. (1986). The Antiproliferative Effect of Calcitriol on Human Peripheral Blood Mononuclear Cells*. *The Journal of Clinical Endocrinology & Metabolism*, *63*(2), 394-400. doi:10.1210/jcem-63-2-394

Mayr, S., Erdfelder, E., Buchner, A., & Faul, F. (2007). A short tutorial of GPower. *Tutorials in Quantitative Methods for Psychology*, *3*(2), 51-59. doi:10.20982/tqmp.03.2.p051

Melnikova, I. (2005). Wet age-related macular degeneration. *Nature Reviews Drug Discovery*, *4*(9), 711-712. doi:10.1038/nrd1827

Millen, A. E., Meyers, K. J., Liu, Z., Engelman, C. D., Wallace, R. B., LeBlanc, E. S., Mares, J. A. (2015). Association Between Vitamin D Status and Age-Related Macular Degeneration by Genetic Risk. JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2015.2715

Millen, A. E., Voland, R., Sondel, S. A., Parekh, N., Horst, R. L., Wallace, R. B., ... & Gehrs, K. M. (2011). Vitamin D status and early age-related macular degeneration in postmenopausal women. *Archives of Ophthalmology*, *129*(4), 481-489. doi:10.1001/archophthalmol.2011.48

Monson, R. R. (1990). *Occupational epidemiology*. CRC press. Retreived from https://www.crcpress.com/Occupational-Epidemiology-Second-Edition/Monson/p/book/9780849349270

Morrison, M. A., Silveira, A. C., Huynh, N., Jun, G., Smith, S. E., Zacharaki, F., ... & Radeke, M. J. (2011). Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. *Human Genomics*, *5*(6), 1. doi:10.1186/1479-7364-5-6-538

National Eye Institute (NEI). (n.d.). Age-related macular degeneration phenotype consensus meeting. Retrieved from http://www.nei.nih.gov/strategicplanning/amd_meeting.asp

National Eye Institute (NEI). (2015). Facts about age-related macular degeneration. Retrieved from http://www.nei.nih.gov/health/maculardegen/armd_facts.asp.

National Institutes of Health Medline Plus. (2016). Diabetes. Retrieved from https://www.nlm.nih.gov/medlineplus/diabetes.html#summary

National Institutes of Health Office of Dietary Supplements. (2014). Vitamin D. Retrieved from https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/

Parekh, N., & Zizza, C. (2013). Life course epidemiology in nutrition and chronic disease research: A timely discussion. *Advances in Nutrition: An International Review Journal*, *4*(5), 551-553. doi:10.3945/an.113.004275

Parekh, N., Chappell, R. J., Millen, A. E., Albert, D. M., & Mares, J. A. (2007). Association between vitamin D and age-related macular degeneration in the Third National Health and Nutrition Examination Survey, 1988 through 1994. *Archives of Ophthalmology*, *125*(5), 661-669. doi:10.1001/archopht.125.5.661

Park, Lee, Woo, Ahn, Shin, Song. (2014). Age-related macular degeneration: Prevalence and risk factors from Korean National Health and Nutrition Examination Survey,

2008 through 2011. *Ophthalmology*, *121*(9), 1756-1765. doi:10.1016/j.ophtha.2014.03.022

Rahman, S., Rahman, T., Ismail, A. A. S., & Rashid, A. R. A. (2007). Diabetes-associated macrovasculopathy: Pathophysiology and pathogenesis.Diabetes, *Obesity and Metabolism*, 9(6), 767-780. doi:10.1111/j.1463-1326.2006.00655.x

Seddon, J. M., Reynolds, R., Shah, H. R., & Rosner, B. (2011). Smoking, dietary betaine, methionine, and vitamin D in monozygotic twins with discordant macular degeneration: Epigenetic implications. *Ophthalmology*, *118*(7), 1386-1394. doi:10.1016/j.ophtha.2010.12.020

Singh, A., Falk, M. K., Subhi, Y., & Sørensen, T. L. (2013). The association between plasma 25-hydroxyvitamin D and subgroups in age-related macular degeneration: A cross-sectional study. *PloS one*, *8*(7), e70948. doi:10.1371/journal.pone.0070948

Smith, W., Assink, J., Klein, R., Mitchell, P., Klaver, C. C., Klein, B. E., ... & de Jong, P. T. (2001). Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology*, *108*(4), 697-704. doi:10.1016/s0161-6420(00)00580-7

Tomany, S. C., Wang, J. J., van Leeuwen, R., Klein, R., Mitchell, P., Vingerling, J. R., ... & de Jong, P. T. (2004). Risk factors for incident age-related macular degeneration: Pooled findings from 3 continents. *Ophthalmology*, *111*(7), 1280-1287. doi:10.1016/j.ophtha.2003.11.010

Wang, J. J., Rochtchina, E., Lee, A. J., Chia, E. M., Smith, W., Cumming, R. G., & Mitchell, P. (2007). Ten-year incidence and progression of age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmology*, *114*(1), 92-98. doi:10.1016/j.ophtha.2006.07.017

Wong, W. L., Su, X., Li, X., Cheung, C. M. G., Klein, R., Cheng, C. Y., & Wong, T. Y. (2014). Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis. *The Lancet Global Health*, *2*(2), e106-e116. doi:10.1016/s2214-109x(13)70145-1

World Health Organization (WHO). (2014). Priority eye diseases. Retrieved from http://www.who.int/blindness/causes/priority/en/

Van Lookeren Campagne, M., LeCouter, J., Yaspan, B. L., & Ye, W. (2014). Mechanisms of age-related macular degeneration and therapeutic opportunities. *The Journal of Pathology*, *232*(2), 151-164. doi:10.1002/path.4266

Zampatti, S., Ricci, F., Cusumano, A., Marsella, L. T., Novelli, G., & Giardina, E. (2014). Review of nutrient actions on age-related macular degeneration. *Nutrition Research*, *34*(2), 95-105. doi:10.1016/j.nutres.2013.10.011