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Self-Reported Tea Consumption and Age-Related Macular Degeneration in U.S. Adults

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ABSTRACT

SELF-REPORTED TEA CONSUMPTION AND AGE-RELATED MACULAR DEGENERATION IN U.S. ADULTS

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Background: Age-related Macular degeneration (AMD) is a multifaceted disease of aging that is currently one of the leading causes of blindness in the US. As aging populations continue to turn to alternatives to prevent age-related diseases, the use of tea antioxidants has been suggested as potential protectors against cellular degradation and oxidation within the retina. To date, no epidemiologic or cross-sectional studies have been conducted regarding the correlation between tea intake and AMD, as it has for other diseases with antioxidant mitigation potential, such as cancer.

Objective: The purpose of this cross-sectional **s**tudy was to examine the correlation between self-reported tea consumption via beverage and the prevalence of AMD in a representative US sample.

Methods: Using data from 1761participants aged ≥40 years in the 2005-2006 National Health and Nutrition Examination Survey (NHANES), we examined the association between self-reported tea intake (hot and cold) ascertained from food frequency questionnaires and AMD. The presence of AMD was defined by graded fundus photography, using logistic model of regression because response variables were binary. Additionally, multiple linear regression analysis was used to develop a model for predicting the presence of AMD based on their age, gender, race, BMI, and smoking, blood pressure and serum cholesterol level, from their level of tea drinking.

Results: There were no significant correlations between incidences or severity of AMD with reported tea consumption. The multiple linear regression analysis for AMD in individuals with tea consumption compared with those with no tea consumption use was 1.052 (95% CI 0.757, 1.460). The only covariates with significant correlations between tea consumption and AMD were age and non-Hispanic white race. The correlation between age and AMD and tea consumption was $p < .0001$, with an odds ratio estimate of 1.088 (95% CI 1.072, 1.104). Self-identification as "non-Hispanic white" and the association with AMD and tea consumption was $p < 0.0013$ (>0.05).

Conclusions: There was no correlation between reported tea consumption (hot or cold) and the incidence of AMD in the sample of US adult population examined. Without controlling for type of tea and standardizing flavonoid content, additional research is needed to continue to examine the effectiveness of tea. Similarly, the use of tea extracts in other preventative models should additionally be explored.

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CHAPTER I

INTRODUCTION

Background

The eye, the organ of sight, has a unique structural and biochemical organization. As vital as vision can be, a report from the World Health Organization (WHO) in 2014 estimated that approximately 285 million people were suffering from visual impairment worldwide[1]. Of that population, 39 million are blind and 246 million suffer from low vision[1]. Age-related macular degeneration (AMD) is the leading cause of blindness worldwide, affecting 30-50 million people worldwide and over two million individuals of all ages and races in the United States. As the name suggests, AMD is primarily a disease of aging with 11% of affected individuals above the age of 80[2]. Coinciding with the growing population of aging adults, the prevalence of AMD is projected to increase over the next decades, with over five million people affected by 2050[1]. The retina is the most metabolically active tissue in the body[3], and its high metabolic rate creates a high oxygen demand and vulnerability to oxidative stress[4,5]. Other factors that make the retina particularly vulnerable to oxidative stress are the high proportion of fatty acids in photoreceptor membranes, chronic exposure to light, and, in the membrane cells, phagocytosis, which gives rise to reactive intermediates[4]. Evidence of oxidative stress is apparent with increasing age, and oxidative damage may be most evident in the location of centralized vision, the macula[4-6]. To that end, many researchers have proposed that age-related changes within the retinal pigment

epithelium (RPE), in congruence with an accumulation of lipofuscin, denote the earliest changes that ultimately lead to AMD[2,4,6].

While the population continues to age, the use of complementary or alternative medicine (CAM) for treatment and prevention of disease has been increasing in popularity[7]. Because there are many risk factors that are not within an individual's control, such as age, genetics and gender, there has been an increased focus on minimizing or eliminating environmental and behavioral risk factors of many diseases[7-9]. Thus, research has intensified on the implications of nutritional intervention in the prevention and treatment of chronic, debilitating diseases[10]. As the adult population continues to increase their lifespan and medicine progresses to allow improved longevity, research continues to refocus on diseases of consequence correlated with the aging population[7,10,11].

As researchers, through controlled studies, have supported evidence for new dietary guidelines to lessen the progression of chronic diseases, AMD has recently received new recommendations for prevention through over-the-counter (OTC) supplementation. There is accumulating evidence that phytochemicals may be involved in the prevention and reversal of age-related eye diseases[12-16]. Knowledge of a relationship between nutrition and age-related eye diseases has been the basis for prevention models for many decades[12,13,16]. Elevated plasma levels of antioxidants have a proven protective benefit against oxidative stress, which has been implicated as the major contributor to the development of each of these ocular disorders[15].

Nutritional therapies involving phytochemical interventions are emerging as potentially effective in reversing the progression of age-related eye diseases such as AMD[11-13,15,16]. However, the exact mechanism of their beneficial effect against the development of ocular disorders is unknown and continues to be explored[11]. With rising public interest in

phytochemicals and health and the increasing use of phytochemicals in CAM therapies, ophthalmologists, physicians, dietitians, and medical researchers need to be better informed about the evidence supporting the specific benefits and risks of particular phytochemicals prior to issuing suggestions to patients[7,10].

Most research performed to date has examined the effects of commonly known antioxidants such as vitamins E, C, and A, and carotenoids (lycopene, lutein, and zeaxanthin) on visual disorders[12,14,17-21]. Limited, yet promising, research trials have been conducted with other phytochemicals with antioxidant and anti-inflammatory properties including flavonoids (ginkgo biloba, bilberry, grape seed extract, green tea), anthocyanins (bilberry, blueberry, black currant), and stilbenes (resveratrol)[19,22-24]. Although antioxidant potential for cancer prevention and treatment has been the primary research focus, other models have considered the similarities between compounds with beneficial properties in both cancer and ocular disorders such as AMD[22,23].

Specific catechins present primarily in green tea, *Camellia sinensis*, are one of those antioxidants that show promising results for antioxidant therapy in prevention and treatment protocols. Epigalloccatechin-3-gallate (EGCG), the main catechin present in tea leaves, is known for its antioxidant, anti-inflammatory, antiviral, and anti-carcinogenic properties[25-27]. A recent study by Lee et al., identified EGCG as an ocular inhibitor of vascular blood vessel growth permeability, which is a defining characteristic of AMD[28]. Matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) play a key role in the processes of extracellular matrix remodeling and microvascular permeability during the angiogenesis present within AMD[2,3,18,28]. Studies have since concluded there exists an inhibitory effect by EGCG on ocular neovascularization and vascular permeability using the retina oriented cells and animal

models induced by VEGF. EGCG treatment has also significantly decreased mRNA and protein expression levels of MMPs in the presence in human retinal pigment epithelial (RPE) cells[29-31]. EGCG also effectively protected RPE from cell death by inhibiting generation of oxygen species (ROS)[29,30-33]. Multiple studies suggest EGCG significantly inhibits proliferation, vascular permeability, and formation in VEGF-induced models of RPE cells *in vitro* which is the closest approximation to human models of the stressed-induced conditions present when AMD is present[34-36]. Furthermore, EGCG significantly reduced vascular leakage and permeability by blood-retinal barrier (BRB) breakdown in VEGF-induced animal models[27].

Additionally, studies involving intraocular injection of EGCG demonstrated a modulatory effect on the retinal photoreceptors, indicating that EGCG may benefit patients suffering from ocular diseases in which oxidative stress is involved[35]. Mechanisms of the catechin's action were described in the study as: "the destruction of oxygen free radicals, oxidative alterations of LDL, and reduction of glutamate toxicity and protein modification"[35]. Studies performed on rat models involving oral administration of EGCG reduced light-induced retinal neuronal death, suggesting that EGCG may be used in preventing photoreceptor cell death[36,37]. Additionally, EGCG has the ability to inhibit RPE cell migration and adhesion, thereby providing potential preventive actions against AMD[38,39]. From this, data suggests catechins such as EGCG would be a compound of future study in the prevention and treatment of diseases such as AMD.

Within human studies, there is a significant gap in research regarding tea consumption and the incidences of AMD. To date there are no human studies investigating the correlation of tea consumption and prevalence of AMD. Because there is an inherent inability to measure functional RPE cells and polyflavonoids' roles in protection within human AMD subjects, we can only rely on *in vitro* and animal studies reporting promising results through RPE protection.

Therefore, correlation studies looking at incidences of AMD (and its progression of ocular disease state) compared to frequency of tea consumption are needed.

Statement of Research Problem

The purpose of this cross-sectional study was to examine the correlation between self-reported tea consumption beverages and the prevalence of AMD in a representative US sample. While some effectiveness of EGCG and associated catechins present within tea has been shown to inhibit VEGF[34-36,39], thus potentially inhibiting the characteristic vascular growth of unwanted blood vessels present when AMD progresses, the association between intake of tea and the presence of AMD or AMD progression has not been studied. While there is extensive research on EGCG and/or tea and cancer prevention, there is little known regarding prevention of AMD outside of the current supplement recommendations.

Research Questions

1. Is there an association between tea intake and macular degeneration?
2. Is there a significant relationship between the degree of macular degeneration and tea intake?
3. Is there a correlation between the consumption of specific types of tea and the incidence of macular degeneration?
4. Is there a significant relationship present among the covariates (gender, age, race, BMI, and smoking, cholesterol and blood pressure statuses), tea consumption and macular degeneration?

Independent Variable

The independent variable was the self-reported intake of tea, both hot and cold. Due to the nature of the food frequency questionnaire, it is not possible to specify the type of tea consumed therefore tea consumption was broken into two primary categories: absence of regular consumption (no tea consumption to 1 cup/week) and regular tea consumption (>1 cup/week of tea). For further analysis, the consumers were coded into monthly, weekly, and daily consumption to determine the degree of association for level of tea intake.

Dependent Variable

The dependent variable was the presence or absence of AMD as measured by fundus photography. Furthermore, the gradation of AMD is also considered, therefore splitting the data into 3 groups: absence of AMD, presence of dry AMD, and presence of wet AMD. Several characteristics of age-related maculopathy are graded in a semiquantitative fashion from stereoscopic 30-degree color fundus photographs, using a grid to define subfields, standard circles printed on plastic to assess size and area, and a specially designed lightbox to allow better discrimination of drusen and maculopathy-related deformities[1].

Hypotheses

1. There is a correlation between tea consumption and the presence of AMD.

H0: There is no relationship between tea consumption and the presence of AMD.

H1: There is a relationship between tea consumption and the presence of AMD.

2. There is a significant relationship between the severity of macular degeneration and tea consumption.

H0: There is no relationship between tea consumption and the severity of AMD.

H1: There is a relationship between tea consumption and the severity of AMD.

3. There is a specific intake of tea consumption correlated with reduced incidences of macular degeneration.

H0: There is no correlation between specific intake levels of tea consumption and incidences of AMD.

H1: There is a correlation between specific intake levels of tea consumption and incidences of AMD.

4. There is a significant relationship present between the covariates (gender, age, race, BMI, and smoking, cholesterol and blood pressure statuses) and tea consumption in the association between tea consumption and macular degeneration.

H0: There is no correlation between the covariates and the relationships of reported tea consumption and incidences of AMD.

H1: There is a correlation between the covariates and the relationships of reported tea consumption and incidences of AMD.

Operational Definitions

The following is a list of operational definitions used throughout the paper.

- **Age-Related Macular Degeneration (AMD):** The presence of AMD in participants without regards to severity levels as determined by NHANES utilizing fundus

photography. These images were graded at the University of Wisconsin, Madison, by 9 experienced graders. After initial diagnosis of AMD, levels of severity were classified as dry AMD (initial) or wet AMD (advanced).

- **Food-Frequency Questionnaire (FFQ):** A questionnaire validated by the CDC in which the respondent is presented with a list of foods and is required to say how often each is eaten in broad terms such as x times per day/per week/per month. Foods chosen are reassessed each decade and can be used to evaluate the participant's total diet.

- **National Health and Nutrition Examination Survey (NHANES):** Cross-sectional survey program of studies designed to assess the health and nutritional status of U.S. adults and children aged 1-84 years, with 85+ years coded separately. The survey is unique in that it combines interviews and physical examinations. The data used for this study was collected from 2005-2006 in adults aged 40 and older who submitted to fundus examination and the FFQ.

- **Tea Consumption:** the self-reported incidence of individuals consuming hot or cold tea beverages (not herbal tea) on the NHANES FFQ. Regular tea consumption is further defined as the self-reported consumption of tea (hot or cold) at a frequency of 1 cup or more per week on average.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

In terms of nutrition, there have been many studies looking at nutrient consumption and interactions and the correlation of AMD[12-16]. Previous researchers have considered many risk factors that influence the development and progression of AMD as well as preventative models. Aside from macronutrient consumption, researchers have focused on the mechanisms of action in AMD progression and the role of antioxidants to delay or suppress them[11-24]. The most commonly studied antioxidants for cellular protection outside of the macula have been those belonging to the flavonoid classification[24]. Because AMD is primarily a disease aging, research into the antioxidant potential of flavonoids in protection against the oxidization and destruction of macular epithelial cells can have further implications for other diseases associated with the aging process[15,19,22]. Recent research suggests that flavonoids may be involved in two major aspects of the diverse processes involved in vision physiology and eye health[24]. Flavonoids may function in visual signal transduction, in ways that are not well understood. Flavonoids may also function in their well-established role as antioxidants, which are particularly important in the eye, where oxidative stress is significant and its damage is implicated in a number of visual pathologies, including macular degeneration[19.24]. Studies showing flavonoid effects on visual

signal transduction or as antioxidants can be put in context once we understand whether flavonoids are absorbed by the eye and, if so, their relative distribution among ocular tissues[19,23,24,28]. The purpose of this literature review is to highlight key areas of research related to AMD and the role of tea flavonoids in future prevention models.

Macular Degeneration

Overview

AMD is an ocular disease that gradually damages the macula, which is the portion of the retina with the highest density of photoreceptors and is responsible for acute vision clarity[5,40]. The macula receives light signals and quickly converts them into chemical and then into subsequent electrical signals that are sent to the brain via the optic nerve[1,5,40]. At the neurologic level, the brain transcodes these electrical signals into the images we see. In the case of AMD, as the photoreceptors in the macula are damaged or prematurely shed, the central field of vision is distorted or lost[5].

The retinal pigment epithelium (RPE) is a single layer of cuboidal cells spaced between the photoreceptors of the neurosensory and the choroidal capillary bed within the retina[4,40]. The RPE cells play important roles in several necessary visual functions such as "retinal chromophore regeneration", functional support of photoreceptors, and phagocytosis of photoreceptor outer segments[40]. Within the retina, the photoreceptors are exposed to widespread oxidative stress in the form of light and oxygen[6]. Consequently, roughly 7-11% of partial photoreceptors are shed each night. After their destruction, RPE cells must engulf, break down

metabolized portions, and remove them[6,41]. Because one RPE cell repairs roughly 30 photoreceptors, the RPE layer has among the highest oxidative loads in the body[41].

In addition, the RPE is involved in maintaining the integrity, function, and nutrition of the photoreceptors because they do not have their own blood supply[41]. Instead, all energy is supplied from the choroidal blood supply, which crosses the Bruch's membrane and enters the RPE layer and photoreceptors[41,42]. Through proper nutritional support the RPE is able to provide efficient turnover of photoreceptors. The largest contributing factor to the formation of deposits in the RPE-Bruch's membrane region are situations of combined inadequate nutrition and the inability to properly break down and remove cellular byproducts[42]. Basal laminar deposits form between the RPE basement membrane and the associated plasma membrane[42]. These deposits are theorized to lead to the formation of drusen, which are established clinical indicators for early AMD[5,40,41]. Drusen are often found between the RPE and the choroid and contain a variety of lipids and proteins, including advanced glycation end products and inflammatory modulators[41-43].

During ocular inflammation, lymphocytes and macrophages migrate to the posterior compartment of the eye and secrete pro-inflammatory cytokines such as interleukin (IL)-1β, interferon (IFN), tumor necrosis factor (TNF), MMPs, and VEGF[44]. These inflammatory mediators can target and impair RPE functions, causing the pathogenesis of diseases in the retina such as uveoretinitis, proliferative vitreoretinopathy (PVR) and, specifically, AMD. Since RPE cells are a component of the outer BRB and are sensitive to pro-inflammatory stimuli, especially TNF, which is secreted by macrophages, as well as vascular endothelial and RPE cells, it is no surprise that AMD is thought be a response of inflammatory markers and BRB breakdown[42,43].

Most studies on early pathogenesis of AMD have focused on oxidative injuries affecting RPE. As previously stated, cumulative oxidant injury to the RPE plays an important role in

development of AMD[35,37,42,45]. In addition, late development of AMD demonstrates accumulation of specific deposits and extracellular matrix (ECM) molecules under the RPE. It is characterized by early degradation of the ECM followed by proliferation and migration of the endothelial cells[28]. Key mediators such as VEGF and MMPs are known to play an important role in this process[28]. MMPs secreted by endothelial cells are hypothesized to play a key role in the processes of ECM remodeling during angiogenesis. MMP-2 and MMP-9 are extracellular proteinases that have been revealed to play an important role in retinal neovascularization and increasing of vascular permeability seen in the late development of diabetic retinopathy and AMD[28]. Specifically, MMP-9 is essential for stable basement membrane function in repair by the central nervous system[28.] In 2006, Drevs and Schneider highlighted the physiological balance of vascular biomarkers such as VEGF and pigment epithelium derived factor (PEDF) as crucial to maintaining normal vasculature[46] and their research has since been extrapolated to our current understanding of AMD and its subsequent treatment.

Classification

Although physiologically there are still many mysteries surrounding the development of AMD, practitioners have classified symptoms into categories in order to define the characteristics of the development of AMD. There are two forms of AMD, commonly referred to as dry and wet. Approximately 90% of AMD patients in the US have dry AMD[5,40]. Dry AMD has three stages, early AMD, intermediate AMD and advanced AMD, which are characterized in part by the size and number of drusen (Figure 1)[5], which begins in the top left corner rotating clockwise with increased severity of the disease progression. Early AMD is indicated by small

(<63μm) and/or no more than three medium-sized (<125μm) drusen, represented in the upper two images of Figure 1. Intermediate AMD is indicated by many medium-sized or one or more large-sized drusen. Advanced dry AMD, which is also termed "geographic atrophy", is categorized by "focal, round depigmentation with sharp margins and, in some cases, visible choroidal vessels."[5] Often there are large drusen liberally distributed along with RPE and photoreceptor death in the macula. Subsequently, those with advanced stages experience significant vision loss[5,40,42]. Typically, early and intermediate are seen in "dry" AMD, whereas "wet" AMD is seen in advanced stage as a progression of the disease[5,40].

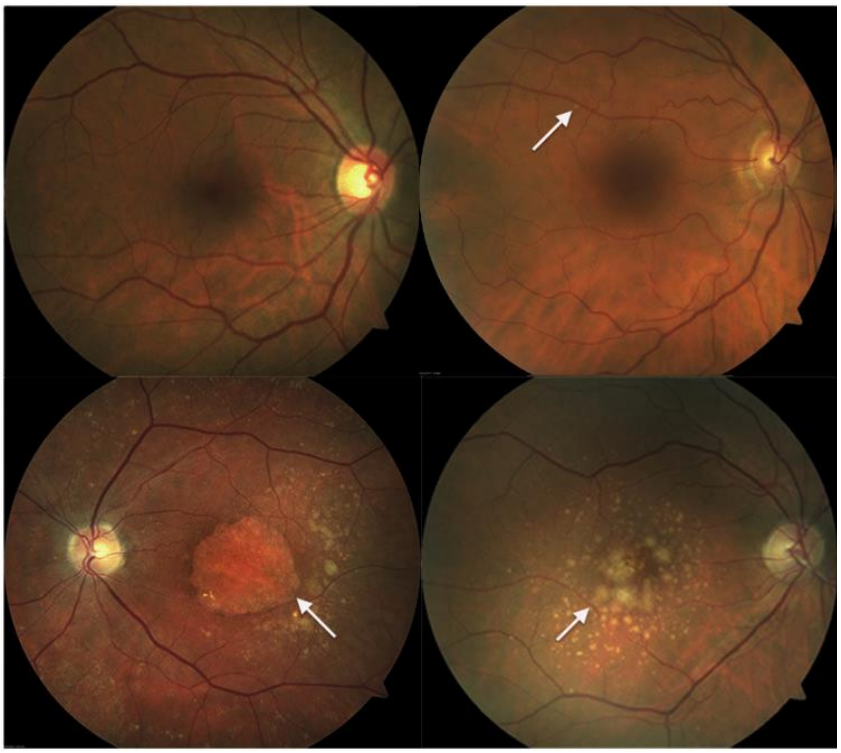

Figure 1: Fundus Photography Displaying Gradation of AMD[40]

Approximately 10% of AMD patients in the US have the more incapacitating wet, or neovascular, form of AMD[5,40]. Wet AMD is categorized by the creation of masses of cells and

fluid termed “exudates” and/or neovascularization of the retina, which is the development of atypical blood vessels, originating from the choroid, that penetrate Bruch’s membrane, causing damage to the RPE and overlying photoreceptors (Figure 2). The abnormal vessels are prone to breaking and leakage, earning the name wet AMD[40]. It is precisely the leakage, or bleeding, that causes the macula to swell and bulge, leading to the characteristic visual distortion reported by patients with wet AMD in which straight lines to appear curved[40].

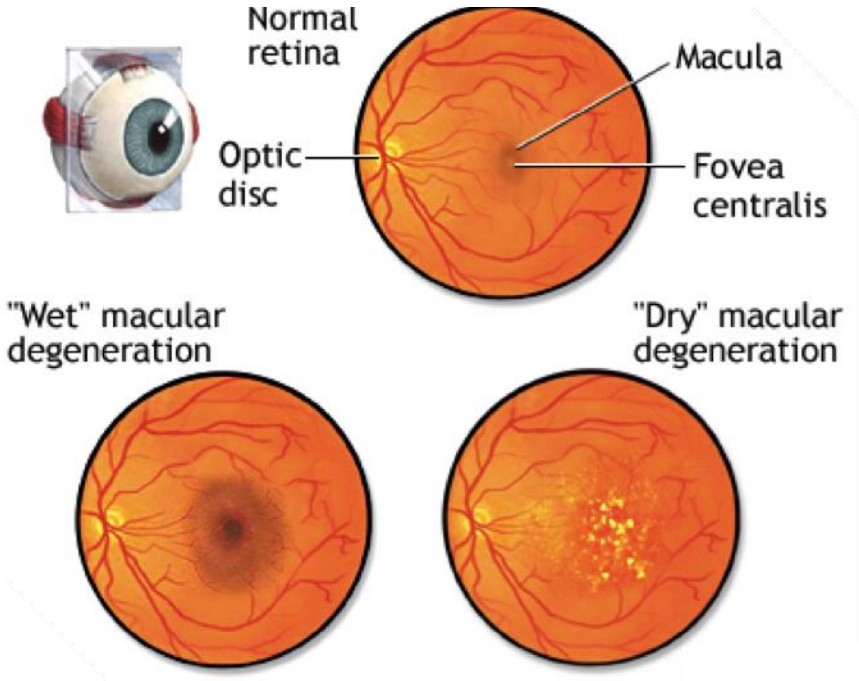

Figure 2: Fundus Images of Dry vs. Wet AMD[5]

Risk Factors

Risk factors for AMD include age, gender, race, family history, genetics, weight and environmental influences such as smoking, physical activity level and diet[5]. As individuals age,

the risk for AMD increases[2,5,40]. Research has also shown a slight gender bias, with women having a slightly higher risk for AMD than men. Non-Hispanic Blacks have less risk for AMD at age 80 than Caucasians, and Asians have a higher rate of dry AMD than Caucasians[5,40]. With regards to genetics, having a first-degree relative with AMD, regardless of the subtype, can increase one's own risk of the disease by 8-22% depending on the study[14]. Also, there are many single nucleotide polymorphisms that have been associated with an increased AMD risk in certain populations. The most widely known and studied polymorphism is found on the Complement Factor H gene[47].

Recently there has been a shift in research looking at modifiable risk factors of AMD. Obesity is a significant risk factor, with investigators suggesting that a 3% reduction in the waist-to-hip ratio decreases risk for AMD by 20%[48]. Coinciding with obesity is a decrease in physical activity[49]. Prior research has found that both AMD and impaired visual acuity are associated with reduced physical activity. One study has recently reported lower physical activity levels among those with impaired visual acuity[50]. Similarly, four studies have examined the association between AMD and physical activity [50-53]. Results from these prior studies are variable, with half of the studies demonstrating that AMD is associated with reduced physical activity levels[50,53].

Diet is also a modifiable risk factor for AMD with research indicating certain nutritional factors may influence the development and/or advancement of AMD. With nutritional analysis significant limitations exist because dietary components may interact with each other, either in a single food or a meal, alteration in intake or elimination of one food may increase intake of another, and the summative effects over time in congruence with other dietary changes are difficult to enumerate[49]. Correlations between single nutrients and AMD are often inconsistent

across studies and it is often difficult to disconnect single aspects of a diet or lifestyle from each other[54]. Examining overall diet quality in relation to AMD has the ability to better account for the relationships among different diet components. Montgomery et al. designed a case control study to evaluate the dietary information of 696 males and females, of which 437 had AMD and 259 were controls, using a 97-item food frequency questionnaire (FFQ) and Health Habits and History Questionnaire (HHHQ)[54]. They found those who were in the highest quartile, compared to the lowest, of diet quality were at significantly decreased odds of AMD.

Similarly, a study of 2005 women, diet quality was measured using a modified Healthy Eating Index (mHEI) score, and a six-point healthy lifestyle score (HLS)[55]. Women in the highest quintile compared to the lowest for the mHEI score had 46% decreased risk for early AMD and those whose diet scores were in the highest quintile had diets significantly lower in fat and higher in median servings of fish, vegetables, dairy, grains and meats or alternatives. Highlighting the previously mentioned modifiable risk factors, the higher quintiles also had more physical activity and fewer years of smoking, lower likelihood of hypertension, lower systolic blood pressure, lower BMI and lower level of serum C-reactive protein (CRP). Supporting their theory, women in this study who had a HLS score of six, which reflected the healthiest of three score components (smoking, physical activity and diet), had 71% lower odds for early AMD compared to those with lower scores[55]. Each study discusses the innumerable covariates (risk factors) involved when investigating the role of disease pathology and progression such as AMD and the correlation of diet. As is the case with most dietary studies, more research is needed.

Treatment

While there are emerging pharmaceutical treatments for dry AMD, the typical management for long-term care is nutritionally related. Currently medical treatments for AMD address only end-stage, exudative AMD and focus on inhibiting the neovascularization that leads to capillary leakage of blood and fluid in the macula[5,22,40]. Managing neovascular AMD is sometimes a difficult, long-term process, with multiple intravitreal anti-VEGF injections, that aim to slow vision loss by preventing vessel formation. Treatments must be instituted early to prevent exudative damage, extensive neovascularization, and secondary scarring[22,56]. Thermal photocoagulation with a laser was the first effective therapy to show promise in neovascular AMD. Unfortunately, thermal laser was only found to be helpful when used on vessels outside of the foveal avascular zone so as to prevent any collateral damage to the crucial foveal photoreceptive cells by the laser. Older techniques focused on foveal neovascularization using a non-thermal laser and a photosensitizing drug that is more selective to blood vessels and causes less collateral photoreceptor damage[56]. Radiation via frequency waves has also been shown to provide benefit in preserving near vision and contrast sensitivity. Other treatments involve anti-angiogenic compounds injected intravitreal or administered periocularly. Another promising molecule is pigment epithelium derived growth factor, which prevents angiogenesis and may improve the health of the retinal pigment epithelium and restore the BRB[22]. At present time, no treatments are available for severe, AMD-associated central vision scarring.

Treatment modalities have been designed to mitigate the intrinsic damage done to the macular pigment and RPE cells. Lutein and zeaxanthin are yellow pigments that are selectively concentrated a thousand-fold greater in the fovea compared to other tissues[57,58]. It is generally

accepted that these pigments protect the delicate macular photoreceptors and improve vision by reducing the amount of light scatter, color abnormalities, direct absorption of high-energy blue light that can injure the macula, as well as through protection against free radicals created from photochemical reactions in the photoreceptor cells (rods and cones) through their antioxidant abilities[57,58]. It is likely that these carotenoids perform each of these functions to protect the retina and preserve central vision[58]. Since we have no drugs that have been shown to prevent or reverse damage to the macula, our bodies rely on dietary intake for the carotenoids necessary to replenish its epithelium.

Dietary and Nutritional Management of AMD

Nutritional management and prevention of AMD can be divided into two approaches: first, increasing dietary antioxidants, and second, augmenting the macular pigment by increasing the intake of xanthophyll carotenoids. Research has shown that the prevalence of AMD is higher in individuals with low antioxidant intake and low lutein intake[20,21]. Clearly, there is overlap between antioxidant and xanthophyll carotenoid intake because carotenoids are able to act as antioxidants, and antioxidants can increase intestinal absorption of lutein and zeaxanthin by protecting these pigmentary carotenoids. As such, this is helpful when considering the scientific literature and counseling patients to make dietary changes.

As mentioned, oxidative stress has been linked to AMD, and the earliest nutritional studies of AMD focused on the effects of antioxidant intake. The most recognized antioxidants include vitamin C, vitamin E, and beta-carotene[59,60]. Vitamin C, ascorbic acid, is a water-soluble, essential nutrient that can scavenge free radicals and can be regenerated (reduced) by

glutathione[4]. Vitamin E, which usually appears in supplements as alpha-tocopherol, is only one molecule in the tocopherol antioxidant family[14]. Also, the tocopherols, and the closely related tocotrienols, all have vitamin E activity. Tocopherols and tocotrienols are fat-soluble nutrients, as are carotenoids. The main compounds with vitamin E activity are alpha-, beta-, gamma-, and delta-tocopherol, and alpha-, beta-, gamma-, and delta-tocotrienols. Nuts, seeds, olive oil, whole-grains, and green leafy vegetables are all excellent sources of tocopherols and tocotrienols. Oxidized vitamin E, which is formed after vitamin E absorbs a free radical, is reduced back to its active form by vitamin C[14]. Both vitamins C and E, as well as beta-carotenes are important because they become more biologically active in the presence of other bioflavonoids[14], of which we will discuss in subsequent sections.

Nutrition-Related Observational Studies

Epidemiological studies such as The Wisconsin Beaver Dam Eye Study, The Australian Blue Mountain Eye Study, The Nurses Health Study, and The Physicians Health Study have explored the relationship between the previously mentioned antioxidants and age-related ocular diseases. Dietary intakes of the carotenoids lutein and zeaxanthin produced protective benefits against cataracts and AMD in Beaver Dam Eye Study and Nurses Health Study. Lutein levels were associated with the decrease in AMD in the Beaver Eye Dam Study[16] and the Nurses Health Study had a 22% decreased risk of AMD with zeaxanthin and lutein intake[49]. Overall, in these four studies the reported findings concerning an association between these nutrients and carotenoids and AMD risk were inconsistent, and the scope of the potential dietary phytochemicals investigated was limited[13-15]. Surveys such as the National Health and Nutrition

Examination Survey (NHANES) have also yielded inconsistent results, with vitamins E and A, having no associated benefits with AMD or cataracts, and a 1 mg/dl serum increase of vitamin C resulting in a 13% reduced risk of AMD[60]. Similarly, the Nurses Health Study and the Health Professionals Follow-up Study, which examined the dietary intake of antioxidants such as vitamins A, E, and C and the carotenoids lutein and zeaxanthin, did not produce strong associations between the amount consumed and risk for glaucoma or AMD[4]. Overall, there has not been strong support for epidemiological studies and the current supplementation recommendations.

Supplementation Trials

A review by Evans and Lawrenson (2012) highlighted the challenges of epidemiologic studies as well as their limitations, and proceeded to discuss several supplementation clinical trials, such as both Age-Related Eye Disease (ARED) studies, the Vitamin E, Cataract and Age-Related Maculopathy Trial (VECAT), and The Alpha-Tocopherol, Beta-Carotene (ATBC) study[12]. Results from the analysis of the effects of antioxidant supplementation on eye health and, just as their epidemiologic counterparts, have yielded inconsistent findings. The beneficial effect of alpha-tocopherol and beta-carotene supplementation on the prevention of AMD was found in the VECAT and ATBC trials[12]. In 2013, the AREDS2 trial was conducted to determine if the AREDS formula could be improved. The primary objective of AREDS2 was to determine if adding lutein and zeaxanthin, DHA + EPA (omega-3 fatty acids), or both to the AREDS formulation further decreases the risk of developing advanced AMD[5,60]. Secondary objectives of the AREDS2 study were to evaluate the effect of eliminating beta-carotene, lowering zinc doses,

or both in the AREDS formulation. ARED original results determined that treatment with an antioxidant combination of vitamin C, vitamin E, beta carotene, and zinc, did produce a 25% reduction in AMD progression[12,59], whereas results from AREDS2 report an 18% reduction in progression to advanced AMD in subjects who received 10 mg lutein and 2 mg zeaxanthin, in addition to an AREDS supplement without beta carotene compared to the original AREDS supplement[60].

The results of meta-analyses and systematic reviews of research conclude that there is a lack of adequately designed supplemental trials to determine whether or not dietary supplementation with carotenoids can reduce the risk of AMD in healthy populations[4,12]. However, the clinical epidemiological AREDS reported that certain dietary antioxidants and mineral supplements reduced the progression of visual loss by up to 28% in patients with moderate to advanced disease, and its predecessor, AREDS2, addressed lutein's beneficial role in supplementation. Foods that were correlated with ocular benefits in both AREDS studies included carotenoids and, of relevance to this research, flavonoids[60]. From this study, the formulation for daily AREDS2 supplementation (currently the only preventative measure for AMD) is as follows: 500mg Vitamin C, 400IU Vitamin E, 80mg zinc, 2mg copper, 10mg lutein, and 2mg zeaxanthin[60].

Phytochemicals

Flavonoid Introduction

At this point in time, more than 8000 polyphenolic compounds have been identified and these flavonoids can be classified into different subclasses, which include flavones, flavonols,

flavanones, flavanols, anthocyanins and isoflavones[23]. Flavonoids are biologically active polyphenolic phytochemicals commonly found in fruits, vegetables, nuts and plant-derived beverages, such as tea or wine. Over the last century, flavonoids or bioflavonoids have been identified as the most common group of plant polyphenols that give color and flavor the compound it inhabits[23,24]. They are secondary metabolites in plants and their biosynthesis takes place via the shikimate and arogenate pathways. The common structural feature of the flavonoids is the flavone nucleus, characterized by a C6-C3-C6 carbon skeleton with the C6 component being aromatic in nature[62]. This basic skeleton may contain numerous substituent groups: hydroxyl groups, generally present at the 4′, 5 and 7 positions; sugars, generally linked with the hydroxyl group positioned at 7; and methyl and isopentyl units. Hydroxyl groups and sugars provide the molecules with hydrophilicity, while methyl groups and isopentyl units specify the lipophilicity of the flavonoids[31,61,62]. In plants, flavonoids are glycosides, meaning there is an attached glucose. Initially they were considered as vitamins and the term "vitamin P" was coined to describe them, although this since been all but abandoned[62]. Flavonoids play an important role in plant physiology, including pigmentation, growth and reproduction.

The composition of flavonoids in different fruit species varies greatly, as does their subsequent color and nutrition. Quercetin, kaempferol, and myricetin are common flavonols, with quercetin being the most predominant one consumed via dietary means[62]. The major polyphenolic elements present in green tea are epicatechin, epigallocatechin, epicate-chin-3-gallate and epigallocatechin-3-gallate. In addition to small amount of catechins, black tea contains thearubigins and theaflavins, which are the polymerised forms of catechin monomers and are the major components formed during enzymatic oxidation and the fermentation process[63].

Flavonoids are reported to possess a wide range of activities in the prevention of common diseases, including CHD, cancer, neurodegenerative diseases, gastrointestinal disorders and others[23-26,63-69]. These effects appear to be related to the various biological/pharmacological activities of flavonoids. A large number of publications suggest immunomodulatory and anti-inflammatory properties of these compounds[23,61,65]. However, after examining many literature reviews, it is clear that the majority of the data is from *in vitro* studies, animal models, or human studies without long-term interventions[61,66]. The largest portion of *in vitro* studies have been carried out with single flavonoids at somewhat "supraphysiological concentrations", and few studies have investigated the anti-inflammatory effects of physiologically attainable flavonoid concentrations in healthy subjects[61]. Thus, there are potential limitations to any study looking at dietary consumption of isolated flavonoids.

In-Vitro Flavonoid Antioxidant Studies

The *in vitro* antioxidant effects of flavonoids and other phenolics have recently been studied using models and ocular cell types that are relevant to visual disorders and their pathologies. Studies of cultured RPE cells were used to explore the role of flavonoids in protection against oxidative damage as well as metabolic responses to oxidative stress *in vitro*[19,20,28,70]. The RPE is vitally important to study because oxidative damage to RPE is implicated in AMD and other visual impairments[3,4,5,40]. Functionally, the RPE forms the BRP and is the site of active transport of materials such as vitamin A (retinol) from blood to the photoreceptors. In 2006, Hanneken and colleagues, studied numerous flavonoids (flavones, flavonols, flavanols, flavanones, and anthocyanidins) and tested for their effect on the survival of

cultured human RPE cells (ARPE-19) after cells were subjected to oxidative stress by the addition of either tert-butyl peroxide (t-BOOH) or hydrogen peroxide (H_2O_2)[19]. Results indicated natural flavones (luteolin and baicalein), flavonols (galangin, fisetin, and quercetin), and a flavanone (eriodictyol) were effective in improving cell survival, whereas flavanols and anthocyanidins were not. The authors indicate that effective clinical treatments after the occurrence of oxidative damage are needed to replicate the current understanding of AMD pathology[19.70]. By selecting the appropriate time frame after oxidative insult and until cells were committed to cell death, the authors showed that specific flavonoids, namely, luteolin, fisetin, and quercetin, were able to protect cells even when applied two hours after treatment with t-BOOH or H_2O_2[19.70]. Conversely, Milbury et al. examined ARPE-19 cells treated with a purified bilberry extract that contained either anthocyanins or non-anthocyanin phenolics before they received an oxidative challenge with H_2O_2. Both the anthocyanin and non-anthocyanin extracts were found to reduce the abundance of ROS[70], suggesting that anthocyanins still may play a role in the reduction of inflammatory markers of AMD.

Flavonoid Ocular Availability

Although flavonoids may affect visual signal transduction and provide antioxidant protection, relatively little is known about their bioavailability to the eye. When Kalt et al. fed blueberries to pigs for 4 weeks, the flavonoid (anthocyanin) concentration measured in the whole eye was detected at sufficient levels[71]. Prior to examination, the pigs were fasted for approximately 18 hours, and no anthocyanins were detectable in plasma, suggesting that anthocyanins had accumulated in the tissues. The bioavailability of flavonoids was studied in a

rat *in situ* perfusion study to determine the extent of their intestinal absorption and intestinal and metabolism within the liver. Crespy et. al concluded the absorption behavior of these flavonoids was influenced more by their lipophilicity than by their structure[72].

Another study reported a significantly higher concentration of anthocyanins in ocular tissues when black currant anthocyanins were administered by intravenous and intraperitoneal means to rabbits and rats suggesting dietary consumption may not be the best way to achieve ocular therapeutic results[73]. The results suggest that flavonoids, including quercetin and various anthocyanins, can cross the BRB after normal feeding in both animal models and after acute administration by intraperitoneal and intravenous means[73]. Even though there are studies displaying the ability of flavonoids to cross the BRB, a major limitation of these studies is the correlation between animal models and human BRBs has yet to be determined. From what we do know about the mechanism of protection in RPE cells and the suggested ocular availability, research continues to evolve in creating adequate animal models[73]. One type of flavonoid that has yet to be studied, aside from animal and *in vitro* studies, is that found in tea. The next section will discuss, in detail, the structure and function of tea flavonoids as well as the research as it relates to AMD.

Tea

Introduction

Tea is a rich source of flavonoids and one of the oldest medicinal compounds of record[25,27]. The tea plant *Camellia sinesis* is native to Southeast Asia but is currently cultivated in over 30 countries around the world. Tea is consumed worldwide, although in greatly different

amounts; it is generally accepted that, next to water, tea is the most consumed beverage in the world, with per capita consumption of roughly 120 mL/d[25]. Of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and less than 2% is oolong tea. Black tea is consumed primarily in Western countries and in some Asian countries, whereas green tea is consumed primarily in China, Japan, India, and a few countries in North Africa and the Middle East. Oolong tea production and the majority of its consumption have primarily been relegated to southeastern China, Taiwan and parts of Japan[27].

Green, black, and oolong teas undergo different manufacturing processes. To produce green tea, freshly harvested leaves are rapidly steamed or cooked over heat to inactivate enzymes, thereby preventing fermentation to create a stable product[27]. The result is a high level of epicatechins in green tea, accounting for its characteristic color and flavor. For the production of black and oolong teas, the fresh leaves are allowed to wither until their moisture content is reduced to an estimated 55% of the original leaf weight, which results in the concentration of polyphenols in the leaves[27,62]. The withered leaves are then rolled and crushed, initiating fermentation of the polyphenols. During these processes, the catechins are converted to theaflavins and thearubigins[27]. Oolong tea is prepared by firing the leaves shortly after rolling to terminate the oxidation and dry the leaves. Normal oolong tea is considered to be about half as fermented as black tea. The fermentation process results in oxidation of simple polyphenols to more complex condensed polyphenols to give black and oolong teas their characteristic colors and flavors[27].

The composition of the tea leaves depends on a variety of factors, including climate, season, horticultural practices, and the type and age of the plant[65,66]. The chemical composition of green tea is similar to that of the leaf. Green tea contains polyphenolic compounds, which

include flavanols, flavandiols, flavonoids, and phenolic acids and account for 30% of the dry weight of green tea leaves[27,65]. Most of the polyphenols in green tea are flavanols, commonly known as catechins. The major catechins in green tea are epicatechin (EC), epicatechin-3-gallate (EGC), epigallocatechin, and epigallocatechin-3-gallate (EGCG). In black teas, the major polyphenols are theaflavin and thearubigin[25,27,65].

Tea Catechins

Metabolism of tea catechins is complex and there has been no definitive study indicating the efficacy of catechins crossing the BRB. From metabolic studies, we do know that after oral administration, green tea catechins are absorbed intestinally, predominately in the jejunum and ileum, with peak absorption occurring at 1.5 to 2.5 hours after consumption. EGCG is thought to be primarily transported across the intestinal epithelial cell layer through paracellular diffusion[61-63]. However, bioavailability is low due to instability under digestive conditions, poor absorption, and rapid metabolism and excretion, leading to only about 5% of consumed catechins appearing in the plasma[63]. EGCG is the only known polyphenol to be mainly in the free form, upwards of 90%, in the plasma whereas EC and EGC are mostly in bound or conjugated form[61]. The general metabolism in the intestine and liver leads to the formation of glucoronides, sulfides and methylated metabolites[74]; thus, aiding the deactivation and breakdown of the biologically active compounds. Green tea catechins are methylated by enzymes and those processes can occur to previously methylated EGCG and vice versa, forming mixed metabolites[63,74]. Tea catechins have also been shown to undergo microbial breakdown in the colon, forming compounds such as

phenolic and benzoic acids, which may be absorbed or discarded via the gastrointestinal system[74].

The biological activity of green tea catechin metabolites have been shown in most cases to be limited compared with the whole structure; however, a small selection of metabolites has been shown to have similar or higher activity than the primary compound. For example, Lu et al. found EGCG-30-glucuronide and EGCG-300-glucuronide metabolites to have similar radical-scavenging ability compared with free EGCG[75]. The major metabolites were also found to have comparable superoxide-scavenging abilities in a later *in vitro* study, as reported in a review of literature by Mazzanti et al.[76]. A limited number of cell-culture studies have also indicated that some catechin metabolites may have a different biological activity to the parent compound[76].

Health benefits of tea catechins have been widely ascribed to one of three areas: antioxidant properties; effects on intestinal microflora and nutrient absorption; and effects on metabolism and metabolic enzymes affecting growth of cancer cells. In a 1998 review, a total of 31 studies and four reviews were examined by Bushman, and it was found most studies showed an inverse association between tea consumption and cancer of the colon, urinary bladder, stomach, esophagus, lung, and pancreas[77]. An empirical link between green tea and its cancer prevention properties was made in the late 1980s and epidemiologic studies show a decrease in incidences of cancer in those who consumed 3 cups of green tea per day or more. However, due to many variables inherent to such studies, a definitive link between green tea and its cancer effects could not be concluded. From the above studies, 10 cups of green tea or 5 cups of black tea per day were recommended. Thus, a possible relationship between high consumption of tea and other diseases involving angiogenesis could exist[77].

Since that review, numerous epidemiological studies provide support for green tea's role in preventing and treating many disorders[76,78-81]. These epidemiological studies suggest a relationship between drinking green tea and positive health outcomes such as reduced incidences of cancer, inflammation markers, hypertension and hypercholesterolemia; however, epidemiological studies are not capable of determining if green tea is directly causing these outcomes. While continuous research does provide valuable insight into real-world effects of tea consumption on human health, many confounding variables exist. These variables include genetics, age, gender, previous dietary habits, physical activity level, health biomarkers, and other comorbid diseases.

Tea and AMD

Because catechins, especially EGCG, have been studied extensively as a potential treatment for a variety of oxidative stress related diseases including cancer and cardiovascular and degenerative diseases, the mechanisms of its capacity to inhibit a variety of inflammatory and angiogenic factors such as tumor necrosis factor (TNF) and VEGF can be translated to other diseases with similar pathology and age-related oxidative damage, such as AMD[82]. As previously stated, the angiostatic potential was hypothesized due to its inhibition of new blood vessel formation in a chick ocular membrane model, and suppression of oxygen-induced retinal neovascularization and corneal neovascularization in animal models[72]. EGCG was found to decrease VEGF receptor phosphorylation in human umbilical arterial endothelial cells and has also been reported to inhibit the MMP-2 and MMP-9 in human umbilical vein endothelial cells[83]. Whether these angiogenic factors, including VEGF, play an important role in RPE cells, and the

precise mechanism by which they do so, is still uncertain[28,82,83]. In a 2014 study, EGCG was found to protect RPE cells from ROS-induced BRB remodeling and angiogenesis through its antioxidative and anti-angiogenic effects[28]. It was also discovered that EGCG protects human retina cells against BRB alteration (breakdown and remodeling), ocular neovascularization, and vascular permeability via suppression of MMP-9 and the consequent VEGF activation in RPE cells[28].

Recent eye studies have also shown that EGCG could significantly protect RPE against cell death induced by oxidative stress[29,38]. In endothelial cells, EGCG has been reported to inhibit leukocyte migration through decreased expression of "adhesion molecules", and decreased leukocyte attachment and migration[32]. Few reports have demonstrated the role of EGCG in decreasing inflammation in RPE, especially with respect to cell adhesion molecule expression and leukocyte movement. A 2015 study evaluated the ability of EGCG to suppress the harmful effects of the pro-inflammatory cytokine TNF and VEGF in RPE cells[29]. The results showed EGCG significantly decreased the TNF-induced increased intracellular ROS level in ARPE-19 cells, and decreased VEGF production indicating that the protective role of EGCG was consistent with its antioxidant property. Even though there is minimal data directly involving RPE cells and the role of tea catechins in VEGF reduction, many individuals still choose to increase their dietary intake of such flavonoids. The increased use of dietary supplements and other CAM therapies has been attributed to several different factors. The main reasons described in previous studies included dissatisfaction with conventional medical practices, the increased costs associated with conventional medicine, desire for self-treatment of one's health, and the prevention/treatment of health conditions[7,85]. Promotion of general health and curiosity were other reasons given for dietary supplement use.

Summary

Due to the complex nature of AMD, it is necessary to continually reevaluate current modalities of prevention and treatment. Because of the rise of older adults looking to CAM therapies, combined with the ongoing research of supplementation (AREDs) for individuals with AMD, it is necessary to determine what other nutrients may play a protective role. Currently more studies are needed to determine the exact mechanisms in which catechins and tea flavonoids exert their effects within RPE cells after crossing the BRB. Future studies should consider examining the different flavonoids and phenols in tea in RPE cells as well as human studies in populations in which tea consumption is high. To date, there are no such correlations made.

CHAPTER III

METHODS

Data Source

The secondary data used in this study is from the 2005-2006 National Health and Nutrition Examination Survey (NHANES). NHANES is a cross-sectional survey conducted by National Center for Health Statistics (NCHS), a division of the Center for Disease Control and Prevention (CDC), which is responsible for providing central health statistics for the United States[84]. It is designed to monitor the health and nutritional status of non-institutionalized civilians in the US. The data is collected on a continual basis and released in two-year increments; it includes interview and examinations of nearly 5000 people per year. The NHANES studies began in the early 1960s and have continued annually. Demographic, socioeconomic, dietary, laboratory tests, physiological measurements, medical, dental and health-related questions are collected, although specific data rotates within those categories are cyclical. One of those perimeters is fundus photography within the laboratory category (repeated for two survey cycles every 10 years)[84].

To select a participant, NHANES used a statistical process to divide the United States into groups that are then further parsed into sections[86]. The process, described on the CDC's website, was as follows:

> Stage 1: Primary sampling units (PSUs) are selected from strata defined by geography and proportions of minority populations. These are mostly single counties or, in a few cases, groups of contiguous counties selected with probability proportional to a measure of size (PPS). Most strata contain two PSUs. Additional stages of sampling are performed to select various types of secondary sampling units (SSUs), namely the segments, households, and individuals that are selected in Stages 2, 3, and 4.
> Stage 2: The PSUs are divided into segments (generally city blocks or their equivalent). As with each PSU, sample segments are selected with PPS.
> Stage 3: Households within each segment are listed, and a sample is randomly drawn. In geographic areas where the proportion of age, ethnic, or income groups selected for over-sampling is high, the probability of selection for those groups is greater than in other areas.
> Stage 4: Individuals are chosen to participate in NHANES from a list of all persons residing in selected households. Individuals are drawn at random within designated age-sex-race/ethnicity screening sub-domains. On average, 1.6 persons are selected per household.[84]

Each participant's identity was kept confidential and given a unique identifier. NHANES uses a stratified multistage sampling design that requires a weighting scheme to most accurately estimate disease prevalence in the US population[84]. These weighted data were used throughout data analysis and included individuals who were 40 years and older and underwent the interview and physical examination portions of the study. The CDC stated the survey was conducted by professional individuals from "varied backgrounds such as social work, the military, education, medical doctors, nurses, health educators, engineers and doctoral degree holders"[84]. The reports of their tests are mailed to the participants. Finally, a cash payment for participating in the survey is given. Participants are expected to participate in an interview that is done by an interviewer at home, by an online interview, and a health examination in a Mobile Examination center.

This research at hand, used publicly available, de-identified data and as a result was considered exempt by the institutional review board (IRB) at Northern Illinois University (NIU) (Appendix A).

Inclusion and Exclusion Criteria

The sample for this study consisted of adults from the NHANES 2005-2006 survey that met the following criteria:

1. Were aged 40 years or older (a requirement of the fundus photography ocular examination).

2. Completed the FFQ questionnaire with questions pertaining to tea consumption.

Exclusions were based on incomplete fundus photography and FFQ. Due to the multifaceted nature of AMD as well as being disease of aging, no further exclusions related to comorbid diseases were made. Therefore, all participants' data from the NHANES 2005-2006 survey who met the above-mentioned criteria was used in the current study.

Data Collection

Data for this study included AMD category, tea consumption, BMI, physical activity level, cholesterol and blood pressure levels, age and gender of participants. The data sets were downloaded from the publically available CDC website and was selected based on the risk factors associated with AMD.

Dependent Variable

AMD

The presence of AMD was determined by NHANES utilizing fundus photography. NHANES investigators performed fundus photography on participants 40 years and older. These images were graded at the University of Wisconsin, Madison, by 9 experienced graders. The graders viewed each fundus image with a high-resolution monitor, using the EyeQLite image processing software and database, and referred to the written protocol and the digital photographic standards to evaluate retinal abnormalities[84]. On a systematic grading, if the first two graders did not agree on a diagnosis, a third individual graded the eye images. If two of these three graders disagreed, the image was evaluated by an adjudicator to make a final decision. The grading was grouped into three categories: "absence of AMD", "dry AMD" and "wet AMD."

Potential confounders included self-reported descriptions from the questionnaire including: gender, age, race, and health-related categories (smoking status, physical activity, blood pressure and cholesterol statuses) and their calculated body mass index (BMI) from the physical examination. Gender was coded as either male or female. Age was represented on a nominal scale from ages 40 to 85 years old. If an individual was older than 85, then they selected the category, "85 or older." Race was divided by the NHANES questionnaire into 5 categories: "non-Hispanic white", "non-Hispanic black", "Mexican American", "other Hispanic", and "other race."

For health-related categories, all information was obtained from the questionnaire portion of NHANES, apart from BMI. Participants' BMI were calculated with the study population's weight in pounds and height in inches after measurement during the physical examination in the mobile unit and converted to the reported units of kg/m^2. The categories for BMI were: underweight ($\leq$18.49 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese ($\geq$30.0 kg/m^2). Smoking status was determined by asking the participant if they were a current smoker with the individuals responding, "yes" or "no." Similarly, the determination of hypertension and hypercholesterolemia for participants was determined by asking if they had been diagnosed with "high blood pressure" or "high cholesterol", respectively, to which they answered "yes", or "no." Lastly, the physical activity measure was obtained from the Physical Activity questionnaire file. Physical activity was categorized into "not enough data", "insufficient physical activity", and "physical activity". Physical activity was derived from the question "days vigorous recreational activities"; those with greater than three days a week of vigorous recreational activities where labeled active. Those with more than 4 days of moderate physical activity, which was derived from the question "days moderate recreational activities," were defined as physically active. Insufficient physical activity was defined that did engage in physical activity but that at the previously stated levels, or reported no physical activity.

Independent Variable

The NHANES data set from 2005-2006 included FFQ dietary recall collected by trained interviewers from participants during their visit to the Mobile Examination Unit. The frequency of tea beverage consumption was assessed by the following question: "Over the past 12 months,

how many cups of hot tea, caffeinated or decaffeinated, did you drink?" The possible responses were: "never", "less than 1 cup per month", "1 to 3 cups per month", "1 cup per week", "2 to 4 cups per week", "5 to 6 cups per week", "1 cup per day", "2 to 3 cups per day", "4 to 5 cups per day", and "6 or more cups per day". The same question was asked to assess the frequency of iced tea consumption. The results of hot and cold tea intake were combined to assess the frequency of total tea intake. Levels of total phenol and catechins in teas vary greatly across types. Since the FFQ does not specify what type of tea it is, aside from the exclusion of strictly herbal teas, further analysis as to the phytochemical nature of tea consumption for each participant was unable to be determined. In a 2002 study, an average of 3 cups of tea per week was sufficient to have detectable levels of phenols and catechins present in participants' serum, with the lowest conditional level of acceptance for frequent intake being more than 1 cup per week[85]. Thus, this study utilized the same measures with >1 cup of tea intake/week coded as "tea consumption" (Table 1) and less than that coded as "no tea consumption."

Table 1

Coding for Tea Consumption

Value Description	Code
None	1
<1 cup per month	1
1-3 cups per month	1
1 cup per week	1
2-4 cups per week	2
5-6 cups per week	2
1 cup per day	2
2-3 cups per day	2
4-5 cups per day	2
≥6 cups per day	2

Data Analysis

All data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC, USA) by the NIU Statistical Consulting Service. For the AMD response variable, we combined the dry (n=132) and wet AMD (n=18) together due to small numbers to maximize power. Thus, in our analysis, we only considered the absence of AMD or presence of AMD. A multivariable logistic regression model was used to determine the odds of an AMD diagnosis among participants consuming tea (hot, cold, or a combination of both). In this analysis, we considered the logistic model because the response variable is binary. We evaluated the data to compare the absence of AMD versus the presence of AMD. We considered the confounding variables of: BMI (underweight, normal, overweight, and obese), type of tea (hot or cold), gender (male or female), activity level (active or sedentary), cigarette smoking (presence or absence), high blood pressure (self-reported presence or absence), high cholesterol (self-reported presence or absence), age, and race. Upon analyzation, 1761 observations were available, which included an analysis of maximum likelihood estimates and odds ratio estimates. For each confounding variable, if the whole vector contained the element zero, the corresponding group was the base for this class (Table 2). All the statistical tests were 2-sided, and $P < 0.05$ was regarded as significant.

For the logistic model, SAS coding was as follows:

Letting Y be the response variable and x be the independent variable, we considered the response variable, Y as a binary variable which can take two values 1 or 0 based on the presence or absence of the category. Let p = Pr(Y= 1| x). Hence the logistic model can be written as: logit(p) = beta_0 + beta_1* x, where logit(p)=log(p/(1-p)) is the logit link.

In our case, we have multiple X. log(p/(1-p)) = beta_0 + beta_1* BMI + beta_2 * ICED + beta_3* HOT + beta_4 * GENDER + beta_5* ACTIVITY + beta_6*SMOKING + beta_7* BP + beta_8 * HCL + beta_9 * AGE + beta_10 * RACE.

Table 2

Binary Coding for Risk Factors

Category	Description	Coding
AMD	No AMD	0
	Dry AMD	1
	Wet AMD	1
BMI (kg/m^2)	BMI < 18.5 (Underweight)	1
	18.5≥BMI ≤ 24.9 (Normal)	2
	25.0≥ BMI ≤ 29.9 (Overweight)	3
	BMI ≥ 30.0 (Obese)	4
Gender	Male	1
	Female	2
Race	Mexican American	1
	Other Hispanic	2
	Non-Hispanic Black	3
	Non-Hispanic White	4
	Other Race - Including MultiRacial	5
Physical Activity	Sedentary	1
	Active	2
Smoking Status (Cigarettes)	Yes	1
	No	2
	Refused/ Don't know/ Missing	3
High Blood Pressure	Yes	1
	No	2
	Refused/ Don't know/ Missing	3
High Cholesterol	Yes	1
	No	2
	Refused/ Don't know/ Missing	3

CHAPTER IV

RESULTS

Descriptive Statistics

Of the 5,526 individuals selected in the two-year time span from the 2005-2006 NHANES survey, a total of 1,761 respondents met eligibility requirements for the current study. The demographic characteristics of the participants in this study with respect to gender, age, race/ethnicity, and presence of AMD are included in Table 3. The mean age was 52.8 ±1.7 years, and gender was represented equally, in both non-AMD and AMD categories. With regards to race, the individuals who identified as "non-Hispanic white" represented the majority (60.82%) of the participants in this study. The smallest percentage of individuals identified as "other race (including multi-racial)" and "other Hispanic" (3.18% and 2.16%, respectively).

Included in the demographics are the participants' self-described health status indicators of BMI, and physical activity, smoking, blood pressure, and cholesterol statuses. The overall mean BMI was 28.73 ± 2.41 kg/m^2 with 29.35 ± 2.07 kg/m^2, and 28.12 ± 3.11 kg/m^2 in the non-AMD and AMD groups respectively. Those with a BMI <18.5 kg/m^2, classified as underweight, were the most underrepresented in this population (1.99%) with and without AMD. A greater percentage of individuals (77.58%) reported some levels of consistent physical activity, than those who classified themselves as "inactive/sedentary." For smoking, blood

pressure, and cholesterol statuses, there were no significant differences in distributions within groups (presence or absence of AMD).

Table 3

Demographic and General Health Characteristics of Participants

Variables	No AMD (n= 1611)	AMD (n=150)	*P*-value
Gender			0.0167
Male	802 (45.54%)	90 (5.11%)	
Female	809 (45.94%)	90 (3.41%)	
Age mean (± SD), in years	*52.8 ±1.7	*57.6 ±2.6	<0.0001
Race			0.0013
Mexican American	225 (12.78%)	17 (0.97%)	
Other Hispanic	36 (2.04%)	2 (0.11%)	
Non-Hispanic White	957 (54.34%)	114 (6.47%)	
Non-Hispanic Black	338 (19.19%)	16 (0.91%)	
Other Race (including multi-racial)	55 (3.12%)	1 (0.06%)	
Body Mass Index			0.1073
Underweight (<18.49)	33 (1.87%)	2 (0.11%)	
Normal	406 (23.06%)	42 (2.39%)	
Overweight	559 (31.74%)	63 (3.58%)	
Obese	613 (34.81%)	43 (2.44%)	
mean (±SD), in kg/m^2	*29.34 (±2.07)	*28.12 (±3.11)	
Smoking Status			0.3114
Yes	865 (49.12%)	87 (4.94%)	
No	746 (42.36%)	63 (3.58%)	
Physical Activity			0.9741
Inactive	359 (20.39%)	33 (1.87%)	
Active	1249 (70.92%)	117 (6.65%)	
High Blood Pressure			0.0511
Yes	727 (41.28%)	83 (4.71%)	.
No	881 (50.03%)	67 (3.80%)	
Refused, Don't know, Missing	3 (0.17%)	0 (0.00%)	
High Cholesterol			0.0545
Yes	665 (37.76%)	60 (3.41%)	.
No	676 (38.39%)	77 (4.37%)	
Refused, Don't know, Missing	270 (15.33%)	13 (0.74%)	

Data represented as frequency of respondents as a number (percentage) of study participants *unless otherwise indicated. P-values calculated using X^2 test for categorical variables. Total Sample Size (N=1761).

Hypothesis One

To test for hypothesis one which stated, "there is a correlation between tea consumption and the presence of AMD," chi-squared analysis was used. This test was used to determine if there was any relationship between nominal variables (tea consumption and AMD). The frequency of one nominal variable was compared with different values of the second nominal variable.

Tea consumption was measured by subjects' reported consumption per day, week and month. Data was categorized as "no tea consumption" (none to 1 cup/week) and tea consumption (>1 cup/week to > 6 cups per day) (Table 1). Tea consumption represented in Table 4 includes both hot and cold tea consumption. For the response variable, we combined the early and late AMD together because the numbers were very small (roughly 17% of total respondents) in each of the two groups and this allowed us to maximize power. Thus, we only compared the absence of AMD to the presence of AMD in the simple frequency of occurrence diagram (Table 4).

Table 4

Frequency of Reported Tea Consumption and Macular Degeneration

Tea Consumption	**Absence of AMD**				**Presence of AMD**			
	Respondents	Total %	Tea %	Absence AMD %	Respondents	Total %	Tea %	AMD %
No Tea Consumption	770	43.73	47.80	91.23	74	4.20	49.33	8.77
Tea Consumption	841	47.76	52.20	91.71	76	4.32	50.67	8.29

There was a higher percentage of individuals (49.33%) who reported inconsistent tea consumption, or no tea consumption, in the AMD group than in the non-AMD (47.8%) group.

Similarly, for the individuals consistently consuming tea, there were similar percentages of individuals in both non-AMD and AMD groups, 52.2% and 50.67%, respectively. Chi-squared analysis of tea intake indicated no significant correlation (0.13, p= 0.720) between the presence AMD and reported tea consumption.

Hypothesis Two

In order to examine whether or not there was a significant relationship between the severity of macular degeneration and tea consumption, logistical regression was used to determine an estimate for confidence intervals in which correlation between large samples could be defined. To determine severity of AMD, respondents were classified into 3 distinct categories: “absence of AMD”, “dry AMD”, and “wet AMD”. In this design the type of tea, hot and cold, were combined into one category. The definition of drinking tea was defined as 1 or more cup per day. Similar to the results from hypothesis one, there was no significant difference in any of the three AMD categories (absence, dry and wet AMD) and tea consumption (Table 5). Of the total 150 individuals with AMD, 18 were diagnosed with the more severe form of AMD, representing only 12% of AMD cases and 1% of the total sample population. From the limited data available from those with wet AMD, the frequency distribution displays the higher proportion of individuals (0.68%) reported frequent tea consumption compared to those did not (0.34%), but this was not statistically significant (p=0.431). Conversely, when comparing tea consumption behaviors in those with dry AMD, there was a higher percentage of individuals who did not drink tea than those who drank tea (3.86% and 3.63%, respectively). Again, it was not statistically significant though (p=0.783).

Table 5

Association Between Tea Consumption and Severity of AMD

Tea Consumption	Absence AMD	Dry AMD	Wet AMD
<1 cup			
Number of Respondents	770	68	6
Percentage of Tea Consumption (%)	91.23%	8.06%	0.71%
1 or more cups			
Number of Respondents	841	64	12
Percentage of Tea Consumption (%)	91.71%	6.98%	1.31%
P-value between Tea Consumption in 3 AMD Categories	0.764	0.783	0.431

Hypothesis Three

To test this hypothesis, "there is a specific intake of tea consumption correlated with reduced incidences of macular degeneration,", again logistical regression was used to produce a type 3 analysis of effects, in order to determine the likelihood of each occurrence and if any level of tea consumption (from none through 6 cups/day and more) were related to severity of AMD. The highest frequency of respondents for both the presence and absence of AMD groups belonged to the individuals reporting no tea intake (n=637, 36.17%) (Table 6). The general frequency distribution is similar between non-AMD and AMD groups, regardless of their tea intake. There was no significant correlation between any of the tea consumption levels and AMD (p=0.6352).

Table 6

Association of the Amount of Reported Tea Consumption and Macular Degeneration

AMD		Reported Tea Consumption										
		No Tea Consumption				Tea Consumption						
		None	< 1 cup /month	1-3 cups /month	1 cup /week	2-4 cups /week	5-6 cups /week	1 cup /day	2-3 cups /day	4-5 cups /day	≥6 cups /day	Total
Absence of AMD	Frequency	576	245	240	97	170	72	96	84	17	14	1611
	Total %	32.71	13.91	13.63	5.51	9.65	4.09	5.45	4.77	0.97	0.80	91.48
	Tea %	35.38	15.21	14.90	6.02	10.55	4.34	5.96	5.21	1.05	0.87	
	Absence AMD %	90.42	93.87	92.31	91.51	92.39	91.14	91.43	89.36	85.0	93.33	
Presence of AMD	Frequency	61	16	20	9	14	7	9	10	3	1	150
	Total %	3.46	0.91	1.13	0.40	0.80	0.40	0.51	0.57	0.02	0.01	8.52
	Tea %	40.67	10.67	13.33	4.67	9.33	4.67	6.00	6.67	2.00	0.67	
	AMD %	9.58	6.13	7.69	8.49	6.67	6.60	8.57	10.64	15.0	6.67	
Total		637	261	260	106	184	79	105	94	20	15	1761
		36.17	14.82	14.76	5.91	10.45	6.02	5.96	5.34	1.13	0.85	100.00

Another finding was the similarity in tea consumption among hot and cold tea drinkers (Table 7). In both hot and cold tea, regardless of the presence or absence of AMD, the highest percentage of respondents (41.62% and 32.03%, respectively) reported no tea consumption. A higher number of respondents drank iced tea (n=537) regularly, as defined by >1 cup of tea/week, when compared to hot tea (n=428). Comparing hot tea and cold tea consumption in the group of individuals without AMD, 1224 respondents reported no consistent iced tea intake (69.5%), whereas 1333 individuals report no consistent hot tea intake (75.7%).

If we look at just iced tea consumption (Table 7), the highest reported category of consumption is 2-4 cups/week (n=195), for both those with AMD (dry AMD (n=8), wet AMD (n=9)) and those without AMD (n=182). Drinking ≥6 cups/day of iced tea had the lowest number of responses in all three AMD categories, with dry and wet having no respondents, and only 11 individuals without AMD. Results from hot tea consumption follow similar trends as the iced tea, with the largest and smallest categories of consumption being 2-4 cups/week and ≥6 cups/day, respectively. The 2-4 cups/week category had a total of 168 (9.5%) respondents with a higher number observed among those without AMD (n=151), compared to 17 participants in the group with AMD. There was a greater proportion of individuals with dry AMD that drink 2-4 cups/week of hot tea (n=16) compared to those drinking cold tea (n=12), although it was not statistically significant. As far as hot tea consumption in the ≥6 cups/day category, there was a markedly lower number in the non-AMD group (n=4) compared to iced tea non-AMD group (n=11), again it was not statistically significant.

Table 7

Association Between the Level of AMD Severity and Types of Tea Consumption

AMD	Iced Tea Consumption										
	No Tea Consumption				Tea Consumption						
Frequency *Percent*	None	< 1 cup /month	1-3 cups /month	1 cup /week	2-4 cups /week	5-6 cups /week	1 cup /day	2-3 cups /day	4-5 cups /day	≥6 cups /day	Total
Absence of AMD	505 *28.68*	245 *13.91*	257 *14.59*	106 *6.02*	182 *10.34*	97 *5.51*	82 *4.66*	104 *5.91*	22 *1.25*	11 *0.62*	1611 *91.48*
Dry AMD	52 *2.95*	14 *0.80*	25 *1.42*	6 *0.34*	12 *0.68*	8 *0.45*	6 *0.34*	8 *0.45*	1 *0.06*	0 *0.00*	132 *7.50*
Wet AMD	7 *0.40*	2 *0.11*	2 *0.11*	3 *0.17*	1 *0.06*	1 *0.06*	0 *0.00*	2 *0.11*	0 *0.00*	0 *0.00*	18 *1.02*
Total	564 *32.03*	261 *14.82*	284 *16.13*	115 *6.53*	195 *11.07*	106 *6.02*	88 *5.00*	114 *6.47*	23 *1.31*	11 0.62	1761 *100.00*

AMD	Hot Tea Consumption										
	No Tea Consumption				Tea Consumption						
Frequency *Percent*	None	< 1 cup /month	1-3 cups /month	1 cup /week	2-4 cups /week	5-6 cups /week	1 cup /day	2-3 cups /day	4-5 cups /day	≥6 cups /day	Total
Absence of AMD	663 *37.65*	254 *14.42*	223 *12.66*	83 *4.71*	151 *8.57*	47 *2.67*	110 *6.25*	64 *3.63*	12 *0.68*	4 *0.23*	1611 *91.48*
Dry AMD	65 *3.69*	14 *0.80*	15 *0.85*	4 *0.23*	16 *0.91*	5 *0.28*	7 *0.40*	4 *0.23*	1 *0.06*	1 *0.06*	132 *7.50*
Wet AMD	5 *0.28*	2 *0.11*	3 *0.17*	2 *0.11*	1 *0.06*	1 *0.06*	1 *0.06*	2 *0.11*	1 *0.06*	0 *0.00*	18 *1.02*
Total	733 *41.62*	270 *15.33*	241 *13.69*	89 *5.05*	168 *9.54*	53 *3.01*	118 *6.70*	70 *3.98*	14 *0.80*	5 *0.28*	1761 *100.00*

Hypothesis Four

To test whether or not there was a significant relationship present between the covariates (gender, age, race, BMI, and smoking, cholesterol and blood pressure statuses) and tea consumption in the association between tea consumption and macular degeneration, the same logistic regression as previous analyses was used because each of the response variables were binary. The covariates that showed significant correlation ($p < 0.05$) were age, race, and reported cholesterol status (Table 8). Age was positively and significantly ($p = <0.0001$) associated with decreased AMD prevalence. Similarly, race ($p=0.0353$) and cholesterol status ($p=0.0362$) had significant correlations with AMD.

To determine an odds ratio for age, race and cholesterol, calculations were made to determine the increased likelihood of variables' effect on a diagnosis of AMD when consuming tea. Upon analysis, it can be stated that the odds of an AMD diagnosis increase by 8.8% each year as one grows older. Additionally, the odds of being diagnosed with AMD in combination with increased tea intake is 0.532 times less for those of the "non-Hispanic white" race category than for rest of the race groups (Table 8). Therefore, non-Hispanic black individual who drink more than 1 cup per week of tea (hot or cold) can be correlated with roughly 46% decreased likelihood in the diagnosis of AMD. Furthermore, when looking at cholesterol status, analysis indicates that the odds of being diagnosed with AMD in combination with regular tea intake is normal cholesterol status is 1.625 times higher when compared to those with high cholesterol status. Individuals with unknown cholesterol status were not statistically significant ($p=0.7454$).

Table 8

Effect of Risk Factors on Macular Degeneration and Tea Consumption

Effect	Degrees of Freedom	Wald Chi-Square	p-value
Gender	1	1.2253	0.2683
BMI	3	1.2722	0.7357
Physical Activity	1	0.5568	0.4556
Smoking	2	0.0091	0.9954
High Blood Pressure	2	0.2226	0.8947
High Cholesterol	2	6.6369	**0.0079**

	Odds Ratio Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Normal Cholesterol	1.625	0.1756	7.6509	**0.0057**
Unknown Cholesterol	0.913	0.2816	0.1055	0.7454

Age	1	128.2256	**<.0001**
Race	4	10.3244	**0.0353**

	Odds Ratio Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Other Hispanic	0.853	0.8114	0.2628	0.6082
Non-Hispanic White	0.927	0.2959	0.0719	0.7885
Non-Hispanic Black	0.449	0.3804	2.752	**0.0971**
Other Race/ Multi-racial	0.313	1.0601	2.0165	0.1556

CHAPTER V

DISCUSSION

The purpose of this study was to examine the correlation between tea consumption and AMD development in US adults. This is the first analysis of its kind that has studied the association between tea consumption and an ocular disorder such as AMD. Drawing upon previous in vitro research that supported the use of specific phytochemicals present in tea with reduction in AMD markers, this research aimed to identify if broadly defined tea consumption provided any correlation in reduction of AMD. The data for this study was obtained from the 2005-2006 NHANES survey composed of a nationally representative sample of US women and men aged 40 and older, therefore, the findings are likely to be generalizable. The present study used a sample population group with diverse racial and social-economic characteristics. In the future, using compounding data over different years and surveys to diversify would increase the natural distribution of the biomarkers and increase the generalizability of the study.

In this representative sample of US men and women, we found that tea consumption was not significantly associated with AMD. Furthermore, there was no association with the severity of AMD, as measured by whether it was wet or dry AMD, and the type and frequency of tea consumed. An attempt was made to rule out any of the confounding variables such as: race, sex, BMI, cigarette smoking, physical activity level, elevated blood pressure, and high cholesterol to study the effects of tea consumption only on AMD development. During this analysis, it was

determined that both age and cholesterol status are significantly correlated with increased risk for development of AMD independent of other confounding factors. Research indicates cholesterol, cigarette smoking status, and blood pressure, respectively are the three biggest contributing factors to development of AMD when age is controlled[5,16]. To date, there are no other studies that have looked at AMD and dietary consumption of tea, therefore we are unable to compare findings with other reports. Subsequently, there are also no long-term studies on consumption levels required from supplemental or non-supplemental sources of tea to reduce VEGF and TNF in the protection of AMD symptomology.

From the literature review, it is known that the composition of the tea leaves depends on a variety of factors[65]. Those considered most influential include their chemical composition with regards to methylation, sulfation, and glucuronic acid metabolites, along with chemical degradation, microbial metabolism, intestinal and hepatic metabolism, membrane permeability, and transporter-mediators impacting their bioavailability[65,66]. Due to the limitations of the FFQ which asked only for the relative frequency of consuming either hot or cold tea, without any other specifications, there is little known about the type of tea that was consumed or how it would be metabolized differently in each individual. That leads to further questions regarding which of the above-mentioned factors could potentially impact the consumed tea's potential efficacy in potential health benefits associated with the reduction of AMD. Due the fact that the metabolism of tea catechins is complex and there have been no definitive studies indicating the ability of catechins to cross the blood-retinal barrier post consumption[73], this research only adds to the questions regarding the efficacy in reduction of AMD by the frequency of tea consumption. Research does show that overall bioavailability is low, with roughly only 5% of

consumed catechins absorbed, due to instability under digestive conditions, poor absorption, and rapid metabolism and excretion, leading to only about 5% of consumed catechins appearing in the plasma[63]. Therefore, with less than 1% (n=16) of the total sample population consuming 6 cups or more of any type of tea on a regular basis, low absorption and bioavailability coupled with less than adequate intake of tea could be reasons why there was no correlation discovered.

Another factor to be considered is not just the amount of tea consumed, but also the preparation and type of tea. Past research tested three different types of tea, white, green, and black tea. Two varieties of each were tested in hot water for two hours, hot water for five minutes, cold water for two hours, and cold water for five minutes. Khan and Mukhtar (2013) determined that the most effective steeping temperature and time was dependent on the type of tea[66]. For white tea, antioxidant properties are affected by time, in which a greater length of time resulted in more antioxidants, and not temperature. In black tea, the highest antioxidant activity resulted from a short hot water steeping of tea leaves, with a reduction of antioxidant behavior observed as length of steeping time increased. Green tea showed temperature sensitivity and time dependence, with prolonged cold steeping of two hours yielding the most antioxidants. Also, the overall antioxidant capacity of white and green tea was found to be greater than that of black tea[66,85,86]. Thus, although, antioxidant activity, measured as hydrogen-donating ability, showed no significant differences among hot or cold teas, there were differences among type of teas, with white tea resulting in the lowest antioxidant ability after preparation times greater than 2 hours for cold water and 10 minutes for hot[85].

Our research was unable to measure the length of steeping or how individuals prepared their tea, therefore, it is very limited in its ability to draw conclusions as to the relative

antioxidant capacities. If individuals are unaware of the best methods of preparation for tea, this may result in lower antioxidant abilities of tea catechins. This could be one possible explanation for the lack of correlation between tea consumption and AMD. Also, without knowing what type of tea was prepared, we are not able to determine what type of catechins are present in the highest amounts after consumption. Because we know that the major catechins in green tea are EGCG and in black teas they are theaflavin and thearubigin[25,27,65], we can discern that the individuals in this study who reported tea consumption (n=917) are getting some combination of those flavonoids' antioxidant abilities, but without further categorization or standardization of preparation and tea type, it levels are unknown at this time.

According to the Tea Association of the United States, tea is the most widely consumed beverage in the world next to water and can be found in almost 80% of all U.S. households, partially due to its flexibility in serving styles and occasions[86]. They estimated that on any given day, roughly 158 million Americans are drinking tea. Our results do not indicate that level of frequency in consumption of tea, however. One possibility of this discrepancy could be due to the age demographics of the tea drinkers in the US. Currently, tea consumption is highest among millennials, with over 87% of those aged 30 and under consuming some type of tea[86]. The data does not indicate what type of tea leaf or how frequently consumption occurs in this age group, however it does state that regardless of age, iced tea consumption is higher than hot tea independent of time of year[86]. Our results, age aside, did confirm this selection preference with a higher proportion of individuals (67.97%) consuming at least some iced tea, compared to hot (58.38%). Since the age of individuals currently consuming the highest levels of tea are below the age of 40 (the starting age of eligibility for this study), it is less surprising that most

individuals were not habitual tea drinkers. Also, because this data from 2005-2006 was collected throughout a two-year span, this study does present a more representative sample of the frequency in which US adults consume tea beverages because it does not discriminate based on time of the year or by seasons. Unfortunately, the FFQ does not give a time as to which individuals are more likely to consume tea, whether it's time of day or type of season. It merely asks participants for an average consumption, assuming they will think about their habits over the course of time, rather than just in the past month.

Other studies have looked specifically at the type of tea and its caffeine content to compare tea's ability to minimize free-radicals[33,64,69], a model that has been used in other studies to replicate conditions of AMD[6]. Those studies have compared the inhibitory effects of green tea and decaffeinated green tea in UV-induced skin carcinogenesis models and found that the decaffeinated products were either somewhat effective but less active, or no result was seen, suggesting that caffeine contributes to the biological activity of green tea[64,69]. However, the decreased effectiveness of decaffeinated green tea may be a result of the decaffeination process, thus reducing green tea polyphenols[69]. This study did not examine the relative caffeine intake associated with tea consumption, nor did it calculate the estimated total caffeine consumption from other beverage sources in addition to tea. Previous studies have indicated caffeine intake can affect weight status and other biomarkers associated with inflammation[87-90]. They posit that caffeine interacts with bioflavonoid absorption, potentially altering absorption. With this being the first study of its kind, it is unknown what effect, if any, caffeine content of the tea consumed had on the incidences of AMD long-term. Because the FFQ does not ask about caffeine content, nor does it describe the type of tea (black, green, etc.), there is no way of knowing, even with the

data representing the frequency of consumption, what the expected biologic activity would be depending on the amount of tea consumed by this study population. This issue of ambiguity within the FFQ is compounded with the problem of the amount of tea needed to be consumed to become significant. As previously mentioned, there are no studies that have determined what level of tea, or what type of tea, should be consumed to reduce VEGF production in humans. Animal models, which we know are problematic due to the differences in the anatomy and functioning of the BRB between other mammals and humans, suggest that over 800mg of EGCG, whether from green tea or supplementation, has been shown to reduce VEGF[79]. This would equate to at least 4 cups per day of green tea, since the average content of EGCG is 50-200mg, depending on the previously mentioned factors of preparation and type of tea[85].

Research also indicates that although tea consumption is on the rise in the US, the number of individuals choosing sugar sweetened tea has also increased in the past two decades[86]. This trend to increase food and beverages marketed under healthy disguises with phrases like "antioxidants" and "good for you" have been increasing over the past two decades[78,86]. Currently, there is no research on the association between sugar sweetened beverages and AMD. However, past research does indicate increased markers of inflammation (plaque and cholesterol deposits, weight, blood pressure, and CRP) are positively associated with increased sugar sweetened beverages, including tea[87]. Currently, there are no studies indicating what, if any, interactions sugar may cause in various types and preparations of tea. Because this study could not specify how much sugar was added and to what type of tea, the effect of sugar on the correlation between tea and AMD is unknown. However, the literature indicates that diets higher

in sugar intake tend to result in higher levels of inflammation[87], and higher inflammatory markers are associated with higher levels of AMD[40,41].

In this population of NHANES participants, the health examination component including fundus photography screening for AMD (dependent variable) was performed after the household interview (FFQ) that inquired about intake of tea during the past month (independent variable). Therefore, it appears implausible that AMD measured in this study would influence the reporting of tea consumption. While the number of participants with AMD was small (n=150), it represented 8.5% of the total sample. In 2010, it was estimated AMD affected over 4.5% of the US population, with over 11% of individuals over age 80 with a diagnosis[5], which is expected to increase in the next decade. One possible explanation for the higher percentage of individuals with AMD in the sample population compared to US population estimates is due to the oversampling from higher risk ethnicities. The highest proportion of respondents were those who identified as non-Hispanic white (n=1071), which research shows are at a higher risk for AMD[16,49,91].

The results of the current study suggested there are increased odds of being diagnosed with AMD with age, regardless of tea consumption. This is not surprising since the retina is the most metabolically active tissue in the body[3] and has the greatest vulnerability to oxidative stress[4,5], which increases with age. In addition, we know from other studies that the bioavailability of tea catechins is very low, especially when crossing the BRB[73] which is confounded with the lack of data on actual flavonoid content consumed by participants in this study. The use of a human population, rather than an animal study, highlights the complexity of

evaluations of various disease states in conjunction with other factors such as diet and environment.

As discussed previously, when examining the impact of the risk factors, it was not surprising to see that there was a higher probability of AMD within the "non-Hispanic white" race. These results agree with those of Fisher et al. (2016) in which they concluded that the overall 8-year age- and sex-standardized incidence of early and late AMD were 4.1% and 2.3%, respectively, with incidences of early and late AMD highest in whites (5.3% and 4.1%)[91]. Without separating severity categories of AMD, this study represented a 6.47% white population diagnosed with AMD which is above the national average. Individuals who identify as "Asian" are among the highest population groups in America who consume tea regularly[86], across age groups, while they also have a lower incidence of AMD[91]. This study was limited in scope because there was not a wide demographic range for ethnicity or race, therefore, we are unable to determine if there was any correlation among the two specific categories. From this data, there was no significant difference in self-ascribed race and consumption of tea, thus we were not able to find the same pattern of tea ingestion that previous research has discovered in US adults.

Diet is also a modifiable risk factor for AMD and this study did not incorporate the additional dietary profiles of participants. Due to the narrow scope of the study in looking only at tea consumption and not the other nutrients, there is room for potential error in possible nutrient interactions. Just as there have been changes to the micronutrients and antioxidants within AREDs formulas for the prevention of AMD, there could be potential interactions among supplements and food intake by individuals consuming tea. Because correlations between single nutrients and AMD are often inconsistent across studies[54], it is often difficult to look at single

aspects of a diet or lifestyle. Examining overall diet quality in relation to AMD has shown that the higher the diet quality, the lower the odds of AMD[54]. Since this study did not investigate the length of time for diagnosis of AMD nor did it look at the quality of the diet, there are several limitations to be addressed in the next section.

CHAPTER VI

LIMITATIONS AND FUTURE RESEARCH

Limitations

A cross sectional study design using secondary data has limitations due to the relative uncertainty regarding the temporal sequence of exposure-outcome relationships and is also subject to recall bias[92] as opposed to prospective studies. Additionally, it is difficult to determine temporal relationships between AMD and diet because this offers a snapshot of the subjects' health status and tea consumption at a moment in time. Again, because they represent a point in time, there is no time sequencing that can be drawn from this study. Even though we have data regarding the prevalence of tea consumption and AMD, we are unable to determine the length of time and the progression of events. Further prospective studies would need to look at sequencing certain diet changes, such as adding more tea consumption, and then following the incidences of AMD. Due to the small sample size of individuals with AMD (n=150), it was difficult to draw significant conclusions from the results with regards to tea consumption. Of the 150 individuals with AMD, only 18 had wet AMD. There was a large percentage of individuals who did not respond to the tea consumption questions or submit to fundus photography (n=3765). The data for this research was obtained from publicly accessible data of an existing dataset (2005-2006 NHANES), restricting the secondary analysis to the available variables in the NHANES dataset. Consequently, the type of tea and the length of time in which individuals have consistently consumed tea were not included into the beverage consumption variable. As previously

mentioned, the flavonoid content is unable to be determined without standardization of tea type and preparation, so correlations between the antioxidant capacity of different tea catechins and AMD was unable to be determined.

Also, additional confounders such as genetic testing for genes that increase disease risk, family history of AMD and length of time of diagnosed AMD could not be assessed. Both the dependent and independent variables were assessed only once and at the same time, so the direction of the association cannot be determined by this analysis. Additionally, all data, other than AMD and BMI, was self-reported so there can be a great deal of error on the part of the participants depending on their comprehension of the questions as well as their truthfulness to responses.

Future Research

Future research should focus on the limitations of this study and extrapolate on the strengths. It would be important to look for diet-controlled subjects, or populations that consume a consistent amount of tea, such as certain regions in Asia. Also, it would be beneficial to have a longitudinal study looking at tea consumption in a specific population, to help minimize the confounding factors of region and climate, over decades to determine if there was any correlation of decreased AMD diagnoses.

One of the biggest limitations of the study was the unknown type of tea. In the future studies should compare the type of tea and the mechanisms of action on ARPE cells to determine the interaction of catechins and flavonoids from a whole food source, rather than isolating specific types. Another possibility would be looking at the interaction of the recommended AREDs formulation and the length of time individuals have taken those vitamins and their

perception of tea consumption. According to previous research, tea is increasingly popular among health-conscious individuals. There may be a greater desire to look at the role of tea in prevention of inflammation prior to the diagnosis of AMD, since this research was not able to determine a correlation in severity level of AMD, nor the development of AMD and tea consumption.

Lastly, more research needs to be done to determine what role tea steeping, temperature, preparation, and time of consumption has on tea bioavailability. By creating standardized testing measures, those variables could be accounted for, but there is no long-term viability for the study and the attrition rates would be too great. Instead, more research should be done to determine urinary clearance rates of bioflavonoids and catechins present in tea to create a recommended guide for consumption of teas to maximize its potency and anti-inflammatory potential.

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APPENDIX A

IRB EXEMPTION FORM

Application for Institutional Review of Research
INVOLVING HUMAN SUBJECTS

Note: Please complete this form thoroughly keeping in mind that the primary concern is the **potential risk** (economic, ethical, legal, physical, political, psychological/emotional, social, breach of confidentiality, or other) to the participants. Provide **copies** of all materials to be used in the investigation. The Institutional Review Board (IRB) must have enough information about the transactions with the participants to evaluate the risks of participation.

Name(s) and employee ID for faculty, Z-ID for students

Lindsey Shaffer, z1641619

Status: ☐ Faculty ☒ Graduate Student ☐ Undergraduate Student

Department:

FCNS

Mailing Address (if not department):

808 Ridge Ave. Wauconda, IL 60084

815-342-7278 **Phone:**

E-mail z1641619@students.niu.edu

Project Title:

Self-Reported Tea Intake and Age-Related Macular Degeneration in U.S. Adults

Proposed Data Collection Start Date: 03/31/2016

Note: Unless the authorized departmental reviewer (e.g., chair or designee) has deemed on the screening form that IRB review is not needed, all projects must receive formal written clearance from the IRB Chair (or an IRB member designated by the Chair) **prior** to the start of data collection.

Type of Project (Check one)

☐ Departmental Research (faculty/student projects not externally funded and not indicated below)

☒ Graduate Thesis/Dissertation (IRB application should be submitted AFTER proposal defense)

Advisor/Committee Chair (& e-mail):Dr. Josephine Umoren, jxu1@niu.edu

☐ Undergraduate Project (Senior thesis/capstone, research rookies, independent study)

Advisor/Committee Chair (& e-mail):

☐ Externally Sponsored Research

A complete copy of the grant proposal or contract must accompany this application form for IRB review to take place.

□□□□□□□Source of Funding:

□□□□□□Title of grant proposal (if different from IRB protocol):

- Name of principal investigator on grant proposal:
- Office of Sponsored Projects file number (Note: this is not the grant number):

OSP#

☐ Other

Specify:

Part I. Purpose and Procedures:

1) Describe the purpose of your study and the reason(s) this study is needed. Include any necessary background information and a description of your hypothesis or your research question.

2) The following items will help the IRB reviewers understand the step-by-step procedures of your study:

2A) Explain the participant **eligibility** and **exclusion** criteria that will be used.

NHANES data from 2005-2006 data set with de-indentified information with completed food frequency questionnaires and fundus photography, aged 40 years and older

2B) Explain the **recruitment** procedures (how will participants learn about the study?). If using the snowballing technique, please explain who contacts potential participants (other participants or the researcher). *Please attach recruitment scripts, flyers, or postings.*

The recruitment was done by NHANES via phone and mailing.

2C) Explain the **consent process** (verbal and/or written procedures for informing participants of the nature of the study and what they will do).
[*Please attach all documents (assent, consent, parent permission) that are appropriate for each group of subjects participating in the study. Consent forms should be prepared for adult participants (age 18 or over). Assent forms should be prepared for minor subjects appropriate to their ages, and permission form(s) for parents or legally authorized representatives should also be prepared. For children too young to comprehend a simple explanation of participation, parental permission is sufficient only if the research will provide direct benefit to the subject, a member of the subject's family, or other children with the same condition as the subject.]*

The consent to participate in NHANES is available on the CDC website.

2D) Describe the **data collection** procedures including what data will be collected, how it will be collected (include a description of any interventions to be used), the duration of participation in the study session(s), and how the session(s) will end.

2E) If applicable, explain the procedures for providing **compensation**.

NA

2F) If applicable, explain the procedures for **debriefing** participants. *Please attach a debriefing script or sheet*

NA

Reminder: Attach copies of all questionnaires, surveys, interview questions, listing of all information/data to be collected, etc. It is the responsibility of the researcher to obtain any relevant permission for copyrighted materials. If the research involves an oral interview or focus group discussion that could evolve as it progresses, include a list of discussion topics and any "starter" questions for each topic that can reasonably be expected to be covered. If a *draft* of a written questionnaire or survey is attached, it should be clearly labeled as such and a final version must be submitted *before* data collection begins.

Part II: Research Participants

3) Participant demographics:

- Gender: M ☐ F ☐ Both ☒
- Estimated age(s):
  40-85
- Are any subjects under age 18? Yes ☐ No ☒
- **Potentially** vulnerable populations (please indicate if any of the following groups are the **target population of the study**)
  - ☐ Pregnant women & fetuses
  - ☐ Prisoners
  - ☐ Decisionally impaired/mentally disabled
  - ☒ Specific ethnic group(s) (list in box):
    Listed in 5 categories in NHANES: "non-Hispanic white", "non-Hispanic Black", "Hispanic", "Other", and "prefer not to answer"

  If any potentially "vulnerable populations" have been indicated above, please explain the necessity for using this particular group, or if specific groups are excluded from the study, please indicate the exclusion criteria used.
  No exclusion criteria was used by this study; it is secondary data
- Target number of participants in the **entire study** (including controls) from start to finish (keep in mind that this is just an **estimate of the total**):
  2000

4) Please explain any outside institutional (i.e., schools, hospitals) approval you will need to obtain and how approval will be sought. Provide scripts, letters, or emails providing any information that will be used to obtain needed approvals/permission. It is the responsibility of the researcher to follow all applicable policies of any outside institution(s).

NA

Part III: Risk/Benefit assessment

5) What knowledge/benefit(s) to the field will be gained from the study?

Understanding a correlation between tea intake and potential covariates with macular degeneration allows for future studies to determine the role of antioxidants and disease prevention

6) What direct benefit(s) are there to the participant(s) (if any) from the proposed research? *[For example, learning a new skill, psychological insight, teaching experience] [Please note that compensation is **NOT** considered a direct benefit.]*

No direct benefit as it is secondary data

7) Describe any potential risks (breach of confidentiality, economic, ethical, legal, physical, political, psychological/emotional, social, or other) to the subjects posed by the proposed research. (Note: Some studies may have "no reasonably foreseeable risks.") Investigators are required to report all unexpected and/or adverse events to the IRB. Therefore, it is important that you list all reasonably anticipated risks because unanticipated adverse events may need to be reported by NIU to OHRP.

no reasonably foreseeable risk

8) Federal regulations require that researchers use procedures that minimize any risks to participants. What procedures will be used to minimize each risk and/or deal with the challenge(s) stated in "7" above?

Continued use of de-indentified data

9) If support services are required to minimize risk of harm to participants, explain what will be provided (list of services available). [*A resource list for the DeKalb area is available on the ORC website – if using this, please provide a copy with your application.]*

NA

10) How do the potential benefits of the study *justify* the potential risks to the participants?

The participants were made aware of the implications for participating in NHANES and the potential to use their data as secondary research

Part IV: Consent Document Variations

11) Will audio, video, or film recording be used?

Yes ☐ No ☒

If **yes**, specify the recording format to be used.

Please keep in mind that specific consent must be sought in the informed consent document(s) by including a **separate signature/date line giving consent** for recording. This is in addition to the signature/date line giving consent to participate in the research project.

12) Will this project require the use of consent/assent documents written in a language other than English? Yes ☐ No ☒

Reminder: If non-English documents will be used, please have the document translator **provide documentation** (email or written) that the translation is equivalent to the English version. [*This can be done after the protocol is approved in order to minimize the number of changes needed.]*

13) Are you requesting a **waiver of a signed** informed consent document?
Yes ☐ No ☒

Please indicate the justification for requesting this waiver:

☐ The only record linking the subject to the research would be the signed consent document and the principal risk of the research would be breach of confidentiality.

☐ The research involves minimal risk to the subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., online surveys).

14) Are you requesting a **waiver/alteration** of some other aspect of the informed consent document?
*[This section is relevant for studies involving **deception**.]*
Yes ☐ No ☒

14a) Please explain which aspects of informed consent will be missing or altered along with a justification for the change.

14b) Please explain how the project meets all of the following criteria:

1) The research presents no more than minimal risk of harm to the participants.

2) The waiver/alteration will not adversely affect the rights or welfare of the participants.

3) The research could not practicably be carried out without the waiver or alteration.

4) Whenever appropriate, the participants will be provided with additional pertinent information after participation.

15) Will any HIPAA protected health information be collected as part of the data? Yes ☐ No ☒

If **yes**, describe the procedures for protecting the information.

[Please provide a copy of your HIPAA disclosure form to be given to participants.]

16) Will any protected school records be collected as part of the data? Yes ☐ No ☒

If **yes**, describe the procedures for protecting the information.

Part V: Confidentiality and Anonymity

17) Will identifying information be connected to the data (even through an identification key linking identities to a pseudonym or code that is kept separate from the data)? Yes ☐ (**confidential** data) No ☒ (**anonymous** data)

18) If you answered **yes to question #17**, describe precautions to insure the privacy of the subjects, and the confidentiality of the data, both in your possession and in reports and publications.

19) If you are collecting your data through an on-line survey tool, will the survey instrument collect email and/or IP addresses with the data?

No ☐ **The survey will be set so that email/IP addresses are NOT collected**

Yes ☐ **IP and/or email addresses WILL be collected with the data**

N/A ☒ **I am not using an online survey tool.**

20) How will the records (data, recordings, and consent forms) be stored? **Also** indicate how long records will be kept and how and when they will be disposed of.

[Note: Signed informed consent documents must be maintained for 3 years following completion of the study.]

Data is available online at anytime for download from CDC website; additional research data will be stored on an encrypted drive

Part VI: Projects Involving Deception *[complete only if your study includes deception]*

21) Describe the deception being used. Be sure to clarify whether this is deception by **omission** (an important aspect of the study is withheld from the participants) or **commission** (the participant is misled about some aspect of the study) or both. *[Complete **item 14** if aspects of consent are missing.]*

22) Why is deception a necessary and unavoidable component of the experimental design?

23) Debriefing of participants will be:

☐ Immediate (directly following the research session) ☐ Delayed

☐ Full (all aspects of deception will be revealed)

☐ Partial (some aspects of deception will remain unexplained)

a) If debriefing is delayed, why is the delay necessary, and when will it occur?

b) If debriefing is partial, why is the partial debriefing necessary? Would the participant be harmed in any way by full debriefing?

c) If debriefing is partial, will full debriefing occur later?

d) Does the presence of deception increase risk of harm to the participants?

e) Is the respondent free to withdraw his/her data after being fully debriefed?

24) Who will provide the debriefing?

Reminder: Please include a copy of your debriefing script/sheet with this application.

Part VII: Credit and Compensation

25) If participants will receive course credit for participation, please describe it below.

26) If participants will receive some other form of compensation for participation, please describe it below.

27) Describe any alternative tasks that will be available for participants to earn the credit or compensation.

Part VIII: Conflict of interest

28) Do any of the researchers conducting this study have any potential conflicts of interest?

[Conflicts of interest may include financial or personal interest, or any condition in which the investigator's judgment regarding a primary interest may be biased by a secondary interest.] Yes ☐ No ☒

29) If **yes** to the above question, please describe the nature of the conflict of interest.

Part IX: Researcher Qualifications

30) In addition to listing the investigators' names, indicate their qualifications to conduct procedures to be used in this study.

31) State the date of completion of the **CITI Human Subjects Protection** training program(s) for the individuals listed in the question above. The required course is "**Social & Behavioral Research - Basic/Refresher, Basic Course.** The required CITI training is accessible from the ORCI website at http://www.niu.edu/orci/human_research/training/index.shtml If you have comparable training, please attach certification verifying this. [*Note: NIU policy requires that research investigators must complete appropriate training before conducting human subjects research*.]

2/26/2014

To be completed by investigator and confirmed by advisor (if student project) and departmental reviewer. Initials indicate all required parties ratify that application is complete:

Checklist of items required to accompany completed application form:

1. _____ Complete grant proposal/contract (for externally funded projects)
2. _____ All surveys, questionnaires, interview questions, or other instruments to be used
3. _____ Subject recruitment/introductory materials
4. _____ Informed consent documents (must select at least one):
5. _____ Consent form for adults (if participants are age 18 or over)
6. _____ Assent form for minors (if participants are under age 18)
7. _____ Parental permission form (if participants are under age 18)

Initial indicating all listed materials are attached and application is complete; INCOMPLETE APPLICATIONS WILL NOT BE PROCESSED. The investigator will be notified of deficiencies in the application via e-mail from the Office of Research Compliance and Integrity (ORCI); if no response is received by the ORCI thirty (30) days the application will be considered withdrawn.

Investigator _____ Advisor (if student project) _____ Department Chair/Designee _____