THE ASSOCIATION BETWEEN CIRCULATING LIPIDS AND SYSTEMIC INFLAMMATION IN INDIVIDUALS WITH MILD COGNITIVE IMPAIRMENT: THE ROLE OF BIOLOGICAL SEX

by

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ABSTRACT

As the average life expectancy increases, the incidence of mild cognitive impairment (MCI), an early stage of memory loss, is expected to increase as well. MCI and cardiovascular disease (CVD) share many of the same risk factors, like consuming an unhealthy diet, hypertension, endothelial dysfunction and arterial stiffness. Moreover, previous studies show a relation between vascular risk factors and the incidence of dementia. Aging is also associated with chronic inflammation, which can lead to vascular dysfunction. The upregulation of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) play a role in a viscous feed-forward cycle that upregulates the production of damaging reactive oxygen species (ROS) that contribute to vascular dysfunction. Moreover, plasma lipid markers are known to play a role in a mechanism that reduces the microstructural integrity of the hippocampus. Because postmenopausal women are known to have an increased risk of developing CVD and Alzheimer's disease (AD), sex differences of inflammatory markers and triglyceride concentrations were explored.

14 adults between the ages of 61 and 84 participated in this study, consisting of 10 females and 4 males. Blood was collected from all participants and clinical variables were measured. Plasma was isolated to measure the concentrations of inflammatory cytokines, and pulse wave analysis was performed to measure arterial stiffness.

Triglycerides were positively associated with markers of inflammation, but no other measured plasma lipid marker had a significant association with a marker of inflammation. There was no significant association between arterial stiffness and markers of inflammation and lipids. Moderate, but non-significant sex differences

were observed, and is something worth exploring further with a larger and more evenly distributed sample size. Understanding the relations between risk factors of AD and CVD enhances our ability to slow the progression of these diseases.

Chapter 1

INTRODUCTION

1.1 AMNESTIC MILD COGNTIVE IMPAIRMENT: ETIOLOGY OF THE CONDITION

Mild cognitive impairment (MCI) is an intermediate state between normal cognition and diagnosed dementia (Peterson, 2016). It is indicative of early stage memory loss without enough loss in functional independence, or memory functionality, to meet the criteria for dementia. As the average life expectancy in developed societies continues to increase, the incidence of MCI and age-related dementia due to Alzheimer's disease (AD) is expected to rise as well (Herbert el al., 2013). Many negative lifestyle factors contribute to the development of MCI, such as sedentary behavior, or consuming an unhealthy diet with high amounts of added sugars (Solfrizzi et al., 2004). These poor lifestyle choices, in turn, lead to the development of cardiovascular risk factors that ultimately increase AD risk. The estimated prevalence of MCI in persons 60 years or older is 20%, and the rate at which MCI progresses to dementia is between 8% and 15% per year (Petersen, 2016). Amnesic MCI is traditionally the prodromal stage of dementia due to AD, but other things, such as carrying one or more copies of the e4 allele on the apolipoprotein allele on the apolipoprotein gene (i.e., APOE4) can increase risk of AD as well (Albert et al., 2011). The APOE4 genotype is known to increase the risk of age-related AD, in part, by impacting arteries and the brain. In certain conditions, this allele is also known to interact with adjacent genes to modify neurodegeneration and metabolism (Yassine et al., 2020); however, the exact mechanisms mediating the transition from MCI to AD are poorly understood. Thus, as the number of older adults continues to rise, it is important to understand the biological markers that are indicative of the condition's etiology and progression.

1.2 CARDIOVASCULAR DISEASE: ITS RELATION TO COGNITIVE AGING

Cardiovascular disease (CVD) is the leading cause of death in the United States and in the world (Mozaffarian et al., 2016). Many of the same risk factors exist for CVD and AD including elevated blood pressure, smoking, diabetes mellitus, and large elastic artery stiffness (Solfrizzi et al., 2004). Previous studies show a positive association between vascular risk factors and the incidence of dementia. For example, subjects with hypertension, diabetes, CVD, and hypercholesterolemia were reported to have a higher risk for the development of AD than their counterparts without cardiovascular risk factors (Li et al., 2011). Moreover, data supports that relative to no treatment, the treatment of some or all cardiovascular risk factors is associated with a decreased risk of developing AD (Li et al., 2011). Thus, it is important to identify and manage vascular risk factors in order to decrease the incidence of MCI and slow the progression of dementia.

Aging is a risk factor for arterial stiffness and a predictor of cognitive decline (Mitchell, 2018). Arterial stiffness is the rigidity of the arterial wall, which leads to an increased pulsatile pressure and an increased workload for the heart (Van Sloten, 2016). In this regard, arterial stiffness interferes with the large elastic artery's ability to accommodate normal blood flow from the left ventricle. This results in excessive pulsatile afterload, which may play a role in left ventricular remodeling, dysfunction, and failure (Jain et al., 2014).

A variety of factors related to elastin fragmentation contribute to the age-related stiffening of the arterial wall. Among others, these factors include the build of calcium bound to elastin in the arterial wall, and the dysregulation of matrix metalloproteinases (Kohn et al., 2015). Previous studies demonstrate that aortic stiffness may contribute to cognitive decline by increasing the transmission of harmful pulsatility in high-flow, low-resistance organs, like the brain. The exposure to excessive pulsatility causes small vessel damage, and cerebral microbleeds, which are associated with reduced cognitive function (Mitchell, 2018; Hughes et al., 2015). In fact, arterial stiffness, as demonstrated by increased PWV, is related to cognitive decline independent of confounding variables like age and education (Hughes et al., 2015).

This study looks at carotid-to-femoral pulse wave velocity (PWV), as a measure of arterial stiffness. PWV measures arterial performance and is an establish predictor of CVD. Carotid-to-femoral PWV is determined noninvasely by measuring the velocity of the pulse wave that travels a known distance from the carotid artery to the femoral artery (Hughes et al., 2015).

1.3 SYSTEMIC INFLAMMATION CAUSED BY PLASMA LIPIDS, VASCULAR DYSFUNCTION, AND THEIR POSSIBLE ROLES IN COGNITIVE IMPAIRMENT/AGING

Cardiometabolic risk factors such as elevated blood triglycerides (TGs), contribute to the increased risk of cardiovascular disease and may also affect the brain. TGs are transported within very low-density lipoproteins (VLDL) for delivery into adipose tissues. This is not only a substrate for obesity (Mayes et al., 1993), but an established risk factor for (CVD). Increased TGs are also associated with reduced microstructural integrity of the hippocampus, which is a brain structure essential for encoding and learning new memories, and then eventually to memory impairment and dementia in the long term. Increased VLDL may have a possible role in this pathway (Sanjana et al., 2020). In this regard, circulating VLDL remnants are known to penetrate the arterial wall, increasing endothelial inflammation in the process (Xie et al., 2017).

Another potential mechanism through which increased lipids may impair brain health is through an increase in oxidative stress (OS) and inflammation. OS is characterized as an increased abundance of reactive oxygen species (ROS) relative to endogenous antioxidant defenses. It is related to inflammation and can give more insight into how blood TGs are related to systemic inflammation (Gill et al., 2012). Increased OS leads to hypertension through endothelial dysfunction and arterial stiffness by reducing the bioactivity of nitric oxide (NO) (Förstermann, 2010). NO is an important paracrine factor that controls vascular tone, limits the oxidation of LDL-C, and decreases the expression of pro-inflammatory genes (Förstermann, 2010). Thus, systemic inflammation caused by the increase in OS from the TGs reduces NO bioavailability and is related to vascular endothelial dysfunction, arterial stiffness, and hypertension (Donato et al., 2009, 2015; Welty, 2013). These mechanisms could attribute to impaired brain integrity and/or cognitive dysfunction.

1.4 PRO-INFLAMMATORY CYTOKINES TNF- α, AND IL-6: THEIR RELATION TO COGNITIVE IMPAIRMENT AND VASCULAR DYSFUNCTION

In addition to lipids, increasing age is associated with chronic low-grade inflammation. This is characterized by an increase in pro-inflammatory cytokines like tumor necrosis factor- α, (TNF- α), and interleukin-6 (IL-6) (Donato et al., 2009). An elevated abundance of pro-inflammatory cytokines increases the production of free radicals, which in turn, drives the production of more pro-inflammatory cytokines, which then drives the production of ROS in a viscous feed forward cycle (Donato et al., 2009). ROS can cause damage to proteins, DNA, and lipids within the body, leading to increased OS, and if not removed, can also cause systemic inflammation (Phaniendra et al., 2015).

Simultaneously, nuclear factor κB (NFκB), a pro-inflammatory transcription factor, binds to promotor regions of DNA and upregulates the transcription of genes that cause inflammation and oxidative stress. Thus, contributing to the upregulation of TNF- α and IL-6 (Donato et al., 2009). This causes inflammation and increases the expression of NADPH oxidase, which upregulates the production of superoxide, a very damaging ROS that causes an increase in OS (Mittal et al., 2014). The increase in OS signals to the immune system that there is damage that needs to be repaired, keeping NFκB activated, thus continuing to upregulate the production of TNF- α and IL-6 (Donato et al., 2009). Moreover, the abundance of OS and inflammation also promotes regional specific remodeling of the intimal, medial, and adventitial layers of the arteries, which contributes to arterial stiffening (Fleenor, 2013).

In young, healthy adults, this viscous feed forward cycle does not occur because they have healthy immune systems. However, with aging some of the down regulatory mechanisms of the immune system are impaired, thus resulting in this feed forward mechanism, and driving endothelial cells into a dysfunctional state (i.e., endothelial dysfunction), and also increasing the stiffness of the large elastic arteries. (Donato et al., 2009; Fleenor, 2013).

It is possible that this feed forward cycle that leads to endothelial dysfunction and arterial stiffness could be occurring in the brain. The increase abundance of plaque can lead to a stroke, and the impaired ability of the blood flow to match the energetic demand of the neurovascular unit, resulting in impaired neuronal and cerebral microvascular function. While the association between inflammation and endothelial function is more clear, less is known about the role of inflammation in arterial stiffness, particularly in people with MCI.

1.5 THE IMPLICATIONS OF BIOLOGICAL SEX

Postmenopausal women have a heightened risk for CVD and are also more likely to develop AD (Rossouw et al., 2002; Henderson, 1997). The steroid hormones estrogen and testosterone, which regulate the development of female and male characteristics respectively, exert protective functions in the brain and vasculature. These functions are known to increase neural functioning, resilience, and reduce aspects of AD development (Pike, 2017). Since aging affects the levels of sex hormones in sex-specific manners, it is important to consider how sex hormones impact the etiology of CVD and AD.

Evidence suggests that decreased estrogen levels after menopause increases the risk for CVD (Rosano et al., 2007). Estrogen is a cardioprotective hormone and an immunomodulator of the inflammatory response in atherosclerosis (Baker et al., 2003). Thus, in response to plummeting levels of estrogen in postmenopausal women, the deposition of plaques in arterial walls increases, likely due to endothelial dysfunction, which in turn increases the risk for CVD (Baker et al., 2003). Moreover, studies support that men with CVD have lower levels of testosterone, and that in some cases testosterone therapy can reduce or reverse the progression of CVD in men (Liu et al., 2003). However, unlike menopause in females, there is no known life event (apart from normal aging), that results in decreased testosterone levels in males.

The greatest risk factors for AD are aging and biological sex. As women age, they are disproportionately affected by AD due to accelerated postmenopausal reductions in estrogen. Resultantly, women show more vigorous development of MCI and higher severity of clinical dementia (Lin et al., 2015; Barnes et al., 2005).

1.6 AIMS OF CURRENT STUDY

I am interested in testing the association between circulating lipid markers and inflammatory cytokines and determining their association with markers of arterial stiffness. Additionally, I am interested in analyzing if these associations differ based on biological sex, as it is well established that postmenopausal women are at greater risk for both CVD and age-related dementia.

1.6.1 HYPOTHESES

My hypothesis is two-fold 1) I expect that blood lipid markers are positively correlated with markers of inflammation and markers of arterial stiffness, and 2) these relations are likely to differ based on biological sex.

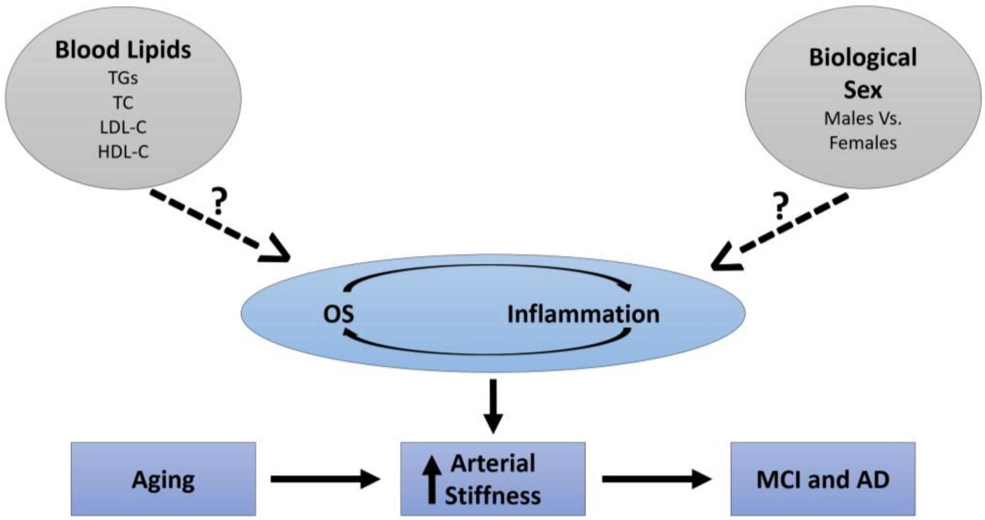

Figure 1. Working hypothesis model. Aging is the primary risk factor for MCI and AD, which is mediated in part by age-related increases in arterial stiffness. Arterial stiffness is partially caused by oxidative stress and inflammation, which is potentially exacerbated by aberrant blood lipids [e.g., triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)] and biological sex.

Chapter 2

MATERIALS AND METHODS

2.1 SUBJECT RECRUITMENT

10 female adults and 4 male adults between the ages of 61 and 84 years old who have cognitive scores that are indicative of amnestic mild cognitive impairment (MCI) were included in this study. These subjects were recruited from an ongoing clinical trial at the University of Delaware (NCT03482167) and only their baseline data were analyzed. In general, subjects were healthy and free of any chronic illnesses and impairments, and CVD. Subjects were excluded from the study if their blood chemistries were indicative of clinically abnormal renal, liver, thyroid and adrenal function; or if they were diagnosed with a major psychiatric disorder like schizophrenia, bipolar disorder, or major depression. Subjects were also excluded if they had dementia or another condition that affects cognition; a concussion in the last two years or three lifetime concussions; systemic medical illnesses or substance abuse or dependence. Additionally, subjects could not be taking medications to treat dementia or drugs known to affect cognition. All procedures were approved by the Institutional Review Board (IRB) at the University of Delaware. Before enrollment, the study rationale, procedures, risks, and benefits were explained to the subjects, and their written, informed consent was obtained. Before their visit, subjects were instructed to fast for at least 12 hours and refrain from taking prescription medications for 24 hours.

2.2 SUBJECT SCREENING

A family history questionnaire was used to obtain information regarding family history of cardiovascular diseases, cancer, diabetes, and Alzheimer’s disease.

Additionally, a menstrual history questionnaire was administered to all female subjects to confirm postmenopausal status. Subjects were asked about their medical history to ensure that they met inclusion criteria and do not have an underlying condition that would disqualify them based on stated exclusion criteria.

2.3 ARTERIAL STIFFNESS ASSESSMENT

Carotid-to-femoral PWV, the gold-standard clinical measure of aortic stiffness in humans, was assessed non-invasively using sequential transcutaneous tonometry of the carotid and femoral arteries. The foot of pressure waves obtained from the carotid and femoral arteries were aligned using the R-wave of an ECG recording in order to calculate the time delay between the carotid and femoral pulse. PWV was calculated as the distance between measurement sites divided by the transit time of the arterial pulse wave.

2.4 BLOOD SAMPLING

Blood was sent out to LabCorp for the analysis of clinical parameters, including a comprehensive metabolic profile, lipid profile, and for determination of APOE4 genotype. Additionally, plasma was obtained from whole blood via centrifugation at 500 x g for 20 minutes at 23°C with the acceleration and break settings set to 1. Plasma was aliquoted and stored in a -80°C freezer for later determination of cytokine expression by enzyme-linked immunosorbent assay (ELISA).

2.5 ENZYME-LINKED IMMUNOSORBENT ASSAY

Plasma samples were thawed and kept on ice until used. All samples were assayed in the same sample run to reduce intra-assay variability. Cytokine concentrations were measured in triplicate using commercially available ELISA kits for TNF-α (R&D Systems; Product #DTA00D) and IL-6 (R&D Systems; Product #D6050). Intra-assay coefficients of variation were 10.1% for TNF- α; and 8.1% for IL-6.

2.6 STATISTICAL ANALYSIS

Bivariate (Pearson) correlations were used to identify whether plasma inflammatory markers (TNF-α and IL-6) were associated with any of the dependent variables (TGs, TC, LDL-C, HDL-C). Bivariate correlations were performed between PWV and both plasma inflammatory markers, and between PWV and the only plasma lipid marker that had a significant correlation with plasma inflammatory markers (TGs). Because of the uneven number of subjects based on biological sex, nonparametric tests (Mann-Whitney) were performed to identify if there's a difference between male and female levels of TNF-α, IL-6 and TGs within the sample. Statistical significance was set at $P < 0.05$. All analyses were performed with GraphPad Prism 9.1 (GraphPad Software Inc., San Diego, CA, USA).

Chapter 3

RESULTS

3.1 SUBJECT CHARACTERISTICS

Subject characteristics are summarized in Table 1. Older adult subjects with MCI were recruited (61–84 yr) and were generally healthy and free of chronic diseases. As a group, all subject characteristics are within normal ranges.

Table 1. Participants' demographic and health-related information

	Females (Mean ± SD)	**Males (Mean ± SD)**	**All (Mean ± SD)**
Sex (F/M)	10	4	10/4
Age (years)	74.1 ± 6.6	73.5 ± 7.0	74 ± 6.5
Height (cm)	158.6 ± 5.0	171.4 ± 6.3*	162.3 ± 7.9
Mass (kg)	75.2 ± 17.5	79.0 ± 4.8	76.3 ± 14.9
Body mass index (kg/m^2)	29.7 ± 6.2	27.0 ± 2.9	28.9 ± 5.5
Carriers of the APOE4 gene	2 (20%)	1 (25%)	3 (21.4%)
TC (mg/dL)	199.2 ± 28.6	184.5 ± 31.4	195 ± 29.0
HDL-C (mg/dL)	67.6 ± 15.8	60.5 ± 12.8	65.6 ± 14.8
LDL-C (mg/dL)	109.4 ± 23.7	108.8 ± 25.1	109 ± 23.0
Triglycerides (mg/dL)	126.8 ± 39.3	82.5 ± 34.2	114.1 ± 42.1
Glucose (mg/dL)	99.2 ± 12.4	102.8 ± 8.2	161.1 ± 6.8
SBP (mmHg)	124.8 ± 17.0	121 ± 9.3	123 ± 15
DBP (mmHg)	67.6 ± 12.8	71.5 ±7.3	69 ± 11

Values are means ± SD and total count with it percentage. Characteristics for all subjects (n = 14). TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure. Mann-Whitney test was performed. α was set at $P < 0.05$ (significant associations indicated by *).

3.2 THE RELATION BETWEEN PLASMA LIPID MARKERS AND MARKERS OF INFLAMMATION

As reported in Figure 2, TGs were positively associated with TNF-α (r = 0.5888, P = 0.0267), and IL-6 (r = 0.5459, P = 0.0435). Total cholesterol (r = 0.1016, P = 0.7296; r = -0.06228, P = 0.8325), low-density lipoprotein cholesterol (r = 0.04298, P = 0.8840; r = -0.1051, P = 0.7208), and high-density lipoprotein cholesterol (r = -0.1265, P = 0.6664; r = -0.2346, P = 0.416) were not associated with TNF-α or IL-6, respectively.

All subjects' (n=14) blood triglyceride concentrations and markers of inflammation were graphed and a line of best fit with 95% confidence intervals were added to visualize the correlations. The only significant relations were between TNF-α and TGs (Fig. 2A), and IL-6 and TGs (Fig. 2B), which show a positive correlation between both inflammatory cytokines and TG. Because two outliers were detected in the analysis of IL-6, a sensitivity analysis was performed removing these outliers from the analysis and the results did not change.

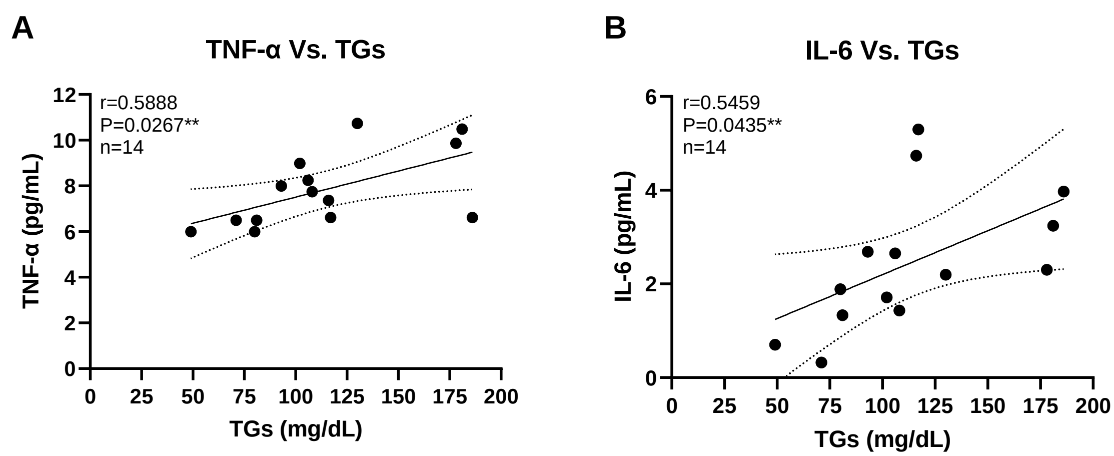

Figure 2. Markers of inflammation and triglycerides. Independent associations between triglycerides (TGs) with TNF-α (*A*) and IL-6 (*B*). α was set at $P < 0.05$. (Significant associations indicated by **).

3.3 THE RELATION BETWEEN PLASMA LIPID MARKERS, MARKERS OF INFLAMMATION, AND ARTERIAL STIFFNESS

Because the association between TNF-α and TGs, and IL-6 and TGs was statistically significant, we further investigated the relation these variables had to arterial stiffness. One subject was excluded from this analysis because their PWV was unable to be measured (n = 13). As reported in table 2, TNF-α ($r = -0.188$, $P = 0.5467$), IL-6 ($r = 0.2836$, $P = 0.3477$), and TGs ($r = 0.1062$, $P = 0.7248$) were not associated with PWV, the measure of arterial stiffness.

Table 2. Correlations arterial stiffness and markers of inflammation and plasma lipid markers

	PWV
TNF-α	
r	-0.188
P value	0.5367
IL-6	
r	0.2836
P value	0.3477
TGs	
r	0.1062
P value	0.7248

Pearson's correlation coefficient between each plasma lipid marker and inflammatory marker. Abbreviations: PWV, pulse wave velocity; TNF- α, tumor necrosis factor- α; IL-6, interleukin-6; TGs, triglycerides. α was set at P < 0.05. No significant associations.

3.4 SEX DIFFERENCES

To explore whether levels of TNF-α, IL-6 and TGs differ by sex, a Mann-Whitney test was performed comparing males (n = 4) and females (n = 10). for each variable. There were no significant difference between male and female plasma concentrations of TNF- α (Fig. 3A), IL-6 (Fig. 3B), or TGs (Fig. 3C); however, there was a trend towards a sex-difference for IL-6 and plasma TGs.

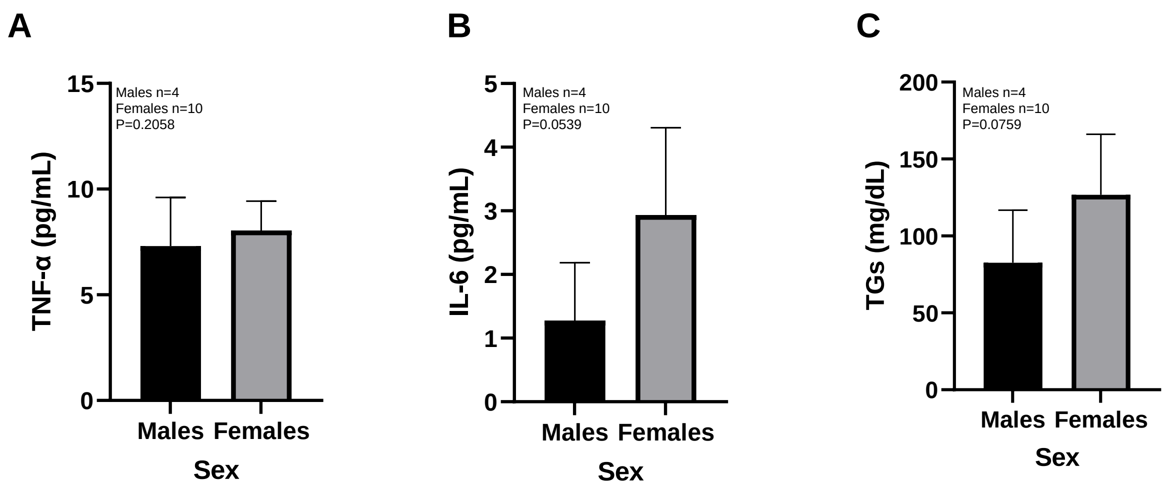

Figure 3. Sex differences between markers of inflammation and plasma triglycerides as determine by Mann-Whitney test. The difference between males and females plasma concentrations of TNF- α (*A*), IL-6 (*B*), and triglycerides (TGs) (*C*). α was set at P < 0.05.

Chapter 4

DISCUSSION

In the present study, we evaluated the relation between plasma lipid markers, markers of inflammation, and arterial stiffness in a group of older adult men and women with amnestic MCI. Using clinical blood reports, measured PWV, and concentrations of plasma pro-inflammatory cytokines obtained through ELISA, we identified that TGs have a significant association with pro-inflammatory cytokines, TNF-α, and IL-6. We further correlated the measures of arterial stiffness with TGs, TNF-α, and IL-6, but did not observe any significant associations. Additionally, plasma concentrations of TGs, TNF-α and IL-6 showed a possible trend towards sex differences that should be evaluated in a larger sample size.

4.1 TRIGLYCERIDES MAY BE POSITIVELY CORRELATED TO MARKERS OF INFLAMMATION, BUT NO OTHER BLOOD LIPID MARKERS SHOW AN ASSOCATION WITH MARKERS OF INFLAMMATION

Measures of plasma pro-inflammatory cytokines are thought to be related to the concentrations of blood lipids (Park et al., 2005). This established relation prompted us to compare the plasma concentrations of TNF-α, and IL-6 with the concentrations of blood lipids (i.e., TGs, TC, LDL-C, and HDL-C). As expected, we found that TGs had a significant and strong correlation to TNF-α and IL-6. However, it remains unknown if elevated TGs drive the upregulation of plasma pro-inflammatory cytokines, or if pro-inflammatory cytokines drive the upregulation of blood TGs.

Interestingly, there is no significant relations between TNF-α and IL-6 with any other tested blood lipid (i.e., TC, LDL-C, and HDL-C). Epidemiological studies have observed that increased circulating HDL-C levels are associated with a decreased risk of developing AD; however, increased circulating LDL-C is associated with an increased risk of developing AD (Button et al., 2019; Chen et al., 2014). Moreover observational studies have revealed that taking statins, cholesterol reducing drugs, may lower the risk of developing AD (Crisby et al., 2002). Thus, further testing should be done to reveal the association between inflammatory markers and cholesterol.

Because chronic inflammation occurs as a result of aging, and pro-inflammatory cytokines are related to blood lipids, age and diet are confounding variables in the study (Donato et al, 2009; Gill et al., 2012). In the future, we suggest controlling for these confounding variables to have a better understanding of how markers of inflammation and blood lipids are related. This is important because age and diet are risk factors for AD and CVD (Solfrizzi et al., 2004) Thus, it is important to understand these relations to slow or stop the progression of AD and CVD. Moreover, our findings suggest that TG-lowering therapies may be effective at lowering inflammation in adults with MCI which may have implications for improving cognitive function and delaying conversion to AD.

4.2 ARTERIAL STIFFNESS SHOWS NO INDICATIONS OF ASSOCIATION WITH TRIGLYCERIDES AND MARKERS OF INFLAMMATION

We were also interested in establishing a relation between arterial stiffness and TGs, TNF-α, and IL-6 because arterial stiffness is a major risk factor for MCI and CVD (Van Sloten, 2016). We chose to test TGs as opposed to the other blood lipid markers, because it was the only one that had a significant correlation to TNF-α and IL-6. Interestingly, a previous study established a significant positive correlation between TNF-α levels and PWV measures (Barbaro et al., 2015). These results are opposite to what we observed. We attribute these differences to a different sample population. Barbaro's group tested subject's normotensive and resistant hypertensive subjects, while our group tested (mostly normotensive) subjects with MCI. These differences suggest that the relation between arterial stiffness and pro-inflammatory cytokines is complex and other health factors influence the association. To gain a better understanding of how PWV is related to pro-inflammatory cytokines, future studies should attempt to control for and analyze possible confounding variables like heart and brain health or examine these associations in individuals with greater baseline arterial dysfunction.

Because the arterial walls are known to stiffen with age, and age is a major risk factor for MCI, it could be possible that subject age had a greater impact on PWV than any blood lipid marker, or marker of inflammation (Mitchell, 2018). Thus, determining the trend between age and PWV in adults diagnosed with MCI, as compared to adults with normal cognitive function, and controlling for age, may provide further insight into how blood lipids and markers of inflammation are associated with CVD and AD.

4.3 THERE MAY BE MODERATE DIFFERENCES BETWEEN MALE AND FEMALE CONCENTRATONS OF TRIGYCLERIDES AND MARKERS OF INFLAMMATION

Our next aim was to determine if the concentrations of TGs, TNF-α, and IL-6 differed between males and females. Because blood lipids and markers of inflammation play a role in the development and progression of AD and CVD, and because postmenopausal women are at a heightened risk to develop both diseases, we predicted that TGs, TNF-α, and IL-6 concentrations would be significantly higher in females than in males. Our prediction is somewhat supported by the data. Although statistically insignificant, we observed that on average, women have higher levels of TGs, TNF-α and IL-6 than men. Moreover, it is important to note the small sample size and unequal distribution between male and female subjects. Because P-values for IL-6 (P = 0.0539) and TGs (P=0.0759) were close to being significant, it is worth running these tests again with a larger and more evenly distributed sample size.

4.4 LIMITATIONS OF THE STUDY

The major limitations of this study are the small sample size (n = 14), the lack of a healthy control group, and an unequal distribution of male and female subjects. We planned to compare TNF-α, IL-6 concentrations and blood lipid markers between subjects with MCI and healthy controls. Unfortunately, the freezer we store blood and plasma samples in broke, so samples from healthy control subjects were unavailable. Additionally, due to the COVID-19 pandemic, the recruitment of subjects for the ongoing clinical trial was significantly delayed and we were not able to achieve as high of a sample size as we originally intended. The small sample size impacted other areas of this study, such as our ability to analyze the influence of APOE4 genotype on markers of inflammation, blood lipids, and arterial stiffness. Another major limitation of this study was the unequal distribution of male and female subjects. We believe that a more equal distribution would have made for a stronger sex comparison.

4.5 CLINICAL RELEVANCE AND FUTURE DIRECTIONS

Previous research supports that reducing the incidence of cardiovascular risk factors is associated with decreased risk of developing AD (Li et al., 2011). MCI is an early stage of dementia, and our ultimate goal is to understand its relation to CVD and cardiovascular risk factors (such as blood lipids, inflammation, and arterial stiffness), so we can suggest early interventions to slow the progression of the disorder. Because women have a higher risk of developing AD and CVD than men, a secondary goal of our study was to determine if and how these risk factors differ based on biological sex. More work needs to be done to determine the differences between men and women, and establish how preventative measures might vary as a result.

One potential cause of elevated TGs is the consumption of excess dietary added sugars. Added sugars, like high-fructose corn syrup, are a large component of a Western diet and are associated with high levels of plasma triglycerides (TGs), which are produced in liver through de novo lipogenesis (Hellerstein, 2002). It is already well-established that consuming a western diet is a risk factor for CVD. Moreover, previous cross-sectional work from our laboratory suggests that elevated blood TGs may decrease hippocampal integrity, impairing the ability to encode and store memories (Sanjana et al., 2020). Thus, the consumption of added sugars may contribute to lipid-induced impairment of brain health, however this hypothesis has not been fully tested in humans. Knowing that elevated TGs and elevated pro-inflammatory cytokines are related in subjects with MCI suggests that a low sugar diet may be beneficial to down-regulate the concentrations of pro-inflammatory cytokines, which, in turn, could be a protective measure against AD and CVD. Future studies should establish the impact added sugars have on cardiovascular and MCI risk factors, like arterial stiffness.

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