THE EFFECTS OF A MEDITERRANEAN VERSUS WESTERN DIET IN C57BL/6 MICE ON INFLAMMATION IN THE BRAIN

By

Emily Van Dyck

Submitted in partial fulfillment of the

requirements for Departmental Honors

in the Department of Biology

Texas Christian University

Fort Worth, Texas

May 8, 2023

THE EFFECTS OF A MEDITERRANEAN VERSUS WESTERN DIET IN C57BL/6 MICE ON INFLAMMATION IN THE BRAIN

Project Approved:

Supervising Professor: Michael Chumley, Ph.D.

Department of Biology

Gary Boehm, Ph.D.

Department of Psychology

Matt Chumchal, Ph.D.

Department of Biology

ABSTRACT

Due to our rapidly aging population, 6.5 million Americans currently have Alzheimer's disease (AD), and this is predicted to increase to almost 14 million in the next 40 years. AD is currently the sixth leading cause of death in America and is characterized by memory and learning loss. There is currently no effective treatment for the pathology of AD, and several studies have begun to analyze different lifestyle choices that can potentially influence the onset of AD. One of these is the effect of diet on AD. AD is more prevalent in western societies, and researchers suggest that this may be due to the typical Western Diet (WD), also known as a "typical American Diet." In contrast, AD prevalence is lower in Mediterranean regions, where they consume a primarily plant-based diet known as the Mediterranean Diet (MED). This research looked to examine the neuroprotective potential of a Mediterranean Diet against AD pathologies and inflammation in mice, compared to mice that consume a Western Diet. Our lab designed two experimental rodent diets, one that mimicked a typical Western Diet, and another that mimicked a typical Mediterranean Diet. Using an Enzyme-Linked Immunosorbent Assay, we examined the lifelong effects of diet on biological markers of AD, including amyloid beta, a protein that aggregates together to form plaques in the AD brain. A pro-inflammatory cytokine, which are associated with increased inflammation, called Tumor Necrosis Factor Alpha (TNF-α) was also analyzed using a MSD Assay. We hypothesized that the consumption of a Western Diet has the potential to increase the development of these AD pathologies, compared to a Mediterranean Diet.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank Dr. Chumley for serving as the primary supervisor for this project. I would also like to thank my other committee members: Dr. Boehm and Dr. Chumchal for their support during this project. This project could not be possible without the hard work and dedication of my mentor, Paige Braden-Kuhle, graduate students Kelly Brice, Viv Lacy, Chelsy Mani, and all of the undergraduate students involved in the Neurobiology of Aging Research Lab for the past two years.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, and the most common form of dementia, one that is currently affecting 35 million people worldwide (Liu et al., 2019). Indeed, over the next forty years, the prevalence of AD is predicted to triple, due to an increase in life expectancy and demographic changes around the world. In addition to the tremendous physical and emotional toll this condition exacts, it is estimated that the yearly cost of AD-related healthcare expenses is approximately $500 billion, and that this number will only continue to increase (Liu et al., 2019). Clinical symptoms of AD include memory and language deficits, decreased ability in executive function, and an overall diminished cognitive ability (Weller & Budson, 2018). As there is currently no cure for AD, its prevalence will continue to be an ever-growing focus of the healthcare system (Barnes, 2011).

Hallmark Pathologies of Alzheimer's Disease

Two key biological markers have been discovered in the brains of AD patients (Coronel et al., 2018). These two markers include amyloid-beta (Aβ) plaque deposition in the extracellular space around neurons, and intracellular, neurofibrillary tangles of hyperphosphorylated tau proteins that form inside dying neurons. Aβ is a protein that originates from a larger molecule known as the amyloid precursor protein (APP). In a healthy brain, APP is a protein that plays an important role in neural growth and cell fate (Coronel et al., 2018). However, under pathogenic circumstances, APP is cleaved by several enzymes, including β and γ-secretase. This cleavage process results in the formation of Aβ peptides that can assemble into insoluble aggregates in the brain (Shi et al., 2022). Accumulation of these aggregates can cause neuronal atrophy (Shi et al., 2022). Postmortem brains of AD patients have increased β-secretase activity leading to the increased formation of Aβ plaques (Barnello et al., 2015). In addition to accumulation of excess

plaques, there is often issues involving the clearance of Aβ that increases the amount of Aβ present. Aβ is typically cleared by multiple pathways involving the proteasome and the lysosome (Baranello et al., 2015). Studies have shown that the more Aβ plaques that are found within a brain, the more severe a person's AD tends to be (Murphy & Levine, 2010).

Furthermore, another key biomarker of AD is hyperphosphorylated tau protein. Tau is a microtubule associated protein involved in neuron homeostasis (Huang, 2009). When it becomes phosphorylated, it loses the ability to bind to microtubules and can lead to destabilization of the microtubules and loss of intracellular transport within the neuron, ultimately leading to neuronal cell death (Huang, 2009). Both the presence of Aβ plaques and accumulation of hyperphosphorylated tau tangles in the brain are indicative of AD pathology and are thought to potentially provoke clinical symptoms of AD, such as memory loss and cognitive dysfunction.

Inflammation, Oxidative Stress & Alzheimer's Disease

Previous work has clearly demonstrated that chronic inflammation and oxidative stress may contribute to the development of AD pathology (Ahmed et al., 2017). Inflammation can occur in two forms: acute or chronic. Acute inflammation is typically seen when the body is infected by a pathogen and is used to eliminate potentially harmful materials in cells, and repair cell damage. Acute inflammation can be beneficial and self-limiting to the body. Chronic inflammation, however, can be harmful to cells and induce tissue damage if left unregulated (Ahmed et al., 2017). Chronic inflammation in the brain can be provoked by several mechanisms, such as prolonged activation of microglia and astrocytes. Notably, microglial cells function as the resident immune cell in the brain, and in the healthy brain, microglia play a protective role by secreting proinflammatory cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α), interleukins (IL) and the chemokine CCL2 (Sun et al., 2022). A key

transcription factor involved in the regulation of inflammation is nuclear factor kappa B (NF-κB). Typically, NF-κB remains inactive until the presence of pro-inflammatory cytokines and pathogens activates it. Once active, NF-κB initiates a phosphorylation cascade within the cell that results in the activation of target genes in the nucleus (Sun et al., 2022). The overexpression of these genes leads to the production of more pro-inflammatory cytokines that, in turn, may exacerbate the nascent AD pathologies in the brain. For example, prior studies have shown that NF-κB activates genes that increase β-secretase expression, and thus have the ability to increase production of Aβ (Sun et al., 2022). Moreover, Aβ can also serve as an inflammatory trigger for microglial cells in the brain. When an individual undergoes chronic inflammation, even in the periphery, these pro-inflammatory triggers lead to overactivation of microglia. Although microglia initially play a protective role in the brain, their overactivation leads to the production of neurotoxic factors and eventual neuronal death.

In addition to inducing a proinflammatory response, overactivation of astrocytes and microglia can lead to the production of reactive oxygen species, which induce oxidative stress in the brain (Fischer & Maier, 2015). Oxidative stress occurs when there is an imbalance of reactive oxygen species (ROS) and antioxidants in the cells and there is more overall ROS are that are present (Trovato et al., 2018). There are several biological markers in the blood in humans with AD that have been associated with oxidative stress, including protein carbonyls (Chen, 2014). In addition to the overabundance of ROS, oxidative stress has also shown to cause a decrease in antioxidant enzymes in patients with AD (Chen, 2014). Excess oxidative stress is directly related to the pathogenesis of AD development as it induces inflammation. The presence of Aβ has also shown to increase several biological markers of oxidative stress (Chen, 2014). Studies have shown that small levels of Aβ exhibit neuroprotective effects against oxidative stress (Chen,

2014). However, in those with AD, the increased presence of Aβ in those with AD has a detrimental effect on neurons and can exacerbates oxidative (Chen, 2014). Oxidative stress has also shown to increase the presence of hyperphosphorylated tau tangles (Chen, 2014). Overall, studies have hypothesized that as an individual's imbalance of ROS and antioxidants progresses, their oxidative stress increases leading to the progression of AD pathology and severity of AD (Chen, 2014).

The Western Diet and Alzheimer's Disease

There are several risk factors involving metabolic syndromes that have been directly associated with the development of AD, due to their influence on the immune system and the brain's oxidative system. One factor that has previously been shown to have significant effects on these metabolic syndromes and the development of AD is diet. A diet that has been the focus of many recent studies is the Western Diet (WD), also known as a "typical American diet". The WD has shown to increase biomarkers of inflammation, such as pro-inflammatory cytokines, and oxidative stress (Schönknecht et al., 2021). The WD is characterized by a high percentage of saturated fat consumption. In mice, a previous study has shown that consumption of a WD leads to a significant increase in hippocampal Aβ (Graham et al., 2016). Previous studies have also repeatedly demonstrated that consuming a diet that is high in saturated fat is associated with the development of cognitive decline, dementia, and AD for people over the age of 65 (Kanoski, 2010). WD is also characterized by the consumption of simple or "refined" carbohydrates. Both simple carbohydrates and saturated fat consumption have shown to increase the incidence rate of AD (Kanoski, 2010). Another study found that consumption of a WD for four weeks resulted in increased levels of Aβ and promoted AD-like pathology in humans (Hoscheidt et al., 2022).

The Mediterranean Diet and Alzheimer's Disease

Conversely, other diets have been shown to potentially reduce one's risk of AD, and could potentially be utilized as a prevention strategy for AD. Notably, the Mediterranean diet (MED) has been associated with a reduced risk of dementia and AD, as well as increased longevity (Trichopoulou, 2003). The MED originates in the areas surrounding the Mediterranean Basin and has origins dating back to ancient civilizations. Although, different countries and cultures vary slightly, the typical diet is plant-based. It relies on high amounts of vegetables, fruits, grains, nuts and fruit and moderate amounts of seafood and dairy. Other meats are rarely consumed and alcohol when consumed is typically red wine (Lăcătușu, 2019). This diet also relies heavily on the use of olive oil and very limited consumption of saturated fat. Instead, there is a high consumption of polyunsaturated fatty acids (PUFAs), such as omega-3. This diet has been proven to have protective effects against various cardiovascular and other deadly metabolic diseases (Lăcătușu, 2019). Recent studies have also demonstrated that adherence to a Mediterranean diet diminishes the onset of AD and can slow cognitive decline (Lăcătușu, 2019). People with AD who follow a Mediterranean diet are also shown to have decreased amounts of Aβ plaques in their brains (Lăcătușu, 2019). In humans, studies have proven that consumption of a MED diet for six months leads to a decrease in cognitive decline (Kaplan et al., 2022).

Additionally, the Mediterranean diet is also composed of high amounts of foods containing antioxidants. Antioxidant rich foods have been shown to reduce biomarkers of oxidative stress that are typically present in the AD brain (Lange, 2019). Additionally, chronic inflammation has shown to be diminished in those with AD who consume a Mediterranean diet due to the high consumption of PUFAs (Lange, 2019). Overall, the Mediterranean diet has been

shown to have a positive effect on several components that contribute to the development of AD in human subjects.

Hypotheses

As previously stated, the WD has been associated with increased risk of AD development in human subjects. Studies in rodents have also shown an increase in amyloid genesis and inflammation. Conversely, the MED has been associated with increased longevity and reduced AD risk in human subjects. Current studies examining the effects of both the WD and MED diets on AD present limitations as they are not usually representative of each "typical" diet in each component. For example, MED research has focused on examining the effects of only one or two dietary factors, such as olive oil, on AD pathologies and inflammation, rather than examining the effects of a whole MED. WD studies typically feature a diet that is high in fat composition, that is unrepresentative of a typical WD.

Therefore, the current study aimed to explore the effects of the typical American diet (WD) in comparison to the typical Mediterranean diet, in mice. We hypothesized that C57BL/6 mice that consume a WD for 6 months will show an increase in the development in the biological markers associated with AD compared to MED diet mice.

MATERIALS & METHODS

Animal Care

This study utilized wildtype, C57BL/6J, male and female mice that were bred in the Texas Christian University vivarium. The mice were weaned at 3 weeks of age and were housed in groups of three to four. The mice lived in a 12-hour light and dark schedule in polycarbonate study boxes. Each mouse had access to one diet, either WD or MED, and water *ad libitum.*

Diet Composition

Both diets utilized in this study were designed to match the macronutrient densities of both typical diets (Figure 1). The WD diet, modeled after the typical American diet, was comprised of 50% kcal from carbohydrates, 35% kcal from fat and 15% kcal from protein. The diet also featured a 15:1 ratio of omega-6 to omega-3 fatty acids, as is seen in a typical American diet. The composition of each macronutrient group featured what is usually seen in a WD. The carbohydrate source included majority corn starch. Protein in the WD was derived from casein and fat kcal consisted of beef fat, safflower oil and butter.

The MED diet was also modeled after the typical Mediterranean diets. The MED diet consisted of 50% kcal from carbohydrates, 35% kcal from fat and 15% kcal from protein. The omega-6 to omega-3 ratio for the MED diet was 2:1. Like the WD diet, each macronutrient consisted of what is seen in a typical MED diet. Carbohydrate sources included brown rice flour and wheat starch. Protein sources included a mixture of soy, fish and egg white protein. Fat sources for the MED diet came from majority olive oil.

	Mediterranean	Western
Protein – 15%	Soy, fish, egg	Casein
Carbohydrates – 50%	Brown rice flour, wheat starch	Corn starch
Fat – 35%	Unsaturated fatty acids, olive oil	Saturated fatty acids, beef fat, butter, safflower oil

Table 1. Composition of Diet Studies

After being weaned at 3 weeks of age, each animal was randomly assigned to either the WD or MED diet. The mice remained on their assigned diet for six months with access to food and water *ad libitum.* Every animal had their total body weight measured weekly. Each animal's food consumption was also monitored weekly by cage and then the mean amount of food consumed of each animal was estimated. A timeline of diet consumption of each animal is shown in Figure 1.

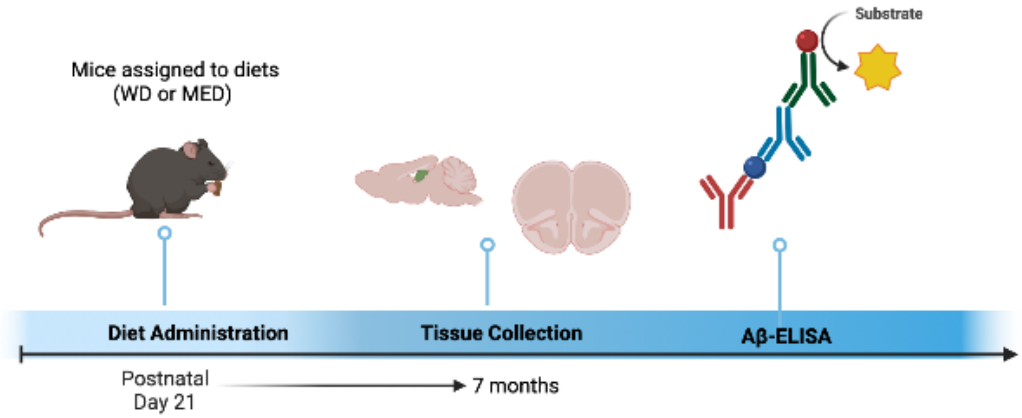

Figure 1: Experimental Timeline

Tissue Collection

At the end of the six months of diet, each animal was euthanized through rapid decapitation. First, blood was collected, placed on ice for 15 minute and kept at room temperature for 30 minutes. Then, the blood was centrifuged at 2000 x g for 10 minutes to

collect the serum, which was then stored at -80ºC. In addition to blood collection, tissue samples of the hippocampus and prefrontal cortex were collected. The hippocampus was stored in 250 μL of PRO-PREP (Bulldog Bio, Portsmouth, NH) along with protease and phosphatase inhibitors (100 μL of 100x concentration per 10 mL of PRO-PREP). The prefrontal cortex was stored in 250 μL of PRO-PREP. Each tissue sample were frozen on dry ice immediately and stored at -80ºC until used again.

Amyloid-Beta ELISA

Before tissue samples could be analyzed, each sample was centrifuged at 15,000 rpm for 40 minutes. From there, clear lysates were collected and protein assays (DC Protein Assay; Bio-Rad Laboratories, Hercules, CA) were performed. After undergoing a protein assay, the protein concentration of each tissue sample was calculated to later use in an enzyme-linked immunosorbent assay (ELISA) looking to measure soluble $A\beta_{1–42}$ in the hippocampus and pre-frontal cortex. Each sample underwent a mouse $A\beta_{1-42}$ ELISA (ThermoFisher Scientific, Waltham, Massachusetts). Initially, each hippocampal lysate was diluted in incubation buffer at a 1:2 dilution due to the protocol stating that protein concentrations should not be greater than 200 pg/mL. Following dilution, the $A\beta_{1-42}$ standard was created using standard reconstitution buffer and serial dilutions. 100 μL of either standard or lysates were placed into the 96-well antibody-coated plate. After incubation, the plates were washed four times with standard wash buffer and treated with $A\beta_{1-42}$ detection antibody. Following this incubation, each well was again washed four times with standard wash buffer and 100 mL of HRP-tagged detection antibody (Anti-Rabbit IgG) was placed in each well. After incubation and another four washes, each well was treated with 100 μL of stabilized chromogen and incubated in the dark. Following the final

incubation period, the plate was treated with stop solution and read with an absorbance of 750 nm.

MSD Assays to Measure Peripheral Inflammation

Using a VPLEX Custom Mouse Cytokine Proinflammatory Panel 1 muliplex kit (Meso Scale Diagnostics, Rockville, MD), the inflammatory mediators, mouse IFN-γ, IL-1, IL-4, IL-6, IL-10 and TNF-α were measured. Serum samples were diluted in a 1:1 concentration with a proprietary diluent. Each sample was washed three times after incubation and antibody detection solution was added to each. After another incubation period and a series of three washes, we added read buffer, and read the electrochemiluminescent signal with a QuickPlex SQ 120 instrument (Meso Scale Diagnostics). Any sample that did not fall within the standard curve of the detection range was not utilized in the study.

RESULTS

$A\beta_{1-42}$ Production Increased in the Prefrontal Cortex of WD Animals

Using an $A\beta_{1-42}$-ELISA, the amount of $A\beta_{1-42}$ present in the prefrontal cortex of mice was measured in pg/mg of total protein present in the sample. A significant increase in the amount of $A\beta_{1-42}$ was found in the males on the Western Diet compared to the males on the Mediterranean diet (Figure 3). Each group contained twelve animals (n = 12), $t(22) = -3.058$, $p = 0.006$. There were two outliers whose amount of $A\beta_{1-42}$ were outside of SPSS interquartile range, and they were removed from the data**set.**

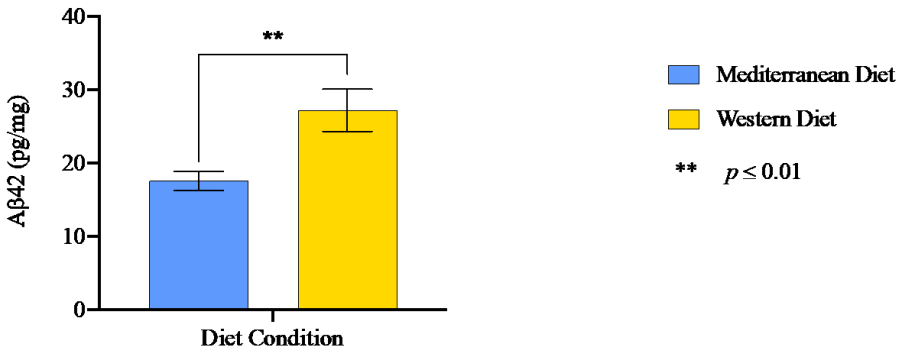

Figure 2: Aβ$_{1-42}$ in the Prefrontal Cortex of Males (pg/mg of total protein). After 6 months of diet, each animal's prefrontal cortex was collected and an Aβ$_{1-42}$-ELISA was performed. A significant increase in Aβ$_{1-42}$ was found in the animals on the Western Diet (n's =12).

In the females, a significant increase in the amount of Aβ$_{1-42}$ present in the prefrontal cortex was seen in the females on the WD compared to females on the MED (Figure 3). Each group contained either eleven or twelve animals (n = 11-12), $t(21) = -2.534$, $p = 0.019$. One animal was removed from the study as an outlier due to being outside of SPSS interquartile range. One animal was also removed as the Aβ$_{1-42}$-ELISA showed a CV over 25%.

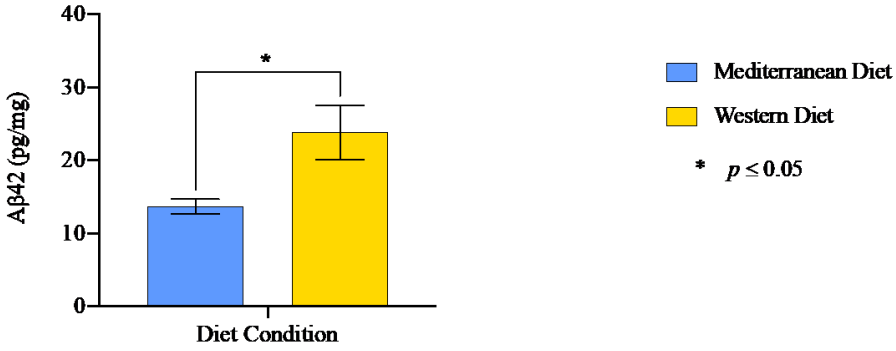

Figure 3. Aβ$_{1-42}$ in the Prefrontal Cortex of Females (pg/mg of total protein) After 6 months of diet, each animal's prefrontal cortex was collected and an Aβ$_{1-42}$-ELISA was performed. A significant increase in Aβ$_{1-42}$ was found in the animals on the Western Diet (n's =11-12).

$A\beta_{1-42}$ Production Increased in the Hippocampus of Males on the WD

An $A\beta_{1-42}$-ELISA was utilized to analyze the amount of $A\beta_{1-42}$ in the hippocampus of mice on the MED and WD in pg/mg of total protein. A significant increase was shown in the amount of $A\beta_{1-42}$ found in the hippocampus of males on the WD compared to MED males. Each diet group contained 15-16 animals (n = 15-16), $t(29) = -2.103$, $p = 0.444$. Three outliers were removed from the data as they were outside of the SPSS interquartile range. Two samples were also removed due to having CVs over 255. This is shown in Figure 4.

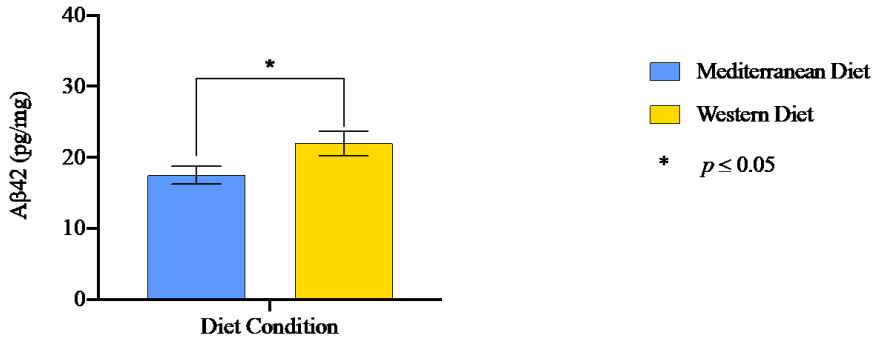

Figure 4. $A\beta_{1-42}$ in the Hippocampus of Males (pg/mg of total protein) After 6 months of diet, each animal's hippocampus was collected and an $A\beta_{1-42}$-ELISA was performed. A significant increase in $A\beta_{1-42}$ was found in the animals on the Western Diet (n's =15-16).

$A\beta_{1-42}$ Production Showed No Change in the Hippocampus of Females

An $A\beta_{1-42}$-ELISA was run to analyze the amount of $A\beta_{1-42}$ (pg/mg of total protein) present in the hippocampus of MED and WD females. There was no significant difference found between the amount of $A\beta_{1-42}$ in female mice on either the WD or MED. Each group contained 12 to 14 animals (n = 12-14), $t(24) = -1.308$, $p = 0.203$. One outlier was removed from the data analysis and two were removed due to CV values over 25%. This data is represented in Figure 5.

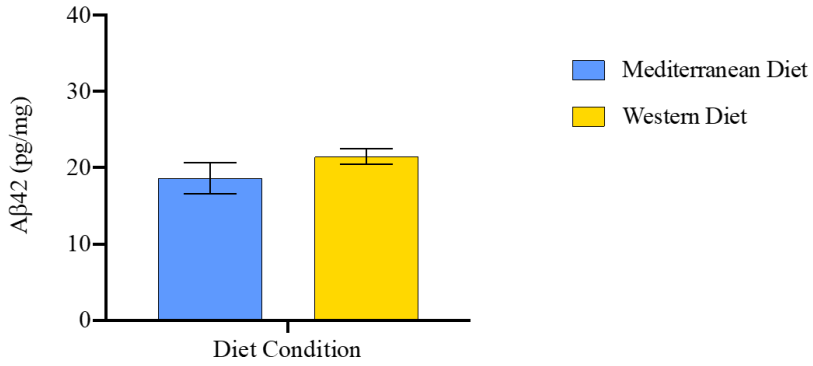

Figure 5. $A\beta_{1-42}$ in the Hippocampus of Females (pg/mg of total protein) After 6 months of diet, each animal's hippocampus was collected and an $A\beta_{1-42}$-ELISA was performed. No significant difference in $A\beta_{1-42}$ was found between animals on the WD or MED. (n's =12-14).

TNF-α Production in the Serum Increased in WD Animals

An MSD Assay was used to analyze cytokines, specifically TNF-α, in the serum of animals both on the WD and MED. A significant increase in TNF-α levels (pg/mL) was found in the males on the WD compared to MED males. Each group contained 16 to 18 animals (n=16-18), $t(32)= -0.3790$, $p < 0.001$ (Figure 6). A significant increase in the levels of TNF-α of the WD females compared to MED females was also found following an MSD Assay. Each group contained 9-12 animals (n=9-12), $t(19) = -3.493$, $p = 0.002$. One sample was removed due to a CV value over 25% and four samples were removed as outliers outside of SPSS's interquartile range (Figure 7)

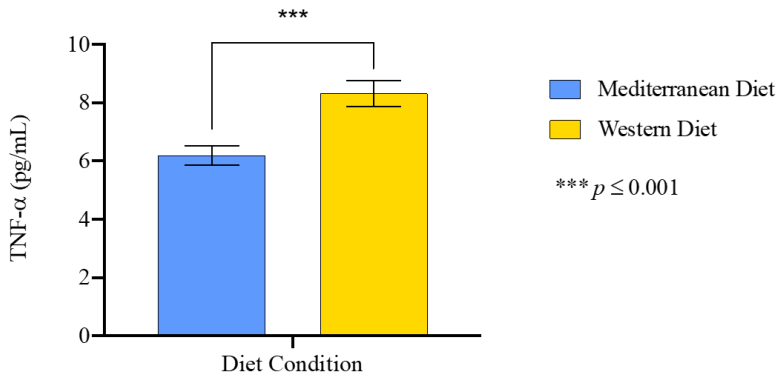

Figure 6. TNF-α Levels in Serum of Males (pg/mL of serum). After 6 months of diet, TNF-α levels were analyzed using an MSD Assay, and a significant increase was shown in WD males compared to MED males (n's = 16-18)

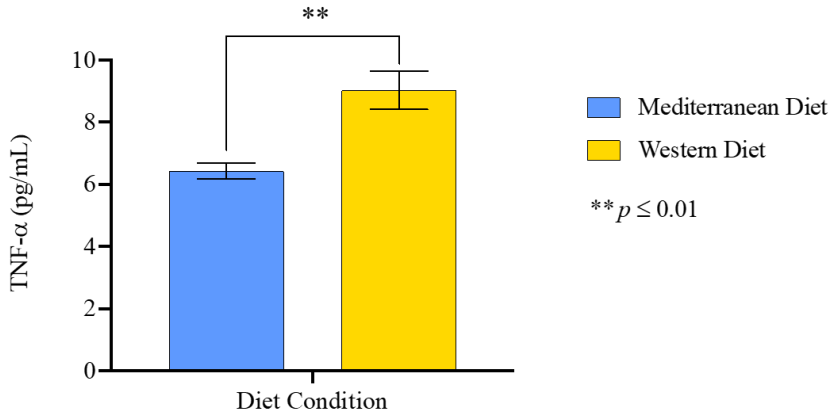

Figure 6. TNF-α Levels in Serum of Females (pg/mL of serum). After 6 months of diet, TNF-α levels were analyzed using an MSD Assay, and a significant increase was shown in WD females compared to MED females (n's = 9-12)**.**

DISCUSSION

It has been hypothesized that there is a link between AD pathology and diet. Previous studies have compared both a Western Diet (WD) and a Mediterranean Diet (MED) and the affect that have on AD pathology. They have shown that a WD promotes inflammation and the development of AD while consumption of a MED diet decreases AD pathology and cognitive

decline (Hoscheidt et al., 2021). Various studies have been conducted to compare these two diets and their effect on AD pathology. However, many of these diets present with limitations as they do not fully encompass a diet that is typical to human consumption. Instead, most studies usually alter one or two components of the diet and analyze the effects. For example, many studies WD usually features a higher macronutrient percentage of fat that is much higher than what a human on a WD consumes. Given these limitations, this study aims to design and compare both a WD and MED that closely mimic what is found in the diets of the humans that consume them. Each diet contained equal percentages of each macronutrient in order to account for potential energy differences. Each macronutrient group of both the MED and WD were comprised of ingredients similar to those found in typical human consumption. C57BL/6 mice were then randomly assigned to either the MED or WD group. After consuming the diet for six months, each group was analyzed to compare the effect of diet on the biological markers associated with AD.

One biological marker of AD pathology is the presence of Aβ plaques in the brain (Liue et al., 2019). This study found that there was a significant increase the amount of $A\beta_{1–42}$ present in the prefrontal cortex of male and female WD mice, compared to male and female Med mice. This is similar to findings of other recent studies that have shown that consumption of a WD diet leads to an increase in both brain inflammation and the presence of Aβ in the brain periphery (Thériault et al., 2016). In the hippocampus, males on the WD diet showed a significant increase in the amount of $A\beta_{1–42}$ present compared to males on the MED. This is consistent with previous studies that have found an increase in hippocampal Aβ in mice following a WD (Hooijmans et al., 2007). However, in the female mice, there was no significant difference found in the amount of Aβ in the hippocampus between the two diet groups. Both the WD and MED females showed similar amounts of hippocampal $A\beta_{42}$ (pg/total mg of protein). Since C57BL/6 mice do not

develop Aβ plaques through aging (Kitazawa, 2012), Aβ was measured through the presence of $A\beta_{1-42}$. Aβ is the soluble amyloid beta peptide that typically comes together in humans to form Aβ plaques in the brain of those with AD (Roher et al., 1993).

This study also aimed to analyze the effect of a MED versus a WD on the presence of the pro-inflammatory cytokine, TNF-α, in the serum of C57BL/6 mice. After conducting an MSD Assay, results showed that there was a significant increase in the amount of TNF-α present in the WD animals, both males and females, compared to MED animals. This increase in WD animals correlates with several other studies that have shown an increase in TNF-α production in mice following consumption of a WD (Li et al., 2018).

CONCLUSION

This study looked to compare the effect of a Mediterranean (MED) versus Western (WD) Diets on Alzheimer's disease (AD) pathology in C57BL/6 mice. Based on previous studies, we hypothesized that mice that consumed a WD Diet would show an increase in the biological markers associated with AD compared to those that consumed a MED Diet. We found that animals that consumed the WD Diet did show a significant increase in AD pathology markers like Aβ and the proinflammatory cytokine, TNF-α. With these results we can confirm our initial hypothesis and conclude that overall, mice on the Western Diet showed an increase in the biological markers associated with AD compared to those on the Mediterranean Diet.

Limitations of this study include a small sample size. Each group of mice contained between 9 to 16 mice. Future studies should aim to replicate and expand these study parameters to draw further conclusions. Future studies can also analyze the effect of a Mediterranean versus a Western Diet on behavior and cognition in these animals, as cognitive decline is a critical

clinical symptom of AD in humans. This study also only analyzed one pro-inflammatory cytokine, TNF-α; however, future studies should aim to analyze both different pro-inflammatory and anti-inflammatory cytokines, and the impact on AD development.

References

Ahmed, S. M. U., Luo, L., Namani, A., Wang, A., X. J., & Tang, X. (2017) Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochimica Et Biophysics Acta (BBA) – Molecular Basis of Disease, 1863(2),* 585-597. https://doi.org/10.2174/138161210793176572

Armstrong R. Risk factors for Alzheimer's disease. Folia Neuropathologica. 2019;57(2):87-105. doi:10.5114/fn.2019.85929.

Baranello, R. J., Bharani, K. L., Padmaraju, V., Chopra, N., Lahiri, D. K., Greig, N. H., Pappolla, M. A., & Sambamurti, K. (2015). Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease. *Current Alzheimer research*, *12*(1), 32–46. https://doi.org/10.2174/1567205012666141218140953

Barnes, Deborah E, and Kristine Yaffe. "The Projected Effect of Risk Factor Reduction on Alzheimer's Disease Prevalence." *The Lancet Neurology*, vol. 10, no. 9, 2011, pp. 819–828., https://doi.org/10.1016/s1474-4422(11)70072-2.

Chen, Z., Zhong, C. Oxidative stress in Alzheimer's disease. *Neurosci. Bull.* **30**, 271–281 (2014). https://doi.org/10.1007/s12264-013-1423-y

Coronel, R., Bernabeu-Zornoza, A., Palmer, C., Muñiz-Moreno, M., Zambrano, A., Cano, E., & Liste, I. (2018). Role of Amyloid Precursor Protein (APP) and Its Derivatives in the Biology and Cell Fate Specification of Neural Stem Cells. Molecular neurobiology, 55(9), 7107–7117. https://doi.org/10.1007/s12035-018-0914-2

Fernández-Sanz P, Ruiz-Gabarre D, García-Escudero V. Modulating Effect of Diet on Alzheimer's Disease. *Diseases*. 2019; 7(1):12. https://doi.org/10.3390/diseases7010012

George Perry, Adam D. cash, Mark A. Smith, "Alzheimer Disease and Oxidative Stress", *BioMed Research International,* vol. 2, Articl ID 542340, 4 pages, 2002. https://doi.org/10.1155/S1110724302203010

Graham, L. C., Harder, J. M., Soto, I., de Vries, W. N., John, S. W., & Howell, G. R. (2016). Chronic consumption of a western diet induces robust glial activation in aging mice and in a mouse model of Alzheimer's disease. *Scientific reports*, *6*, 21568. https://doi.org/10.1038/srep21568

Huang, Han-Chang and Jiang, Zhao-Feng. 'Accumulated Amyloid-β Peptide and Hyperphosphorylated Tau Protein: Relationship and Links in Alzheimer's Disease'. 1 Jan. 2009: 15 – 27.

Hoscheidt, S., Sanderlin, A. H., Baker, L. D., Jung, Y., Lockhart, S., Kellar, D., Whitlow, C. T., Hanson, A. J., Friedman, S., Register, T., Leverenz, J. B., & Craft, S. (2022). Mediterranean and Western diet effects on Alzheimer's disease biomarkers, cerebral perfusion, and cognition in mid-life: A randomized trial. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *18*(3), 457–468. https://doi.org/10.1002/alz.12421

Hooijmans, C. R., Rutters, F., Dederen, P. J., Gambarota, G., Veltien, A., van Groen, T., Broersen, L. M., Lütjohann, D., Heerschap, A., Tanila, H., & Kiliaan, A. J. (2007). Changes in cerebral blood volume and amyloid pathology in aged Alzheimer APP/PS1 mice on a docosahexaenoic acid (DHA) diet or cholesterol enriched Typical Western Diet (TWD). *Neurobiology of disease*, *28*(1), 16–29. https://doi.org/10.1016/j.nbd.2007.06.007

Kaplan, A., Zelicha, H., Yaskolka Meir, A., Rinott, E., Tsaban, G., Levakov, G., Prager, O., Salti, M., Yovell, Y., Ofer, J., Huhn, S., Beyer, F., Witte, V., Villringer, A., Meiran, N., B Emesh, T., Kovacs, P., von Bergen, M., Ceglarek, U., Blüher, M., … Shai, I. (2022). The effect of a high-polyphenol Mediterranean diet (Green-MED) combined with physical activity on age-related brain atrophy: the Dietary Intervention Randomized Controlled Trial Polyphenols Unprocessed Study (DIRECT PLUS). *The American journal of clinical nutrition*, *115*(5), 1270–1281. https://doi.org/10.1093/ajcn/nqac001

Kitazawa, M., Medeiros, R., & Laferla, F. M. (2012). Transgenic mouse models of Alzheimer disease: developing a better model as a tool for therapeutic interventions. *Current pharmaceutical design*, *18*(8), 1131–1147. https://doi.org/10.2174/138161212799315786

Martin, Elodie, Boucher, Céline, Fontaine Bertrand & Delarasse, Cécile. *Distinct Inflammatory Phenotypes of Microglia and Monocyte-Derived.* 08 Oct. 2016 ...https://onlinelibrary.wiley.com/doi/10.1111/acel.12522.

Murphy, M. Paul and LeVine III, Harry. 'Alzheimer's Disease and the Amyloid-β Peptide'. 1 Jan. 2010 : 311 – 323.

Lăcătușu, Cristina-Mihaela, et al. "The Mediterranean Diet: From an Environment-Driven Food Culture to an Emerging Medical Prescription." *MDPI*, Multidisciplinary Digital Publishing Institute, 15 Mar. 2019, https://doi.org/10.3390%2Fijerph16060942.

Lange, Klaus W. "Mediterranean Diet and Alzheimer's Disease." *Movement and Nutrition in Health and Disease*, 2019 https://www.movement-nutrition.de/article/view/13.

Li, X., Liu, Y., Wang, B., Chen, C., Zhang, H., & Kang, J. X. (2018). Identification of a sustainable two-plant diet that effectively prevents age-related metabolic syndrome and extends lifespan in aged mice. *The Journal of Nutritional Biochemistry 51*, 16-26. doi:10.1016/j.jnutbio.2017.09.003

Liu, y., Nguyen, M., Robert, A., & Meunier, B. (2019). Metal Ions in Alzheimer's Disease: A key Role or Not? *Accounts of Chemical Research, 52(7), 2026-2035.* https://doi.org/10.1021/acs.accounts.9b00248

Roher, A. E., Lowenson, J. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E., & Ball, M. J. (1993). Beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(22), 10836–10840. https://doi.org/10.1073/pnas.90.22.10836

Roman Fischer, Olaf Maier, "Interrelation of Oxidative Stress and Inflammation in Neurodegenerative Disease: Role of TNF", *Oxidative Medicine and Cellular Longevity,* vol. 2015, Article ID 610813, 18 pages, 2015. https://doi.org/10.1155/2015/610813

Schönknecht, Y. B., Crommen, S., Stoffel-Wagner, B., Coenen, M., Fimmers, R., Stehle, P., Ramirez, A., & Egert, S. (2021). APOE ε4 Is Associated with Postprandial Inflammation in Older Adults with Metabolic Syndrome Traits. *Nutrients*, *13*(11), 3924. https://doi.org/10.3390/nu13113924

Sun E, Motolani A, Campos L, Lu T. The Pivotal Role of NF-kB in the Pathogenesis and Therapeutics of Alzheimer's Disease. *International Journal of Molecular Sciences*. 2022; 23(16):8972. https://doi.org/10.3390/ijms23168972

Shi, M., Chu, F., Zhu, F., & Zhu, J. (2022). Impact of Anti-amyloid-β Monoclonal Antibodies on the Pathology and Clinical Profile of Alzheimer's Disease: A Focus on Aducanumab and Lecanemab. *Frontiers in aging neuroscience*, *14*, 870517. https://doi.org/10.3389/fnagi.2022.870517

Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *The New England journal of medicine*, *348*(26), 2599–2608. https://doi.org/10.1056/NEJMoa025039

Trovato Salinaro, A., Pennisi, M., Di Paola, R. *et al.* Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms. *Immun Ageing* **15**, 8 (2018). https://doi.org/10.1186/s12979-017-0108-1

Weller, Jason, and Andrew Budson. "Current Understanding of Alzheimer's Disease Diagnosis and Treatment." *F1000Research*, F1000 Research Limited, 31 July 2018, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073093/.