2023

Sex-specific effects of high-fat and ketogenic diet on inflammatory responses in the hippocampus

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Undergraduate Honors Thesis of

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January 2023

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Preface

When I began at NSU, I had no idea that I would completely fall in love with the field of neuroscience. Dr. Robison, my very first neuroscience professor at NSU, heavily contributed to igniting this spark in me. Excited to get involved in research, I approached Dr. Robison with the wish to work with her on her research. She took me in without hesitation, guiding me as I learned new techniques and even agreeing to be my Honors Thesis Advisor.

I started my journey in the lab from humble beginnings, reading papers on fascinating topics and labeling countless microscope slides. Since starting my Honors Thesis, I have had numerous opportunities to present my research at conferences, develop my interprofessional skills, and work alongside amazing researchers like Dr. Speth from the College of Pharmacy in the Health Professions Division Building and Dr. Robison in the Center for Collaborative Research and the Parker Building. Through this research project, our lab placed first in the poster competition at the 2023 Society for NeuroSports Conference, and I witnessed the logo I designed represent the 2023 Undergraduate Student Symposium. The skills I have gained on this journey aid in my aspiration to profoundly impact patients' lives, advocating for them as their physician and as a researcher.

Reflecting on this experience, I have developed a deeper appreciation for research. The late nights spent in the Zombie Lab or immersed in an Excel sheet have all been worthwhile for the memories I have made through this Honors Thesis. I am forever grateful for this incredible opportunity.

Abstract

Poor diet and metabolic diseases (obesity, Type 2 diabetes) are associated with increased risk of neurodegenerative and neuropsychiatric disorders, including Alzheimer's disease, anxiety, and depression. Studies indicate that inflammation in the hippocampus could be one mechanism linking these conditions. Previous findings on inflammation, specifically glial activity in response to a high-fat diet, indicate sex differences in microglial responses in the hippocampus. The ketogenic diet is characterized by a high-fat, low-carbohydrate, and moderate-protein diet. While the ketogenic diet is very high in fat content, it may also possess neuroprotective properties against brain aging and neurodegenerative disorders, as well as boost mood and cognitive function. The aim of the experiment is to examine further the sex-specific effects of a high-fat diet (HFD) and a translationally relevant ketogenic diet (KD) on inflammation in the hippocampus, with a specific focus on astrocytes. Male and female C57BL/6J mice were fed high-fat, ketogenic, or low-fat control diets starting at 9 weeks of age and remained on the diet for ~5 months. Mice were assessed for weight gain, adiposity, ketone body levels, and diabetic status using glucose tolerance testing. Immunofluorescence was performed using a GFAP antibody for the analysis of astrocytes in the hippocampus. In males, there was no effect of diet on GFAP labeling in any subregion of the hippocampus. However, in female KD mice, there was a decrease in GFAP percent area covered in the CA1 and CA2 regions of the hippocampus, with similar but non-significant trends in other hippocampus regions (CA3 and dentate gyrus). These findings suggest that KD affects hippocampal astrocytes in a sex-specific manner,

though further research is necessary to evaluate the downstream and functional effects of these changes.

Acknowledgements

To my incredible mentor Dr. Lisa Robison, I would not be where I am on this journey without your guidance. I hope you know how impactful and inspiring you have been in my life. As I move forward in my academic and professional life, I will always carry the lessons and skills you have imparted on me deep inside my heart.

To the Farquhar Honors College and Dean Andrea Nevins, thank you for granting me this precious opportunity to explore my passion for neuroscience with funding and support.

To my lab mates, I extend my heartfelt gratitude for your invaluable support in the completion of this thesis. Your contributions were essential to the success of this project.

To my best friends, this journey wouldn't have been the same without you two. From dissecting brains to presenting at conferences, we have grown so much since we first met as freshmen in Dr. Robison's Introduction to Neuroscience class.

And finally, to my family, I cannot put into words how much your love and encouragement mean to me. Thank you for being there for me every step of the way.

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Sex-specific effects of high-fat and ketogenic diet on inflammatory responses in the hippocampus

Chapter 1

1. Introduction

Neuroinflammation is an immune response triggered by injury, infection, disease, or stress in the nervous system. The immune response of the central nervous system (CNS) is orchestrated by microglia. These resident immune cells act as macrophages, responsible for immune surveillance and the production of inflammatory mediators (DiSabato et al., 2016). Astrocytes, another glial cell type in the CNS, perform metabolic, structural, homeostatic, and neuroprotective tasks in the brain. Activated astrocytes participate in neuroinflammatory responses by producing pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 via the NF-κB signaling pathway (Jiang et al., 2022).

Previous findings suggest that one potential cause of widespread increased neuroinflammation is poor diet and resulting metabolic disease. A review by Milanova et al. reported a synergistic effect on microglial activation by aging and obesity induced by a high-fat diet in multiple brain areas (2021). One area that has been investigated is the hippocampus, an integral brain region that plays a crucial role in learning and forming new memories. Studies suggest that both short-term and long-term consumption of a high increases microglial activation in the hippocampus (Ma et al., 2018; Spencer et al., 2017; Thirumangalakudi et al., 2008; Tramullas et al., 2016). This is concerning, as studies indicate that inflammation in the hippocampus contributes to the pathogenesis of neurodegenerative disorders like Alzheimer's disease and other dementias (Ekdahl et al. 2003).

Of note, some studies suggest that the effects of diet and metabolic disease on hippocampal inflammation and microglial responses may occur in a sex-specific manner (Kang et al., 2018; Robison et al., 2020; Schwarz et al., 2012). It is possible that these sex differences in microglial responses and resulting functional changes in the hippocampus could contribute to sex differences in the prevalence of Alzheimer's disease, as well as other neurological conditions involving the hippocampus. Interestingly, not only are women at greater risk for these diseases (Alzheimer's disease facts and figures, 2020; Halbreich et al., 2007; Kessler et al., 1994), but metabolic disease also appears to be associated with greater risk for these conditions in women compared to men (Arroyo et al., 2004; Chatterjee et al., 2016; Jacka et al., 2010; Whitmer et al., 2007).

The ketogenic diet is a dietary intervention consisting of various levels of macromolecules, specifically low-carbohydrate (<5%), high-fat, and moderate protein. Though the ketogenic diet was initially recommended to treat severe epilepsy under medical supervision, this diet gained popularity in recent years as it promised quick weight loss. With a normal diet, the human body acquires energy through the conversion of glucose to pyruvate, a process known as glycolysis. Under conditions of nutrient deprivation such as carbohydrate restriction, the body enters ketosis, producing ketones bodies as a source of energy. There is evidence to suggest that the ketogenic diet plays an anti-inflammatory role in the brain due to central and peripheral inflammatory mechanisms (Ziying et al., 2022). While studies have been conducted on inflammation in the hippocampus and the effects of the ketogenic diet on the human body, further research is required to examine the sex-specific effects of high-fat and ketogenic diets on inflammation in the hippocampus.

Here, we examined further the sex-specific effects of chronic administration (~2-8 months of age) of high-fat diet (HFD) and ketogenic diet (KD) on inflammation (immunofluorescence labeling of astrocytes) in several subregions of the hippocampus in male and female mice. These findings on the sex-specific effects of a ketogenic diet and how they differ from a high-fat diet will help gain a deeper understanding of the effects of diet on the brain.

Chapter 2

2. Methods

2.1 Animals & Experimental Design (with Zachery Lawrence)

Experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and protocols were approved by the Institutional Animal Care and Use Committee at Nova Southeastern University (Davie, FL, USA). Male and female C57BL/6J mice (n = 10 per group) were obtained from Jackson Laboratories (Bar Harbor, ME). At approximately 9 weeks of age, mice were placed on a high-fat diet (60% fat, 20% carbohydrates, 20% protein; D12492, Research Diets Inc., New Brunswick, NJ), ketogenic diet (80% fat, 5% carbohydrates, 15% protein; D06040601, Research Diets, Inc., New Brunswick, NJ), or control low-fat diet (LF; 10% fat, 70% carbohydrates, 20% protein; D12450J, Research Diets Inc., New Brunswick, NJ). Mice remained on the diet until they were euthanized at ~32 weeks of age. A timeline of the experiment is shown in **Figure 1**.

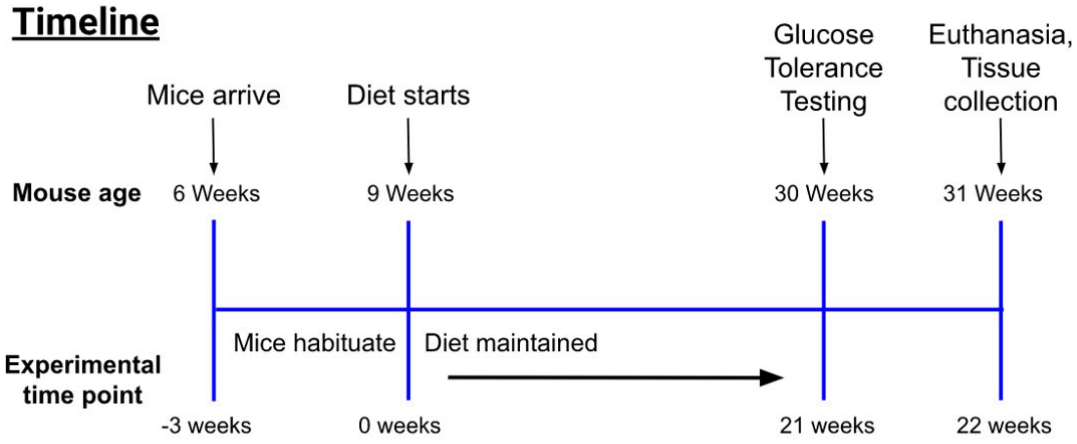

Figure 1. Experimental Design. Mice started the diet intervention at 9 weeks of age and the diet was maintained for 22 weeks. Near the end of the study, mice were assessed for ketone body levels and glucose tolerance. Mice were euthanized at 31 weeks of age and tissue was collected for further analysis.

2.2 Glucose Tolerance Test (with Zachery Lawrence)

Glucose tolerance testing (GTT) was conducted to assess diabetic status of the mice following ~5 months on the diets. Mice were fasted overnight, and baseline glucose levels in blood collected from a tail snip of <1 mm were measured by glucometer. Each mouse received 2g/kg of glucose via intraperitoneal injection. Blood glucose levels were measured again at 15, 30, 60, and 120 min post-glucose administration.

2.3 Assessment of Ketosis (with Zachery Lawrence)

Blood samples obtained from the tail vein through a tail snip were analyzed using a blood ketone meter approximately 2 to 4 hours into the dark cycle to determine if mice were in a state of ketosis.

2.4 Tissue Collection & Cryosectioning (with Zachery Lawrence)

Mice were perfused with ice cold 0.9% saline under deep isoflurane anesthesia. Brains were removed and fixed overnight in 4% paraformaldehyde at 4°C, followed by cryoprotection in 30% sucrose for at least 72h at 4°C. Brains were preserved in OCT and stored at -80°C until ready for cryosectioning. Brain sections were cut at a thickness of 40 µm at -17°C. Sections were refrigerated in cryopreserve until immunofluorescence was conducted.

The procedures below this point were performed by Shalet James only.

2.5 Immunofluorescence

Immunofluorescence was conducted for the analysis of astrocytes in the hippocampus. Brain sections were washed with PBS with 0.1% sodium azide 3 times for 5 minutes, permeabilized in 0.3% TPBS with sodium azide for 1h at room temperature, blocked with 4% donkey serum in 0.3% TPBS with sodium azide for 1h at room temperature, and incubated at 4°C overnight with primary antibodies diluted in blocking buffer. For the analysis of astrocytes, rabbit anti-glial fibrillary acidic protein (GFAP) antibody (1:1000; AB5804, Millipore) was utilized. Sections were washed 3 times for 5 min following primary antibody incubation. Fluorescent secondary antibody (Alexa Fluor 594 donkey anti-rabbit; 1:300; A21207, Invitrogen) was diluted in blocking buffer and applied at room temperature for 2 hours, followed by 3 washes for 5 min each. Sections were mounted on glass slides with mounting media and DAPI stain applied, coverslipped, and sealed. Slides were stored at 4°C until ready to be imaged under the microscope. Images

of the dorsal hippocampus were taken under 10× magnification using the Olympus IXplore fluorescence microscope.

2.6 Immunofluorescence Analysis

All immunohistochemistry analyses were performed in coronal sections across the dorsal hippocampus, noting the distance from bregma for each slice. Measures were taken in the hippocampus of each hemisphere and a series of left and right values were averaged for each slice to create an overall hippocampus average. Using coronal sections, the dorsal portion of the hippocampus generally included four sections from approximately −1.2 to approximately −2.06 mm from bregma.

GFAP images were analyzed with ImageJ software (NIH) using a custom macro for image thresholding, which included background subtraction followed by auto thresholding using the "Triangle" option. Regions of interest (ROIs) of set shape and size were placed in CA1, CA2, CA3, and the dentate gyrus of the hippocampus to quantify the average percent area covered by cells positive for GFAP (**Figure 2**). All measurements were performed blinded, with the experimenter unaware of the identity of the treatment group from which the sections originated.

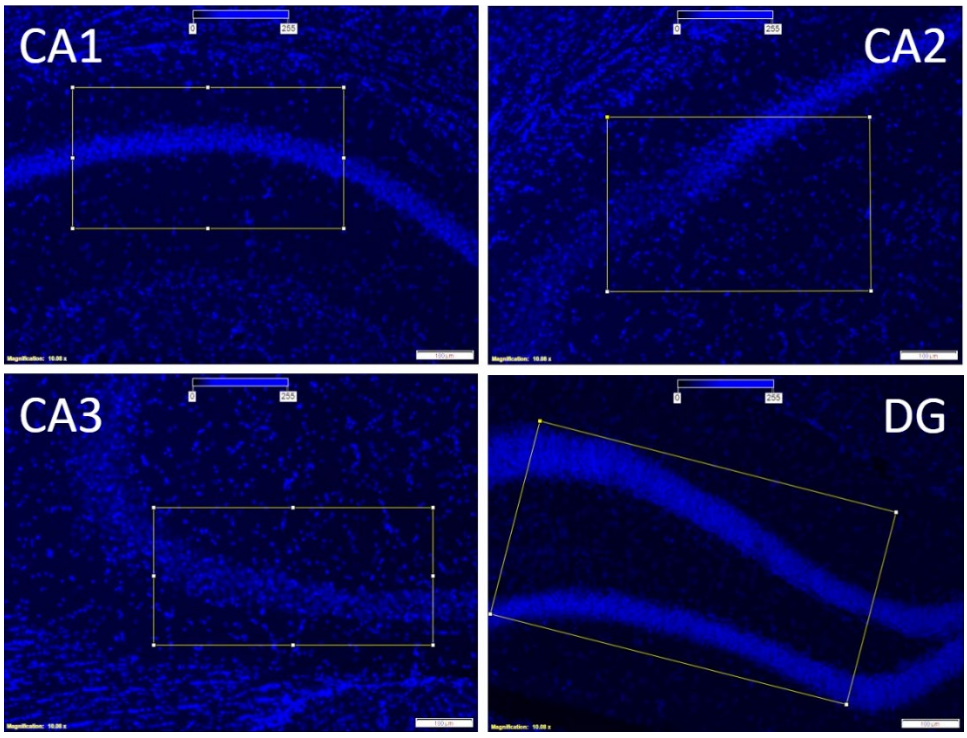

Figure 2. Regions of Interest in the Hippocampus. Representative images depict the placement of regions of interest, characterized by standardized size and shape, within each subregion of the hippocampus for the quantification of GFAP-positive cells.

2.7 Statistical Analysis

Two-way ANOVAs were used to assess group differences separately in each subregion of the hippocampus, followed by Holm-Šidák's correction for multiple comparisons [diet (LF vs. Keto vs. HF) x sex (Males vs. Females)] and Dunnett's multiple comparisons. Data was analyzed using PRISM version 10 software (GraphPad Software Inc., San Diego, CA, USA) and are represented as the mean+SEM. Statistical significance was set at $p<0.05$.

Chapter 3

3. Results

3.1 Body Weight

C57BL/6J mice were fed an HFD, KD, or LF control diet for 22 weeks starting at 9 weeks of age. Body weight in male and female mice was assessed before and throughout the diet intervention. To compare the difference in body weight between baseline measurements and final weight, the percentage of weight gain was calculated over the length of the study as seen in **Figure 3**. As expected after 22 weeks of the diet intervention, body weight increased over time among all diet groups in both males and females [**Figures 3A (males) & 3B (females)**]. A two-way ANOVA was performed to analyze the effect of diet on weight gain over the course of the entire dietary intervention (**Figure 3C**). A two-way ANOVA found a significant main effect of diet [$F(2, 54) = 38.70$, $p<0.0001$], with HFD and KD mice gaining more weight than LF diet mice ($p<0.0001$ for both). There was also a trend of HFD mice gaining more weight than KD mice ($p = 0.093$). A Holm-Šidák's multiple comparisons test revealed that within males, HFD ($p<0.0001$) and KD ($p<0.0001$) mice experienced more weight gain than LF. Within females, HFD ($p<0.0001$) and KD ($p = 0.0017$) mice also experienced significantly more weight gain than LF. There was also a significant main effect of sex [$F(1, 54) = 7.458$, $p = 0.0085$], with males gaining more weight than females. Male KD mice gained more weight than female KD mice ($p = 0.0246$), and male HFD mice gained more weight than female HFD mice ($p = 0.0199$). The diet x sex interaction [$F(2,54) = 1.823$, $p = 0.1714$] was not significant.

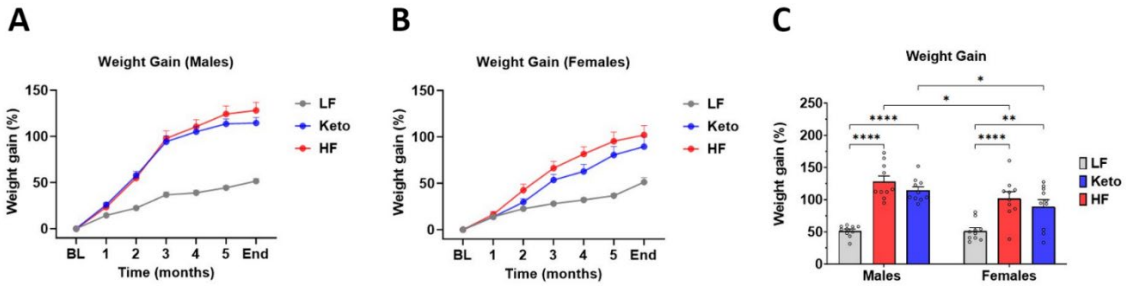

Figure 3. Weight Gain. Figures A & B: Weight Gain Over Time (Males & Females). Weight gain percentage was calculated using final and initial body weights at baseline and each month of the study. **Figure C: Total Weight Gain.** Weight gain percentage, from the beginning to the end of the diet intervention, in male and female mice on HFD, KD, and LF diets. **** $p<0.0001$, ** $p<0.01$, * $p<0.05$.

3.2 Food Intake

Measurements of food intake were acquired biweekly for all cages of mice, and caloric intake was calculated based on mass of food consumed and energy density of each food type. A two-way ANOVA was performed to analyze the effect of diet on food intake, as measured by caloric consumption (kcal) (**Figure 4**). A two-way ANOVA found a significant main effect of diet [$F(2, 6) = 168.5$, $p<0.0001$] and a significant diet x sex interaction [$F(2, 6) = 11.75$, $p = 0.0084$]. The main effect of sex was not significant [$F(1, 6) = 0.051$, $p = 0.8288$]. A Holm-Šidák's multiple comparisons test revealed that, in males, HFD mice consumed a greater amount of kcal than KD ($p = 0.0003$) and LF ($p<0.0001$) mice. Female HFD ($p<0.0001$) and KD ($p<0.0001$) mice consumed a significantly greater amount of kcal compared to LF female mice. Female KD mice had significantly greater average food intake than male KD mice ($p = 0.0065$).

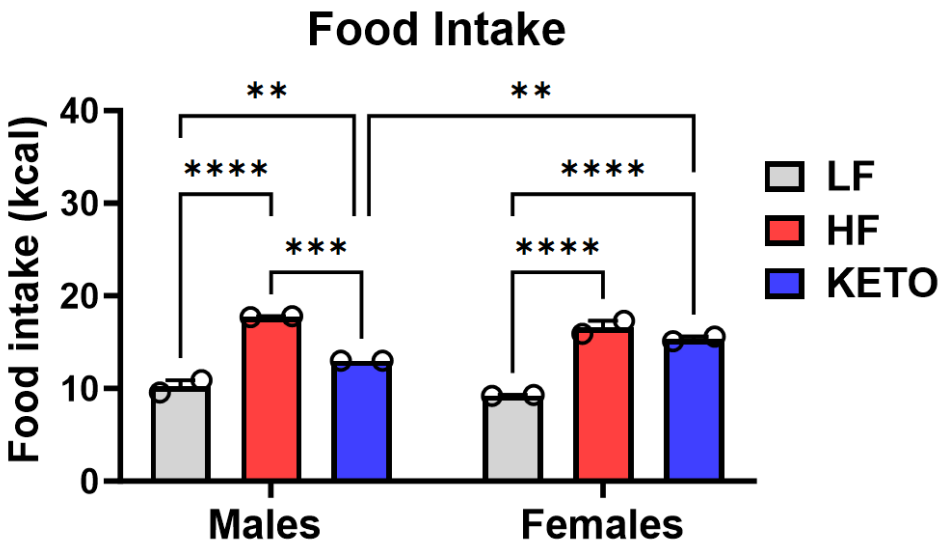

Figure 4. Food Intake. Measurements in kcal of average daily food intake in male and female HFD, KD, and control LF diet mice. **** p<0.0001, *** p<0.001, ** p<0.01.

3.3 Glucose Tolerance

Glucose tolerance testing (GTT) was performed to assess diabetic status in mice. Blood glucose levels were measured prior to glucose injection to retrieve average fasting blood glucose levels (**Figure 5A**). The area under the curve for GTT was calculated as a measure of cumulative glucose exposure (**Figure 5B**). Following the measurement of fasting blood glucose levels, subsequent blood glucose readings (in mg/dL) were recorded at regular time intervals after initial glucose injection (**Figures 5C & 5D**).

A two-way ANOVA found a significant diet x sex interaction [$F(2, 53) = 3.679$, $p = 0.0319$], a significant main effect of diet [$F(2, 53) = 28.58$, $p<0.0001$] and a significant main effect of sex [$F(1, 53) = 36.07$, $p<0.0001$] on fasting blood glucose levels. A Holm-Šidák's multiple comparisons test revealed that, in males, HFD mice exhibited higher

fasting blood glucose than KD ($p = 0.0014$) and LF ($p<0.0001$) mice. Additionally, KD male mice exhibited significantly higher fasting blood glucose than LF male mice ($p = 0.0018$). In females, HFD mice displayed higher levels of fasting blood glucose than LF ($p = 0.0014$) and KD ($p = 0.0012$) mice. Both HFD and KD males displayed significantly higher levels of fasting blood glucose than HFD ($p<0.0001$) and KD ($p<0.0001$) females, respectively.

Glucose tolerance was assessed by calculating glucose area under the curve (AUC) following a glucose tolerance test. A two-way ANOVA found a significant main effect of diet [$F (2, 53) = 30.25$, $p<0.0001$]. A Holm-Šidák's multiple comparisons test revealed that male HFD ($p = 0.0006$) and KD ($p = 0.0006$) mice displayed increased cumulative glucose exposure compared to LF mice. Female mice exhibited similar trends, wherein HFD ($p<0.0001$) and KD ($p<0.0001$) mice showed increased cumulative glucose exposure compared to LF mice. The main effect of sex [$F (1, 53) = 0.9149$, $p = 0.3431$] was not significant.

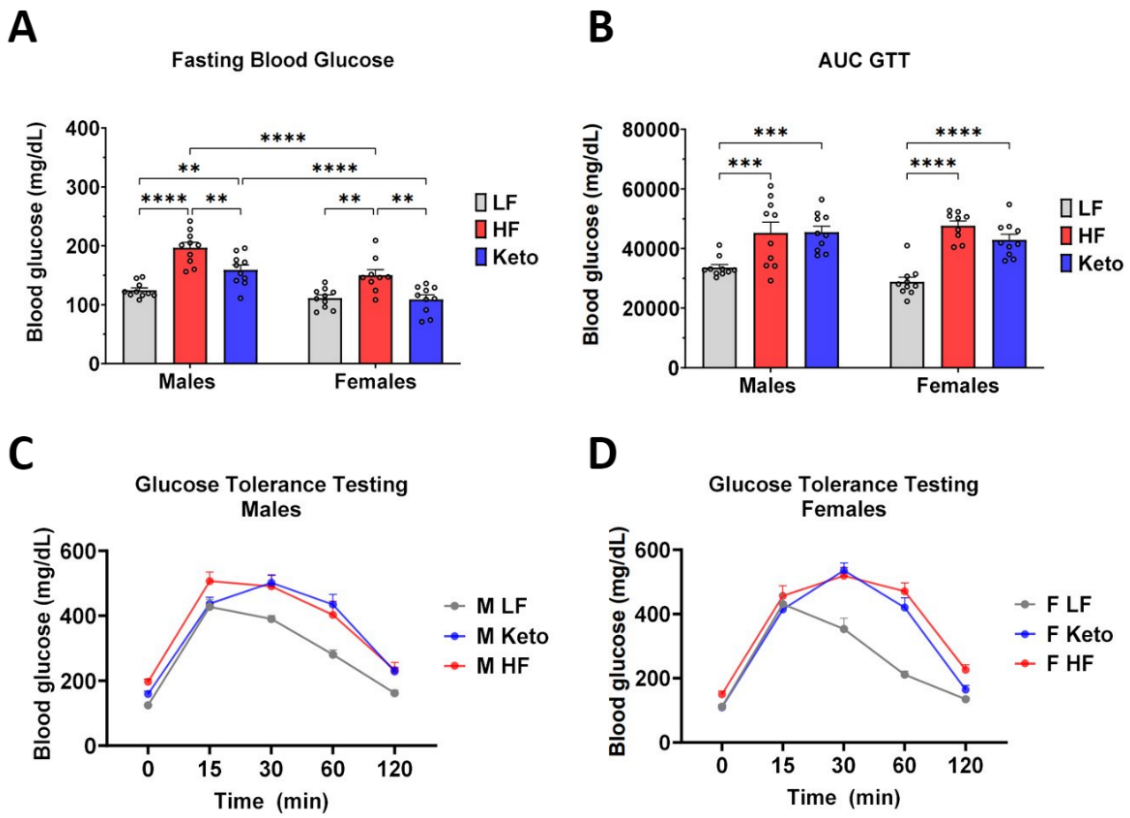

Figure 5. Fasting Blood Glucose and Glucose Tolerance Test. Figure A: Fasting Blood Glucose. Baseline blood glucose levels were measured in mice by glucometer after fasting overnight. **Figure B: Glucose Tolerance Test Area Under the Curve.** Area under the curve was calculated to determine glucose tolerance. **Figures C & D: Blood Glucose Over Time.** Mice underwent overnight fasting, and fasting blood glucose was assessed. Subsequent measurements were taken by glucometer at 15, 30, 60, and 120 minutes post intraperitoneal injection of 2g/kg of glucose for both males (C) and females (D). **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$.

3.4 Ketone Levels

Ketone body levels were examined to characterize the state of ketosis induced by the ketogenic diet. A two-way ANOVA found a significant main effect of diet [$F(2, 53) = 22.49$, $p<0.0001$], wherein KD mice demonstrated greater levels of ketone bodies than HFD and LF (**Figure 6**). A Holm-Šidák's multiple comparisons test revealed that male

KD mice displayed greater ketone body levels than male HFD ($p = 0.0051$) and LF ($p = 0.0051$) mice. Similar trends were exhibited by female mice. Female KD mice displayed greater ketone body levels than female HFD ($p<0.0001$) and LF ($p<0.0001$) mice. The main effects of the diet x sex interaction [$F (2, 53) = 0.9443$, $p=0.3954$] and sex interaction [$F (1, 53) = 0.09623$, $p = 0.7576$] were not significant.

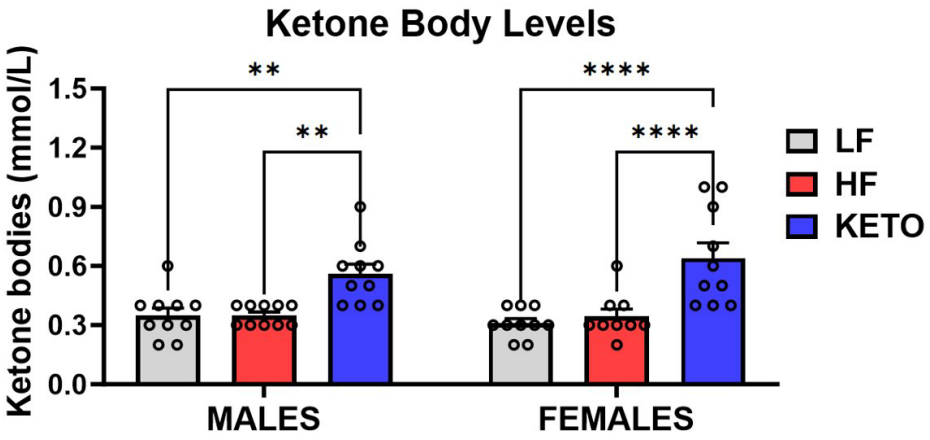

Figure 6. Ketone Body Levels. Ketone body levels were measured with a blood ketone meter from blood collected via tail snip (<1 mm) to assess nutritional ketosis. KD mice exhibited greater ketone body levels in comparison to HFD and LF mice as observed by ketone body levels greater than 0.5 mmol/L. **** $p<0.0001$, ** $p<0.01$.

3.5 Immunofluorescent Detection of Astrocytes

Immunofluorescence with an antibody against GFAP was used to detect the effects of high-fat and ketogenic diets on astrocytes in multiple regions of the hippocampus. Percent area positive for GFAP labeling was assessed in the CA1, CA2, CA3, and dentate gyrus of the dorsal hippocampus (~ −1.2. to ~ −2.06 mm distance from bregma). A two-way ANOVA with follow-up Dunnett's multiple comparisons found that, in males,

neither HFD nor KD affected the percent area covered by GFAP in any subregion of the hippocampus ($p > 0.70$). However, in females, some regions of the hippocampus showed significant differences in percent area covered by GFAP based on the consumed diet (**Figure 7**). In particular, the ketogenic diet caused a reduction in GFAP percent area covered in the CA1 ($p = 0.0287$) and CA2 ($p = 0.0439$) regions of the hippocampus. Similar trends were seen in CA3 ($p = 0.1883$) and DG ($p = 0.1191$), however, these did not reach statistical significance.

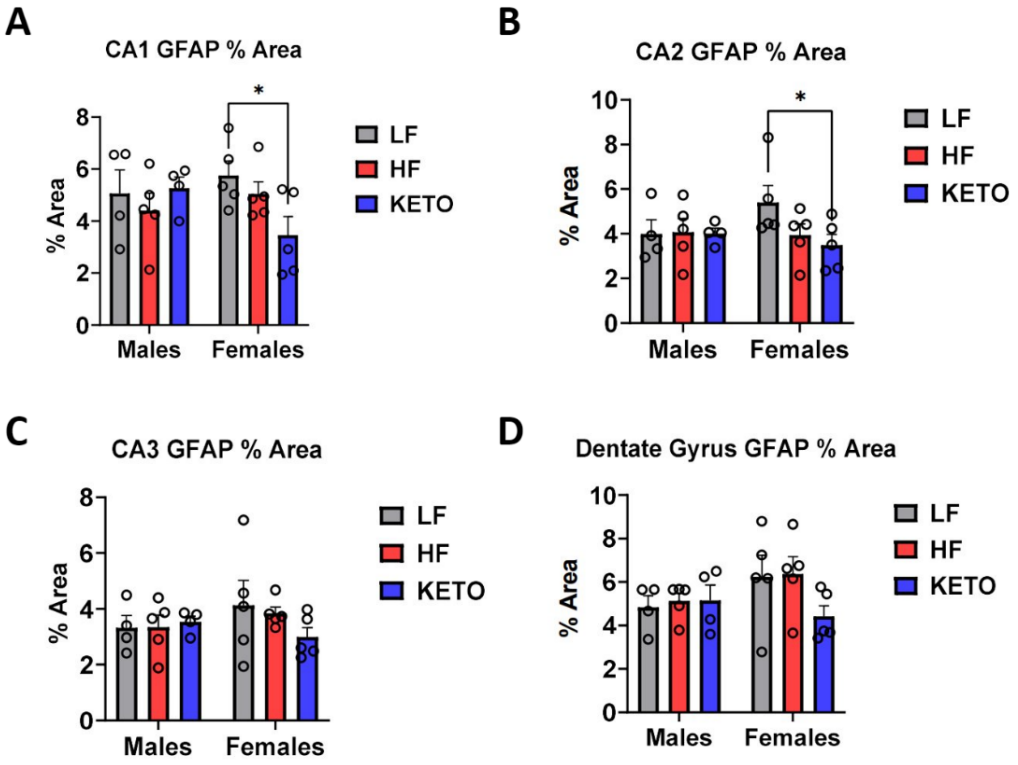

Figure 7: Immunofluorescent Detection of Astrocytes in the Hippocampus. Immunofluorescence was performed using an antibody against GFAP as a marker of astrocytes in several subregions of the hippocampus. Percent area covered by GFAP+ cells in CA1 **(A)**, CA2 **(B)**, CA3 **(C)**, and DG **(D)** of the hippocampus was quantified. * $p<0.05$

Chapter 4

4. Discussion

Research shows that inflammation in the hippocampus, a crucial brain region involved in learning and memory, contributes to neurodegenerative disorders like Alzheimer's and related dementias (Rao et al., 2022). One contributor to neuroinflammation across several brain regions, including the hippocampus, is the chronic consumption of a poor diet and resulting metabolic disease; these are also known to contribute to an increased risk for dementia (Morris et al., 2014). There is evidence to suggest that sex might mediate the relationship between high fat diet and various aspects of neuroinflammation (Abi-Ghanem et al., 2023; Robison et al., 2020), however, less is known about the potential effects of a ketogenic diet on hippocampal inflammatory markers that may occur in a sex-specific manner. This study aimed to investigate the impact of high-fat and ketogenic diets on hippocampal inflammation, focusing on sex-specific differences in reactive astrocytes. Both high-fat and ketogenic diets resulted in metabolic disturbances in males and females, though females were less negatively affected by the ketogenic diet compared to males. The principal finding of this study is that the ketogenic diet resulted in reduced GFAP immunolabeling (reactive astrocyte marker) in the CA1 and CA2 regions of the hippocampus in females only, while similar but non-significant trends were seen in the CA3 and dentate gyrus. Both the high-fat and ketogenic diets had no significant effect on GFAP percent area covered in males. Taken together, these findings suggest that despite the metabolic impairments observed in both sexes, the HF and

ketogenic diets do not seem to induce overt neuroinflammation as assessed by astrocyte reactivity.

The HFD resulted in increased weight gain and glucose intolerance in male and female mice, as reported in previous studies (Robison et al., 2019; Salinero et al., 2018). The ketogenic diet, containing 80% fat and 5% carbohydrates, induced a mild state of ketosis and weight gain in both males and females, though males were susceptible to the obesity-promoting effects compared to females. Mice on both HFD and KD also became prediabetic (glucose-intolerant). These findings may be attributed to the relatively high percentage of carbohydrates in the ketogenic diet regimen used in this study (5% carbohydrates), though this is still generally considered to be within the range of an acceptable ketogenic diet. Many other studies on the effects of ketogenic diets in rodents use a diet formulation with a lower percentage of calories from carbohydrates (~3% down to nearly 0% carbohydrates) (Kennedy et al., 2007; Roberts et al., 2017), which we believed would have less translational relevance.

Astrocytes, the predominant glial cells in the brain, play a pivotal role in regulating glutamatergic transmission as they assume primary responsibility for the majority of glutamate uptake and metabolism (Figley & Stroman, 2011). In response to central nervous system injuries and neurodegeneration, GFAP gene activation plays a critical role in astrocyte cell activation or astrogliosis (Yang & Wang, 2015). In this study, GFAP was utilized as an indicator of astrocytic activation. The ketogenic diet led to decreased GFAP immunolabeling in the CA1 and CA2 regions of the hippocampus in females only; this trend was also seen in the CA3 and DG subregions but did not reach statistical

significance. Previous research indicates that diet-induced astrocyte activation occurs in a brain region-specific manner (Guillemot-Legris et al., 2016). In a study conducted by de Paula et al., GFAP labeling was found to increase in the hippocampus of male Swiss mice on a high-fat diet only after 4 weeks suggesting that astrocyte activation is implicated during the progression of hippocampal dysfunction (2021). Interestingly, our study in C57BL/6 male and female mice on a 22-week HFD found no significant effect of an HFD on GFAP labeling in the hippocampus. Furthermore, a study conducted on middle-aged 37-week-old male Wistar-Kyoto rats fed a 30-week HFD also observed a significant decrease in astrocytes in the substantia nigra and locus coeruleus (Chou et al., 2022). These findings suggest that astrocyte activation occurs in a brain-region and time-dependent manner. Further research with additional markers of neuroinflammation in multiple brain regions and diverse diet durations is necessary to understand mechanisms of the high-fat and ketogenic diets in inflammatory responses in the brain.

The HFD and KD had no significant effect on GFAP percent area covered in males. A similar study performed by Cano et al. found that while a 32-week HFD in C57BL/6J male mice resulted in longer astrocyte projections, it did not trigger astrogliosis in the CA3 region of the hippocampus (2014). Another study conducted on 12-week HFD male C57BL/6 mice also reported finding no significance in hippocampal GFAP+ areas between the Chow and HFD groups. Moreover, the study reported decreased total process lengths and numbers of branch points of the GFAP+ astrocytes in the CA1 and CA3 regions of the HFD male mice (Tsai et al., 2018). Together, these findings suggest diet-induced morphological changes in astrocytes in the hippocampus, which was not assessed here. In a study conducted by Gzielo et al., no significant differences were found for

GFAP expression in hippocampi between ketogenic diet and normal diet Wistar male mice (2019). This might be attributed to the animals being in a non-pathological state, in contrast to the majority of studies in the field (Gzielo et al., 2019). Ultimately, astrogliosis in different regions of the brain in HFD and KD mice arises from a complex interplay between numerous environmental factors in addition to age and sex.

Chapter 5

5. Limitations and Future Directions

Limitations were encountered during the research process. The mice subjected to the ketogenic diet reached a state of mild ketosis and experienced weight gain, contrary to the anticipated outcome of the ketogenic diet. Future studies should consider adjusting the macronutrient ratio for the ketogenic diet to ensure ketosis. Further work is required to investigate differences in other markers of neuroinflammation such as tumor necrosis factor-α, interleukin-1β, interleukin-1β, and nitric oxide across different regions of the brain or other organs including the prefrontal cortex, hypothalamus, and liver. Additional studies should also assess the potential for the ketogenic diet to be used as preventive or adjunctive therapy for various neurodegenerative and neuropsychiatric conditions.

Chapter 6

6. Conclusions

Despite metabolic impairments, the ketogenic diet reduced immunolabeling markers of reactive astrocytes in several subregions of the hippocampus in females. However, the high-fat and ketogenic diets had no significant effect on astrocytic activation in males. Our research demonstrates that diet exerts a complex influence on neuroinflammation. Further research in a sex-specific manner is needed to fully understand the role of the diet in inflammatory responses in the brain and evaluate the efficacy of the ketogenic diet.

Chapter 7

7. References

Abi-Ghanem, C., Salinero, A. E., Kordit, D., Mansour, F. M., Kelly, R. D., Venkataganesh, H., Kyaw, N. R., Gannon, O. J., Riccio, D., Fredman, G., Poitelon, Y., Belin, S., Kopec, A. M., Robison, L. S., & Zuloaga, K. L. (2023). Sex differences in the effects of high fat diet on underlying neuropathology in a mouse model of VCID. *Biology of sex differences, 14*(1), 31. https://doi.org/10.1186/s13293-023-00513-y

Arroyo, C., Hu, F. B., Ryan, L. M., Kawachi, I., Colditz, G. A., Speizer, F. E., & Manson, J. (2004). Depressive symptoms and risk of type 2 diabetes in women. *Diabetes care, 27*(1), 129–133. https://doi.org/10.2337/diacare.27.1.129

Cano, V., Valladolid-Acebes, I., Hernández-Nuño, F., Merino, B., Del Olmo, N., Chowen, J. A., & Ruiz-Gayo, M. (2014). Morphological changes in glial fibrillary acidic protein immunopositive astrocytes in the hippocampus of dietary-induced obese mice. *Neuroreport, 25*(11), 819–822. https://doi.org/10.1097/WNR.0000000000000180

Chatterjee, S., Peters, S. A., Woodward, M., Mejia Arango, S., Batty, G. D., Beckett, N., Beiser, A., Borenstein, A. R., Crane, P. K., Haan, M., Hassing, L. B., Hayden, K. M., Kiyohara, Y., Larson, E. B., Li, C. Y., Ninomiya, T., Ohara, T., Peters, R., Russ, T. C., Seshadri, S., … Huxley, R. R. (2016). Type 2 Diabetes as a Risk Factor for Dementia in Women Compared With Men: A Pooled Analysis of 2.3

Million People Comprising More Than 100,000 Cases of Dementia. *Diabetes care, 39*(2), 300–307. https://doi.org/10.2337/dc15-1588

Chou, M. C., Lee, H. C., Liu, Y. C., Yen, P. S., Liu, C. K., Chen, C. H., Hsieh, T. H., & Chen, S. L. (2022). Long-Term High-Fat Diet Consumption Depletes Glial Cells and Tyrosine Hydroxylase-Containing Neurons in the Brain of Middle-Aged Rats. *Cells, 11*(2), 295. https://doi.org/10.3390/cells11020295

de Paula, G. C., Brunetta, H. S., Engel, D. F., Gaspar, J. M., Velloso, L. A., Engblom, D., de Oliveira, J., & de Bem, A. F. (2021). Hippocampal Function Is Impaired by a Short-Term High-Fat Diet in Mice: Increased Blood-Brain Barrier Permeability and Neuroinflammation as Triggering Events. *Frontiers in neuroscience, 15,* 734158. https://doi.org/10.3389/fnins.2021.734158

DiSabato, D. J., Quan, N., & Godbout, J. P. (2016). Neuroinflammation: the devil is in the details. *Journal of neurochemistry, 139* Suppl 2, 136–153. https://doi.org/10.1111/jnc.13607

Ekdahl, C. T., Claasen, J.-H., Bonde, S., Kokaia, Z., & Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proceedings of the National Academy of Sciences, 100*(23), 13632–13637. https://doi.org/10.1073/pnas.2234031100

Figley, C. R., & Stroman, P. W. (2011). The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional

neuroimaging signals. *The European journal of neuroscience, 33*(4), 577–588. https://doi.org/10.1111/j.1460-9568.2010.07584.x

Galland L. (2010). Diet and inflammation. *Nutrition in clinical practice: official publication of the American Society for Parenteral and Enteral Nutrition, 25*(6), 634–640. https://doi.org/10.1177/0884533610385703

Guillemot-Legris, O., Masquelier, J., Everard, A., Cani, P. D., Alhouayek, M., & Muccioli, G. G. (2016). High-fat diet feeding differentially affects the development of inflammation in the central nervous system. *Journal of neuroinflammation, 13*(1), 206. https://doi.org/10.1186/s12974-016-0666-8

Giugliano, D., Ceriello, A., & Esposito, K. (2006). The effects of diet on inflammation. *Journal of the American College of Cardiology, 48*(4), 677–685. https://doi.org/10.1016/j.jacc.2006.03.052

Gzielo, K., Soltys, Z., Rajfur, Z., & Setkowicz, Z. K. (2019). The Impact of the Ketogenic Diet on Glial Cells Morphology. A Quantitative Morphological Analysis. *Neuroscience,* 413, 239–251. https://doi.org/10.1016/j.neuroscience.2019.06.009

Halbreich, U., & Kahn, L. S. (2007). Atypical depression, somatic depression and anxious depression in women: are they gender-preferred phenotypes?. *Journal of affective disorders, 102*(1-3), 245–258. https://doi.org/10.1016/j.jad.2006.09.023

How to identify activated microglia. Proteintech Group. (n.d.). Retrieved October 6, 2022, from https://www.ptglab.com/news/blog/how-to-identify-activated-microglia/?utm_source=content%2B&utm_medium=email

Jacka, F. N., Pasco, J. A., Mykletun, A., Williams, L. J., Hodge, A. M., O'Reilly, S. L., Nicholson, G. C., Kotowicz, M. A., & Berk, M. (2010). Association of Western and traditional diets with depression and anxiety in women. *The American journal of psychiatry, 167*(3), 305–311. https://doi.org/10.1176/appi.ajp.2009.09060881

Jiang, Z., Yin, X., Wang, M., Chen, T., Wang, Y., Gao, Z., & Wang, Z. (2022). Effects of Ketogenic Diet on Neuroinflammation in Neurodegenerative Diseases. *Aging and disease, 13*(4), 1146–1165. https://doi.org/10.14336/AD.2021.1217

Kang, S. S., Ebbert, M. T. W., Baker, K. E., Cook, C., Wang, X., Sens, J. P., Kocher, J. P., Petrucelli, L., & Fryer, J. D. (2018). Microglial translational profiling reveals a convergent APOE pathway from aging, amyloid, and tau. *The Journal of experimental medicine, 215*(9), 2235–2245. https://doi.org/10.1084/jem.20180653

Kennedy, A. R., Pissios, P., Otu, H., Roberson, R., Xue, B., Asakura, K., Furukawa, N., Marino, F. E., Liu, F. F., Kahn, B. B., Libermann, T. A., & Maratos-Flier, E. (2007). A high-fat, ketogenic diet induces a unique metabolic state in mice. *American journal of physiology. Endocrinology and metabolism, 292*(6), E1724–E1739. https://doi.org/10.1152/ajpendo.00717.2006

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S., Wittchen, H. U., & Kendler, K. S. (1994). Lifetime and 12-month prevalence of

DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Archives of general psychiatry, 51*(1), 8–19. https://doi.org/10.1001/archpsyc.1994.03950010008002

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M., & Lamb, B. T. (2018). Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's & dementia, 4,* 575–590. https://doi.org/10.1016/j.trci.2018.06.014

Ma, Y. L., Xia, J. L., & Gao, X. (2018). Suppressing Irf2bp2 expressions accelerates metabolic syndrome-associated brain injury and hepatic dyslipidemia. *Biochemical and biophysical research communications, 503*(3), 1651–1658. https://doi.org/10.1016/j.bbrc.2018.07.095

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological psychiatry, 65*(9), 732–741. https://doi.org/10.1016/j.biopsych.2008.11.029

Morris, M. C., & Tangney, C. C. (2014). Dietary fat composition and dementia risk. *Neurobiology of aging, 35* Suppl 2, S59–S64. https://doi.org/10.1016/j.neurobiolaging.2014.03.038

Peruga, I., Hartwig, S., Thöne, J., Hovemann, B., Gold, R., Juckel, G., & Linker, R. A. (2011). Inflammation modulates anxiety in an animal model of multiple sclerosis. *Behavioural brain research, 220*(1), 20–29. https://doi.org/10.1016/j.bbr.2011.01.018

Rao, Y. L., Ganaraja, B., Murlimanju, B. V., Joy, T., Krishnamurthy, A., & Agrawal, A. (2022). Hippocampus and its involvement in Alzheimer's disease: a review. *3 Biotech, 12*(2), 55. https://doi.org/10.1007/s13205-022-03123-4

Roberts, M. N., Wallace, M. A., Tomilov, A. A., Zhou, Z., Marcotte, G. R., Tran, D., Perez, G., Gutierrez-Casado, E., Koike, S., Knotts, T. A., Imai, D. M., Griffey, S. M., Kim, K., Hagopian, K., McMackin, M. Z., Haj, F. G., Baar, K., Cortopassi, G. A., Ramsey, J. J., & Lopez-Dominguez, J. A. (2017). A Ketogenic Diet Extends Longevity and Healthspan in Adult Mice. *Cell metabolism, 26*(3), 539–546.e5. https://doi.org/10.1016/j.cmet.2017.08.005

Robison, L. S., Albert, N. M., Camargo, L. A., Anderson, B. M., Salinero, A. E., Riccio, D. A., Abi-Ghanem, C., Gannon, O. J., & Zuloaga, K. L. (2020). High-fat diet-induced obesity causes sex-specific deficits in adult hippocampal neurogenesis in mice. *ENeuro, 7*(1). https://doi.org/10.1523/eneuro.0391-19.2020

Schwarz, J. M., Sholar, P. W., & Bilbo, S. D. (2012). Sex differences in microglial colonization of the developing rat brain. *Journal of neurochemistry, 120*(6), 948–963. https://doi.org/10.1111/j.1471-4159.2011.07630.x

Sofroniew, M.V., Vinters, H.V. Astrocytes: biology and pathology. *Acta Neuropathol 119*, 7–35 (2010). https://doi.org/10.1007/s00401-009-0619-8

Spencer, S. J., D'Angelo, H., Soch, A., Watkins, L. R., Maier, S. F., & Barrientos, R. M. (2017). High-fat diet and aging interact to produce neuroinflammation and impair

hippocampal- and amygdalar-dependent memory. *Neurobiology of aging, 58*, 88–101. https://doi.org/10.1016/j.neurobiolaging.2017.06.014

Thirumangalakudi, L., Prakasam, A., Zhang, R., Bimonte-Nelson, H., Sambamurti, K., Kindy, M. S., & Bhat, N. R. (2008). High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. *Journal of neurochemistry, 106*(1), 475–485. https://doi.org/10.1111/j.1471-4159.2008.05415.x

Tramullas, M., Finger, B. C., Dinan, T. G., & Cryan, J. F. (2016). Obesity Takes Its Toll on Visceral Pain: High-Fat Diet Induces Toll-Like Receptor 4-Dependent Visceral Hypersensitivity. *PloS one, 11*(5), e0155367. https://doi.org/10.1371/journal.pone.0155367

Tsai, S. F., Wu, H. T., Chen, P. C., Chen, Y. W., Yu, M., Wang, T. F., Wu, S. Y., Tzeng, S. F., & Kuo, Y. M. (2018). High-fat diet suppresses the astrocytic process arborization and downregulates the glial glutamate transporters in the hippocampus of mice. *Brain research, 1700*, 66–77. https://doi.org/10.1016/j.brainres.2018.07.017

2020 Alzheimer's disease facts and figures. (2020). *Alzheimer's & dementia: the journal of the Alzheimer's Association,* 10.1002/alz.12068. Advance online publication. https://doi.org/10.1002/alz.12068

Whitmer, R. A., Gunderson, E. P., Quesenberry, C. P., Jr, Zhou, J., & Yaffe, K. (2007). Body mass index in midlife and risk of Alzheimer disease and vascular dementia.

Current Alzheimer research, 4(2), 103–109. https://doi.org/10.2174/156720507780362047

Yang, Z., & Wang, K. K. (2015). Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends in neurosciences, 38*(6), 364–374. https://doi.org/10.1016/j.tins.2015.04.003