A Diet High in Saturated Fat and Sucrose Increases Susceptibility to Aspects of Alzheimer's Disease in Aging Mice

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Abstract

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Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain and is the most common form of dementia in older adults. Prominent behavioral manifestations of AD include memory impairments and decline in other cognitive and non-cognitive domains. Clinical data suggest that the pathophysiological processes of AD begin more than a decade prior to the diagnosis of dementia. Therefore, in addition to increasing age, other factors could impose significant risks for developing AD. Diets rich in saturated fats and sugar (HFS) have been implicated in increasing risks for age-related diseases including AD but studies linking AD to HFS diets have been limited and at times controversial. A model of AAV vector (Aβ42 and pTau) mediated early stage AD in aging C57BL/6 mice was used to determine the impact of a HFS diet on the development of AD-associated cognitive impairment and neuropathology. Mice were started on the HFS diet and administered AAV-AD vectors intravenously at 20 months of age and followed for three months when they were tested for cognitive and non-cognitive behavior, white blood cell counts, and physical performance. Mice were then euthanized, and tissues collected for immunohistochemistry. The HFS diet generally increased cognitive impairment, anxiety, and incoordination and decreased strength in mice with AAV-AD in a sex-dependent manner. Neuronal Aβ42 showed increased density in the presence of AAV-AD and in association with decreased density of microglia and astrocytes. In conclusion, observations from this project suggest that behavioral and performance phenotypes may be associated with AD neuropathology but in a diet and sex dependent manner. The HFS diet is suspected of increasing neuronal susceptibility to AAV-AD in aging C57BL/6 mice, especially females, suggesting that a metabolically stressful diet could potentially increase the risk for neurodegeneration and development of cognitive decline and behavioral dysfunction.

Table of Contents

Acknowledgements
Abstract
Introduction
Materials & Methods
Results
Discussion
Conclusion

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Introduction

The field of geroscience aims to understand, at the cellular and molecular level, the interconnections between aging and disease/disabilities, with a focus on understanding the mechanisms by which aging contributes to most chronic diseases [1,2]. The geroscience concept posits that chronic diseases share the same underlying multiple processes involved in aging [3,4]. Given that the major risk factor for sporadic Alzheimer's disease (AD) is aging, AD is thus a centerpiece for geroscience [5]. AD is a progressive, degenerative disorder of the brain and is the most common form of dementia in older adults [6,7,8]. Prominent behavioral manifestations of AD include memory impairments and decline in other cognitive domains. Clinical data suggest that the pathophysiological processes of AD begin more than a decade prior to the diagnosis of dementia [9,10]. Therefore, in addition to increasing age, other risk factors are genetic, epigenetic, environmental, and lifestyle. Among lifestyle factors, nutrition is a major consideration, especially diets that result in increased weight gain with increasing age.

Different diets have been extensively studied to determine the effects on normal physiological function. Diets rich in saturated fats and sugar (HFS) have been implicated in increasing risks for age-related diseases including AD but studies linking AD to HFS diets have been limited and at times controversial [11,12,13,14]. It is known that obesity and the generation of excess fat from HFS diets can lead to the development of comorbid diseases such as type II diabetes, cardiovascular disease, and nonalcoholic fatty liver disease [15,16,17,18,19]. In addition, HFS diets can lead to advances in disease development for conditions that are commonly associated with normal aging [15,16,18,20,21]. AD is a perfect example of this as it is generally considered to be developing for 10 to 15 years before any clinical signs are apparent [11,16,22]. Because AD is an age-related disease, pathways of aging can be candidates for studying effect of HFS diets. Inflammation is a well-validated pathway associated with aging [23]. Increasing stress levels have negative effects on normal levels of inflammation that are seen in aging populations, and lead to increased rates of illness, autoimmune diseases, and adverse effects from detrimental diet consumption [11,24,25,26]. Increased consumption of fats and sugars can cause recovery timelines for illness, fatigue, and other stress-related responses to increase compared to similar groups that consume a healthier diet [27].

Continued consumption of a diet that is disadvantageous for normal physiological functioning can trigger increased levels of adipocytes tissue macrophages that express high levels of pro-inflammatory cytokines and molecules. Continued consumption of a HFS diet can lead to increased levels of tumor-necrosis factor receptors, interleukin receptors, as well as impairment in metabolism and insulin sensitivity [26]. These changes can lead to increased neurodegeneration and the development of additional low-grade inflammation, causing worse health outcomes for patients with AD [14,22].

As HFS diets are consumed, normal physiological functioning becomes dysregulated leading to impaired functioning for metabolism, insulin reactivity, and energy generation [28]. These factors in turn can lead to increased generation of reactive oxygen species (ROS) which can lead to increased mitochondrial damage, DNA damage, and cell death due to [28]. As normal mitochondrial functioning decreases, communication with apoptotic cells also decreases leading to diseased cells creating pockets of decreased communication for healthy tissues [7,14,22]. Loss

of clearance caused by increased rates of damage in cells can lead to increased severity for disease progression, inflammatory response, and associations with common-aging paradigms [23,29,30,31]. These increases in mitochondrial damage lead to further neurodegeneration, because neurons no longer have the energy output to maintain functionality. As ROS damage to the mitochondrial increases in severity, impacts on organ systems start exhibiting additional stain. For example, as damaged sections for the brain increase, clinical signs start becoming notable such as increasing gaps in memory formation, decreased mobility, and confusion [22,14,7,32,27].

Through the use of an adenovirus vector with pathogenic AD components simulating an early-stage model of AD, the objective of this project was to investigate how AD progression is impacted by a HFS diet, and how a physically stressful diet impacts cognitive and noncognitive impairment. Mice were tested to determine physical and behavioral changes created by changes in diet. Pathological analysis was performed to determine the presence of Aβ42 and pTau in the brain. Observations from the project suggest that a metabolically stressful diet could potentially increase the risk for cognitive decline and behavioral dysfunction in female mice with early stage AD.

Materials & Methods

Mice and Experimental Plan.

Male and female C57BL/6 mice (19 to 20 months of age) were obtained from the National Institute on Aging Aged Rodent Colony and housed 2 to 5 per cage in a specific pathogen free mouse facility at the University of Washington. They were allowed to acclimate to the facility for 14 days before starting the experimental procedures. The room was on a continuous 12-hour light and 12-hour dark cycle with a consistent temperature of 72-74 degrees F. Caging was an Allentown ventilated system. All mice were initially fed standard rodent chow ad lib. An automatic water system delivered water treated by reverse osmosis.

Prior to the start of the experiment, body weight and feed consumption were measured, and tail blood was collected from each mouse. Mice were randomized to a group and injected intravenously via the retro-orbital route with an adeno-associated viral (AAV) vector control (mCherry; SHAM) or experimental (human origin p301L Tau and Aß-42; AAV-AD). Mice were closely monitored three times post-injections. The first time was two hours after receiving the injections, the next was six hours post injection, and then the last time the mice were specifically monitored for adverse reactions after the injection was the morning after the injections to ensure nothing adverse occurred overnight. For the duration of the study mice were monitored daily. After one week, each group of mice were further divided into two groups. One group was fed a HFS diet (male n=30; female n=29) and the other was continued on regular chow diet (male n=29; female n=26).

Feed consumption, body weight and body composition were measured weekly on the same day. Feed was weighed using a digital scale prior to being placed in the cage to determine how much they were receiving, and then taken out and weighed the following week to determine how much was consumed. Feed was checked daily excluding weekends to ensure that sufficient feed was provided. Mice were weighted using a digital scale expressing units in grams. Body fat mass and

lean muscle mass were determined using quantitative magnetic resonance (QMR) imaging [33]. The machine was calibrated prior to each use using a canola oil rod, and then calibrated as needed during the experiment. Body composition was measured three times for each mouse. Body weights taken on the same day were used for fat and lean muscle mass calculation.

Three months after infection with AAV-AD or AAV-SH, mice were euthanized with CO_2 as the primary method, and then cervical dislocation as the secondary euthanasia method. Target tissues were collected for formalin fixation and paraffin block formation or freezing in a -80 freezer for cellular and molecular analyses. These tissues included the brain, heart/lung, liver/kidney, spleen/pancreas, smooth muscle, as well as white and brown adipose tissue.

AAV Vectors.

An adeno-associated viral (AAV) vector was used to mimic human Alzheimer's disease (AD) through expression of amyloid beta 42 (Aß-42) and phosphorylated tau (pTau). The AAV vector was a non-replicating parvovirus with a capsid PHP.eB with an affinity for neuronal cells. To ensure access to the brain, this virus was engineered to cross the blood-brain barrier (BBB) through the LY6A receptor. C57BL/6 mice have increased numbers of LY6A receptors and thus, it is likely that our engineered AAV vector will reach the brain in this mouse strain. Three AAV vectors were created. One carried the Aß-42 sequence, another carried the pTau sequence, and a third carried an empty sham sequence called mCherry. Each vector was constructed with a different promoter to enhance targeting. The Aß-42 vector had an EF-1α promoter, pTau a CAG promoter, and mCherry an hSyn promoter. Each vector target a different promotor to ensure individual expression of the target vector. The EF-1α promotor is one of the strongest promotors for various mammalian cell lines, while the CAG promotor drives strong gene expression. The difference in targets is primarily driven to allow for cell expression of Aß-42 and Tau to be more visible. Mice were injected with $5x10^{11}$ genomic copies of Aß-42 and pTau each or $1x10^{12}$ genomic copies of mCherry via retro-orbital injection. Aß-42 and pTau were mixed together to limit injections necessary for the experiment. Each vector was prepared in 80 µL of sterile saline per mouse.

Diets.

Mice were fed one of two diets that were randomly assigned to cohort cages after injection of the AAV vectors. The high fat and sucrose diet (HFS) was obtained from Bioserv (S1850 Mouse Diet, Paste, Gamma Irradiated) and contained lard, sucrose, casein, maltodextrin, and a vitamin-mineral mixture (Table 1). The regular chow (RC) diet was obtained from Picolab (Rodent Diet 20, 5053, Irradiated) and contained corn, soybean, wheat, fish meal, and a vitamin-mineral mixture. Feed consumption was averaged across the cage for the number of mice in the cage. Mice were fed ad lib for the duration of the experiment.

Diet	Nutrients	Kcal/g
HFS	Protein	0.82
	Carbohydrate	1.48
	Fat	3.24
RC	Protein	0.76
	Carbohydrate	1.91
	Fat	0.41

Table 1. Nutritional composition of the diets.

Cognitive and Noncognitive Behavioral Assays.

The spatial navigation task (Box maze) is a behavioral assessment that looks at short-term memory processing depending on the experimental design [37,38]. The task uses a primary chamber with 7 blocked holes and one open escape hole that leads to a non-stressful rest chamber. During trials for the mice, the procedure room was kept pitch black with an overhead light shining directly into the primary chamber to prompt the mouse to escape. Mice completed a total of 4 trials with each trial having a total time of 120 seconds. Escape (primary latency) was recorded when the mouse had all 4 paws in the escape hole leading to the rest chamber. If the mouse failed to escape during that time, it was shown the escape hole and prompted to escape the primary chamber. Mice were allowed to rest in the rest chamber for 30 seconds before being placed back in their home cage. The primary and rest chamber were cleaned with 70% ethanol between each trial to eliminate odors that could interfere with the test, and the primary escape hole was cleaned with soap and water between cages. Escape attempts using the dummy holes, and rearing were also recorded.

The Y maze analyzes short-term memory in mice to determine cognitive impairment and abnormal patterns of behavior [39]. Mice were brought to the procedure room to acclimate for 30 minutes before running the experiment. The Y maze is placed on a platform with arms designated as left, right, and center. The center arm is positioned facing the scientist recording the experiment in a dimmed room, and each mouse starts facing the wall of the right arm of the maze. Each mouse is run for one 5 minute trial. The Y maze is cleaned between each mouse with 70% ethanol. Data collected for each trial are the number of rearings (defined as front feet leaving the ground), arm entries, and time spent in each arm. Data from the Y Maze were calculated as percent alternations, rearing patterns, time spent in each arm, and the number of alternations [39].

$$Percent\ Alternation\ = \frac{Total\ number\ of\ alternations}{Number\ of\ arms\ entered}\ x\ 100\%$$

The Photobeam Activity System (PAS) is an open field assessment system that uses photobeams that detect lateral and vertical movements of a mouse in a chamber that can then be analyzed to determine changes in normal behavioral functioning [40]. Using associated computer programs, individual mouse data was collected and processed in a variety of formats to express horizontal and vertical movement for behavioral analysis. Mice were brought to a procedure room and acclimated for 30 minutes. PAS was performed in the same procedure room for each cohort of mice, with the room being semi-dark while the mice were running the experiment. The chamber used was cleaned with 70% ethanol to eliminate odors and sterilize the chamber between each mouse. Each mouse performed one trial for a total time of 5 minutes.

Performance tests

Rotarod is a physical performance assay that looks at coordination and balance [41]. Mice were acclimated in the procedure room for 30 minutes prior to starting the experiment. Mice were trained on the rotarod the day before actually running the experiment. Training included setting up the procedural room in the same capacity as it would look when the experiment was actually

run. Mice were run a total of two times, and allowed to run until they fell of the rotarod. A run wouldn't count if a mouse fell off prior to the machine starting. Up to four mice were placed on the rotarod at a time, with a trial considered successful if they were able to remain on the rotating rod for at least 20 seconds. If they failed to make it to the 20 second mark it was considered a failed run. Each mouse performed 3 trials with the ability to perform an additional two trials if they had run failures. After a trial was complete mice were allowed to rest for at least 15 minutes before starting their next trial. Between trials the rotarod was cleaned with a chlorine-based disinfectant, Clidox (1:18:1). Data were analyzed for time spent on the rotarod against body weight.

Grip strength is a physical performance assay looking at the total pull force of the mouse [42]. Mice were brought to the procedure room to acclimate for 30 minutes. Mice were tested in a series of 5 experiments, and forelimb grip strength was the only grip that was tested. If a mouse was able to get a hindlimb on the grip bar the test was reset. The grip strength machine was cleaned with 20% ethanol between each mouse.

Immunohistochemistry (IHC) and Digital Imaging.

Brains from mice were formalin fixed for 72 hours before being embedded into paraffin blocks for further analysis. Six brains were selected from each of the 8 cohorts of mice (female AAV-AD HFS, female SHAM HFS, female AAV-AD RC, female SHAM RC, male AAV-AD HFS, male SHAM HFS, male AAV-AD RC, male SHAM RC). Blocks were sectioned at 4 µm per slice and placed on a charged glass slide. Slides were allowed to dry for at least 24 hours before performing immunohistochemistry (IHC). IHC was performed using kits from Abcam (HRP/DAB Rabbit Kit: ab64261, HRP/DAB Mouse Kit: ab64259). Day 1 starts with rehydration of the histology slides in a stepwise series running from xylene, 100% ethanol, 95% ethanol, 70% ethanol, and deionized water for a total running time of 70 minutes. Slides were then placed in a hot water bath with an antigen retrieval consisting of either Sodium Citrate (pH 6.0) or Tris-EDTA (pH 9.0) for 20 minutes per manufacturing recommendation or titration analysis from in-lab analysis (See table 2). Slides were placed in a cool water bath for 10 minutes, and then washed in wash buffer (1x TBST; Tris-buffered saline with Tween20) for an additional 10 minutes. A hydrophobic barrier pen was used to outline the tissue, and then a hydrogen peroxide buffer block was applied to the tissue. Slides were washed in wash buffer for 20 minutes, before buffer was replaced. Slides then had a protein block applied that reduces non-specific binding of the target antibody, and then were washed for 20 minutes as previously described. Antibody was diluted in the wash buffer at previously proven titration amounts (See table 3). Slides incubated with the diluted antibody for 24 hours in a 4°C fridge. Day 2 starts with a 20 minute wash in wash buffer. Biotin, a generic secondary antibody specific to the species that the primary antibody binds to, is placed on the slides, and then washed off for 20 minutes as previously described. Streptavidin was then applied to the slides, and then washed off with wash buffer for 20 minutes. DAB was then applied in a fume hood for 1 minute. Slides were then placed in a 50/50 mix of wash buffer and deionized water for 5 minutes, and then in deionized water for 5 minutes. Slides were then dehydrated in a reverse-series for 2 minutes per solution going from 70% ethanol, 95% ethanol, 100% ethanol, and then xylene. Once the dehydration step completed the series, coverslips were placed on the slides using a toluene solution. Slides dried for 24 hours before having photos taken with a confocal microscope for further analysis and imaging.

Antibody	Day	Step	Change from Manufacturer Protocol
HT7	1	Protein Block	Additional Mouse-on-Mouse protein block; 30 minutes
	1	Hydrogen Peroxide	20 Minutes
	1	Protein Block	20 Minutes
	2	Anti-Goat	30 Minutes
	2	Anti-Streptavidin (Secondary Antibody)	30 Minutes

Table 2. Titrations different from manufacturers recommendation.

Antibody	Company	Inventory Code	Dilution
H31L21	Invitrogen	700254	1/500
HT7	Invitrogen	MN1000B	1/100
GFAP	Invitrogen	PA1-10019	1/2000
IBA1	Abcam	ab178846	1/1000

Table 3. Antibodies used for IHC in select tissue sections.

Photos of the brain were taken with target areas being frontal cortex and hippocampus with possible associations with the development of AD. Images were taken using a confocal microscope on 20x magnification with a digital imaging software package (Nikon Instruments Inc.). Images were then uploaded to an open-source image processing software, ImageJ (https://imagej.net/ij/ , version 1.54h). Background subtraction was performed before adjusting the contrast to show only positive DAB staining on the images to determine the percent of the image that had positive DAB staining. Threshold in ImageJ was then automatically computed to determine the percent density of DAB staining in the previously specified sections of brain tissue.

Statistical Analysis.

Statistical analysis was performed for all of the experiments, with analysis dependent on which statistical measure would fit the experiment best. One-way ANOVA was used to analyze the behavioral and performance data. This method was used to determine effects of a dependent variable (AAV-AD) against dependent variables (control injections and diet). For the performance tests, the average of each mouse trial was used for analysis. IHC experiments were analyzed using the Wilcoxon-Sign Rank test. P-values were expressed underneath each figure legend.

Results

The HFS diet increased body weight and fat mass equally in AAV-AD and AAV-Sham mice compared to mice fed regular chow.

Weight from baseline was calculated to determine how body weight changed over the course of the experiment (Figure 1). Across the 12 weeks of the study, both male and female HFS fed mice showed increased body weight compared to the RC fed mice that stayed the same weight as the beginning of the study. Changes in fat mass showed no difference between the mice fed the RC diet for both male and female mice.

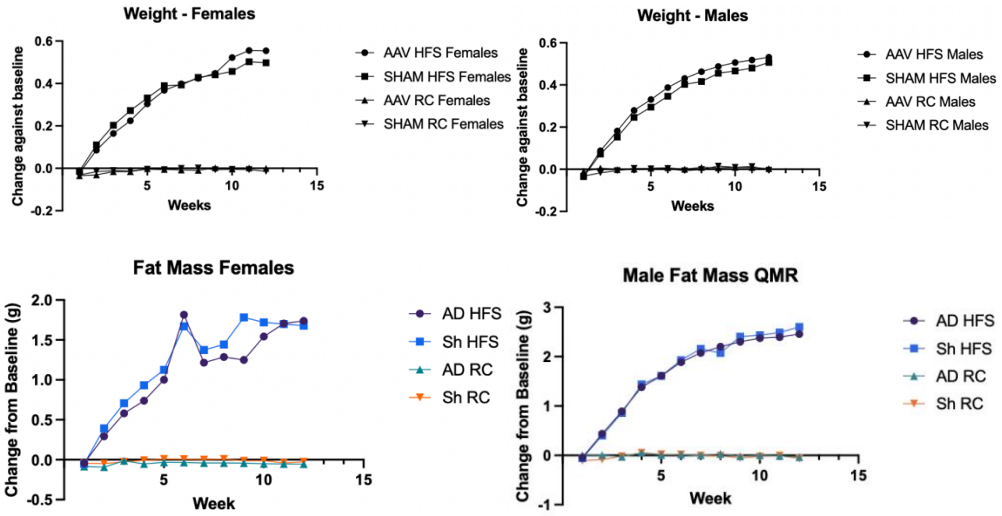

Figure 1. Weight. Percent change in body weight is shown in the top two graphs for 12 weeks for both AAV-AD and AAV-Sham males and females fed the high fat sugar (HFS) diet compared to AAV-AD and AAV-Sham males and females fed regular chow (RC) diet. N=10-19 per cohort. **Body fat mass.** Body fat mass was measured by quantitative magnetic resonance (QMR) imaging and is shown in the bottom two graphs. The pattern for changes and cohort numbers were the same as for body weight.

The HFS diet increased cognitive impairment and anxiety in mice infected with AAV-AD in a sex-dependent manner.

Female mice with AAV-AD on regular chow (RC) diet were not cognitively impaired compared to female mice with AAV-Sham (Sh) on RC diet when tested in the spatial navigation task (Figure 2). However, female mice with AAV-AD on the high fat sugar (HFS) diet were slower to find the escape hole in trial 3 compared to female mice with AAV-AD fed RC diet. Because there was no difference in finding the escape hole between AAV-Sh mice fed either diet, the extended delay in latency seen in AAV-AD females fed the HFS diet suggests the HFS diet may be associated with cognitive impairment in the presence of AAV-AD. In contrast, there was no significant difference in males with AAV-AD on the RC diet and males with AAV-Sh on the RC diet as well as males with AAV-AD on the HFS diet and males on the AAV-AD fed the RC diet suggesting males were less susceptible to developing cognitive impairment in the presence of AAV-AD. There was no significant difference between any of the groups for both male and female mice in the Y-maze percent alternation data (data not shown).

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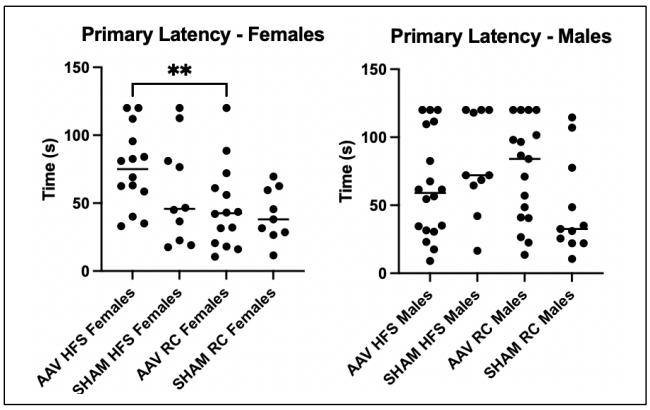

Figure 2. Primary latency time to find an escape hole was measured by a spatial navigation task and is shown for both females and males. Escape times look at differences in trials 2 and 3 to determine learning curve changes. The lower the time, the faster the mice learned to escape. Significance was plotted using one-way ANOVA for comparison between trials 2 and 3 latency times. **$p \leq 0.05$. N=10-19 mice per cohort.

The photobeam activity system (PAS), also referred to as the open field test, was used to assess anxiety in this study. In new environments normal responses in mice are to decrease distance traveled to assess the environment, reduce travel speed to be more aware of predators, and increase freezing action. Freezing in mice was defined as stopping for longer than 3 seconds during the five minute testing period. Lack of freezing for mice showed increased rates of anxiety, as freezing is an innate process that allows mice to assess the environment they are located in for danger and threats to their well-being. The time of each freezing event was then added as a total freezing duration for each mouse. Female mice showed no significant differences in freezing durations (Figure 3). Male AAV injected HFS fed mice showed significant increases against AAV RC mice. Additionally, SHAM injected HFS fed mice had lower periods of freeze durations compared to the AAV injected HFS fed mice. SHAM injected RC fed mice showed increased freeze durations compared to the AAV injected RC mice. There was no significant difference in distance traveled for the female mice regardless of diet or AAV vector. Male mice with AAV-AD fed RC diet showed increased travel distance compared to males with AAV-Sh fed RC diet. However, males with AAV-AD fed the HFS diet had similar travel distances as males with AAV-AD fed RC diet suggesting that the HFS diet did not affect travel distance.

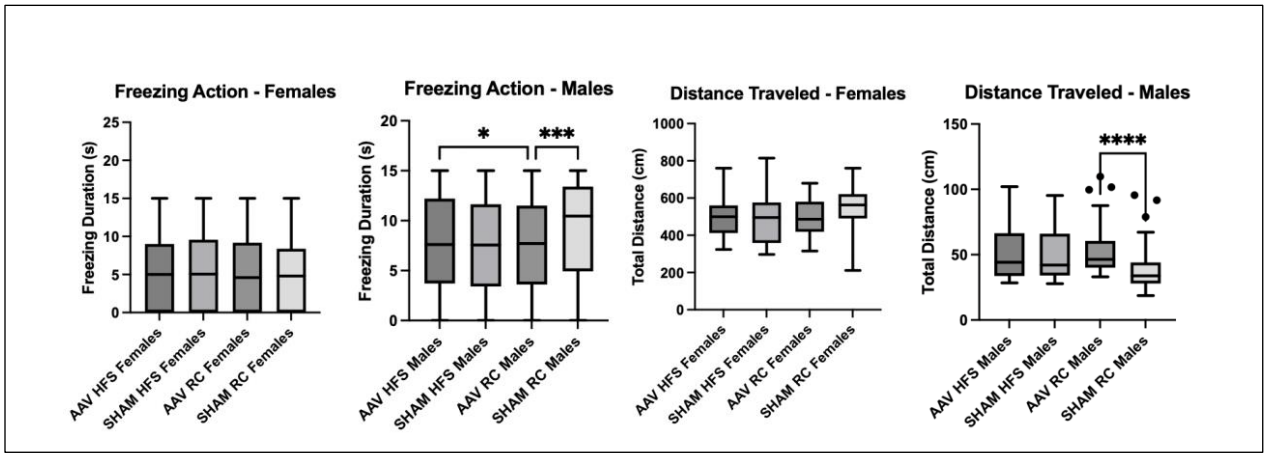

Figure 3. Anxiety-like behavior was assessed in mice using the open field photobeam activity system (PAS). Freezing time is shown for females and males in the first two graphs. Total distance traveled over 5 minutes is shown for females and males in the last two graphs. Significance was plotted using two-way ANOVA. **, ***, ****p≤0.05, 0.01, and 0.005 respectively. N=10-19 mice per cohort.

Mice with AAV-AD fed the HFS diet showed incoordination and decreased strength.
Rotarod measures an animal's ability to remain on a rotating rod testing their coordination. The time that mice were able to maintain their balance on the rotating rod was divided by their weight taken prior to the start of the experiment. There was no difference in the ability of males and females with AAV-AD fed RC to stay on the rotating rod compared to mice with AAV-Sh fed RC (Figure 4). However, both males and females with AAV-AD fed the HFS diet were unable to stay on the rod as long as mice with AAV-AD fed RC diet suggesting the HFS diet might be associated with dysfunctional coordination in mice with AAV-AD.

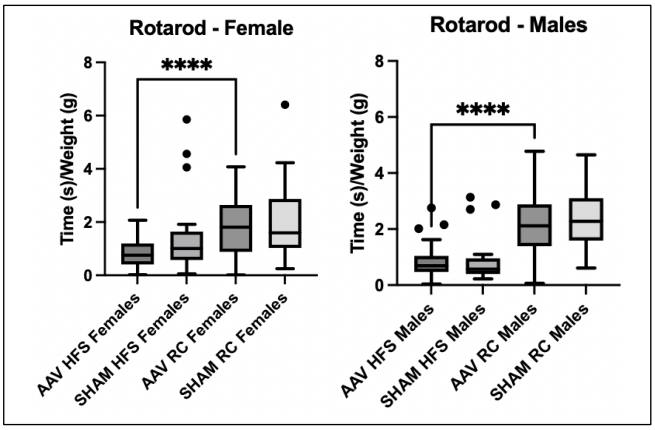

Figure 4. Coordination and balance were assessed in mice using a rotarod apparatus. The amount of time staying on a rotating rod is shown for females in the first graph and males in the second graph. Significance was plotted using one-way ANOVA.****p=<0.0001. N=10-19 mice for each cohort.

Grip strength was analyzed using forelimb strength for both male and female mice. Prior to performing the experiment, mice were weighed. Forelimb force for each mouse was divided against their body weight to get force per gram. Female mice with AAV-AD on regular chow (RC) did not have a decrease in muscle strength compared to female mice with AAV-SH on RC diet (Figure 5). However, female mice with AAV-AD on the HFS diet had weaker grip strength compared to female mice with AAV-AD mice fed RC. Because there was no difference in grip strength between AAV-Sh mice fed either diet, the decreased grip strength seen in AAV-AD females fed the HFS diet suggests the HFS diet may be associated with muscle weakness in the presence of AAV-AD. Males with AAV-AD on RC diet also did not show any differences in grip strength compared to male mice with AAV-Sh fed RC. However, in contrast to females, males with AAV-AD fed the HFS diet did not show any differences in grip strength compared to male mice with AAV-AD fed RC diet, suggesting that the HFS diet was probably not linked to muscle strength in males with AAV-AD.

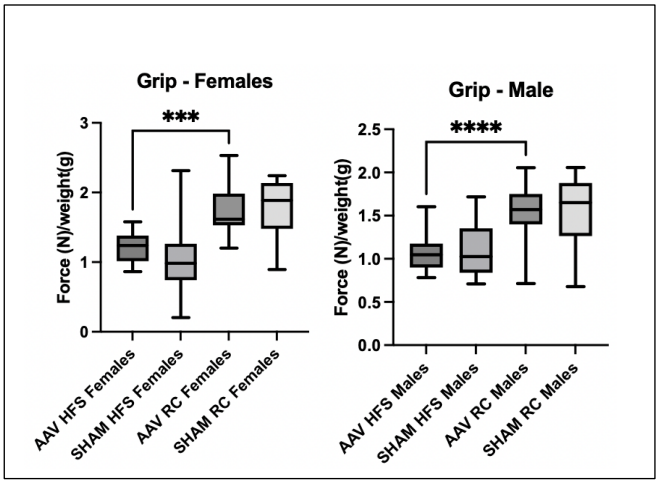

Figure 5. Muscle strength was measured using a paw grip meter. Force in grams standardized by body weight is shown for females in the first graph and males in the second graph. Significance was plotted using one-way ANOVA.****p=<0.0001. N=10-19 mice for each cohort.

Aβ42 and phosphorylated tau showed increased density in the hippocampus of AAV-AD female mice.

Females with AAV-AD fed the RC diet had similar density of Aβ42 in the hippocampus compared to females with AAV-Sh fed the RC diet (Figure 6). Females with AAV-AD fed the HFS diet had increased density of Aβ42 in the hippocampus, but not the frontal cortex, compared to female mice with AAV-AD fed the RC diet, suggesting the HFS diet may be associated with expression in the hippocampus in the presence of AAV-AD. However, the higher than expected density in AAV-Sh mice detracts from this observation. Males with AAV-AD fed the HFS diet showed a nonsignificant trend of increased density of Aβ42 in the hippocampus and frontal cortex compared to male mice with AAV-AD fed the RC diet. These observations suggest that the HFS diet may be associated with increased susceptibility of female mice to expression of Aβ42 in the hippocampus. Similar conclusions cannot be made for males.

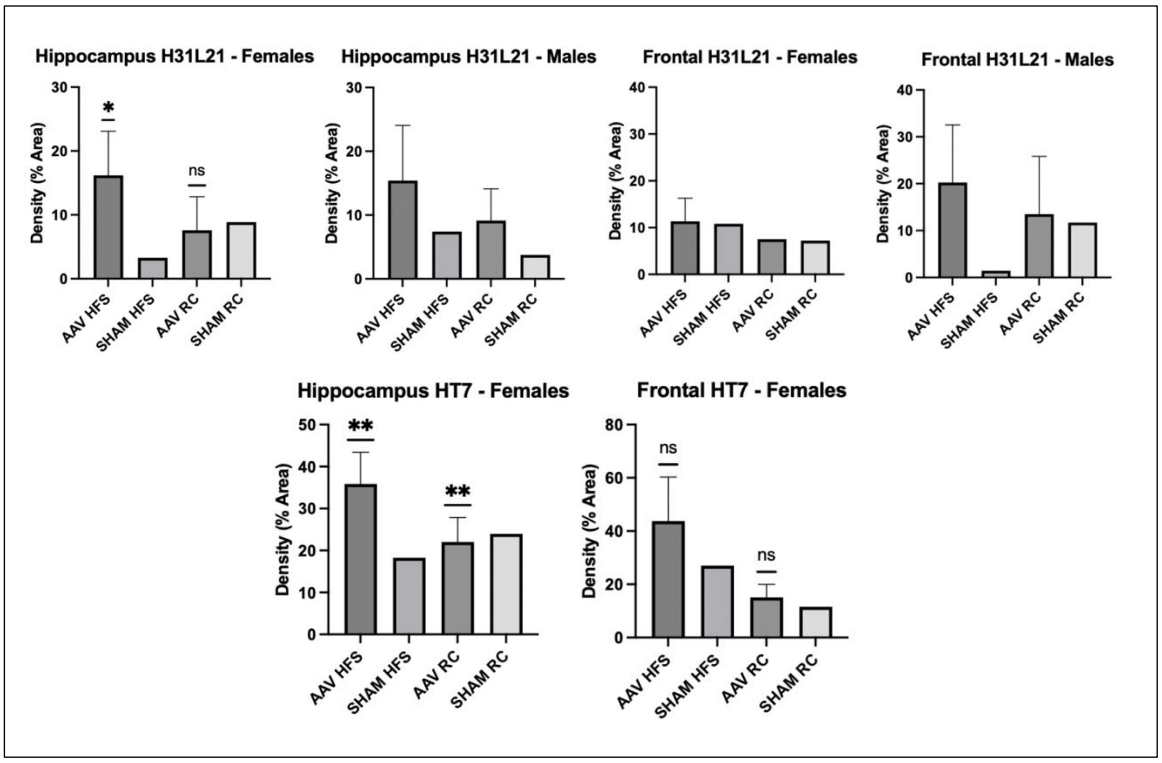

Figure 6. Expression of Aβ42 and pTau in the hippocampus and frontal cortex was determined using immunohistochemistry and antibody H31L21 specific for human Aβ42 and antibody HT7 specific for human Tau. The top row shows staining density of H31L21 in the hippocampus of females and males and in the frontal cortex for females and males. The bottom row shows staining density of HT7 in the hippocampus and frontal cortex for females. Consistent data for males was not generated for HT7 staining. Statistical significance was performed using the Wilcoxon-sign rank test. *, **p≤0.05, 0.01. N= 10-19 mice per cohort.

The HFS diet may have had a suppressive effect on microglia and astrocytes in the presence of AAV-AD in the hippocampus.

IBA1 is a marker for microglia, which are immune cells in the brain. Females with AAV-AD fed the HFS diet had decreased density of IBA1 in the hippocampus compared to females with AAV-AD fed the RC diet (Figure 7), suggesting AAV-AD may be associated with a decreased presence of microglia at least in the hippocampus. However, the high background in AAV-Sh mice fed either diet preclude any definitive conclusions. Similar high background staining was observed for males. Staining for astrocytes using a GFAP-specific antibody showed that females with AAV-AD fed the HFS diet had decreased density for GFAP in the hippocampus but increased density in the frontal cortex compared to females with AAV-AD fed the RC diet, Similar results were seen in the hippocampus but not the frontal cortex of males. As with the IBA1 stain, high backgrounds were seen in male and female AAV-Sh mice fed either diet precluding any definitive conclusions.

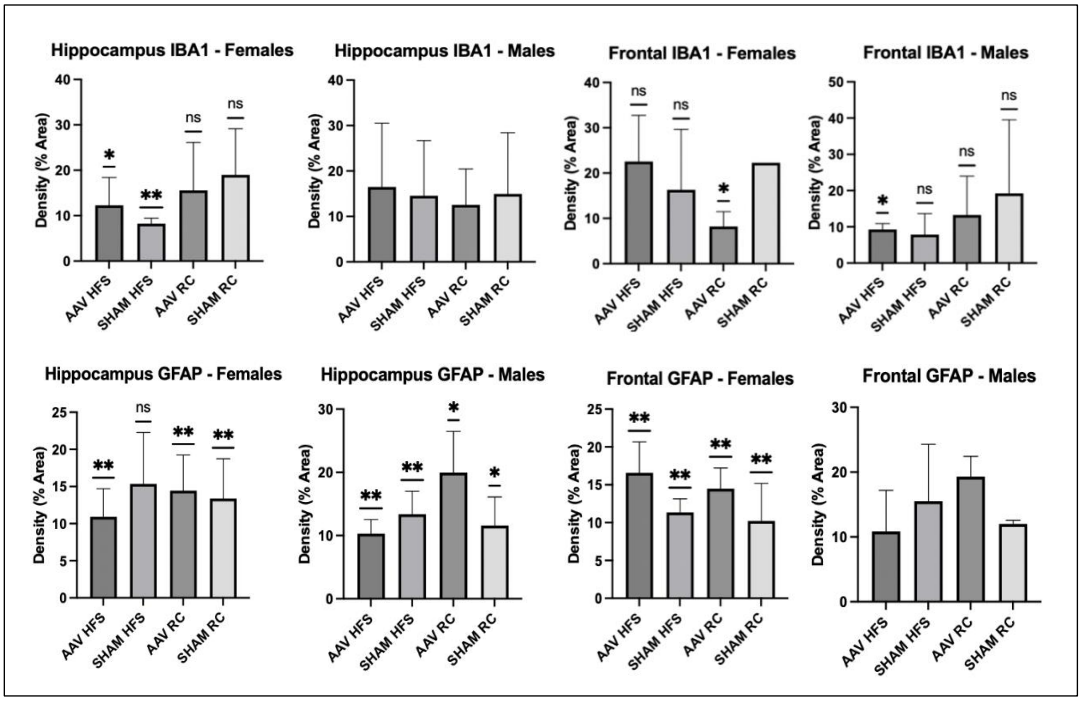

Figure 7. Expression of IBA1, a marker for microglia, and GFAP, a marker for astrocytes, in the hippocampus and frontal cortex was determined using immunohistochemistry and an antibody specific for microglial IBA1 and an antibody specific for astrocytic GFAP. The top row shows staining density using Image J of IBA1 in the hippocampus and frontal cortex of females and males. The bottom row shows staining density using Image J of GFAP in the hippocampus and frontal cortex of females and males. Statistical significance was performed with the Wilcoxon-sign rank test. *, **$p \leq 0.05, 0.01$. N= 10-19 mice per cohort.

Discussion

This project sought to determine if a diet high in saturated fat and table sugar could have effects on behavior and neuropathology in aging mice with AAV-mediated Alzheimer's disease. The AAV-AD model has been previously characterized as an early stage of AD with relatively mild behavior changes and evidence of mild to moderate neuropathology that are sex dependent (46). Since AD is an age-related disease, the AAV-AD model in aging mice was the logical choice to help address the objectives of the project. Therefore, it was critically important to be able to duplicate the characteristics of early stage disease previously reported by the Jackson paper (46). In this regard, cognitive and noncognitive and performance phenotypes showing increased cognitive impairment, increased anxiety, decreased coordination, and decreased muscle strength in female and male mice with AAV-AD fed the high fat and sucrose diet were consistent with an association with the presence of AAV-AD. In order to determine beyond a reasonable doubt that the model was being driven by the neuronal presence of Aβ42 and phosphorylated tau (pTau), evidence of expression of these two pathogenic components in areas of the brain was necessary. Female mice with AAV-AD fed the HFS diet showed higher density of Aβ42 in the hippocampus, while males with AAV-AD on the HFS diet generally only showed a trend. This may have been due to high background staining in sham mice. The bottom line is that the model of early stage AD was only tentatively confirmed in females and only trending for males.

A second important issue to establish was the absence of any influence of the AAV-AD and AAV-Sham vectors on food intake. There was a significant increase in body weight and fat mass for both male and female groups that consumed the HFS diet compared to mice fed the RC diet, as expected, but no differences within each group fed the HFS diet or the RC diet.

Cognitive assessments were performed using a spatial navigation task and the Y maze. These two behavioral tests have been well documented to show cognitive impairment in mice [39]. Female cohorts on average showed faster learning times for box maze compared to the male cohorts. AAV-Sham in both sexes fed the RC diet had faster escape times than AAV-AD mice fed the RC diet, which suggests that the influence of the HFS diet had a negative effect on cognitive abilities of older animals with AAV-AD. There was little difference in learning ability between the HFS fed female mice, while AAV HFS diet-fed males with AAV-AD showed increased latency compared to the AAV-AD RC diet fed males. AD-HFS females also showed increased learning time to escape from the box maze compared to the AD-RC females. Male AD-RC showed increased learning times for the spatial navigation task compared to their Sh-RC cohort.

Noncognitive behavior was tested using the open field photobeam activity system (PAS). Primary analysis showed that male and female mice had sex differences in freezing action and distance traveled. Typical function for mice show hesitation in movement patterns, referred to as freezing, in an unfamiliar environment to check for dangers that could lead to harm for the animal [29,40]. Freezing action was not significant for female mice, but was for male mice. AAV-Sh RC males had the greatest levels of freezing time compared to the AAV-AD males showing normal safety measures that are innate to mice. AAV-AD HFS males saw decreased freezing times compared to the AAV-AD RC males. Associations with freezing time showed that a HFS diet decreased freezing time, and was associated with adverse neurological functioning, because stress and anxiety responses were no longer associated with normal behaviors. Distance traveled showed that AAV-Sh male mice traveled less than AAV-AD RC mice, suggesting that AAV-Sh mice fed the RC diet have better associations with decision-making in a new environment. There was no significant difference in male mice fed the HFS diet. Changes in freezing action and distance traveled can show increased rates of anxiety associated with parameters that affect normal behavior in both male and female mice. Overall, male mice showed differences in these metrics of behavior where female mice showed no difference.

Performance tests measured incoordination and muscle strength. Female mice with AAV-AD fed the RC diet showed decreased coordination on the rotarod compared to the AAV-Sham mice. When comparing differences in AAV-AD females, the mice that consumed the HFS diet showed decreases in coordination and balance on the rotarod compared to the RC diet-fed females that received the same AAV vector. AAV-AD males fed the HFS diet had significant decreases in their ability to maintain their balance on the rotarod compared to their RC diet cohort. These sex differences suggest that females are more impacted in their ability to maintain their physical abilities compared to male mice. Changes in muscle strength also showed significant differences. Females showed the same patterns with grip strength that were seen with the rotarod suggesting that the HFS diet impacted physical strength compared to female mice fed the regular RC diet. Male mice also showed similar results with grip strength that were seen with rotarod, suggesting that adverse diets have less of an impact on their physical abilities (42).

Nonneuronal cells are critically important for maintaining brain health with increasing age and instilling resilience to age-related diseases such as AD. Unfortunately, the high background staining seen with antibodies specific for IBA1 and GFAP used for this project preclude any definitive conclusions.

In summary, observations from this project showed there were sex differences associated with the consumption of a HFS diet in relation to AAV-AD neurodegeneration and mild cognitive impairment and dysfunctional behavior. Female mice with AAV-AD fed the HFS diet seemed to be more susceptible than male mice to decreased cognitive and noncognitive abilities, and decreased physical performance. Overall, the HFS diet increased some aspects of neuronal susceptibility to AAV-AD in aging mice suggesting that a metabolically stressful diet might be associated with an increased risk for neurodegeneration and development of cognitive decline and behavioral dysfunction.

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