HIGH FAT DIET CONSUMPTION AND ITS ASSOCIATION WITH PARENCHYMAL ARTERIOLE STRUCTURE AND COGNITION

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ABSTRACT

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Obesity/overweight has been identified as a major risk factor for the development of vascular cognitive impairment with dementia; a form of dementia with a vascular origin. Obesity at young age increases the risk of cognitive impairment in later life that is associated with cerebral hypoperfusion. Reductions in the diameter of cerebral parenchymal arterioles (PAs) could reduce cerebral blood flow and lead to cognitive decline. In this study, we hypothesized that a high fat diet (HFD) would lead to a decline in cognition and inward remodeling of PAs. 3-week-old male and female Sprague Dawley rats were fed a HFD containing 36% fat or control diet containing 7.2% fat for 23-28 weeks. Data are presented as the mean ± SEM, HFD vs Control, n=3-16 in each group. At euthanasia, the male HFD group weighed more than its control while no significant differences were observed in the females (Male: 559±12.4g vs 475 ±9.12g, p=<0.0001, Female: 303±7.60g vs 264± 5.9g, p=0.474). HFD rats had more abdominal fat in both males and females (Male: 16.1 ± 0.957g vs 6.55 ±0.427g, p=<0.0001, Female: 13.2 ±1.55g vs 3.93 ± 1.08g, p=<0.0001). PAs were collected and their structure was assessed using pressure myography. In females, there was a significant decrease in outer and lumen diameter (p=0.0009, p=0.0007), in vessel area (p=0.0005), wall area (p=0.0028), and lumen area (p=0.0008) in PAs from the rats fed a HFD. Cognitive function was evaluated and there were no significant differences as well as no significant differences in blood flow, inflammation, neurogenesis, or synaptic markers. Thus, this HFD led to alterations in body weight and abdominal fat weight as well as PA structure in females without significantly affecting cognition.

This thesis is in dedicated to the memory of my grandmother Khadija,
for her kind heart and selflessness.
Your light will always shine bright.

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INTRODUCTION

I. Statement of the Problem

Obesity has become a global epidemic and the incidence of obesity is increasing at an alarming rate (3). To account for differences in height, the body mass index (BMI) is used to assess if an individual is overweight or obese. It is defined as the person's weight divided by the square of height in meters and a BMI ranging from 25.0 to 30.0 falls in the overweight range while a higher BMI above 30.0 is considered to be obese (1, 6, 38). Almost 40% of US adults and 18.5% of children are obese, and approximately 40% of women and 35% of men are obese (1). Overweight or obese can also be defined as having excess fat as a result of an imbalance in energy intake and output in the body (6). Obesity is associated with comorbidities including hypertension, type 2 diabetes, non-alcoholic fatty liver disease, sleep apnea, dyslipidemia, and childhood obesity specifically increases the risk of cardiovascular diseases in later life (3, 14). A high percentage of obese children carry this obesity into adulthood, and parental obesity increases the risk of adult obesity in those children as well. Childhood obesity is a consequence of several factors including genetic, environmental, and ecological factors such as family, and community, as well heritable factors which are responsible for 30-50% of the variation in adiposity (14). Several environmental factors increase "obesogenic" states including increased caloric consumption which is often associated with reduced physical activity and increased time doing sedentary activities (35). These environmental factors have led to the observed shifts in worldwide obesity rates. Correlation studies show obesity rates began to increase in the 1980s and 1990s and it was directly linked to the changes in diet and lack of exercise (14, 35).

Obesity has been established as leading risk factor for dementia development (8). According to Yamashiro *et al.*, visceral fat accumulation alone is associated with cerebral small vessel disease (4) which is a leading cause for vascular cognitive impairment, affecting information processing speed and executive function, gradually leading to vascular cognitive impairment with dementia (VCID) (5, 12).

II. Vascular Cognitive Impairment with Dementia (VCID)

The prevalence of dementia increases from 2-3% in 70-75 year olds to 20-25% among those aged 85 years and older. VCID, a form of dementia with a vascular origin, is more prevalent in men than women (18, 39). The costs of Alzheimer's and other dementia forms are increasing. In the United States alone, total payments in 2018 were estimated at approximately $277 billion (17). Dementia affects 4-5 million older adults in the United States having a social and economic impact on patients, families, and government programs. Overall dementia cases are expected to increase by 3-fold by the year 2050 (33). In recent years, it has become clear that a portion of these patients suffer from VCID (18). VCID is the 2nd cause of dementia just after Alzheimer's disease. It occurs when damage to blood vessels takes place threatening blood and oxygen supply to the brain, leading to reduced protein synthesis, reduction in synaptic plasticity, cell death and as a consequence, slowed thinking, loss of executive function, thinking, and disrupted execution of tasks (7, 41). Individuals with VCID exhibit reductions in cognition as well as difficulty with motor function; specifically, VCID patients have a slow gait and poor balance.

Obesity has been shown to trigger VCID by decreasing blood supply to the brain and is also associated with brain atrophy, which is a reduction of volume in certain brain regions like the hippocampus. A reduction in hippocampus volume predicts cognitive decline and dementia

(19). The accumulation of white adipose tissue as a result of this disease also leads to systemic inflammation and contributes to increased circulating levels of pro-inflammatory cytokines such as TNFα and IL-6. A mechanism by which systemic inflammation is linked to cognitive decline is via TNFα secreted from adipose tissue (19). In a study conducted by Pohl *et al*., they showed that high fat diet (HFD) rats challenged with lipopolysaccharides in their diet had elevated levels of TNFα and IL-6 (46). Elevated levels of cytokines like IL-6 have been shown to disrupt neural circuits involved with cognition and memory and are associated with the increase in dementia (72). Microglia are the primary mediators of the central nervous system's immune defense system and are able to release pro-inflammatory cytokines. In brain regions involved in cognition and memory, exacerbated microglial expression has been observed (19). In a study conducted in 24-month-old mice treated with a HFD, hippocampal microglial activation was exacerbated (66). Astrocytes are the most abundant glial cells within the central nervous system and respond to all forms of insults. They produce cytokines that drive inflammatory processes in the hypothalamus and in HFD mice, astrocytes from the CA3 region of the hippocampus had longer and less abundant projections. Further, in obese Zucker rats, there were increased Glial fibrillary acidic protein (GFAP) immunoreactive astrocytes throughout the hippocampus (19) indicating the increased astrocytic proliferation.

The vascular effects of obesity play a key role in the development of VCID in aged people by inducing atherosclerosis in large cerebral arteries and alterations at the cerebral microcirculation like blood brain barrier (BBB) disruption, neuroinflammation, exacerbation of neurodegeneration, microvascular rarefaction, cerebral micro-hemorrhages, and ischemic neuronal dysfunction and damage. (19, 34, 40, 73). Previous studies from our lab, show that obesity induced by a high fat diet in male Sprague Dawley rats leads to middle cerebral artery

(MCA) remodeling and vascular inflammation as well as white matter injury, which is a risk factor for cognitive decline. The remodeling that took place in the MCA was a thickening of the vascular wall as well as a reduced diameter of the artery (9).

III. The Cerebral Circulation and Parenchymal Arterioles

The brain is one of the most highly perfused organs in the body requiring 15-20% of total cardiac output. Due to this high metabolic demand and the brain's lack of energy stores, the brain requires a constant blood supply (20). This supply comes through the left and right carotid arteries and the left and right vertebral arteries. The carotid arteries supply blood to the cerebrum and the vertebral arteries supply the basilar artery and branches of the vertebral and basilar arteries supply the cerebellum and the brain stem. The basilar artery joins the left and right carotid arteries to form the circle of Willis. There are three main arteries that branch from this ring and they are the anterior, middle, and posterior cerebral arteries which then further branch into smaller arteries and arterioles (21). Pial arteries extend from all the large cerebral arteries to supply the surface of the brain. Some branches of the posterior cerebral artery (PCA) supply the hippocampus and midbrain while the anterior artery branches supply the parietal cortex (20).

The pial arteries lie on the surface of the brain and the parenchymal arterioles (PAs) branch from the pial arteries and dive perpendicularly into the brain. The capillaries arise from PAs. PAs are unique in comparison to other arterioles in the brain because they lack branching, thus, an occlusion of one PA, can lead to ischemia that is sufficient to cause cognitive impairment (22, 23). The PAs are encased by microglia and astrocytes and PAs are major regulators of the neurovascular coupling process, which is the mechanism that allows for the matching of local cerebral blood flow (CBF) and local neuronal activity (20). Due to the PAs' critical role in CBF control, their structure and function in a disease state must be elucidated.

IV. Arterial Structural Remodeling in Obesity

The term remodeling refers to the structural changes in the vascular wall that takes place in response to a disease state, injury, and aging which can be associated with hemodynamic changes. There are several types of remodeling that can take place; remodeling can be classified as outward or inward reflecting an increase or a decrease in the diameter of the vessel. It can also be hypertrophic defined as an increase in the wall to lumen ratio, eutrophic defined having as no change in the wall to lumen ratio, or hypotrophic defines a reduction in the wall to lumen ratio (10). Arterial remodeling has been studied in hypertension and obesity. In addition to studies from our lab assessing MCA structure, other studies have been conducted to assess the effect of genetic obesity on artery structure (48). In order to do so, the Zucker rat was used. The Zucker rat is a rodent model that is insensitive to leptin due to a mutation in the leptin receptor. In these studies, young and old Zucker rats were compared with the finding that older Zucker rats developed hypertension that was linked to MCA remodeling (49). In a subsequent study, to confirm that the MCA remodeling was the result of hypertension, rats were treated with hydrochlorothiazide which is a thiazide diuretic anti-hypertensive drug. Lowering blood pressure in the Zucker rats prevented the MCA remodeling suggesting that in this case the remodeling was blood pressure and not obesity dependent (49). Obesity's effect on artery structure and cerebral perfusion have not been extensively studied and this study aims to focus on this gap. From hypertension studies, alterations in artery structure can reduce cerebral perfusion and increase cerebrovascular resistance (48).

V. The Neurovascular Unit

In the 1960s, the discovery of brain activity increasing blood flow to activated areas was realized and as a result the concept of neurovascular coupling (NVC) or functional hyperemia

was developed (24, 26). The neurovascular unit (NVU) is a concept that was designed in 2001 at the Stroke Progress Review Group meeting of the National Institute of Neurological Disorders and Stroke which emphasized the relationship between the brain and vessels (15). The NVU refers to the network of active neurons and the cerebral vasculature, and is composed of vascular cells, neurons, pericytes, astrocytes, and glial cells and these cells work together to control NVC. NVC links the metabolic demands of neurons to cerebral blood flow by integrating signaling between all components of this unit (20,24, 25). The composition of the unit varies depending on the location of the artery on the cerebrovascular tree. At the PA level, the NVU is made up of capillary networks, vascular cells, glia, astrocytes, pericytes, and neurons. Rapid communication to and from the cerebral vasculature, neurons, and astrocytes takes place to regulate constriction and dilatory pathways allowing for CBF to change based on oxygen and nutrient demand. (26, 92).

VI. Aldosterone and the Mineralocorticoid Receptor (MR)

Aldosterone levels are elevated in obese and overweight patients. It is the primary steroid hormone with the ability to activate the MR. It is synthesized from its precursor cholesterol through a series of enzymatic reactions occurring in the mitochondria and endoplasmic reticulum (ER) of the adrenal zona glomerulosa (16). The final, rate limiting, step of aldosterone synthesis is catalyzed by aldosterone synthase which is encoded by the CYP11B2 gene. Aldosterone synthesis has two major controllers and they are angiotensin II (AngII) and serum potassium levels, and a secondary regulator, adrenocorticotrophic hormone (ACTH) (27). Aldosterone mainly acts in the collecting duct of the kidney to regulate salt and water balance as well as extracellular fluid volume. It also regulates blood pressure through sodium and water reabsorption (27, 28, 93). In addition to aldosterone, the MR can also be activated by cortisol in

humans and corticosterone in rodents. 11β-Hydroxysteroid dehydrogenase-2 (11β-HSD) is responsible for converting cortisol to inactive cortisone in humans and corticosterone to 11-dehydrocorticosterone in rats. Cortisol can activate the MR while cortisone cannot, therefore, this enzyme is able to prevent the activation of the MR by cortisol which circulates at higher concentrations than aldosterone (28, 49, 79).

The MR is a member of the steroid-thyroid-retinoid superfamily of ligand-dependent transcription factors (29). Once aldosterone binds to the MR, it translocates to the nucleus, binds to specific DNA sequences and triggers transcription of target genes (94). The MR is found in a wide range of tissues and cell types in the body including vascular smooth muscle cells (VSMC), endothelial cells, cardiac myocytes, macrophages, neurons, microglia, and astrocytes (28). In vessels, aldosterone can activate the MR, regulate tone and remodeling of the vascular wall and this occurs by VSMC proliferation, hypertrophy and vascular fibrosis (31). Aldosterone has the ability to affect the endothelium dependent dilatory or constriction mechanisms. Aldosterone infusion in rats has been shown to impair endothelium-dependent relaxation and was associated with increased oxidative stress which was reversed by MR antagonism (30, 32).

Clinical studies show a link between high BMI and elevated levels of aldosterone, and weight loss in obese patients leads to reductions in aldosterone levels. The increased prevalence hyperaldosteronism and obesity suggests that aldosterone may be a mechanistic link between adiposity and cardiovascular risks and studies conducted by Whaley-Connell *et al* confirms this link (50). In other words, since there is an increase in adipocytes in obese patients and adipocytes produce aldosterone, aldosterone levels will be elevated. Moreover, it was discovered that adipocytes have functionally active aldosterone synthase and produce aldosterone regulated by

AngII and is responsible for regulating adipocyte differentiation in an autocrine manner and vascular function in a paracrine manner (51).

In a study conducted by Pires *et al*., the role the MR plays in cerebral artery remodeling was studied in male SD rats on a HFD or control diets treated with the MR antagonist canrenoic acid for 17 weeks. The MCA structure was analyzed and a change in the structure of the MCA and increase in white matter injury were observed. Treatment with the MR antagonist reduced white matter injury and prevented MCA remodeling. Furthermore, mRNA expression levels of doublecortin were analyzed and they were reduced as a result of the HFD. Treatment with the MR antagonist normalized these levels (9). Studies conducted by Diaz-Otero *et al*., showed that the endothelial MR plays a role in PA and posterior cerebral artery remodeling in AngII induced hypertension and MR antagonism by eplerenone prevented these changes (2). All these studies highlight the significant role the MR and its primary activator; aldosterone, play in remodeling of cerebral arteries.

VII. Leptin

Leptin is a hormone produced by the expression of the *lep* gene (36). Leptin is primarily produced in adipocytes, therefore the concentration of leptin in the body is proportional to the total body fat mass (37). Leptin's actions are mediated by binding to the Lepr-A isoform of the leptin receptor which is a single transmembrane-spanning protein of the class I cytokine receptor family (38). Leptin plays several roles in the body, all beginning with it binding to Lepr-A in the blood once it's secreted from adipocytes (37, 38). The main function of leptin is to reduce food intake and increase energy output and it does so by binding and activating the leptin receptor Lepr-B in the hypothalamus (38). It gets transferred through the BBB via the short leptin receptor isoform to the hypothalamus where it mediates most of its actions (13, 36, 37).

Circulating leptin levels in the blood serve as a measure of energy reserves and direct the CNS to adjust food intake and energy output. Outside the hypothalamus, leptin acts on the mesolimbic dopamine system which is involved in motivation for and reward of feeding and the nucleus of the solitary tract of the brainstem contributing to satiety (38).

Obese patients have high levels of plasma leptin. Leptin plays different role in males and females. Females typically have three to four times more leptin than in males and this is only exacerbated in overweight and obese women. The origin of this difference is yet to be discovered but strong evidence suggests that testosterone in males controls leptin levels by reducing subcutaneous adipose mass (55). Leptin is also an important regulator of aldosterone production. In studies conducted by Faulkner *et al.*, they found leptin to be directly implicated in aldosterone secretion in males and female by showing that leptin infusion increases aldosterone plasma levels in controls and diet-induced obese (DIO) mice (53). In additional studies conducted by this group, in a model of leptin hypersensitivity, the PTP1b knockout mouse, female mice had elevated levels of aldosterone as well as increased adrenal CYP11B2 expression levels showing that leptin is a direct regulator of aldosterone synthesis and acts directly on the adrenal glomerulosa via calcium dependent mechanisms (5).

MATERIALS AND METHODS

This thesis addresses three specific aims to test the central hypothesis: *High fat diet feeding leads to PA remodeling, cerebral hypoperfusion, neuroinflammation, and cognitive impairment in male and female Sprague Dawley rats.*

Model Validation: Baselines for dough diet fed HFD model

AIM I: To study the impact of high fat diet feeding on PA structure in males and females.

AIM II: To study the impact high fat diet feeding on neuroinflammation and cerebral perfusion.

AIM III: To study the impact of high fat diet on memory and changes in cognition.

I. Animals

All animal procedures were performed in agreement with Michigan State University animal use guidelines. The experimental protocol was approved by the Michigan State University Institutional Animal Care & Use Committee and was in accordance with the "Guide for the Care and Use of Laboratory Animals" from the National Academy of Sciences, Institute for Laboratory Animal Research. A total of 32 males (n=16 control, n=16 HFD) and 16 female (n=8 control, n=8 HF) Sprague Dawley rats were used in this study. All the rats were obtained from Invigo. HFD rats were maintained on HFD diet (BioServ containing 20.5% protein, 36.0% fat, and 35.7% carbohydrates), while control rats were maintained on control diet (Bioserv containing 20.5% protein, 7.2% fat, and 61.6% carbohydrates) from three weeks of age. Rats were euthanized between 27-31 weeks of age. The rats were housed in pairs and weight gain was tracked weekly. Animals were fasted overnight prior to euthanasia, final body weights were

measured, blood was collected via cardiac puncture, and organs were collected. Fasting blood glucose was measured. One male control, two female controls, and one HFD female were excluded from the abdominal fat weight analyses due to the lack of measurement of body weight. Additionally, the n number for the fasting blood glucose is smaller due to this variable not being measured in some rats as a result of experimental error.

II. Blood Pressure Measurement

Blood Pressure was measured using tail-cuff plethysmography (CODA-6, Kent Scientific, Torrington, CT) a week before euthanasia. One control and two HFD males were excluded from the final analysis due to lack of measurements obtained resulting from experimental error.

III. Measurement of Plasma Aldosterone and Leptin

ELISA assays were used to measure plasma aldosterone and leptin (Enzo Life Sciences, Ann Arbor, MI). Assays were conducted on one cohort of male rats while one control and one HFD female rats excluded from the final analysis due to dilution errors in the experiment.

IV. Measurement of Cerebral Blood Flow

Magnetic Resonance Imaging

To assess cerebral perfusion, a Burker 7T BioSpec 70/30 USR with arterial spin labeling was used. 3%-5% isoflurane in 100% O_2 was used to induce anesthesia, core temperature of the rat was maintained at 37°C, and breathing was monitored and maintained at 20-80 breaths per minute by altering the isoflurane to maintain its levels between 1%-2%. From the perfusion scans, total perfusion was obtained and expressed in ml/g/min. The formula used to calculate the adjusted T1 value was (0.9/T1) * (Control-Labeled/2*Control) (95). The brain regions measured

for this study were the cortex to serve as a control region and the CA1 region of the hippocampus due to its importance in long term memory formation (these studies were performed in collaboration with Dr. Robert Wiseman, Department of Physiology, Michigan State University). Six male control and HFD were randomly selected to undergo this test.

V. Pressure Myography

Pressure myography was used to assess PA structure. To isolate PAs from the brain, a section of the cortex from around the MCA was dissected and the pia along with the MCA was separated from the brain section. PAs that branch from the MCA were isolated and transferred to the pressure myograph chamber. The arteriole was bathed in calcium free physiological salt solution (PSS) supplemented with 1% Bovine serum albumin (BSA) and 10μM diltiazem to induce dilation of the arterioles. Two 30-40 μm glass micropipettes were used to cannulate the PA. After cannulation, the vessel was equilibrated at 37°C in zero Ca^{2+} physiological salt solution containing 141.9mmol/L NaCl, 4.79mmol/L KCl, 1.79mmol/L $MgSO_4 \bullet 7H_2O$, 109mmol/L HEPES, and 59mmol/L Dextrose. The arteriole was pressurized by gradually increasing the pressure. The pressure was started at 3mmHg and increased up to 120mmHg in 20mmHg increments and the arteriole was allowed to equilibrate at each pressure for 5 minutes after which the outer and lumen diameter was recorded. MyoVIEW (Danish Myo Technology (DMT), Aarhus, Denmark) was the program used to track these changes (11). Three control male rats and one HFD male were excluded from the final analysis due to inaccurate reading obtained from the software. Additionally, data for five controls and two HFD females were not obtained due to errors in arteriole dissections and cannulation due to experimental errors.

VI. Analysis of Inflammatory Markers mRNA Expression

Real-Time PCR (qRT-PCR)- Adrenal glands and the brain

Total RNA was extracted from a posterior section of the brains of obese and control rats between the cerebellum and the MCAs using Trizol. The sections were selected after PA extraction took place, therefore, the exact area varied from rat to another. RNA was reverse transcribed using VILO reverse transcriptase. TAQMAN-specific probes were used for the PCR to assess the mRNA expression of doublecortin (DCX), synaptophysin, brain-derived neurotrophic factor (BDNF), tumor necrosis factor (TNFα), interleukin 6 (IL-6), glial fibrillary acrid protein (GFAP), and ionized calcium binding adaptor molecule 1 (Iba1) as changes in these markers were expected to change as a result of HFD feeding. Additionally, the adrenal glands were collected to look at mRNA expression of CYP11B1 and CYP11B2 mRNA. β2-microglobulin (β2M) was used for normalization for all these experiments. mRNA expression is expressed as the fold change from control using the $2^{-\Delta\Delta Ct}$ method. These experiments were only conducted on a portion of the samples resulting in a lower n number for the males. Additionally, some samples were lost throughout the experiment due to dilution and pipetting errors in males and females.

VII. Open Field Testing

In order to assess anxiety levels and locomotor activity (44), the rats were placed in a square arena and allowed to explore for 30 minutes. The amount of time spent in the center, corners and sides was expressed as a percentage of total time present in the arena. Additional parameters including total distance moved, velocity, rest time, and movement time were calculated.

VIII. Novel Object Recognition Testing

This test was used to assess learning and recognition memory (43). Rats were acclimated to the testing chamber which was an open box with dark walls for 10 minutes per day for three days. On the testing day, two identical objects were placed in the box and the rat was placed facing away from them. The rats were allowed to explore the arena and objects for 10 minutes before being removed to their home cage. After a retention time of 90 minutes, the rats were placed back in the same manner after one of the objects was switched with a new novel object, the rats were allowed to explore these objects for 5 minutes. The objects used were a wooden sphere and a wooden cube. Their movement was tracked using EthoVision XT (Noldus, Wageningen, Netherlands). A rat was considered exploring the object when the nose-point was touching the object. The total exploration time of the novel object was calculated and used to assess recognition memory (80).

IX. Barnes Maze Testing

The Barnes maze test was used to test spatial learning and memory (45). All experimental sessions were recorded by a video camera placed above the apparatus and analyzed with EthoVision XT. On the first day, the rats went through a habituation session where the animals were placed under a large clean beaker and pulled to the escape hole and given time to escape. Subsequently, the rats had two training days where a 4,000 Hz sound was used as an aversive stimulus while the rat explored and the trials lasted 120 secs or until the animal reached the escape hole. If the rat did not reach the hole, they were guided by the experimenter. After the rats reached the escape hole, they remained there for 60 secs before being returned to their home cage. The test day was conducted 48 hr after their last training session. The procedure was

similar to the previous days but the escape hole was covered and the rats were evaluated for 120 secs. At the beginning of each session, the animals were placed under an opaque container at the center of the maze and the animals began exploring once the container was removed (81). During testing, some animals fell off the table, therefore, they were removed from the final analysis of the latency to first.

X. Statistical Analysis

All the following data are presented as mean ± standard error of the mean (SEM). Blood pressure, mRNA expression, aldosterone concentration, leptin concentration, and behavioral tests data were analyzed by One-way ANOVA followed by Bonferroni-adjusted t-tests for post-hoc comparison. Plasma aldosterone and leptin were quantified using a standard curve, and reported as concentrations in pictograms per milliliters (pg/mL). All passive structure data were analyzed with two-way repeated measures ANOVA, followed by Bonferroni-adjusted t-tests for post-hoc comparison. In all cases, a *p*-value of 0.05 or less was deemed significant. All statistical analyses were performed with GraphPhad Prism 7.0 software (GraphPad, San Diego, CA).

RESULTS

I. Validation of the Overweight Model

Body weights in male and female HFD rats increased as they aged. At week 10, differences began to appear between the HFD and control groups. (Figure 1a). At the time of euthanasia, each rat was weighed and HFD males had significantly higher body weight than their controls while the females HFD did not significantly change in comparison to their controls (Male: 559 ± 12.4 vs 475 ±9.12g, p=<0.0001, Female: 303 ±7.60 vs 264 ± 15.9g, p=0.474). Additionally, male controls and HFD rats weighed significantly more than both female groups (Figure 1b). Both male and female HFD rats had significantly higher abdominal fat weight than their controls (Male: 16.1 ± 0.957 vs 6.55 ±0.427g, p=<0.0001, Female: 13.2 ±1.55 vs 3.93 ± 1.08g, p=<0.0001) (Figure 2a). Fat weight was expressed as percentage of the body weight, as expected, the female HFD rats had a higher percentage of abdominal fat to body weight than the male HFD rats with a significance of p=0.0004 (Figure 2b). The males had significantly elevated glucose levels in comparison to their controls while the female blood glucose levels were not changed (Male: 183 ± 5.27 vs 155 ±7.24 mmol/L, p=0.0193, Female: 138 ±8.18 vs 125 ± 9.90 mmol/L, p>0.999). When comparing males to females, HFD males had significantly higher blood glucose levels than the HFD females (Figure 3). There were no significant differences observed in systolic or diastolic blood pressure measurements between the groups (Figure 4a, 4b).

Leptin levels were significantly elevated only in male HFD rats while no change took place in the females (Male: 3190 ± 557 vs 961 ±267 pg/mL, p=0.0006, Female: 1283 ±170 vs 626 ± 171 pg/mL, p>0.999). In comparison to the HFD males, HFD females had significantly lower leptin levels with a significance of p=0.0090 (Figure 5). Using qRT-PCR, we measured

CYP11B1 and CYP11B2 levels from the adrenal glands and normalized to β2M. CYP11B1 is the gene responsible for the production of 11-beta-hydroxylase which helps produce cortisol in humans and corticosterone in rodents. CYP11B2 is the gene responsible for producing aldosterone synthase which helps produce aldosterone. Only CYP11B2 was increased in HFD males while no changes took place in the females. CYP11B1 (Male: 1.99 ± 0.372 vs 0.860 ±0.185, p=0.174, Female: 1.89 ±0.532 vs 2.03 ± 0.954, p=>0.999), CYP11B2 (Male: 1.93 ± 0.325 vs 0.742 ±0.151, p=0.0094, Female: 0.718 ±0.149 vs 0.871 ± 0.285, p=>0.999) (Figure 6a, 6b). Despite the elevation in CYP11B2, aldosterone levels remained unchanged at weeks 12 and 24 of age. Only a significant difference was observed between HFD males and HFD females at week 12 where the females had significantly lower plasma aldosterone levels (Figure 7a, 7b).

II. Cerebral Perfusion

Using arterial spin labeling MRI, cerebral perfusion was measured in male HFD and control rats. Specifically, perfusion was measured in the cerebral cortex and the CA1 region of the hippocampus. There were no significant differences between controls and HFD animals to either region indicating that cerebral perfusion was not affected by the HFD (Figure 8c, 8d). Perfusion was not measured in female but will be in future studies.

III. Parenchymal Arteriole Structure

Using pressure myography, the structure of the PAs was analyzed in male and female HFD rats and controls. In Figure 9a, the female HFD group PA outer diameter was significantly reduced compared to control. Similar trends were observed with the lumen diameter parameter (Figure 9b), vessel area, wall area, and lumen area (Figure 10a, 10b, 10c). Wall thickness, wall to lumen ratio (Figure 11a, 11b), strain, stress, and stiffness (Figure 12a, 12b, 12c) on the other hand were not significantly different between HFD and control females. All these parameters

were also measured in PAs from male rats fed the HFD and controls and no significant changes took place in any parameter (Figures 9-12). However, there is a trend of increase in the HFD female wall to lumen ratio and a trend of reduced stress as well. These results indicate that female PAs are undergoing inward remodeling due to the observed reduction in PA diameter and a trend of hypertrophic remodeling due to the wall to lumen ratio change. The male PAs are not changing as a result of this diet.

IV. pRT-PCR

RT-PCR was used to compare the expression of DCX, a marker of new and immature neurons, and synaptophysin, a synaptic marker, in brain tissue from HFD and control rats. There were no significant differences in mRNA expression levels of DCX (Figure 13). Synaptophysin mRNA expression was not affected by the HFD in male or female rats (Figure 14). BDNF is a marker for neuronal support, its mRNA expression was assessed and no differences were observed between the groups (Figure 15). The mRNA expression of active microglia using Iba-1 as a marker was used and there were no significant changed in any group (Figure 16). GFAP is a marker for astrocytes and this was not significantly changed in any groups (Figure 17). Inflammatory markers TNFα and IL-6 were measured and there were no significant differences observed between the groups (Figure 18 and 19).

V. Behavior and Memory

To test anxiety and locomotor activity, the open field test was utilized. The amount of time spent in corners and on the sides of the arena versus the center was assessed. As expected, all the control rats spent the majority of the time in the corners and sides and that did not differ in the HFD rats. There was a significant difference between male and female HFD rats with the females spending more time in the center with significance of $p=0.0431$ (Figure 20a, 20b). The

velocity, total distance traveled, average rest area, and average movement time were all assessed as well. In comparison to the males, all the females moved at a significantly higher velocity than the males, they traveled further distance, they spent more time moving and less time resting than the males (Figure 21a, 21b, 21c, 21d).

Using novel object testing to assess learning and recognition memory, the percent of the time spent exploring the novel object was quantified. Between the male control and HFD rats, there were no significant differences and similarly in the females, no differences were observed (Male: 43.3 ± 3.67 vs 52.2 ±4.51 p=0.629, Female: 44.3 ±4.28 vs 44.5 ± 4.35 p=>0.999) (Figure 22).

The Barnes maze test was used to measure spatial learning and memory. The results obtained from this test are puzzling, indicating that the male HFD rats had a lower latency to finding the escape hole than their control counterparts. These trends were confirmed in the learning curve, indicating that on probe day, the male HFD rats had a lower latency to first than the controls in comparison to the training days and the differences between the latency to first from day to day were significant in the HFD males but not in the controls (Figure 23a, 23b). There were no significant differences in the distance moved or velocity in males and females on probe day (Figure 24a, 24b).

VI. Correlations

Correlations were conducted to determine relationships between the parameters measured in this study in males and females. There was a significant positive correlation determined in this model between body weight and fasting plasma glucose, leptin and body weight, leptin and % abdominal fat weight, leptin and fasting plasma glucose, and leptin and CYP11B2. (Figure 25a – 25e). No correlations between body weight/ percent abdominal fat weight and blood pressure,

inflammatory markers, or neurogenesis markers were observed. Furthermore, a correlation between leptin and aldosterone levels at week 12 and euthanasia were not significant.

FIGURES

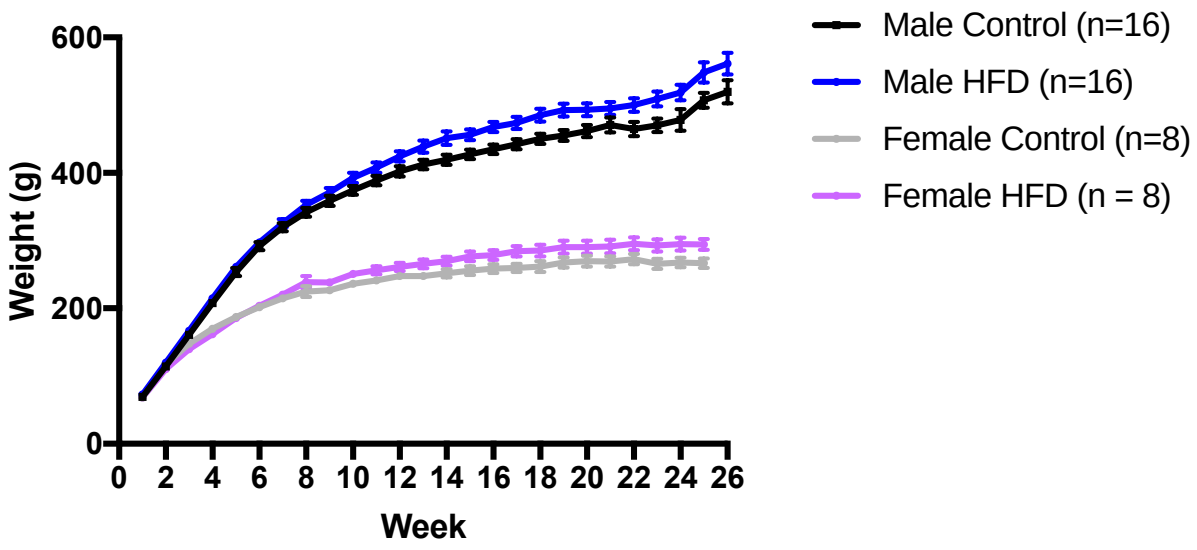

Figure 1A. Body Weight Average per Week in Obese and Control SD Rats. HFD increases body weight in male SD rats. Body weight in grams was measured once a week as the rats aged from 3 weeks old until euthanized.

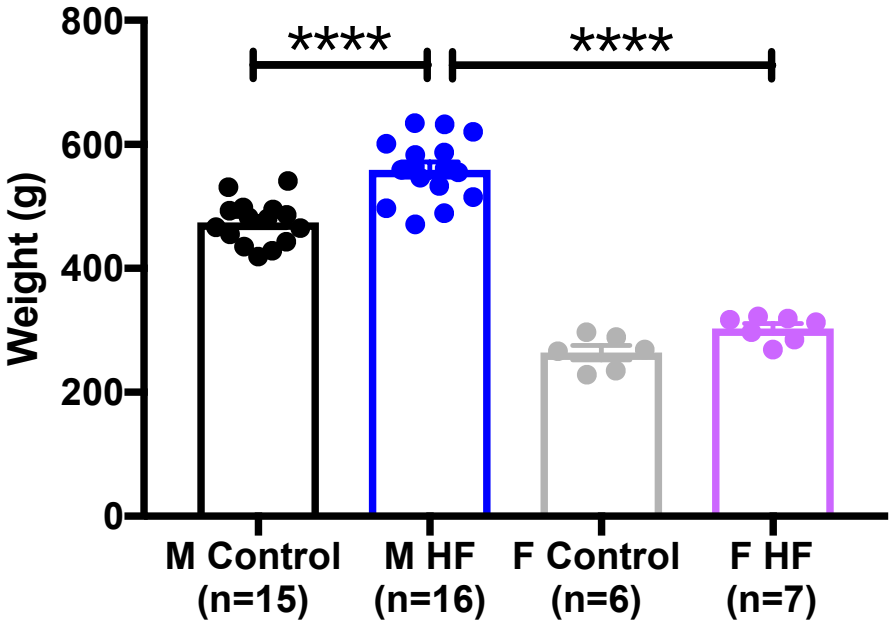

Figure 1B. Final Body Weights in Obese and Control SD Rats. Final body weight at euthanasia was measured. Data are represented as mean ± SEM. ****p<0.0001 by one-way ANOVA.

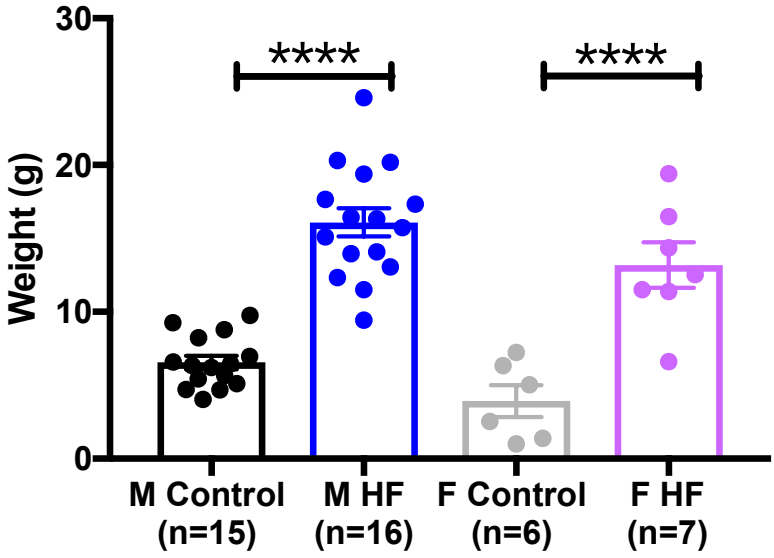

Figure 2A. Abdominal Fat Weight. Abdominal fat was increased in male and female HFD rats. It was collected and weighed in grams at euthanasia. HFD led to significantly higher abdominal fat weight in males and females in comparison to their controls. Data are represented as mean ± SEM. ****p<0.0001 and ***p<0.001 by one-way ANOVA.

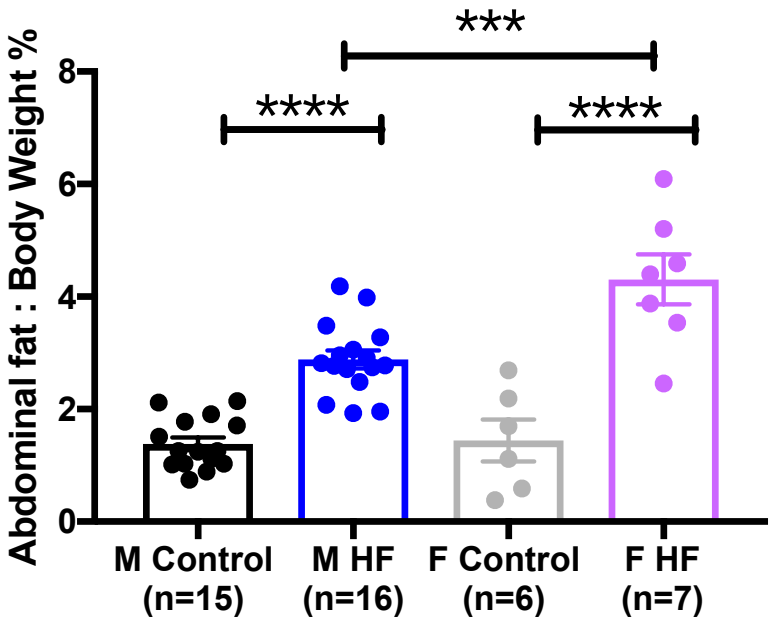

Figure 2B. Percent Abdominal Fat Weight to Body Weight. The % abdominal fat weight was higher as well. It was also higher in females than in males. Data are represented as mean ± SEM. ****p<0.0001 and ***p<0.001 by one-way ANOVA.

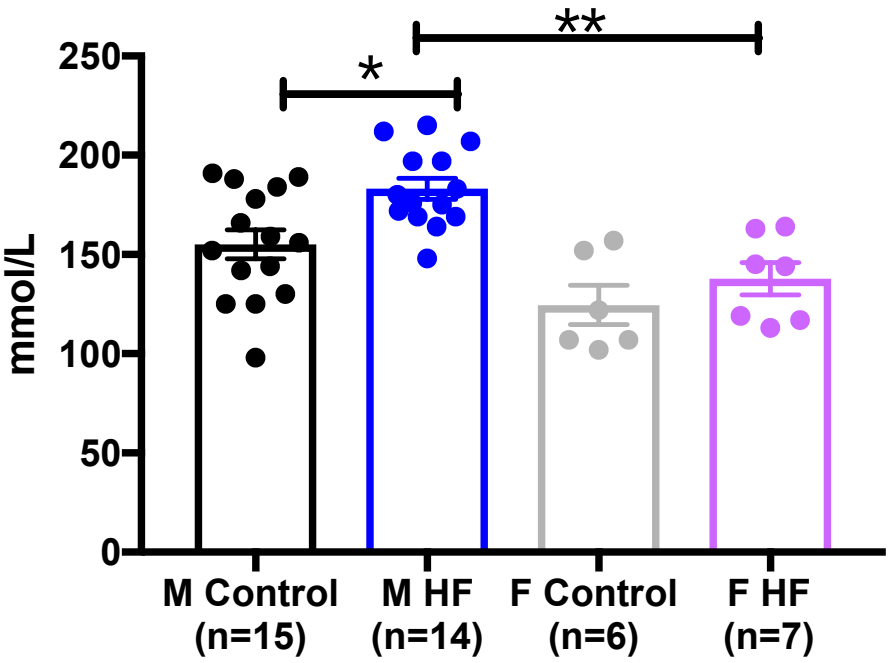

Figure 3. Fasting Blood Glucose. Blood glucose was measured from blood collected via cardiac puncture. It was significantly increased in male HFD rats but was not changed in females. It was significantly lower in HFD females compared to HFD males. Data are represented as mean ± SEM. **p<0.01 and *p<0.05 by one-way ANOVA.

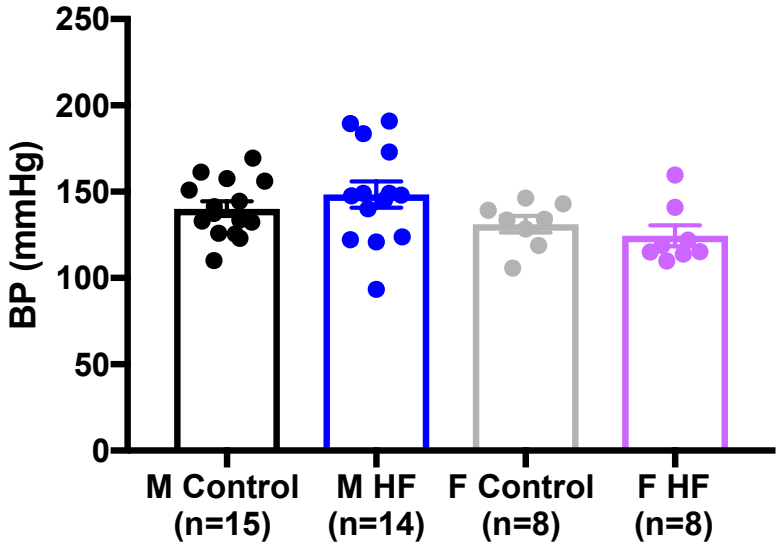

Figure 4A. Systolic Blood Pressure. Systolic pressure was measured via tail cuff plethysmography in male and female rats. There were no changed detected. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

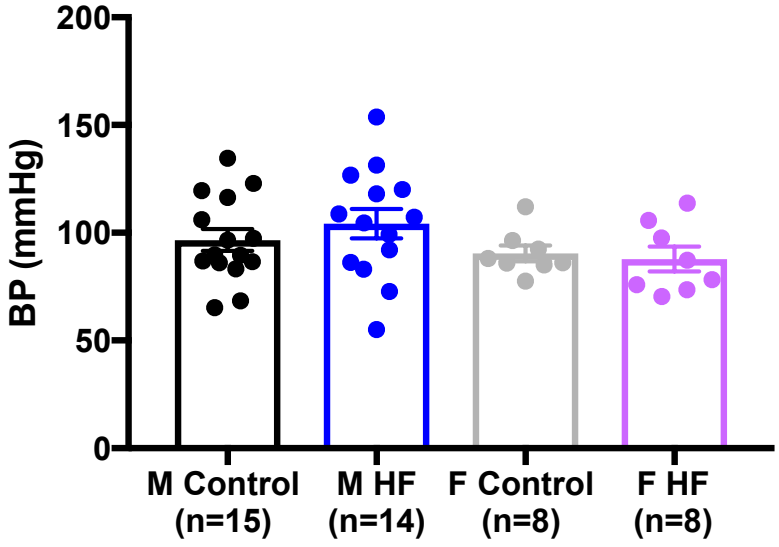

Figure 4B. Diastolic Blood Pressure. Diastolic pressure was measured via tail cuff plethysmography in male and female rats. There were no changed detected. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

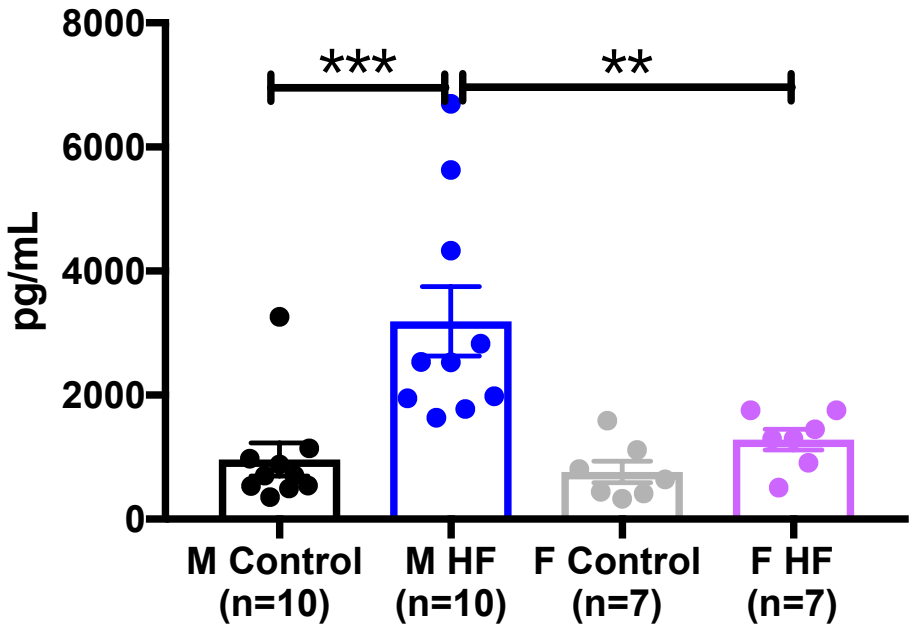

Figure 5. Leptin ELISA. Fasting plasma leptin levels were measured via ELISA assay. Leptin was significantly increased in male HFD rats in comparison to their controls. It was significantly reduced in HFD females in comparison to HFD males. Data are represented as mean ± SEM. ***p<0.001 and **p<0.01 by one-way ANOVA.

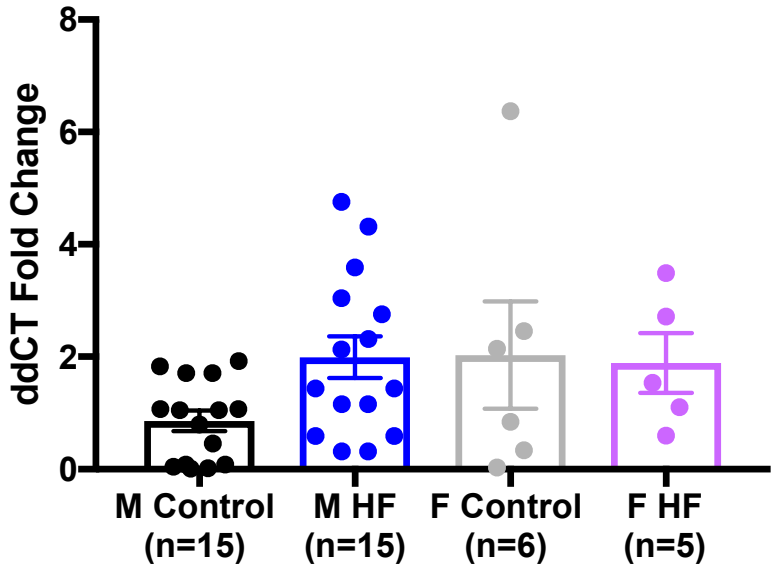

Figure 6A. CYP11B1. qRT-PCR was utilized to measure adrenal levels of CYP11B1 and there were no significant changes in any group. Data are represented as mean ± SEM. By one-way ANOVA.

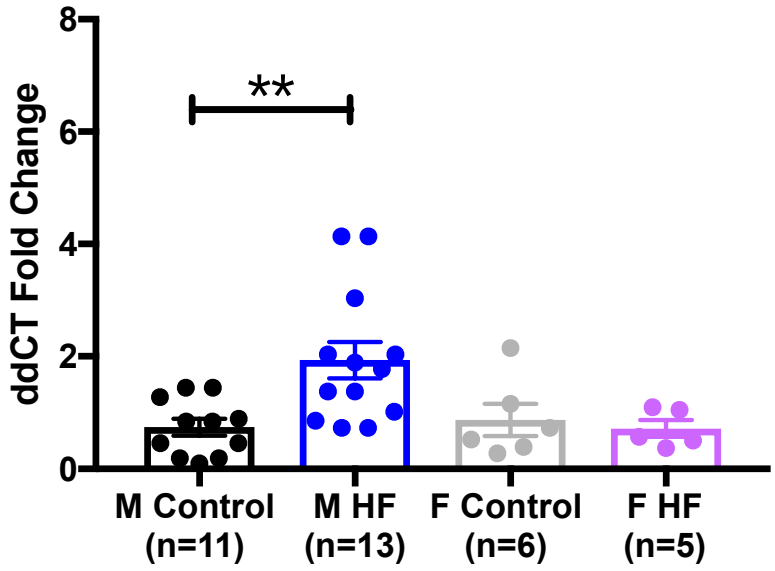

Figure 6B. CYP11B2: qRT-PCR was utilized to measure adrenal levels of CYP11B2. CYP11B2 levels were increased in male HFD rats in comparison to their controls while no change took place in the females. Data are represented as mean ± SEM. **p<0.01 by one-way ANOVA.

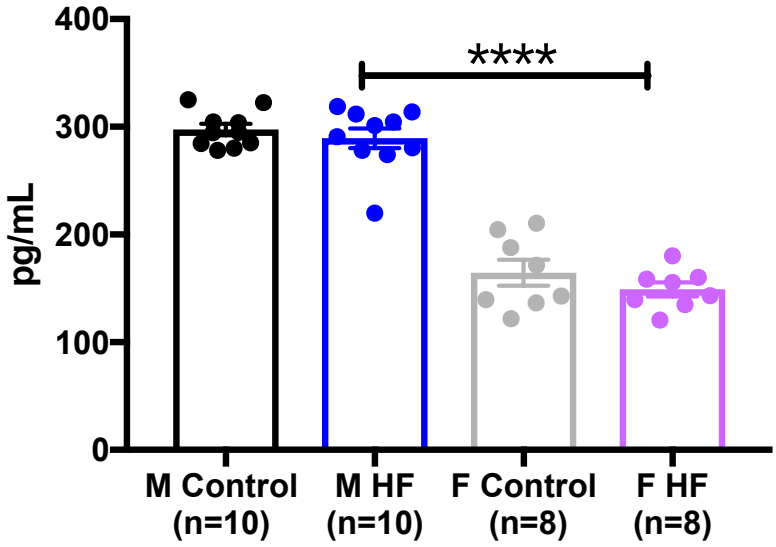

Figure 7A. Aldosterone 12 Week. ELISA assays were used to measure plasma aldosterone levels. At 12 weeks, blood was collected via tail vein and no change in plasma aldosterone took place in males or females. Data are represented as mean ± SEM. ****p<0.0001 by one-way ANOVA.

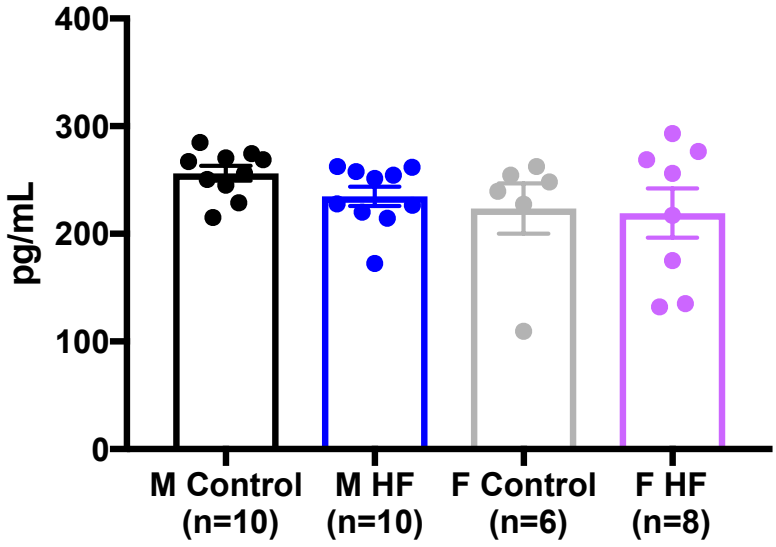

Figure 7B. Aldosterone Euthanasia. At euthanasia, blood was collected via cardiac puncture and no change in plasma aldosterone took place in males or females. Data are represented as mean ± SEM. By one-way ANOVA.

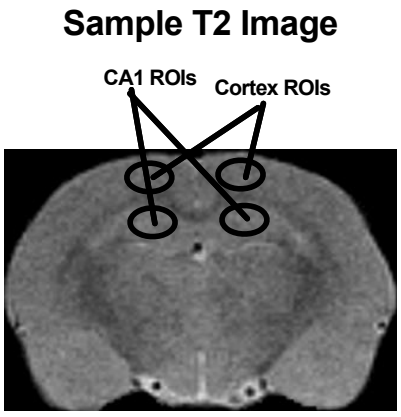

Figure 8A. Sample T2 Image. Perfusion was assessed using MRI with arterial spin labeling. A representative image of the T2 slice.

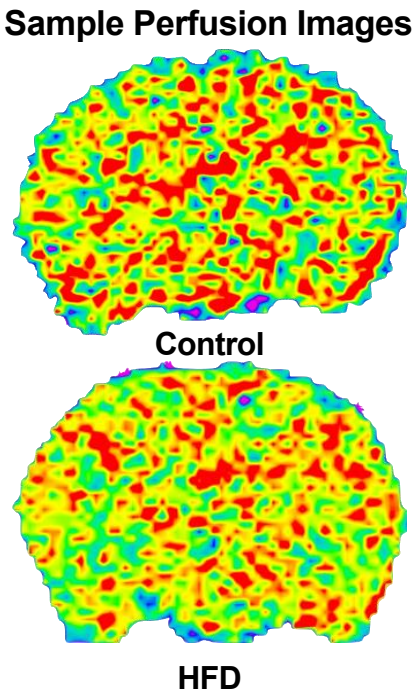

Figure 8B. Sample Perfusion Images. A representative image of total perfusion to the brain slice of a HFD and a control rat.

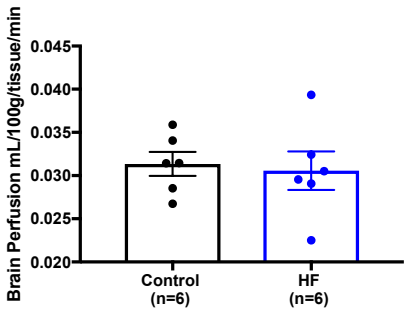

Figure 8C. MRI-Average Perfusion to CA1 Region of the Hippocampus. Cerebral perfusion was not changed in male HFD rats in either the CA1 region of the hippocampus. Data are represented as mean ± SEM. Analyzed by Student's t-test.

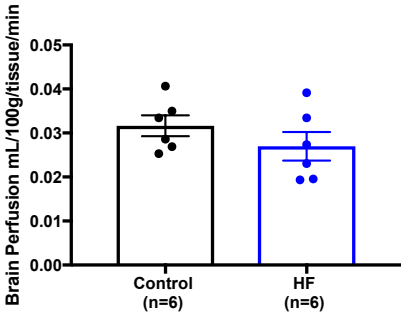

Figure 8D. MRI-Average Perfusion to Cortex
or Cerebral perfusion was not changed in male HFD rats in the cortex in comparison to their controls. Data are represented as mean ± SEM. Analyzed by Student's t-test.

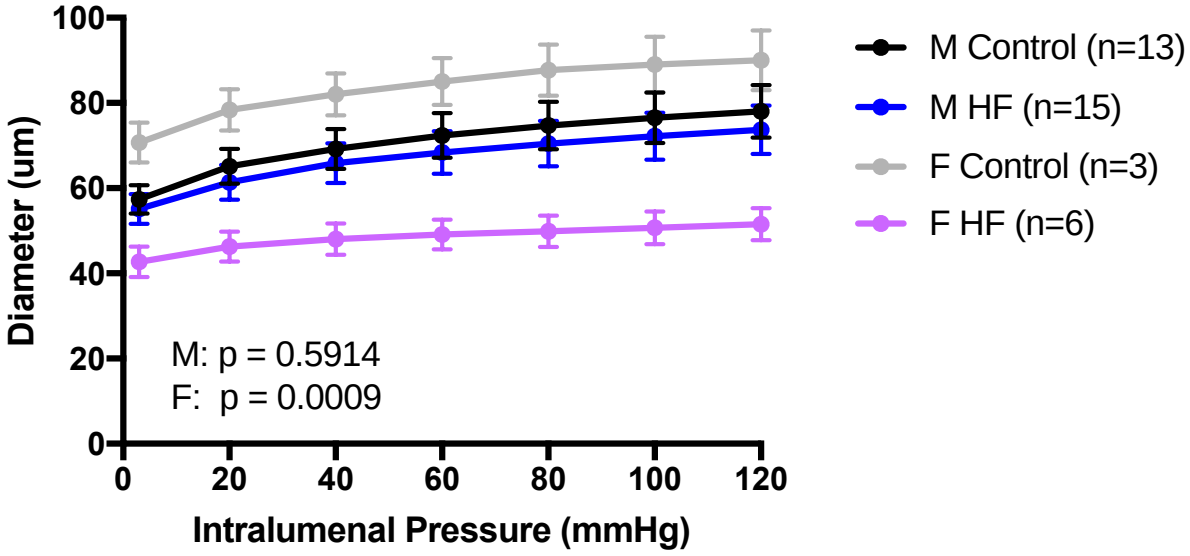

Figure 9A. Outer Diameter. HFD leads to parenchymal arteriole structure changes only in females on a HFD. The outer diameter of the female HFD arterioles were reduced in comparison to their controls while not changed in the males. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

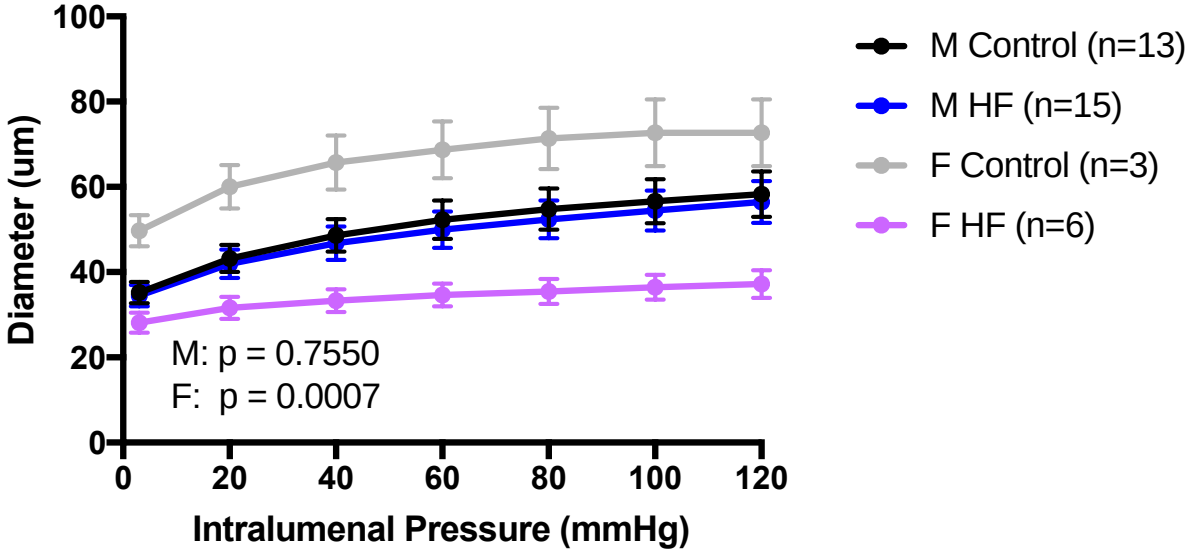

Figure 9B. Lumen Diameter. The lumen diameter of the female HFD arterioles were reduced in comparison to their controls while not changed in the males. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

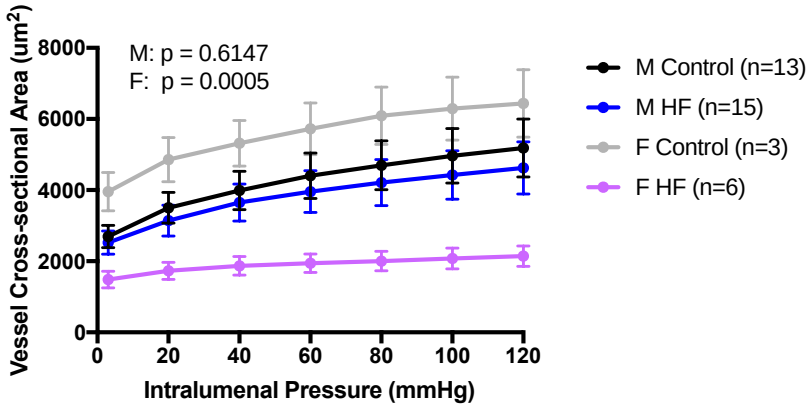

Figure 10A: Vessel Area. HFD leads to parenchymal arteriole structure changes only in females on a HFD. The vessel area of the female HFD arterioles were reduced in comparison to their controls while not changed in the males. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

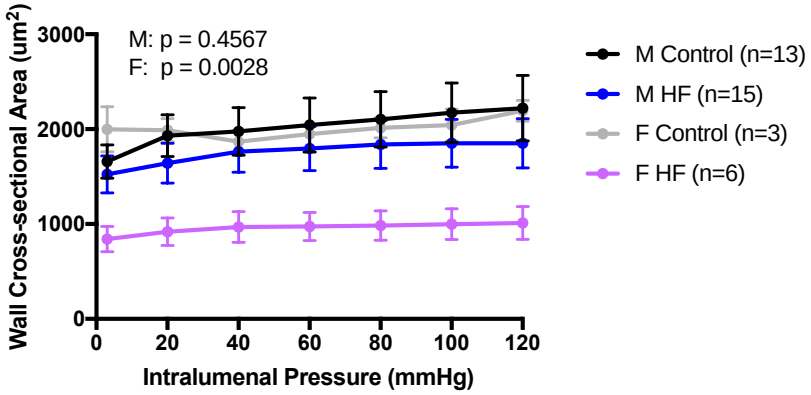

Figure 10B: Wall Area. The wall area of the female HFD arterioles were reduced in comparison to their controls while not changed in the males. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

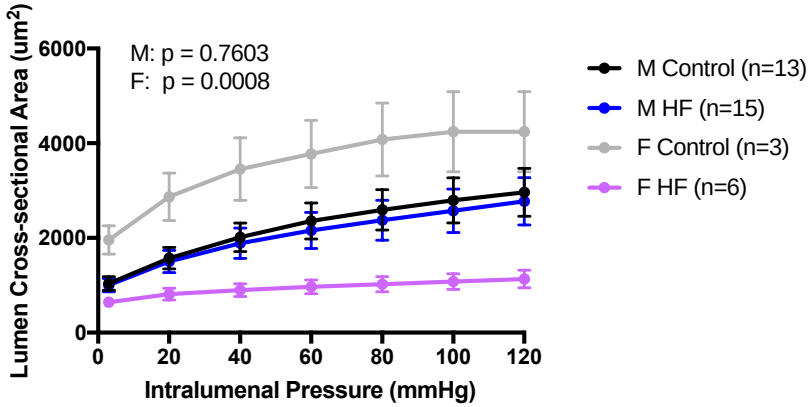

Figure 10C. Lumen Area. The lumen area of the female HFD arterioles were reduced in comparison to their controls while not changed in the males. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

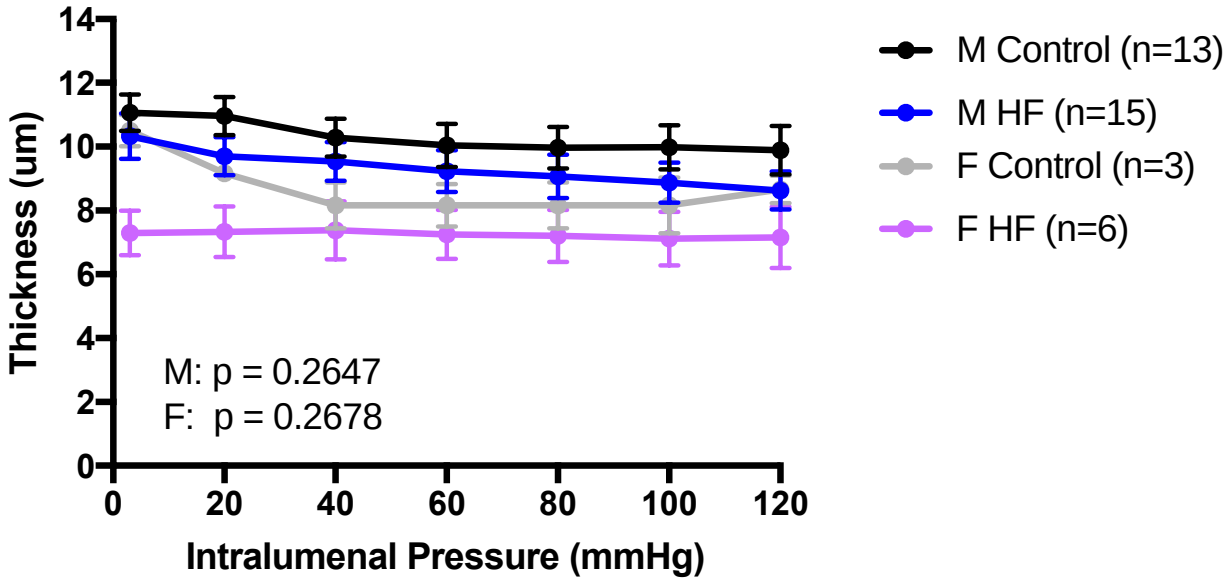

Figure 11A. Wall Thickness. HFD leads to parenchymal arteriole structure changes only in females on a HFD. The wall thickness of the male and female HFD arterioles were not changed in comparison to their controls. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

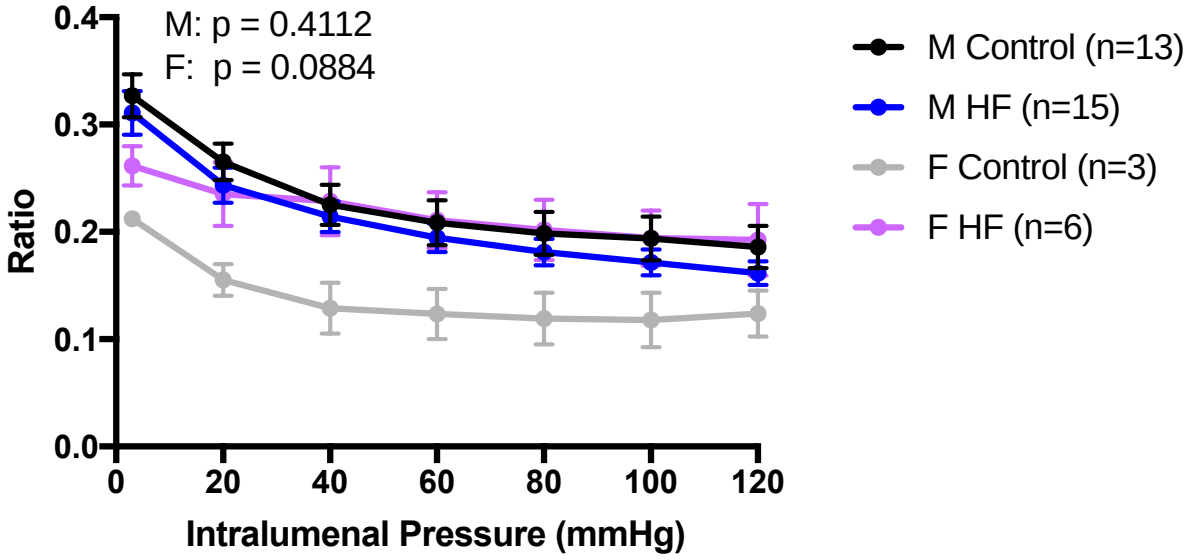

Figure 11B. Wall to Lumen Ratio. The wall to lumen ratio of the male and female HFD arterioles were not changed in comparison to their controls. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

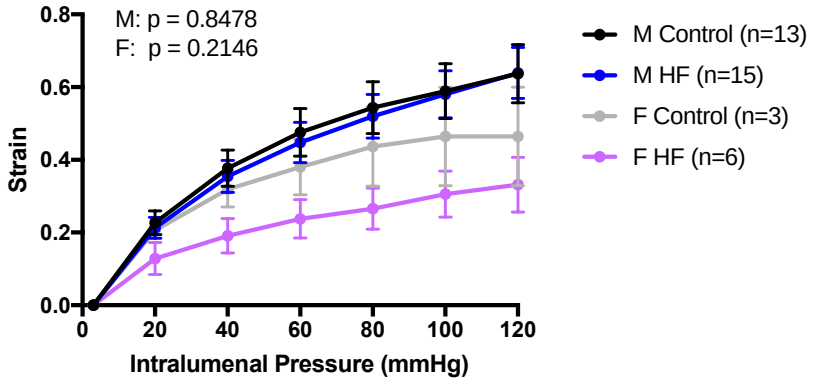

Figure 12A. Strain. HFD leads to parenchymal arteriole structure changes only in females on a HFD. The strain was not changed in comparison to their controls. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

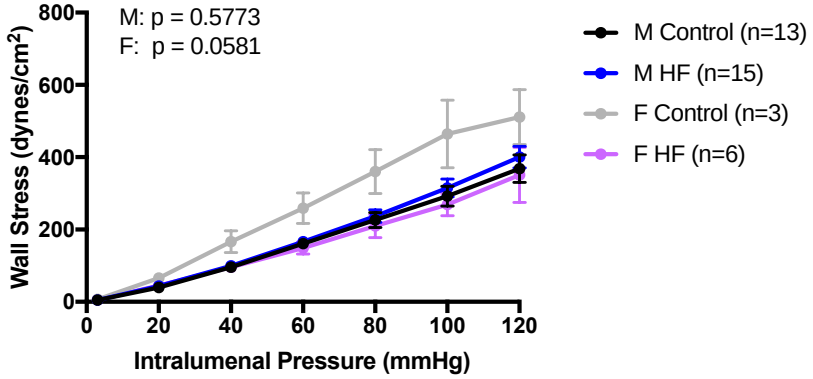

Figure 12B. Stress. The stress was not changed in comparison to their controls. Data are represented as mean ± SEM. Analyzed by two-way ANOVA.

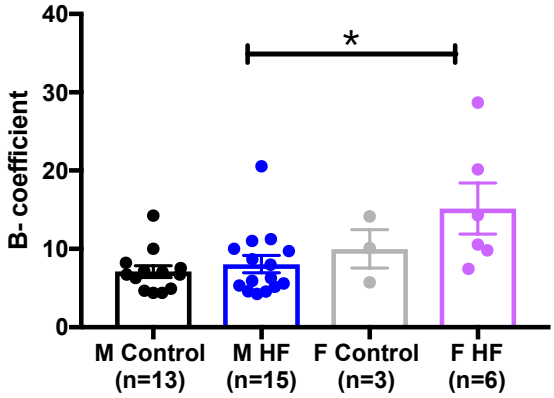

Figure 12C. Wall Stiffness. The wall stiffness of the male and female HFD arterioles were not changed in comparison to their controls. Data are represented as mean ± SEM. **p<0.01by one-way ANOVA.

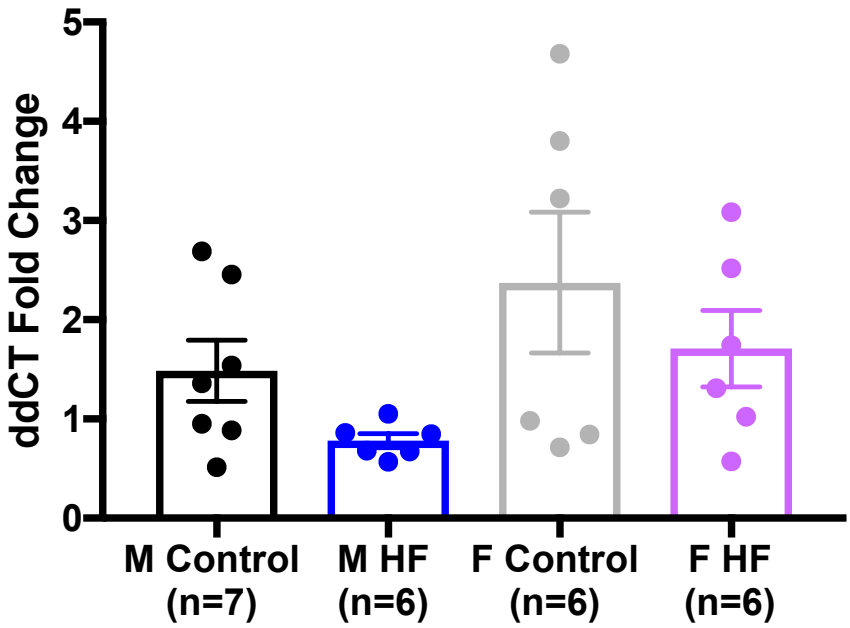

Figure 13. Doublecortin mRNA Expression. qRT-PCR was used to measure the neuronal marker doublecortin in brain sections of males and females. It was not significantly reduced in male or female HFD rats in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

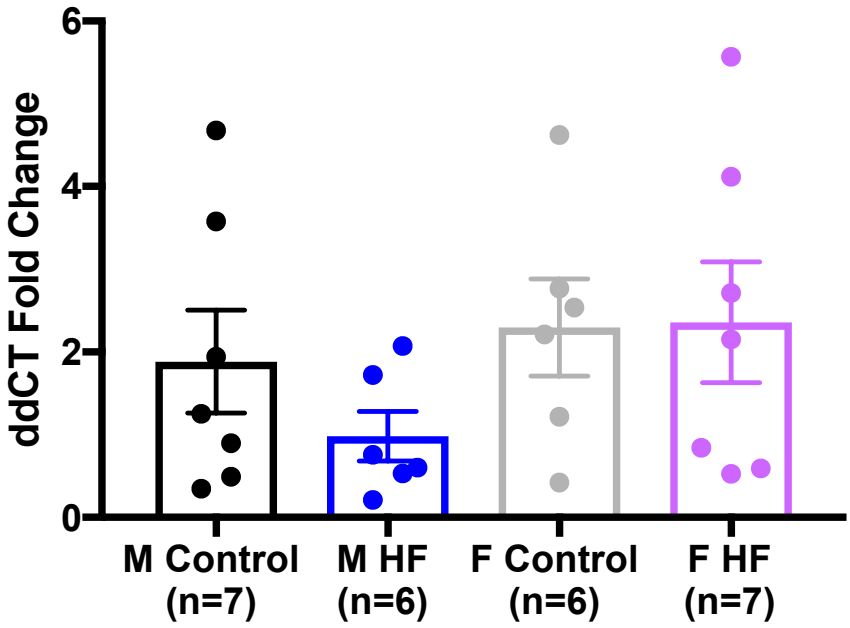

Figure 14. Synaptophysin mRNA Expression. qRT-PCR was used to measure the synaptic marker synaptophysin in brain sections of males and females. It was not significantly changed in males or females in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

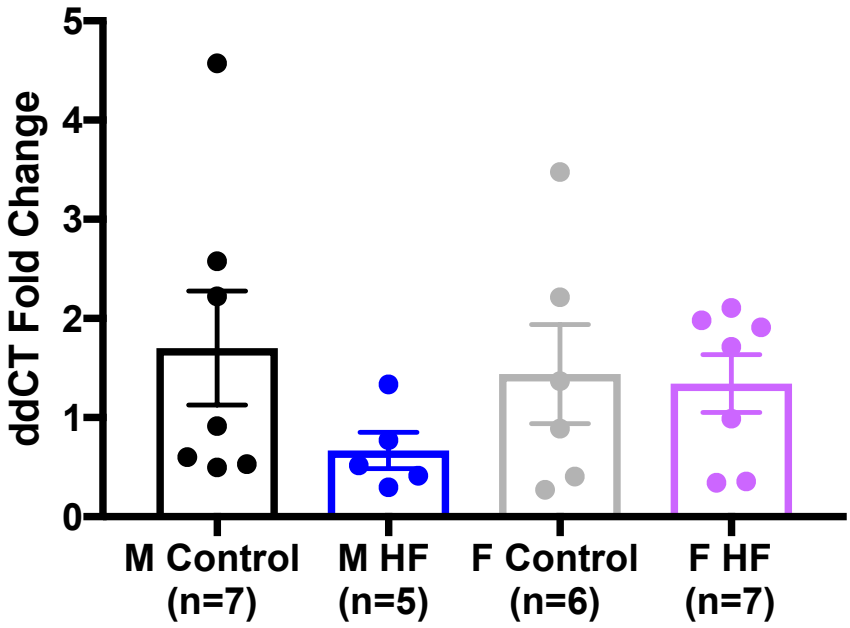

Figure 15. BDNF mRNA Expression. qRT-PCR was used to measure the neuronal support marker BDNF in brain sections of males and females. It was not significantly changed in males or females in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

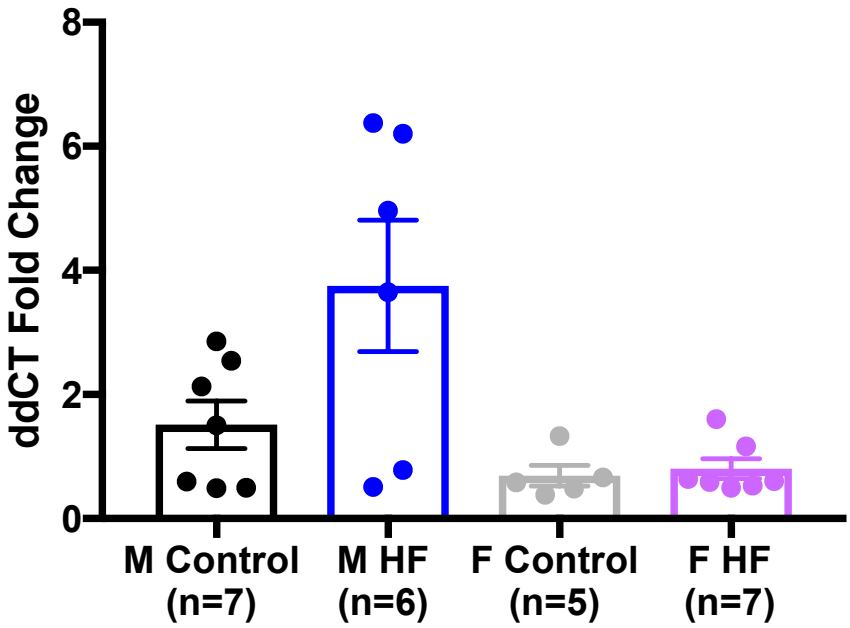

Figure 16. Iba1 mRNA Expression: qRT-PCR was used to measure the microglial marker Iba-1 in brain sections of males and females. It was not significantly changed in male or female HFD rats in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

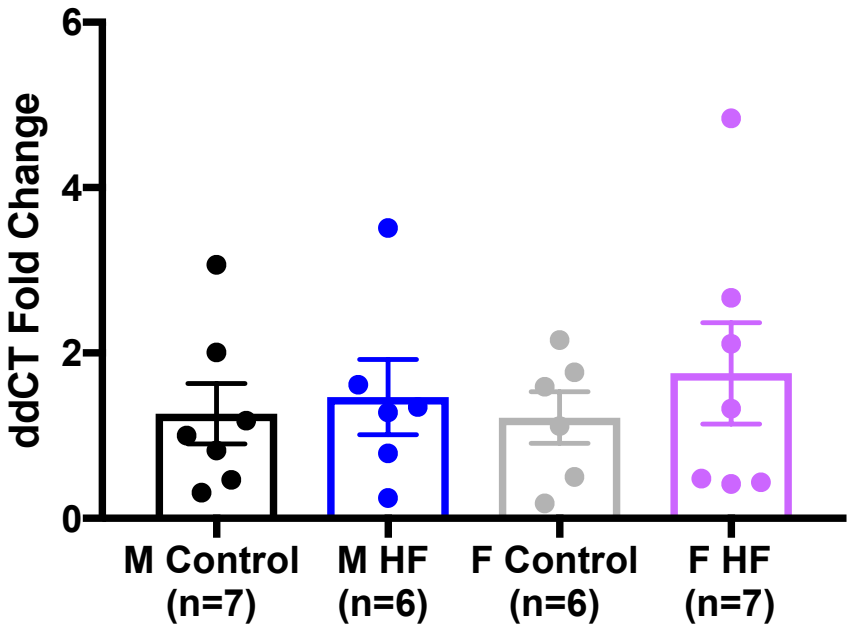

Figure 17. GFAP mRNA Expression. qRT-PCR was used to measure the astrocyte marker GFAP in brain sections of males and females. It was not significantly changed in males or females in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

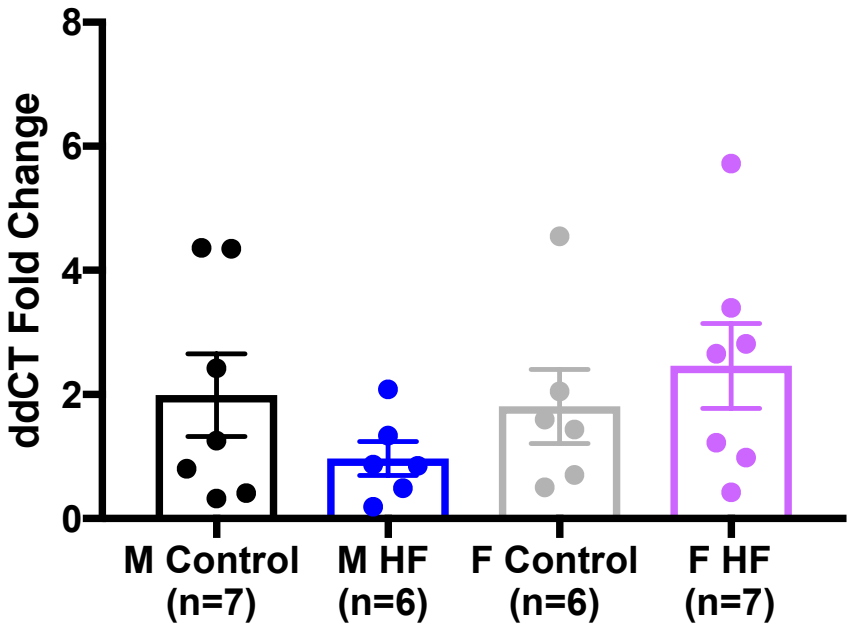

Figure 18. TNFα mRNA Expression. qRT-PCR was used to measure the inflammatory marker TNFα in brain sections of males and females. It was not significantly changed in males or females in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

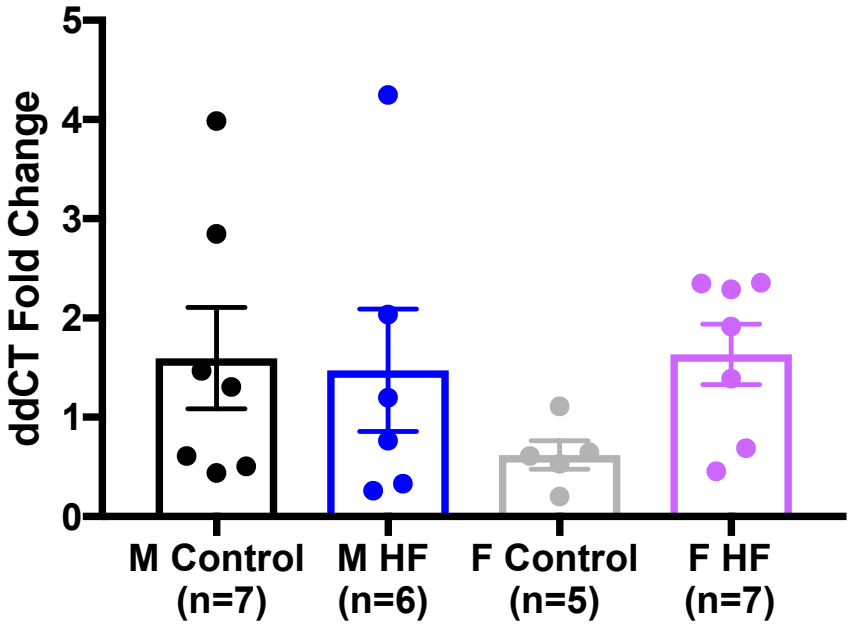

Figure 19. IL-6 mRNA Expression. qRT-PCR was used to measure the inflammatory marker IL-6 in brain sections of males and females. It was not significantly changed in male or female HFD rats in comparison to their controls. Data are represented as mean ± SEM. Analyzed by non-parametric one-way ANOVA.

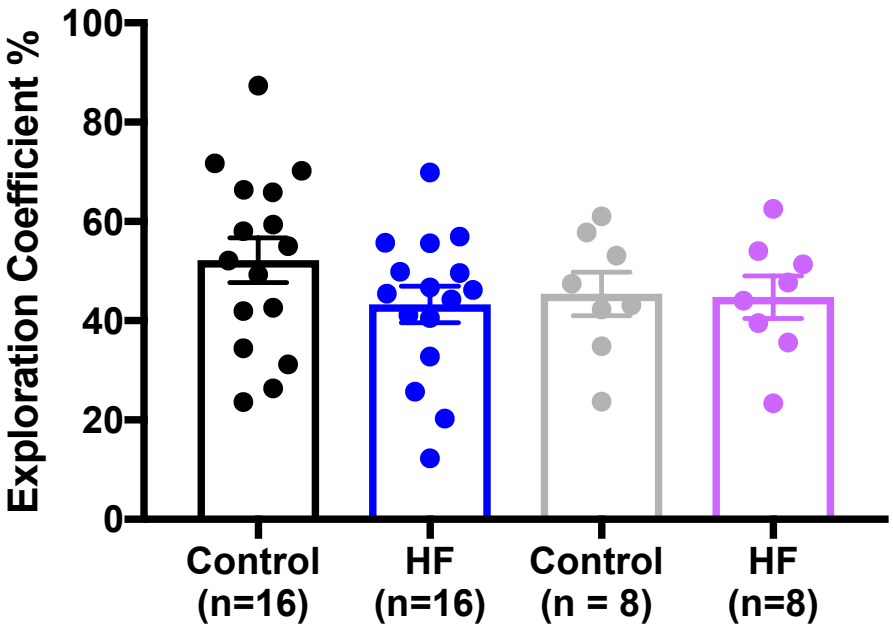

Figure 20. Novel Object Percent Exploration. The Novel object recognition test was used to assess learning and recognition memory. It was not significantly changed in HFD males or females in comparison to their controls. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

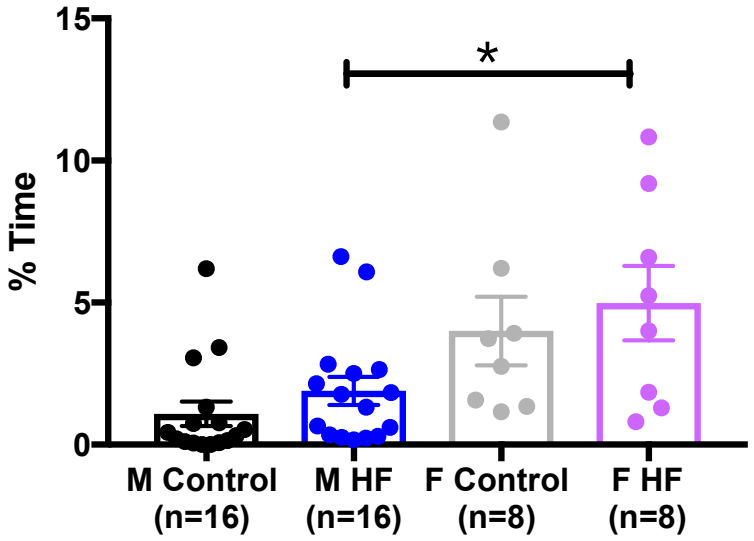

Figure 21A. Open Field Percent Time in Center. The open field test was used to assess anxiety and locomotor activity. There were no significant differences in the time spent in the center in male or female HFD rats in comparison to their controls. HFD Females in comparison to HFD males spend less time in the corners. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

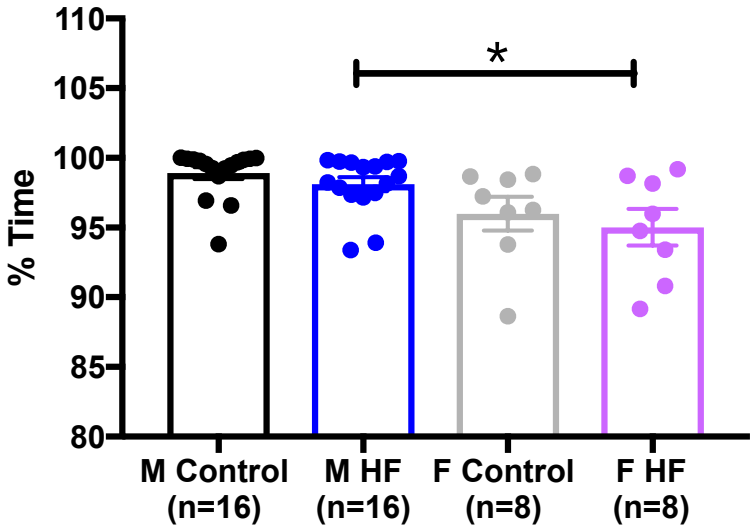

Figure 21B. Open Field Percent Time in Corners and Sides. There were no significant differences in the time spent in the corners in male or female HFD rats in comparison to their controls. HFD Females in comparison to HFD males spend less time in the corners. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

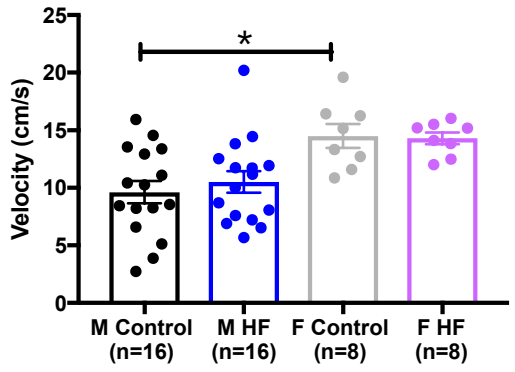

Figure 22A. Open Field Velocity. The open field test was used to assess anxiety and locomotor activity. There were no significant differences in the velocity in male or female HFD rats in comparison to their controls. Females in comparison to males moved at higher velocity. Data are represented as mean ± SEM. *p<0.05 by one-way ANOVA.

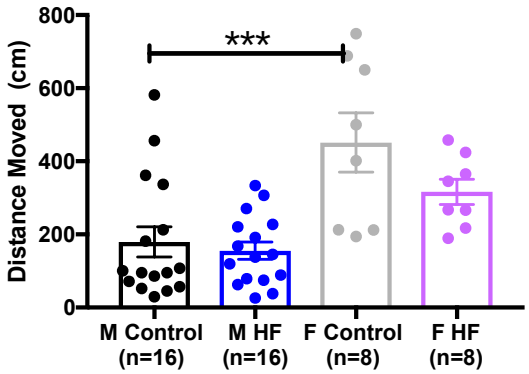

Figure 22B. Open Field Total Distance Traveled. There were no significant differences in the total distance traveled in male or female HFD rats in comparison to their controls. Females in comparison to males traveled further distance. Data are represented as mean ± SEM. ***p<0.001 by one-way ANOVA.

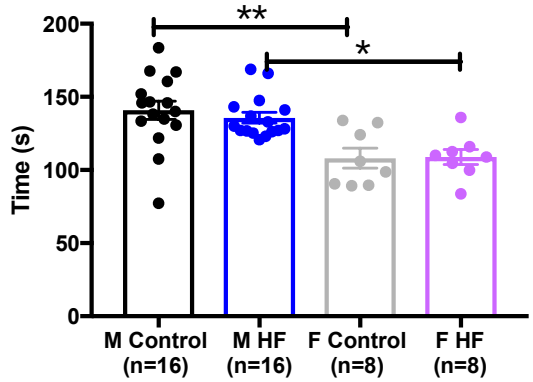

Figure 22C. Open Field Average Rest Time. There were no significant differences in the average rest time in male or female HFD rats in comparison to their controls. Females in comparison to males spent less time resting. Data are represented as mean ± SEM., **p<0.01, and *p<0.05 by one-way ANOVA.

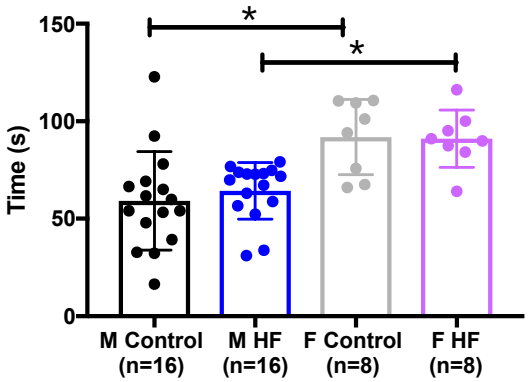

Figure 22D. Open Field Average Movement Time. There were no significant differences in the average movement time in male or female HFD rats in comparison to their controls. Females in comparison to males spent more time moving. Data are represented as mean ± SEM. *p<0.05 by one-way ANOVA.

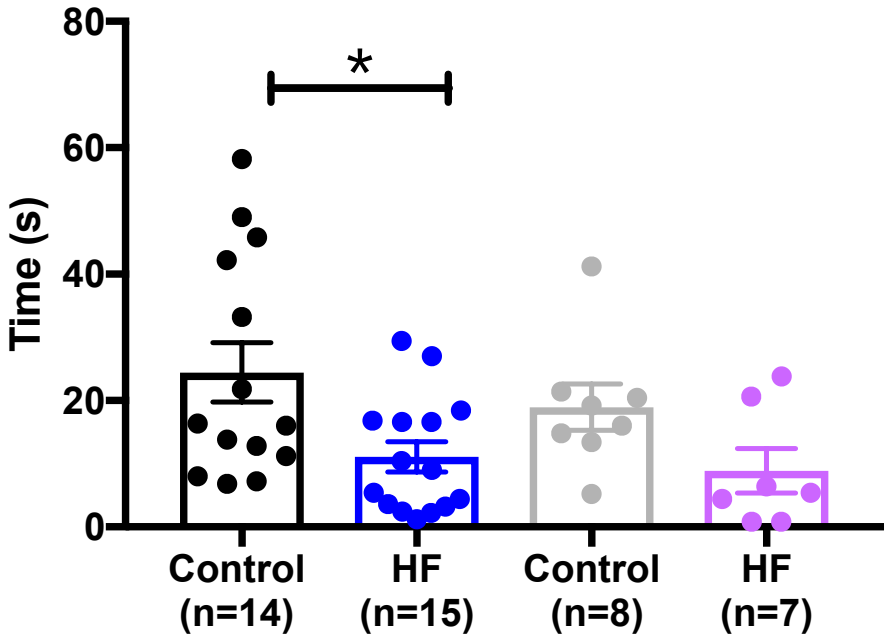

Figure 23A. Barnes Maze Probe Day Latency to First. The Barnes maze test was used to assess spatial learning and memory. The latency to first was reduced in male HFD rats in comparison to controls and not changed in the females. Data are represented as mean ± SEM. *p<0.05 by one-way ANOVA.

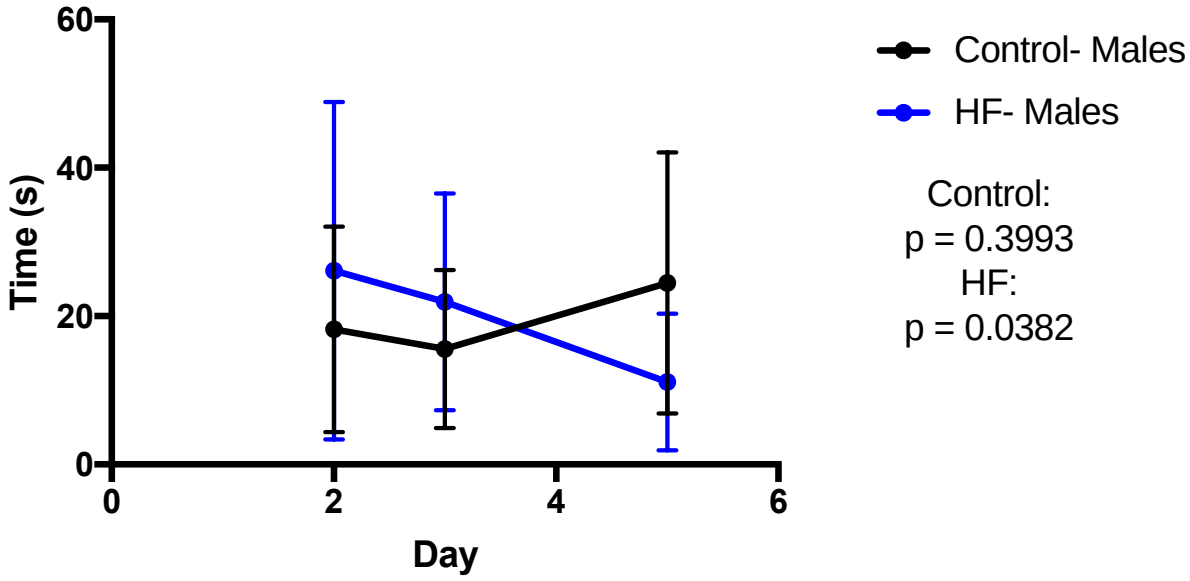

Figure 23B. Barnes Maze Latency to First Learning Curve. The learning curve for the male groups shows reduced latency to first on probe day. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

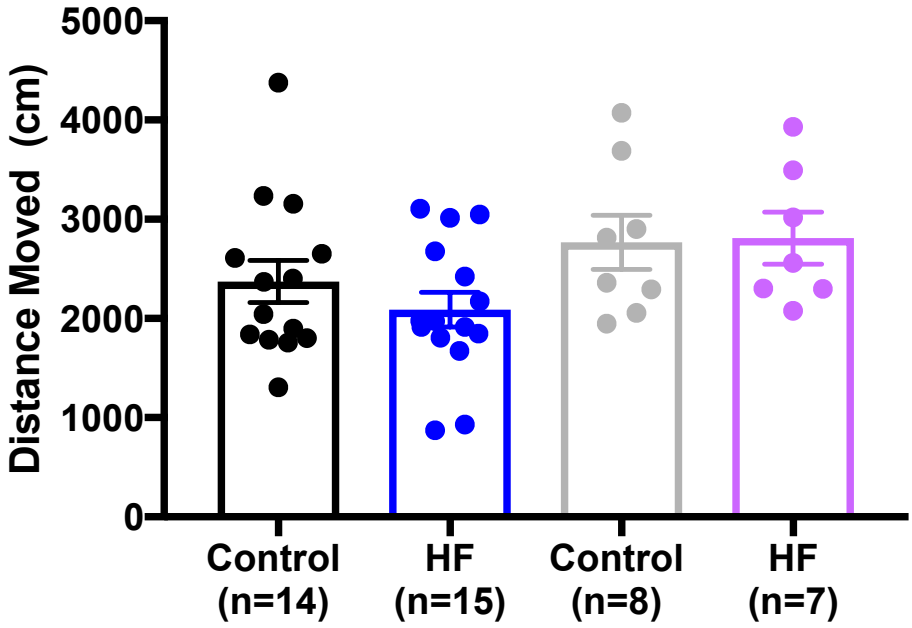

Figure 24A. Barnes Maze Probe Day Distance Moved. The Barnes maze test was used to assess distance moved and there were no significant differences between the groups. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

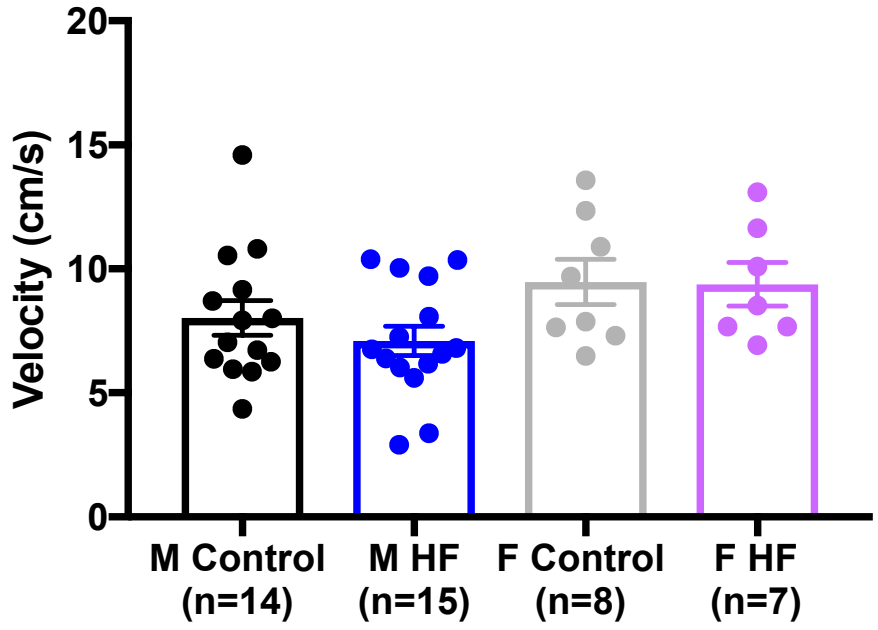

Figure 24B. Barnes Maze Probe Day Velocity. The Barnes maze test was used to assess velocity and there were no significant differences between the groups. Data are represented as mean ± SEM. Analyzed by one-way ANOVA.

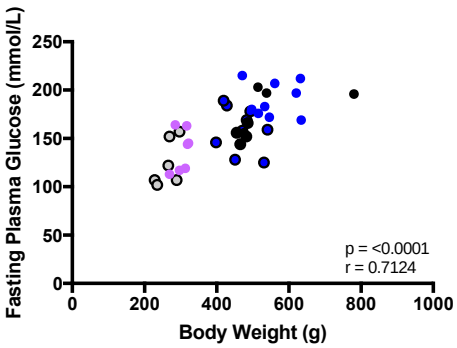

Figure 25A. Body : Fasting Blood Glucose. Correlations were conducted to determine relationships between fasting plasma glucose levels and body weight. There were significant positive correlations in this relationship.

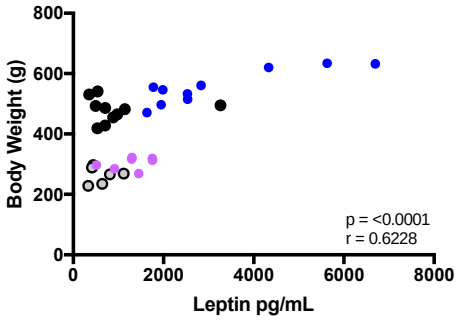

Figure 25B. Leptin : Body Weight. There were significant positive correlations in this relationship.

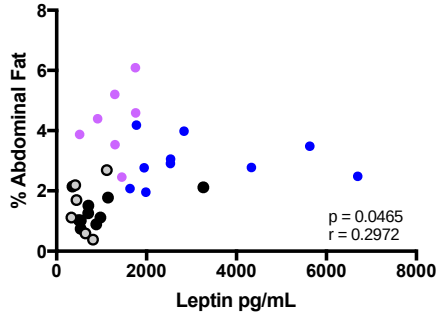

Figure 25C. Leptin : % Abdominal Fat Weight. There were significant positive correlations in this relationship.

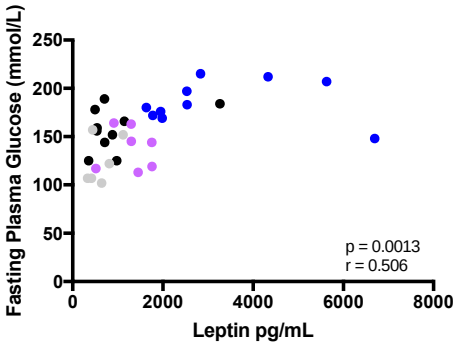

Figure 25D. Leptin : Fasting Blood Glucose. There were significant positive correlations in this relationship**.**

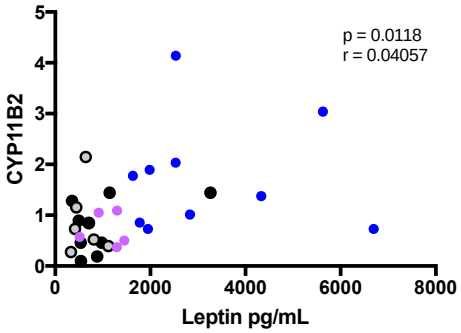

Figure 25E. Leptin : CYP11B2. There were significant positive correlations in this relationship.

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DISCUSSION

In this study, a HFD rat model was used to assess the effects of HFD on parenchymal arteriole structure, cerebral perfusion, inflammation, neurogenesis, and memory in males and females. The effects of the diet were confirmed by measuring different physiological parameters such as aldosterone, leptin, and blood glucose. The HFD led to significant alterations in PA structure in females and did not change PAs in males. Furthermore, this HFD did not lead to alterations in any other parameters in males or females. Therefore, this HFD did not lead to alterations in memory or cognition.

I. Validation of the Model

The SD rats were obtained at 3 weeks of age and placed on high fat diet containing 36% fat until 27-31 weeks of age. As they aged, the rats were weighed weekly until euthanasia. The control and HFD rats did not significantly change in weight until week 10 when the separation in weight began to take place. As expected, the HFD male rats gained more weight than the controls, but unexpectedly, the female HFD rats did not significantly differ in weight in comparison to the controls. Each rat's final body weight was measured on the day of euthanasia and was averaged. This model was previously used in a study conducted by Northcott *et al* and in that study, their HFD rats had significantly elevated body weight, abdominal fat weight, increased mean arterial pressure (MAP) as well as hyperaldosteronism (82). In this study, the male HFD rats had significantly elevated body weights which confirmed that the HFD was leading to the desired result and was similar to the results previously obtained from this HFD diet in Northcott's study. Furthermore, the males had significantly higher body weights than the females.

In addition to body weight, the abdominal fat weight was collected from each rat and weighed. As expected, both male and female HFD rats had significantly increased fat weight in comparison to their individual controls. The HFD females had a significantly higher percentage of abdominal fat weight to body weight in comparison to the HFD males. In this section of the study, some animals were not weighed so they were excluded from the body weight analysis as well as the abdominal fat weight analyses. In a recent study conducted by Pétrault *et al*., in male C57Bl6/J mice fed a HFD containing 40% saturated fat or a control diet containing 3% fat, they looked at the link between cerebrovascular dysfunction and cognitive impairment and the role visceral adiposity played. They found significant elevations in visceral adipose tissue weight with their HFD group gaining significantly more weight at 3, 9, and 12 months of age. Interestingly, they did not have any significant differences at 6 months of age whereas in our study, we see significant differences in fat tissue accumulation. As for body weight, they saw significant differences between their groups until the age of 12 months. Our animals were not aged for that long so we cannot make that direct comparison (91). The results obtained from our study are in keeping with human studies that show that for the same BMI, women typically present with ~10% higher body fat in comparison to men throughout the entire life span (55). In another study conducted in 18-84-year-old males and females, they found as men and women aged, they experienced increases in BMI as well as visceral fat accumulation. Overall, men gained more visceral fat weight than women did. However, as the men aged further, they experienced a decrease in the rate of BMI and visceral fat accumulation while the women's rates remained constant. In other words, the men's rate of fat gain decreased to eventually match the rate of gain seen in the women, while the women maintain a constant rate of fat change in adulthood (57). This supports the sex differences observed in our studies. It is not yet clear as to

what is driving these differences, but studies suggest that estrogen and testosterone play a role in fat distribution in both sexes (56). Furthermore, these results suggest that the male rat is an overweight model rather than obese. In other diet induced obesity models like the cafeteria diet-induced obesity rat model, significant weight differences between controls and HFD rats begin to take place as early as week two of feeding. In a study conducted to look at the effects of the cafeteria-diet induced obesity on cognitive impairment, their HFD rats gained approximately 163g of weight in comparison to their controls (83) while in our studies, there was a gain of 84g.

Obesity is associated with diseases like type II diabetes so blood glucose was measured to determine the likelihood of developing this disease. Fasting plasma glucose was significantly elevated in male HFD rats in comparison to their controls while the females did not change. Additionally, plasma glucose was significantly higher in HFD males than females. According to the study conducted by Pétrault *et al*., male overweight and obese mice had significantly higher fasting plasma glucose levels than normal weight controls (91) matching the results obtained from this study. According to another study conducted in 100 obese and 100 normal weight men and women, serum fasting glucose was significantly elevated in males and females in the obese group which indicated that the obese subjects were prone to develop cardiovascular and metabolic disease (58). In another study conducted in male and female Wistar rats aged 10 weeks, they were fed a HFD containing 26% fat or a control diet containing 2.9% fat. Male and female HFD rats and controls had similar glucose levels regardless of the significant fat accumulations observed (59). In our studies, only males had significantly elevated fasting blood glucose which could indicate the development of insulin resistance but further data on insulin levels is needed to make this conclusion. Further, we observed a lower plasma glucose level in the female HFD rats when compared to HFD males even at baselines suggesting that females

start with a lower plasma glucose levels. In a study conducted in 126 adults aged 17 to 25 years old, blood sugar, blood pressure, and cholesterol levels were measured in men and women. They found that all these parameters were lower in women (67). A positive correlation between body weight and fasting plasma glucose was also observed in this present study suggesting a relationship between the two parameters. In a previous study conducted in this rat model, blood glucose was significantly elevated in the male rats as well which matches the results obtained from our study (82). In other studies conducted in humans, blood glucose levels and insulin resistance increase with increasing BMI (84).

Obesity/overweight increases the risk of hypertension development so blood pressure is another factor that can be altered as a result of HFD feeding. Therefore, we measured blood pressure but there were no significant changes observed between our groups. In a study conducted in 10-week-old male and female Wistar rats on a HFD diet, obesity is suggested to be a predictor of cardiovascular risk in males and females. In their studies, body weight was increased by 49% in females and 21% in males. They further indicated that obesity alone, independent of the blood pressure increase they observed, leads to increased arterial stiffness (59). Other studies using the Sprague Dawley strain of rats have shown systolic blood pressure to be constant in controls and HFD fed rats which is consistent with the results of this study (60). In our lab, previous studies used radiotelemetery transmitter catheters placed in the femoral artery were used to measure blood pressure in the same rat model used for this study, and the results obtained differed from those obtained in this study. In their study, blood pressure was significantly elevated in the HFD rats (82). The difference in this result could be due to the different method utilized to study this parameter. In a study conducted to compare radiotelemetry and tail-cuff methods, they found that although reliable systolic blood pressure measurements

were made from both methods, in some cases, the tail-cuff failed to accurately detect elevate blood pressure and despite the invasive nature of the radiotelemeter, it produced more consistent measurements (96). Therefore, further measurements should be taken and a more reliable method should be utilized.

Leptin and aldosterone are the two major hormones that are affected as a result of HFD feeding. Leptin is made in adipocytes and its main function in the body is to reduce food intake and increase energy expenditure. Leptin levels are increased in the body as a result of obesity (51). As expected, leptin in the HFD fed males was significantly elevated in comparison to its controls but unexpectedly, it was not significantly changed in the females HFD groups despite the significant increase in abdominal fat weight. In overweight/obesity, abdominal fat weight was increased and adipocyte size and number typically increase and therefore leptin levels were expected to be higher. Human and animal studies show that females produce more leptin than males (54, 85) and given the percentage of abdominal fat weight to body weight, we expected to see higher leptin in the females but that was not the case. In studies conducted in men and women, leptin levels rose 3.4-fold more rapidly as a function of BMI in women than men and this hyperleptinemia was associated with insulin resistance (62). It is not clear as to why leptin levels do not reflect what is seen in the literature in this study. It is possible that errors were made during the experiment and therefore, further assays need to be conducted with a larger sample size. In correlations conducted between leptin and body weight, leptin and percent abdominal fat weight, as well as leptin and fasting blood glucose, significant positive correlations were observed confirming the relationships that exist between these parameters.

Leptin has been proposed to be a regulator of aldosterone release in males and females (53). This suggests that leptin plays a role in regulating CYP11B2 levels which codes for

aldosterone synthase. Therefore, CYP11B2 mRNA expression levels were expected to be elevated in the HFD rats. As expected, CYP11B2 mRNA expression was significantly elevated in HFD males but unexpectedly, it was not changed in the females which is consistent with the lack of increase in leptin levels. A correlation was also observed between leptin levels and CYP11B2 in this model. We collected blood from the rats via tail vein at 12 weeks of age and cardiac puncture at the time of euthanasia and aldosterone levels were measured using an ELISA assay. At 12 weeks of age, there were no significant difference between the male control and HFD rats. Similarly, there were no significant differences between the female control and HFD rats. At 12 weeks, a sex difference was observed between the male and female HFD group where aldosterone was significantly lower in females than in males and at euthanasia age, the levels were not different in any groups. The results from this study are similar to published reports showing aldosterone levels increase in correlation with leptin levels in hyperleptiemic mice that were obese but only in the case of the males (53). Further studies confirmed these findings by measuring aldosterone and adrenal CYP11B2 levels in models of obesity where leptin was depleted and as a result of this depletion, plasma aldosterone levels and CYP11B2 did not increase (53). In comparison to previous studies conducted in this rat model, aldosterone levels at time of euthanasia were similar in the male HFD rats. In the controls however, the aldosterone levels measured in this study were significantly higher than previously reported (82).

The adrenal glands were weighed in all groups, and there were no significant differences between the weights, therefore, differences in adrenal weights cannot be attributed to any observed differences in CYP11B1, CYP11B2, or aldosterone levels in the males and females. Although aldosterone levels were increased in the females from week 12 to euthanasia age, the methods of blood draw were different and therefore, the two sets of aldosterone measurements

should not be compared to each other in a direct manner. CYP11B1 mRNA expression levels were also measured since it codes for 11-beta-hydroxylase which is the rate limiting enzyme for corticosterone production, and corticosterone can be converted to aldosterone. Those levels were not expected to be changed and indeed, there were no significant differences in the males and females or between the sexes.

II. Cerebral Perfusion:

Cerebral perfusion is critical for normal brain function and memory, therefore, we utilized arterial spin labeling to measure cerebral perfusion to the cortex and CA1 region of the hippocampus. The CA1 region is vitally important for representing space in the environment. Overall, the hippocampus consolidates short term memory to long term memory and is a major region affected in VCID. Regions of interest (ROIs) were selected from both hemispheres of the brain utilizing the T2 maps for guidance. The cortex served as a control region to compare to the CA1 region. Perfusion was not changed to either location of the brain. Some studies conducted in humans have shown correlations between high BMI and reduced blood flow velocity (BFV) in the MCA and men had lower BFV than women. They also measured cerebrovascular resistance which was increased with BMI in men and women (63). In the study conducted by Pétrault *et al*., they looked at perfusion of the MCA using laser Doppler laser flowmetry and observed a reduction in the HFD group. They also used MRI to measure perfusion to the hippocampus and other regions. Unexpectedly, they found that perfusion to the hippocampus was higher in overweight and obese mice in comparison to the normal weight (91). Their differences in perfusion appeared between 6 to 12 months so the older age can contribute to the differences observed in our results.

In our data, there is some variability observed and this could be due to a few factors. The rats were anesthetized with isoflurane which is a vasodilator. This could interfere with baseline perfusion. The temperature and breaths per minute were monitored throughout the experiment and any fluctuations can lead to alterations in flow. Furthermore, the slice of brain chosen for analysis slightly differed in location in anterior and posterior position which could lead to the observed variability as well. The ROI sizes and locations selected for analysis in the CA1 region and cortex were kept the same in an attempt to minimize variability so this can be excluded as a factor of the variability. Additionally, any fluctuations in the anesthesia rats, heart rate, or breaths per minute that take place in the animal can contribute to this variability.

III. PA Structure:

PA structure was assessed using pressure myography in male and female control and HFD rats. It was hypothesized that PAs would undergo remodeling in HFD males and females. The parameters obtained from these experiments are outer diameter and lumen diameter. Parameters including wall thickness, vessel area, wall area, lumen area, wall to lumen ratio, strain, stress, and stiffness were subsequently calculated. Previous studies in our lab in AngII hypertensive mice have shown PAs to have inward remodeling (2) and other studies have shown the MCA to have an increase in wall thickness opposing the effect seen in PAs (9). Other studies conducted by Osmond *et al* also assessed MCA remodeling in the obese Zucker rat (OZR) at different ages, the MCA exhibited inward remodeling with a small increase in wall thickness, but this change was associated with the hypertension that develops with age in the Zucker rat and not with obesity (64). In the current study, male control and HFD rat PAs did not have any significant changes in structure while the females had significant changes. The function of these arterioles was not studied here, but alterations in the function of PAs can explain potential

compensation mechanisms that may take place to prevent reductions in cerebral perfusion. In a study conducted in hypertensive rats, enhanced arteriole tone and astrocyte signaling was observed in order to protect PA dilation and preserve the function of the NVU (97). Studies conducted in our lab in MCAs show the artery undergoes inward hypertrophic remodeling. In this present study, the female PAs are undergoing inward remodeling evidenced by the reduction in outer and lumen diameter, as well as the wall, lumen, and vessel areas. It is also trending towards hypertrophic remodeling shown by the trend of increase in the wall to lumen ratio. The function of PAs in this HFD state will be addressed in future studies

In the study conducted by Pétrault *et al*., they looked at vascular reactivity of the MCA which is a parameter not measured in this study, but they found evidence of impaired endothelium dependent relaxation in their HFD mice aged 9 to 12 months which could contribute to reductions in cerebral perfusion. They also observed this relaxation to be lower in PAs in the hippocampus and prefrontal cortex regions of the brain (91). In our study, we used pressure myography to assess PA structure. The function of the PAs can be altered without a change in structure which can explain potential changes in perfusion but this needs to be further studied and will be addressed in future work.

IV. Neurogenesis, synaptic markers, and inflammation:

PAs dive into the brain from the pial arteries and as they do, they become encased in astrocytes and microglia. Communication takes place between PAs and their surrounding environment which can happen in both directions meaning that PAs can communicate to microglia and astrocytes (65, 86) or microglia and astrocytes can initiate that communication to the PAs (66). There were no significant differences in any markers measured in this study, and there were no sex differences observed. DCX is associated with precursor cells and immature

neurons in the brain. A decrease in this marker is an indicator of early neuronal damage and therefore has the potential to reduce memory (52). In a study conducted in juvenile and adult HFD C57BL6/J mice, a HFD diet containing 24% fat was administered to 3-week-old and 12-week-old mice. Neurogenesis in the hippocampus was evaluated by determining the number of immature neurons using DCX as a marker. The number of DCX-positive cells was significantly reduced in juvenile HFD mice but not adult HFD mice. In addition, they found no differences in blood glucose between age matched controls and HFD mice but they observed a reduction in glucose in the older mice in comparison to the young mice. (68). Therefore, it is possible that in the current study we missed the time when DCX expression might have been reduced because we only sampled at one time point in adult rats.

Synaptophysin is a synaptic vesicle protein encoded by the SYP gene. Reductions in the levels of this protein due to changes in the gene expression can lead to reduced spatial learning (61). In a study conducted in C57Bl6J mice, a group of low fat diet and HFD mice were treated for one, two, or three months. Their results showed that SYP levels were only decreased after 3 months of HFD feeding. Their groups did not express any significant changes in blood glucose (69). The diet they used is composed of different percentages of fat than the diet used in our study which could explain the differences in the results obtained.

Inflammatory makers were also measured. Inflammation is associated with cerebral small vessel disease (CSVD) and elevations in pro-inflammatory markers such as IL-6 promote macrophage activation leading to increased BBB permeability and ultimately CSVD (72). Inflammation is the method by which the body protects itself from injury and through that response, pro-inflammatory cytokine production is increased in the body. Cytokines are proteins secreted by immune cells and work to regulate the immune response. They are a mechanism by

which communication between immune cells takes place. They bind to receptors on the surface of cells and determine the actions of these cells (87). TNFα is produced in microglia of the brain in response to various pathological processes such as ischemia and its production corresponds to other cytokines like IL-6. Additionally, TNFα activates glial cells regulating tissue remodeling, and scar formation and it promotes inflammation by stimulating capillary endothelial cell pro-inflammatory responses (70). It is also synthesized and released in the brain by astrocytes and some populations of neurons (71). IL-6 plays a critical role in normal homeostasis of neuronal tissue and its absence leads to reduced glial activation in brain injury, while IL-6 overproduction in the brain leads to neurodegeneration. In the brain IL-6 is secreted by microglia, endothelial cells, and neurons (72). There were no significant differences in mRNA expression from IL-6 or TNFα between HFD and control fed male or female rats and no sex differences were observed.

In addition to inflammation, BDNF expression levels were measured. BDNF is a neurotrophic factor encoded by the BDNF gene and is a protein involved in enhancing the survival and function of neurons. It also promotes the growth and proliferation of new neurons and synapses (88). There were no significant differences observed in the mRNA expression of BDNF in male or female HFD or control diet rats. In studies conducted on 2-month, 6-month, and 2-year old female SD rats maintained on a high fat and refined sugar diet, hippocampal BDNF mRNA expression levels were reduced in the HFD group at all ages in comparison to the controls (42). These results differ from the results found in our study as the females did not have reduction in BDNF and the differences in diet could play a role. In another study conducted on male Wistar rats, three different diets were administered; a high fat, high carbohydrate, and a high fat pair-fed diet for 6-weeks, BDNF levels were not altered in any treatment (74). Similarly to our male data, their HFD group gained significantly higher weight than the other groups.

However, in their study, the rats were maintained on the HFD for a longer period of time and the diet was different. Their HFD contained 43% fat while the one utilized here contains 36% fat. This suggests that the rats should be maintained on the diet for a longer period of time or a different diet would achieve more prominent results.

Additionally, we used the microglial marker Iba-1 to assess microglial numbers and activation. Similarly, GFAP was also measured to provide an estimate of astrocyte mRNA expression levels. Iba-1 levels were not significantly changed in male or female HFD rats in comparison to their controls. Microglia serve as the resident immune cells of the brain and work to restore CNS function and astrocytes serve a critical function in structuring the brain and play active roles including the secretion of neurotransmitters (89). These cells express GFAP and in our groups, there were no difference in males or females and no sex differences were observed. In a study conducted in adult male C56BL/6J placed on a HFD, obesity was associated with increased activation of microglia but not astrocytes. Specifically, obese mice had more microglia expression with features of activation in the hippocampus. Levels of GFAP-positive astrocytes was not changed in obese mice in the hippocampus leading to the conclusion that obesity leads to changes only in microglial activation in the hippocampus (75).

V. Behavior and Memory:

The open field test was utilized in this study to assess anxiety like behavior and locomotor activity. Rodents avoid light and open spaces, so they are naturally less likely to spend more time in the center of an arena and likely spend more time in the corners and on the sides. A change in that can be seen as a reduction in anxiety like behavior and an indicator of altered executive function (44). Throughout this study, the female control and HFD rats had overall reduced anxiety than the males evidenced by the lower percentage time spent in the corners and

more time in the center, increased velocity, increase in total distance traveled, reduced rest time, and increased movement time than the males. In a study conducted in 4-week-old male and female SD rats, locomotor activity was assessed using the open field test and they found that females moved more than the males (90). Thus, their results match those found in our study. Further, in the study conducted by Pétrault *et al*., they assessed motor activity in an acimeter and found no significant differences between their control and HFD male groups. They further divided their HFD group into a normal weight group, overweight, and obese groups and found a significant reduction in distance travelled and an increase in time rest in their obese group (91). Their results match those obtained from this study showing no differences in locomotor activity in males between the controls and HFD group. However, the method utilized to obtain these measurements was different and could require further investigation to confirm the results.

Utilizing two additional behavioral tests, we were able to measure different aspects of memory and executive function. The brain regions involved in memory formation are the hippocampus, prefrontal cortex, cerebellum, and amygdala. Through the novel object recognition test, we assessed learning and memory alterations (43). In a rat brain, the perirhinal cortex plays an important role in recognition memory, and the ability to evaluate a previously encountered item such as the familiar object is dependent on proper function of the medial temporal lobe (43). According to our results, there were no significant differences in memory in these groups suggesting that this diet is not impacting recognition memory or learning and there were no sex differences observed. Similar to this study, Pétrault *et al* used the novel object recognition test to look at visual recognition memory and unlike our results, they found the HFD had reduced novel object recognition index. However, this observation took place after 12 months on the diet (91) whereas ours took place after 6 months. Therefore, it is possible that with a longer feeding

period, we will observe similar results. An unexpected result was the low exploration coefficient percent for the control males and females. In a study conducted in male Long-Evans rats maintained on a HFD from weeks 6-14, novel object recognition test results have shown that rats explore the novel object ~70% of the time (77). Another study conducted in mice show control mice exploring the novel object 80-90% of the time (78) while our studies show less than 60% of the time and at this time, we do not have a complete understanding of this. An interesting positive correlation was observed between increased fasting plasma glucose concentration and increased exploration coefficient in HFD males. This is a puzzling result as we would expect to see the adverse effects of high plasma glucose to reduce the novel object exploration percent indicating a reduction in memory function. In a study conducted in 210 cognitively health individuals with baseline blood glucose levels between 3.2 and 6.1 mmol/l underwent neuropsychological tests and blood glucose measurements as well as MRI scans. They found higher blood glucose levels to be associated with lower white and grey matter volumes and was associated with poorer cognitive performance. Furthermore, they observed a gender difference with the men exhibiting worse cognitive performances than the women and suggested a role for estrogen protection in women (47).

The Barnes maze test is used to assess spatial learning and memory and is a hippocampal-dependent task where the animals learn the relationship between distal visual cues and finding a fixed escape hole location (45). The main parameters obtained from this test are the latency to first (defined as the amount of time it takes a rat to find the escape hole), the velocity, and the distance traveled during testing. The results from this test are somewhat puzzling at this point because the HFD rats appear to have better memory function than the control rats we do not understand why that is the case. On probe day, the latency to first was measured. In males,

the HFD groups had lower times than their controls indicating that the HFD rats found the escape hole faster which is an indication of improved memory which was not expected. This was also confirmed by the learning curve for males which showed latency to first to be significantly lower in the HFD rats on probe day in comparison to the training days. As for the velocity, it was compared between the controls and HFD rats on probe day and there were no significant differences observed and similarly the distance moved. In the study conducted by Pétrault *et al.*, they utilized the Barnes maze test to assess spatial reference memory and found that it was not altered by diet or weight category which differs from the results from this study and the reason for this difference is unknown.

In this study, there were some differences observed between males and females and a potential explanation for this is the presence of estrogen in females. A study was conducted on C57BL/6J male mice, intact female mice, ovariectomized female, and ovariectomized female mice supplemented with estrogen placed on a HFD or control diet to identify the effect of estrogen. Males and ovariectomized female mice gained weight in the form of abdominal adipose tissue. Males and ovariectomized female mice had significantly higher leptin level than intact females and ovariectomized mice supplemented with estrogen. This suggests a direct correlation between estrogen and abdominal fat weight as well as estrogen and leptin levels (76). In other studies, estrogen has been shown to improve hippocampal-dependent cognitive behavior performances. Specifically, estradiol administration in overiectomized rats has been shown to decrease anxiety and depressant behavior as well as enhance performance in hippocampus-dependent tasks such as water maze navigation and reward-motivated plus maze (77).

LIMITATIONS

There were several limitations in this study. The first limitation was the diet used, which in this study, a dough diet containing 36% fat. It is possible that with a different diet containing a lower percentage of fat or in pellet form, a more profound difference in weight would be observed at earlier age. Furthermore, a pellet diet would allow for easier access to the food. Although, the HFD rats were placed on a calorically higher diet, the food consumption was not tracked therefore, the amount of food consumed could have affected some of the final results. In addition, three different male rat groups were utilized for this study to ensure a powered n-value, while only one set of females was used, so increasing the number of female rats per group would enhance the validity of the results particularly the PA structure analysis. Cerebral perfusion in females was not measured so adding this data would enhance the validity of these results. Additional behavioral tests to measure other areas of cognition would have been useful. The object location memory task is similar to the novel object recognition test but would also identify alterations in spatial memory and discrimination when an object is relocated. The Y maze spontaneous alteration test would measure the willingness of the rodent to discover new spaces and would serve to further validate the open field test results. In addition to studying PA structure, analyzing PA constriction and dilation pathways would provide further insight into the effect of HFD on PA function and would be helpful to determine if structure is sole factor behind the observed changes in memory, inflammation, and neurogenesis.

CONCLUSIONS

The studies conducted in this thesis highlight evidence that HFD feeding from young age increases the susceptibility to PA remodeling and that there are sex differences that exist. In males, the HFD lead to increased adipose tissue weight, increased % abdominal fat and increased leptin while in females, adipocyte and % abdominal fat was increased but vascular remodeling of PAs was observed while nothing else changed. Therefore, we conclude that this HFD leads to inward hypotrophic remodeling in female PAs but not in male PAs. The disconnect between PA structure and lack of change in cognition in the females could suggest that PAs could have an enhanced dilator capacity and therefore, there are no consequences on memory, inflammation, synaptic markers, or neurogenesis. The mechanism by which that happens is yet to be elucidated. The lack of change in the males, indicates that this HFD does not exert a specific difference on the PAs regardless of the changes in leptin levels and the increased body weight. In addition, studies show that midlife obesity is associated with late-life dementia and Alzheimer's disease (19) but in this study, we aimed to look at lifelong obesity starting at childhood. The results obtained from this study could indicate that obesity from childhood to adolescence is not associated with the risks of developing dementia.

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