CHRONIC CONSUMPTION OF A HIGH-FAT DIET: INVESTIGATION OF NEGATIVE CONSEQUENCES

by

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For my friends and family, whose support motivates me to reach my endeavors

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ABSTRACT

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Chronic consumption of a high-fat diet is a lifestyle factor that increases the risk for cognitive impairment (Granholm et al., 2008; Greenwood & Winocur, 2005; Mattson, 2004; Winocur & Greenwood, 2005). A high-fat diet appears to facilitate cognitive impairment through the promotion of insulin resistance (Greenwood & Winocur, 2005; Stranahan et al., 2008; Winocur & Greenwood, 2005). A gap in the literature is an established timeframe of the progression and underlying mechanism, which study in animals would better afford. Furthermore, A limited number of studies have investigated the relationship between a high-fat diet and behavioral dysregulation such as anxiety and depression. The 1st aim of the study was to determine if consumption of a high-fat diet leads to cognitive impairment and behavioral dysfunction at 3, 8, or 13 weeks of consumption. The 2nd aim was to determine if cholesterol levels and HBP activity are aberrantly increased in specific regions in mice that display feeding induced cognitive/behavioral dysfunction. Consumption of the experimental specialty diets produced a number of significant behavioral effects. These significant effects began to emerge after only 3 weeks of low-and high-fat feeding with increased anxiety-like behavior displayed higher in the high-fat diet group for the Elevated Plus Maze and Open Field Test. There was increased thigmotactic behavior and floating in the low-fat diet group in the Morris Water Maze (MWM) task, therefore making cognitive assessment uninterpretable. This pattern in the behavioral tasks were more robust in the 8 week group and alleviated in the 13 week group. There was only a significant difference in depression-like symptoms in the Forced Swim (FS) Task in the 3 week group. Cholesterol analysis is still under review in Dr. Elmendorf's lab to correlate cholesterol levels and cognitive/behavioral impairment.

CHAPTER 1 INTRODUCTION

1.1 Lifestyle Impact on Brain Function

Chronic consumption of a high-fat diet is a lifestyle factor that increases the risk for cognitive impairment (Granholm et al., 2008; Greenwood & Winocur, 2005; Mattson, 2004; Winocur & Greenwood, 2005). A high-fat diet consists of saturated fats, hydrogenated fats and refined carbohydrates (Granholm et al., 2008; Kaplan & Greenwood, 1998). A high-fat diet appears to facilitate cognitive impairment through the promotion of insulin resistance (Greenwood & Winocur, 2005; Stranahan et al., 2008; Winocur & Greenwood, 2005).

This relationship was founded in Alzheimer Disease (AD) research investigating early modifiable risk factors such as insulin resistance and Type 2 diabetes (T2D) (Kilander, Nyman, Boberg, Hansson, & Lithell, 1998; Muller et al., 2007; Nazaribadie et al., 2013; Rasgon et al., 2011; Ronnemaa et al., 2008; Schrijvers et al., 2010; Yaffe, Blackwell, Kanaya, Davidowitz, Barrett-Connor & Krueger, 2004; Young, Mainous, & Carnemolla, 2006). Insulin resistance is associated with a reduced glucose metabolic rate and subtle cognitive impairment with early stages of T2D (Janson et al., 2004). Advanced stages of insulin resistance are associated with greater hippocampal and amygdalar atrophy in T2D patients, which have been associated with lower verbal memory performance in elderly patients (den Heijer et al., 2003). The development of insulin resistance starts years before diagnosis of T2D, and even before prediabetes (Mason, Hanson, & Knowler, 2007; Tabak et al., 2009; Weyer, Bogardus, Mott, & Pratley, 1999). Moreover, cognitive and related affective behavior decline are widely observed in prediabetes and T2D patients, suggesting insulin resistance or an accompanying metabolic derangement may be responsible for an increased risk of AD (Kilander, Nyman, Boberg, Hansson, & Lithell, 1998; Muller et al., 2007; Nazaribadie et al., 2013; Rasgon et al., 2011; Ronnemaa et al., 2008; Schrijvers et al., 2010; Yaffe, Blackwell, Kanaya, Davidowitz, Barrett-Connor & Krueger, 2004; Young, Mainous, & Carnemolla, 2006).

Cell data demonstrate that conditions that mimic the high-fat feeding milieu stimulate cholesterol biosynthesis via increasing hexosamine biosynthesis pathway (HBP) activity (Bhonagiri et al., 2011, Habegger et al., 2012, Penque et al., 2013). Unpublished in vivo data support these cell data showing that in the setting of high-fat feeding peripheral tissue (muscle,

fat) and brain (cerebrum) cholesterol levels are increased. Emerging evidence suggests that deregulated cholesterol metabolism in the peripheral nervous system may be coupled to and/or a determinant of the development of insulin resistance (Stranahan et al., 2008; Winocur & Greenwood, 2005). The changes in the peripheral nervous system have also been implicated in elevation in brain cholesterol that is important for cognitive processing. The underlying relationship between high-fat diet induced changes in peripheral and brain cholesterol remains unknown.

Since World War II, western society has seen a trend in the overconsumption of diets containing excess fat (Granholm et al., 2008). Of growing concern is that a history of high-fat diet consumption has been associated with behavioral dysregulation (Gainey et al., 2016; Sharma, Fernandes, & Fulton, 2013). All in all, investigating the behavioral and underlying biological mechanism(s) associated with high-fat consumption will be essential to understanding risk factors associated with prediabetes, T2D, cognitive decline, and perhaps even AD.

1.2 High-Fat Diet & Cognitive Impairment

Cross-sectional and longitudinal studies indicate that high-fat consumption in various age groups leads to cognitive impairment (Eskelinen et al., 2008; Kalmijn et al., 2004; Kanoski & Davidson, 2011; Solfrizzi, Panza, & Capurso, 2003). A study by Eskelinen et al. (2008) investigated the relationship between midlife dietary fat intake and cognitive impairment. Their design was a longitudinal population-based study in which samples were collected at midlife (50.2), and an average follow-up was 21 years (71.1). They found that saturated fat (SFA) intake (milk products & butter) were associated with poorer global cognitive function and prospective memory.

Other studies have experimentally manipulated dietary fat and examined the effects on cognition. Two studies looked at young males that controlled their diets and measured the effects on cognition (Beilharz, Maniam, & Morris, 2015; Morris, Beilharz, Maniam, Reichelt, & Westbrook, 2015). Holloway et al. (2011) investigated the effects of a high-fat diet (75% Fat) to see if any alterations occurred in healthy subjects. Male subjects (n=16) were randomly assigned for five days to consume either the high-fat diet (75% Fat) or a standard diet (23% Fat). It was a crossover design, so after a 2-week washout period, subjects consumed the opposite diet. A nutritionist educated the subjects on healthy eating and developed a prospective food diary that

started 3 days prior to testing. Each subject was required to follow the prescribed meal plans. A post-hoc dietary analysis was done using a computer-based program. In order to assess cognitive function, the individuals did the Cognitive Drug Research (CDR) computerized assessment battery to measure attention and episodic memory, as well as a self-report to evaluate their mood and alertness. They also conducted a Rapid Visual Information Processing (RVIP) task that assessed complex attention and working memory. It was found that consuming the high-fat diet for five days impaired attention and speed of retrieval, as well as depressed the subjects' mood (Holloway et al., 2011).

A similar study using sedentary male subjects (i.e., physical exertion less than 2 hrs. in a week) revealed a detrimental cognitive effect of inactivity. In this study, the subjects were given a balanced diet for 3 days, and then switched to either a high-fat diet (74%) or a standard diet (17.2%) for 7 days. A written dietary plan was given to them, each subject recorded the food consumed each day and had an ongoing phone interview to monitor compliance. A post hoc dietary analysis was done to confirm compliance. To assess cognitive functions, they were given the CDR computerized assessment battery to measure attention, episodic memory, and two self-reports to measure mood and alertness. They also conducted a RVIP task that assessed complex attention and working memory. As a result, there was a decrease in reaction time and attention (Edwards et al., 2011).

Finally, a recent study in prepubertal children related saturated fats and dietary cholesterol to cognitive flexibility (Khan, Raine, Drollette, Scudder & Hillman, 2015). The children between ages 7-10 were assessed by a dietician for dietary intake in a 24-hour food recall with parental help. Using the Color-Shape Task Switching Paradigm, which utilizes visuospatial attention and reaction time to determine cognitive flexibility, assessment of cognition was made. As a result, they did a partial correlation between diet variables and switch task performance (adjusting for age, sex, SES, VO2 max, and BMI). The study demonstrated that the saturated fats and dietary cholesterol decreased affected their cognitive flexibility (Khan et al., 2015). Collectively, these data from these studies provide strong human evidence that high-fat diet negatively impacts cognition.

1.3 High-Fat Diet & Animal Models

In strong agreement with the clinical data, high-fat feeding of animals has been demonstrated to impair cognition. A significant gap, however, in our understanding is an established timeframe of the progression and underlying mechanisms, which study in animals would better afford. Therefore, the utilization of animal models to measure the progressive effects of high-fat feeding on cognition and behavior would be of translational value to advancing understanding human brain disorders. Moreover, parallel cellular/molecular analyses of various brain regions would be possible and provide insight into mechanisms of disorder.

Scientists have demonstrated that chronic consumption of excess fat induces cognitive impairment in rodents within 1 week (Beilharz, Maniam, & Morris, 2016). This high-fat feeding impairment appears to persist during longer (e.g., 8 months) dietary interventions (Stranahan et al., 2008). Although the literature indicates high-fat consumption leads to cognitive impairment, different dietary parameters, as well as animals used, preclude establishment of a timeframe of cognitive decline. A study by Gainey et al. (2016) compared 6-week-old C57BL/6J (6J) mice, using a control group with a low-fat diet (10% fat), and an experimental group consuming a high-fat diet (60%), and investigated cognitive and anxiety symptoms. They tested cognitive and behavioral dysfunction with separate groups at 1 week, 3 weeks, or 6 weeks of consumption history, with each time point having a low-fat control and a high-fat diet group. They found that after 1 week of high-fat consumption, mice had impaired memory as assessed with the Novel Object Recognition (NOR) test. The 3-week consumption history high-fat diet group performed worse in the object learning recognition tasks and the 6-week group showed increased anxiety symptoms in the Open Field Test (OF) and Elevated Zero Maze Task (EZM). This indicates that consumption history differentially affects the outcome of specific tasks, and cognitive and behavioral symptoms vary across consumption history. Understanding a timeframe for the development of high-fat diet-induced memory impairment will help advance understanding of the development of early cognitive impairment.

Dietary animal studies have given us insight into specific brain regions that are more vulnerable to high-fat feeding. These regions include the hippocampus, frontal cortex and thalamus (Cordner & Tamashiro, 2015; Greenwood & Winocur, 2005; Kaplan & Greenwood, 1998; Pistell et al., 2010; Stranahan et al., 2008; Winocur & Greenwood, 2005). Hippocampal-dependent tasks, which are important for spatial learning and memory, were the most vulnerable to a high-fat diet

(Granholm et al., 2008; Winocur & Greenwood, 2005; Stranahan et al., 2008). The most common hippocampal-dependent tasks include the Morris Water Maze (MWM), Barnes Maze, Radial Arm Maze (RAM), T and Y maze, and NOR; rodents given a high-fat diet in these tasks typically showed poorer performance (Cordner & Tamashiro, 2015). Reversal learning in the MWM and Barnes Maze are considered prefrontal cortex- and striatum-dependent, which is also affected by a high-fat diet (Cordner & Tamasiro, 2015). Other deficits such as procedural learning, short-and long-term memory, and general intellectual functioning show poorer performance as a consequence of high-fat consumption (Greenwood & Winocur, 2005; Kaplan & Greenwood, 1998; Pistell et al., 2010; Stranahan et al., 2008; Winocur & Greenwood, 2005). The MWM and RAM are the most common behavioral task used to assess cognitive impairment due to a high-fat diet. The MWM & RAM was found to be hippocampal dependent, more specifically by NMDA receptors. Cordner & Tamashiro (2015) found that several studies gave NMDA antagonist to rodents and performance on the MWM or the RAM was impaired. The Barnes maze, T and Y-Maze, and NOR tasks are other hippocampal dependent task that has shown impairment due to a high-fat diet, except they are not dependent on NMDA receptors. Lesion studies have given insight into brain regions involved in these behavioral tasks: Lesions of the hippocampus has found to impact the MWM, RAM, and Barnes Maze. Furthermore, modifications to the MWM, RAM, and Barnes Maze to measure reversal learning have shown deficits when there are lesions in the prefrontal cortex and striatum.

Lesion studies and transgenic mice have shown that the T-Maze is dependent on the hippocampus, septum, prefrontal cortex, basal forebrain, thalamus, striatum and cerebellum (Cordner & Tamashiro, 2015). Lesion on the NOR task show that the hippocampus is important for recall of an object's place otherwise known as Object Recency, whereas lesions to the prefrontal cortex and perineal cortex is involved in novel object preference. Arnold et al. (2014) found that a high-fat diet is associated with abnormal neuroanatomic integrity of hippocampal CA3 dendrites and spines, evidence was shown more with impaired working memory using the T-maze. Furthermore, cortical and hippocampal regions showed the development of insulin resistance demonstrating insulin signaling is disrupted. All in all, high-fat consumption has been reported to impair these specific behavioral tasks, which are brain region dependent. Therefore, it shows evidence for specific brain regions are more vulnerable to high-fat consumption.

A majority of high-fat diet studies are conducted in rats, with limited number utilizing mice in recent years. Characterization of high-fat diet effects in mice would seem important given the vast potential of mutant mouse models to help inform the mechanisms by which high-fat diet leads to behavioral impairment. 6J mice were first utilized in high-fat feeding in 1988 (Surwit et al., 1988). According to Alexander, Chang, Dourmashkin & Leibowitz (2006), the target goal is to have an animal model that reflect physiological changes similar what is seen in humans. Inbred strains such as the 6J mice are helpful since they can be genetically specific and modified to reflect human pathology (Alexander, Chang, Dourmashkin & Leibowitz, 2006; King, 2012). 6J mice were found to be susceptible to the high-fat effects, which have led to the development of obesity, hyperinsulinemia and altered glucose homeostasis (Alexander, Chang, Dourmashkin & Leibowitz, 2006; Winzell & Ahren, 2004). Also, mice have a shorter generation time, which allows longitudinal studies to be more efficient (King, 2012).

While high-fat feeding studies with mice provide an essential experimental tool to understand etiological aspects of obesity-related disease, a large majority of these feeding studies utilize a diet that provides ~60% of total calories from fat (Arnold et al., 2015; Gainey et al., 2016; Kleine et al., 2016), which exceeds Western Society's upper limits estimated to be at 45% of total calories from saturated fats (Stranahan et al., 2008). Furthermore, Pistell et al. (2010), using the Stone T-Maze spatial learning and memory task with high-fat fed (40% and 60% kcal from saturated fat) 6J mice, found impairment with consumption of the 60%, but not at 40% of a high-fat diet. Notwithstanding, however, many aspects of that study (e.g., mouse strain, diet duration, etc.) could explain the lack of an effect of a diet that is closely akin to that eaten in Western society on metabolic and brain health.

1.4 High-Fat Diet & Emotional Dysregulation

There are a limited number of studies investigating the relationship between high-fat diet and behavioral dysregulation such as anxiety and depression. A recent study by Gainey et al. (2016) investigated both cognitive and anxiety-like behavior in independent groups of 6J mice at 1, 3, or 6 weeks of high-fat diet consumption. Anxiety-like behavior was exhibited at the 6-week timeframe using the OF and EZM task. Similarly, Sharma and Fulton (2013) found that 12 weeks of a high-fat diet (58%) induced anxiety and depressive like behavior in obese 6J mice using the EPM, OF, & FS test. A gap in the literature exists in assessing anxiety and depressive symptoms

reflective of high-fat consumption of a Western diet. Some studies show that palatable foods (i.e., high-fat foods) alleviate depression and anxiety symptoms for a short period, further reinforcing consumption during stressful events (Maniam & Morris, 2010; Maniam, Antoniadis, Le & Morris, 2016). Sharma et al. (2013) used 6J mice and placed them on a 6-week high-fat diet (58% fat); they exhibited signs of anhedonia, anxiety, and sensitivity to stressors during withdrawal. These studies provide evidence that a high-fat diet can influence behavioral dysregulation. As presented later, studies currently underway support the hypothesis that chronic consumption of a high-fat diet (45%) reflective of a Western diet may exhibit anxiety and depressive symptoms.

1.5 High-Fat Diet & Insulin Resistance

Studies suggest that chronic consumption of a high-fat diet leads to cognitive impairment through the promotion of insulin resistance (Greenwood & Winocur, 2005; Stranahan et al., 2008; Winocur & Greenwood, 2005). Winocur & Greenwood (2005) compared Zucker fa/fa rats that are insulin resistant with rats fed a high-fat diet for 3 months and found similar impairments on the cognitive tasks compared to their respective controls. They theorized that high-fat induced cognitive impairment may be due to insulin resistance and decreased glucose uptake in the brain. So they did another study with the same design and they found baseline scores showing impairment, but the next day half the group was given an intraperitoneal injection of glucose (100 mg/kg BW) or saline (equal amounts) and found improvement in the glucose treatment group (Winocur & Greenwood, 2005). In an earlier study, Greenwood and Winocur (1996) found that saturated fatty acid was a major contributor to cognitive impairment when rats were fed a diet of saturated fats, monounsaturated fats, or polyunsaturated fats. Evidence suggests that high-fat consumption at 45% in mice and humans are equivalent since they have similar physiological changes during the development of insulin resistance (Arnold et al., 2015; Bhonagiri et al., 201l; Fisher-Wellman, et al., 2016; Habegger et al., 2012; Penque, Hoggatt, Herring, & Elmendorf 2013). A study by Lee et al. (2011) showed high-fat diet induces insulin resistance as early as 3 days. These studies indicate a relationship between a high-fat diet and insulin resistance and suggest that insulin resistance or a similar etiological factor for both insulin resistance and brain dysfunction cause cognitive decline.

1.6 High-fat Diet: Cholesterol-Insulin Resistance Interaction in Periphery

Chronic consumption of a high-fat diet has been implicated in contributing to abnormal cholesterol metabolism, which has been linked to insulin resistance and impaired cognition (Suzuki et al., 2010). The blood-brain barrier is known to regulate cholesterol influx from the circulation, and the cellular cholesterol demand of the brain depends on the regulation of underlying cholesterol biosynthesis in the peripheral nervous system (Bjorkhem & Meaney, 2004; Dietschy & Turley, 2004). A high-fat diet induces a response in the adipose tissue and skeletal muscle that increases plasma membrane (PM) cholesterol content (Habegger et al., 2012, Ambery et al., 2017). These and other studies found that the excess membrane cholesterol causes insulin resistance in these tissues by reducing cortical actin filaments that are essential for the insulin-stimulated glucose transporter (GLUT4)-mediated glucose transport (Bhonagiri et al., 201l; Habegger et al., 2012a; Penque, Hoggatt, Herring, & Elmendorf 2013). Human skeletal muscle data also show that insulin-stimulated glucose disposal is inversely related to membrane cholesterol content (Habegger et al., 2012). Strikingly, lowering the excess membrane cholesterol to levels seen in insulin-sensitive skeletal muscle fully restores insulin sensitivity (Bhonagiri et al., 201l; Habegger et al., 2012a; Habegger et al 2012b).

Mechanistically, in vitro data suggest that membrane cholesterol accumulation results from increased glucose flux through the hexosamine biosynthesis pathway (HBP) (Bhonagiri et al., 201l; Habegger et al., 2012a; Penque, Hoggatt, Herring, & Elmendorf 2013), a pathway well-recognized to cause insulin resistance and play a key role in the etiology of T2D (Buse, 2006; McClain & Crook, 1996). Marshal, Bacote, & Traxinger (1991) first demonstrated that HBP activity was involved in the development of insulin resistance in adipose tissue and skeletal muscle (Fig. 1). Glucose entry into the HBP is catalyzed by the first and rate-limiting enzyme: GFAT, which converts fructose-6-phosphate and glutamine into glucosamine-6-phosphate (GlcN-6-P). GlcN-6- P is subsequently metabolized, culminating in the production of UDP-N-acetylglucosamine (UDP-GlcNAc), the high-energy substrate for O-GlcNAc transferase (OGT), a nuclear and cytosolic enzyme that catalyzes the addition of GlcNAc to serine/threonine residues (Kreppal, Blomberg, & Hart, 1997; Lubas, Frank, Krause, & Hanover, 1997). This posttranscriptional modification modulates the activities of signaling proteins, regulates most components of the transcription machinery, effects cell cycle progression and regulates the targeting/turnover or functions of many other regulatory proteins (Hart, 2014). In transgenic mice,

overexpression of OGT and GFAT lead to insulin resistance (Cooksey et al., 1999; Herbert et al., 1996; McClain et al., 2002).

In vitro study suggests that increased HBP activity is responsible for the increase cholesterol accumulation by increasing the O-linked N-acetylglucosamine modification of the transcription factor Sp1, leading to transcriptional activation of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis (Fig. 2) (Bhonagiri et al., 201l; Habegger et al., 2012; Penque, et al, 2013). Blocking HBP or Sp1 from attaching to DNA has been shown to reduce cholesterol accumulation and GLUT4/glucose transport dysregulation in cultured cells (Bhonagiri et al., 201l; Habegger et al., 2012; Penque, et al., 2013).

1.7 High-Fat Diet: Central Nervous System

Brain cholesterol is primarily made *in situ* within the brain (Granholm et al., 2008; Ferris et al., 2017; Suzuki et al., 2010). However, evidence suggests that elevations in cholesterol, insulin and glucose in the peripheral nervous system may influence brain cholesterol synthesis within the brain, and that this may in turn be linked to Alzheimer's-like neurobiological changes. Diabetes research has shown evidence that during pre-diabetic stages, there is an elevation of brain cholesterol, whereas, there is a depletion of brain cholesterol when an individual is diabetic (Ferris et al., 2017; Ismail et al., 2017; Refolo et al., 2000; Suzuki et al., 2010).

A study by Refolo et al. (2000) investigated the link between cholesterol metabolism and AD. They wanted to test if increased cholesterol had an effect on amyloid accumulation using hemizygous, double-mutant PSAPP transgenic mice (Crossbreed between $APP_{K670N,\ M671L,}$ and $PS1_{M146V}$), which contain the familial human risk genes APP and PSI. They fed the transgenic mice a high-fat diet for 7 weeks and showed cholesterol was elevated in both the peripheral and central nervous system. Elevated cholesterol was shown to increase amyloid accumulation by 3 measures: sandwich ELISA, IP/MS, and immunohistochemical/image analysis of serial sections. This supports our model that increased HBP activity occurring in fat/muscle causes peripheral insulin resistance and that this may be occurring at the same time increased HBP activity in the brain is contributing to cognitive decline.

The prevailing hypothesis has been that elevated brain cholesterol comes about by the movement of peripheral cholesterol across the blood-brain barrier after its synthesis in the periphery. There is a gap in the literature with respect to how cholesterol becomes elevated in the

brain; indeed, even though the prevailing view is that peripheral cholesterol can get into the brain, the blood-brain barrier is not thought to allow cholesterol to get across. According to Suzuki et al. (2010), it is theorized that insulin in the peripheral nervous system increases and is able to cross the blood brain barrier via a receptor-mediated transport. So as insulin increases in the peripheral nervous system, it will travel to the brain and influence insulin signaling that will increase de novo brain cholesterol biosynthesis. Another theory to explain the elevated brain cholesterol is by Ferris et al. (2017), who hypothesized that there is an alteration in the astrocytes with affected SREBP2 mediated cholesterol synthesis. Lastly, although the blood brain barrier blocks cholesterol in the peripheral nervous system, there is an active cholesterol metabolite named 27 Hydroxycholesterol (27-OH), and it is thought to cross the blood brain barrier. Excess 27-OH will increase brain cholesterol and reduce brain glucose uptake (GLUT4 expression impaired) (Ismail et al., 2017). Preliminary data we have supports the hypothesis that elevated brain cholesterol comes about by *de novo* HBP-mediated synthesis of cholesterol in the brain as a consequence of high-fat diet consumption.

In conclusions, there is a lack of an established timeframe of the progression of cognitive and behavioral dysfunction. Behavioral dysregulation, such as anxiety and depression symptoms during chronic consumption of a high-fat diet, is limited. Characterization of high-fat diet effects in mice are important given the vast potential of mutant mouse models to help inform the mechanism(s) by which high-fat diet leads to cognitive and behavioral impairment. Also, an established correlation between cognitive, behavioral, and physiological changes can help develop a better picture of these relationships. All in all, specific aims presented next were designed to fill in significant gaps in the literature by developing a longitudinal study to assess the progression of cognitive and behavioral dysfunction and associated brain cholesterol, and to delineate mechanisms of brain cholesterol buildup.

1.8 Specific Aims

1. Determine if consumption of a high-fat diet leads to cognitive impairment and behavioral dysfunction at 3, 8, or 13 weeks of consumption. I hypothesized that increased consumption duration will increase cognitive and behavioral impairment.

2. Determine if cholesterol levels and HBP activity are aberrantly increased in specific brain regions in mice that display feeding-induced cognitive/behavioral dysfunction. I hypothesized that vulnerable brain regions will have elevated cholesterol levels as a result of aberrantly increased HBP activity.

CHAPTER 2 METHODS & MATERIALS

2.1 Aim 1 General Design

A longitudinal experimental design was used to study time-related changes in cognitive performance and affect behavior. 5-week old male C57BL/6NJ (6N) mice obtained from Jackson Laboratory were used in this study. The mice were randomly assigned to one of two groups: either a low-fat or high-fat group. Following a 1 week acclimation period on the low-fat diet, to acclimate to the taste of palm oil and prevent any positive or negative contrast that may occur to switching feed, the mice were 6 weeks old and either switched to a high-fat diet or were continued on the low-fat diet.

Separate groups of 6N mice were tested at 3-, 8-, or 13 weeks of high-fat or low-fat diet consumption. Preliminary data indicated that these time points were able to assess the progression of cognitive impairment, and there were differences in performance between feeding conditions in the MWM (not graphed). I hypothesize that chronic consumption of the high-fat diet will increase anxiety-like behavior in the EPM and OF tests, increase depression-like-behavior in the forced swim (FS) test, and impair cognitive performance in the MWM. I anticipate that some or all of these effects might emerge as a function of time on the high-fat diet, which makes the longitudinal design an important aspect of the experiment.

2.2 Mouse Model

6N mice express nicotinamide nucleotide transhydrogenase (NNT, an enzyme that protects against reactive oxygen damage known to harm pancreatic insulin secretory processes) that is not expressed in the widely used 6J substrain. 6N mice have been shown to display insulin secretion and fasting hyperinsulinemia under high-fat feeding conditions similar to the human condition (Fisher-Wellman, et al., 2016). Use of the 6N mice affords a more precise model of insulin resistance in humans during high-fat feeding (Fisher-Wellman, et al., 2016). A total of 60 mice were used [10 mice per subgroup x 3 subgroups (3, 8 or 13 weeks) x 2 diets (LF and HF) = 60].

2.3 Specialty Food

The low-fat diet contains 20% kcal from protein, 70% kcal from carbohydrates, and 10% kcal from fat (DO1030107, Research Diets, Inc.). This low-fat, as well as the high-fat, diet represented modified forms of the standard low-fat (D12450B) and high-fat (D12451) diets from this supplier, with adaptations regarding type of fat (palm oil instead of lard) and carbohydrates, to better mimic the fatty acid/carbohydrates of the average diet in Western societies. At 6 weeks of age, the group designated for a high-fat diet were switched to the HF feed containing 20% kcal from protein, 35% kcal from carbohydrates, and 45% kcal from fat (D01030108) for 8 weeks. This diet mimics the percent of saturated to monounsaturated to polyunsaturated fatty acids (40:40:20). The group designated for the low-fat diet continued on this diet for the designated timeframe.

2.4 Body Weight Measurement

The body weight of all mice was measured during the first and last week of testing with an Ohaus CS200-001 Portable Compact Scale. Body weight of each mouse was measures in grams. This allowed us to measure any weight difference due to aging and high-fat diet consumption.

2.5 Behavioral Assessment

2.5.1 Elevated Plus Maze

The EPM is an assessment of anxiety-like behavior in rodents (Walf & Frye, 2007). The apparatus is configured in a plus shape (+) and comprised of two open arms (34.6 cm x 7.3 cm) and closed arms (34.6 cm X 7.3 cm) elevated 74.3 cm from the ground. Room lighting consisted of a mix of red light with ambient light near the apparatus to allow the open arms to be 40 lux and the closed arms 12 lux. This maze assesses anxiety-like behavior due to the mouse's natural response to avoid open areas and find closed areas. Therefore, recording the number of open arm entries and the amount of time spent in the open arms has been validated as a measure of anxiety in the mice (Walf & Frye, 2007). Drugs that are anxiogenic reduce the amount of time in open arms, whereas anxiolytic drugs increase the time spent in open arms. The mice were placed in the center of the maze and their behavior recorded for 5 minutes, and the amount of time spent and entries into the open arms were recorded using a video camera, and manually scored.

2.5.2 Open Field Test

The OF measures general locomotor activity and anxiety-like behavior in rodents. The mice were placed in a Plexiglas box (40 cm x 40 cm x 30 cm) that contains photocell beams along the perimeter to detect the location of the rodent vertically and horizontally and the distance traveled is measured. The chamber is an enclosed apparatus that is lit by a light and is ventilated with a fan in the back wall. The mice were placed in the center of the chamber and recorded for 30 minutes. Assessment for total distance and amount of time in the center of the box compared to peripheral areas is measured. It is natural for rodents to spend time on the outer edges if they are anxious, called thigmotaxis. Thigmotactic behavior is recorded if they were in the center of the locomotor box with a length 20 cm x width 20 cm. When mice spend time in the center location, it is a sign they are less anxious. Certain drugs such as benzodiazepines have an anxiolytic effect in mice and the center time is increased.

2.5.3 Morris Water Maze

The MWM is a behavioral task to assess spatial learning and memory. The pool is 125cm in diameter and a circumference of 785.4 cm. The pool was filled to within 25 cm of the rim of the tank. The temperature of the pool was 24- 26 °C water which was made cloudy by adding non-toxic white paint. This experiment assesses spatial learning and memory in the first phase, reference memory in the second phase, and visual acuity or weight difference in the third phase.

The first phase of MWM experiment was the hidden platform task and assessed spatial navigation, and the rodent's ability to quickly find the platform. The mice rely on cues in its environment to remember where the platform is located. The mice were placed in the water (24-26^{o}C) and swam to a submerged platform (Hidden) within 60s. The location to drop the mouse into the pool was randomly chosen and not in the same quadrant as the hidden platform or the platform's adjacent sides. The mice were tested in four trials per day, at 60s each, over 7 days. The hidden platform did not move from its location across trials or days. Furthermore, software technology (HVS image, Hampton, UK) recorded the movement of the mice in order to determine path lengths thigmotaxis, speed and floating.

On day 7, after the last hidden platform trial, the mice completed a probe trial that assessed reference memory to see if the mice have learned the location of the platform. The platform was

removed, and the mice were placed into the pool and monitored for swim search behavior measured as preference for target location along with measured of path length, thigmotaxis, floating and swim speed. The stronger the tendency for the mouse to swim in the area where the platform was previously hidden. The stronger the spatial bias acquisition in the hidden platform training.

Lastly, the mice were tested in a visible platform trial. useful for assessing any general performance deficits including any visual impairment, effects of weight difference, motivational differences or other possible deficits due to high-fat consumption other than spatial cognition. This test consists of the platform being placed above the water line, and black tape is used on the edges of the platform to make it visible to the mouse swimming in the tank. The visual platform was randomly placed in a different quadrant at each trial, and the mice were placed in the pool in a location that was not in the same quadrant as visible platform. Measurements such as latencies, path lengths, thigmotaxis, floating and speed were recorded for each trial.

2.5.4 Forced Swim Test

The FS test is utilized to assess depression-like behavior. The mice were placed in a 2000 ml beaker (Height 19.3cm & Diameter 13.1cm) with water (24-26°C) filled to 1400 ml that is inescapable for 6 minutes and scored for immobility. Mice showing depressive-like behavior expressed a higher amount of time spent being immobile. The amount of time spent immobile was recorded for the last 4 minutes of the 6-minute test; data show that in the initial two minutes of the task, mice are very active and react to placement into an inescapable apparatus. This task is considered not fully reflective of human depression, however, there is considerable predictive validity when assessing antidepressant medication used to treat depression in humans. Antidepressants have been shown to reduce immobility time in rodents; this gives credibility for assessment of depressive symptoms.

2.6 Aim 2 Cholesterol Analysis

After the behavioral assessments, brains of all mice were extracted, and the cerebral cortices, hippocampi, and cerebella were isolated, frozen in 2-methylbutane, and stored in microcentrifuge tubes (Fisherbrand). These isolated brain regions were delivered to the Elmendorf

lab for cholesterol content and HBP activity measures. Differences between the low-fat and high-fat groups and between the 3, 8, and 13 week groups will be determined.

Briefly, lysates of the isolated brain regions will be prepared via polytron homogenization in a detergent-free HES buffer [20 mM HEPES (pH 7.4), 1 mM EDTA, and 255 mM sucrose containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml pepstatin, 10 μg/ml aprotinin, and 5 μg/ml leupeptin]. The detergent-free lysate is used to measure cholesterol using the Amplex Red Cholesterol Assay Kit as routinely performed (Penque et al., 2013). To accurately determine the total protein content of the detergent-free HES lysate, a well-mixed sample of the lysate will be mixed with NP40 detergent and the protein amount in the solubilized lysate will be determined with the Bradford protein assay. Cholesterol content in each sample will be normalized to the total protein amount in each sample. The solubilized lysates will also be used to measure mRNA/protein (e.g. HMGR) expression, as well as O-GlcNAc modification of Sp1 as routinely performed in the lab to assess the HBP-mediated cholesterolgenic response [Bhonagiri et al., 2011; Habegger et al., 2012; Penque et al., 2013].

2.7 Statistical Analysis

2.7.1 Body weight

An unpaired t-test was used to compare body weight differences between the low-fat diet and high-fat diet group during behavioral testing at each consumption history time point (3, 8. & 13 weeks).

A Pearson correlation coefficient was calculated to assess a relationship between specific consumption history groups' body weight and behavioral tasks in the Elevated Plus Maze (Total entries, open and closed entries, and open and closed times), Open Field Test (Total distance, center distance, and center time), Morris Water Maze (Latencies, path lengths, speed, thigmotactic behavior and floating) and Forced Swim Test (Immobility). Significance was determined at $p<0.05$.

2.7.2 Elevated Plus Maze

An unpaired t-test was used to compare the open arm and closed arm; more specifically, entries and time in the open and closed arms was analyzed in the high-fat diet group and low-fat

diet (control) group. A one-way ANOVA was used to compare across consumption history groups. Where a significant difference was measured, a Sidak's multiple comparisons test was used to compare individual groups. Significance was determined at $p<0.05$ in all measurements.

2.7.3 Locomotor/Open Field Test

For the Locomotor/Open Field Test the mean ±SEM of general locomotor activity and time spent in the center were compared using an unpaired t-test between the high-fat diet group (treatment) and low-fat diet (control) group. A two-way ANOVA was used to compare across consumption history groups and feeding conditions. Significance was determined at $p<0.05$ in all measurements.

2.7.4 Morris Water Maze

For each duration of feeding, a two-way mixed ANOVA with diets as a grouping factor and training as a repeated measure was used to compare mean daily latencies, path lengths, thigmotaxis, floating and speed during the acquisition and also for the visible platform training. During the probe trial, a two-way mixed ANOVA with diet as a grouping factor and quadrant as a within-subjects factor was used to assess preference for the location by measuring time in seconds. An independent groups t-test was used to compare differences in Probe Trial thigmotactic behavior (time and path), speed and floating. Significance was determined at $p<0.05$ in all measurements.

2.7.5 Forced Swim Test

Time spent immobile in the high-fat diet (treatment) group and low-fat (control) group was compared using an unpaired t-test. One-way ANOVA was used to compare across consumption histories for each feeding condition. Significance was determined at $p<0.05$ in all measurements.

2.7.6 Cholesterol Analysis

A two-way ANOVA will be used to find a main effect difference between feeding conditions and consumption history group of cholesterol levels at each specific brain region (hippocampus, cerebral cortices & cerebellum). Significance will be determined at $p<0.05$. An unpaired t-test will be used to measure individual groups and brain regions.

CHAPTER 3 RESULTS

3.1 Elevated Plus Maze: Anxiety Measures

3.1.1 Week 3

An independent groups t-test was conducted to compare open and closed arm entries and time between the low-fat and high-fat diet group in order to assess differences in anxiety behavior. It was predicted that the high-fat diet group would have less entries and time in the open arm and increased entries and time in the closed arms. In the week 3 group, there was not a significant difference in open or closed arm entries between the low-fat and high-fat groups [$t(18) = 0.8155$, $p = .4255$, for open arm entries; $t(18) = 0.6821$, $p = .5039$, for closed arm entries], (Fig. 3A, Fig. 3B). There was not a significant difference in time spent in the open arm and closed arm between the low-fat and high-fat groups [$t(18) = 0.2514$, $p = .8043$, for open arm time; $t(18) = 0.1344$, $p = .8946$], (Fig. 3C, Fig. 3D). The high-fat diet group did not show higher anxiety behavior as indicated by similar entries and time into the open and closed arm compared to the low-fat diet.

3.1.2 Week 8

An independent groups t-test was conducted to compare open and closed arm entries and time between the low-fat and high-fat diet group in order to assess differences in anxiety behavior. It was predicted that the high-fat diet group would have less entries, and time in the open arm and increased entries and time in the closed arms. In the week 8 group, there was not a significant difference in open or closed arm entries between the low-fat and high-fat groups [$t(18) = 1.789$, $p = .0905$, for open arm entries; $t(18) = 0.1089$, $p = .1552$, for closed arm entries], (Fig. 4A, Fig. 4B). There were no significant differences in time spent in the open arm and closed arm between the low-fat and high-fat groups [$t(18) = 1.621$, $p = .1224$, for open arm time; $t(18) = 1.4$, $p = .1784$], (Fig. 4C, Fig. 4D). The high-fat diet group failed to show higher anxiety behavior as indicated by similar entries and time into the open and closed arm compared to the low-fat diet.

3.1.3 Week 13

An independent groups t-test was conducted to compare open and closed arm entries and time between the low-fat and high-fat diet group in order to assess difference in anxiety behavior. It was predicted that the high-fat diet group would have less entries and time in the open arm, and increased entries and time in the closed arms. In the week 13 group, on average, there was not a significant difference in open or closed arm entries between the low-fat and high-fat groups [$t(18) = 1.24$, $p = .2309$, for open arm entries; $t(18) = 0.94$, $p = .3597$, for closed arm entries], (Fig. 5A, Fig. 5B). There were no significant differences in time spent in the open arm and closed arm between the low-fat and high-fat groups [$t(18) = 0.8671$, $p = .3973$, for open arm time; $t(18) = 1.501$, $p = .1507$], (Fig. 5C, Fig. 5D). The high-fat diet group failed to show higher anxiety-like behavior as indicated by similar entries and time into the open and closed arm compared to the low-fat diet.

3.1.4 Across-group comparison

An independent groups t-test was conducted to compare open arm entries and time between consumption history groups in order to assess differences in anxiety behavior. It was predicted that increased consumption duration would have less entries and time in the open arm due to increased anxiety behavior. Consumption duration had a significant effect on the number of entries in the low-fat group [main effect of consumption history, $F(2, 27) = 8.184$, $p = .0017$]. Using the Sidak's multiple comparisons test to analyze individual groups, the 3 week group had a higher number of entries compared to the 8week group $t(27) = 3.72$, $p = .0028$ and 13 week group $t(27) = 3.238$, $p = .0095$ (Fig. 6A). In contrast, when analyzing the low-fat diet group on time spent in open arms, there was not a significant difference between consumption duration (Fig. 6B). The low-fat diet group at 3 weeks displayed less anxiety-like behavior as indicated by the higher number of entries compared to the 8week and 13week group.

An independent groups t-test was conducted to compare open and closed arm entries between consumption history groups in order to assess differences in anxiety behavior. It was predicted that increased consumption duration would have less entries and time in the open arm. Consumption durations had a significant effect on the number of entries in the high-fat diet group [main effect by consumption history, $F(2, 27) = 10.83$, $p = .0004$]. The 3week group had a higher

number of entries compared to the 8week group t(27) =4.279, p=.0006 and the 13week group t(27) = 3.724, p = .0027 (Fig. 6C). Similarly, consumption duration had a significant effect on the time spent in the open arms in the high-fat diet group [main effect by consumption duration, F(2, 27) = 5.268, p = .0117]. Using the Sidak's multiple comparisons test to analyze individual groups, the 3week group had a higher time spent in the open arms compared to the 8week group t(27) = 3.148, p = .0119 (Fig. 6D). The high-fat diet group at 3 weeks displayed less anxiety-like behavior as indicated by the higher number of entries and time compared to the 8week and 13week group.

3.2 Locomotor Data (Open Field Test): Anxiety & General Activity

3.2.1 Week 3

An unpaired t-test was conducted to assess general locomotor activity (total distance) and anxiety behavior (center distance and time) between the low-fat diet group and the high-fat diet group. It was hypothesized that the high-fat diet group will display less general locomotor activity and increased anxiety behavior. There was no significant difference between the low-fat and high-fat diet groups in total distance, center distance, and percentage distance traveled in the center (Fig. 7A, Fig. 7B, Fig. 7C). There was not a significant difference in time spent in the center or percentage of time in the center between the low-fat diet group and the high-fat diet group (Fig. 7D, Fig. 7E). This indicates that the feeding conditions did not differ in general locomotor activity or anxiety behavior in the OF test.

3.2.2 Week 8

An unpaired t-test was conducted to assess general locomotor activity (total distance) and anxiety behavior (center distance and time) between the low-fat diet group and the high-fat diet group. It was hypothesized that the high-fat diet group will display less general locomotor activity and increased anxiety behavior. On average, the low-fat diet group traveled more compared to the high-fat diet group t(18) = 2.651, p = .0162 (Fig. 8A). The low-fat diet group and high-fat diet group did not have a significant difference between in the distance traveled in the center or the percentage of distance in the center (Fig. 8B, Fig. 8C). There was not a significant difference in time spent in the center or percentage of time spent in the center between the low-fat diet group and the high-fat diet group (Fig. 8D. Fig. 8E). This indicates that the high-fat diet group had a

lower general locomotor activity compared to the low-fat diet group. There was no difference in anxiety-like behavior between feeding conditions as indicated by center distance and time.

3.2.3 Week 13

An unpaired t-test was conducted to assess general locomotor activity and anxiety behavior between the low-fat diet group and the high-fat diet group. It was hypothesized that the high-fat diet group will display less general locomotor activity and increased anxiety behavior. On average, the low-fat diet group traveled more compared to the high-fat diet group $t(18) = 2.829$, $p = .0111$(Fig. 9A). The low-fat diet group traveled more in the center of the locomotor box compared to high-fat diet group $t(18) = 2.829$, $p = .0111$ (Fig. 9B). The low-fat diet group spent a greater percentage of time in the center compared to high-fat diet group $t(18) = 2.809$, $p = .0116$ (Fig. 9C).

On average, the low-fat diet group spent a higher amount of time in the center compared to high-fat diet group $t(18) = 2.584$, $p = .0187$ (Fig. 9D). Analyzing the percentage of time spent in the center, the low-fat diet group spent a higher percentage of time in the center compared to the high-fat diet group $t(18) = 2.584$, $p = .0187$ (Fig. 9E). The high-fat diet group had less general locomotor activity as indicated by less total distance. The high-fat diet group showed anxiety behavior has indicated by less distance and time traveled in the center of the locomotor box. This confirmed our hypothesis that the high-fat diet group would have less general locomotor activity and increases anxiety behavior.

3.2.4 Across-group comparison

A two-way ANOVA was conducted to analyze general locomotor activity between feeding conditions and consumption histories. When analyzing total distance, there was a significant main effect on consumption history [main effect by consumption history, $F(2, 54) = 183$, $p<.0001$]. There was a significant main effect on feeding condition [main effect by feeding condition, $F(1, 54) = 16.51$, $p = .0002$] (Fig. 10A). There were significant differences in general locomotor activity as indicated by total distance between feeding conditions and consumption history groups.

A two-way ANOVA was conducted to analyze general locomotor activity between feeding conditions and consumption histories. When analyzing center distance, there was a significant main effect on consumption history [main effect by consumption history, $F(2, 54) = 20.89$, $p<.0001$]. There was a significant main effect on feeding condition [main effect by feeding

condition, F(1, 54) = 7.728, p = .0075] (Fig. 10B). There were significant differences in anxiety-like behavior as indicated by center distance between feeding conditions and consumption history groups.

A two-way ANOVA was conducted to analyze general locomotor activity between feeding conditions and consumption histories. When analyzing center time, there was a significant main effect on consumption history [main effect by consumption history, F(2, 54) = 183, p = .0006]. There was a significant main effect on feeding condition [main effect by feeding condition, F(1, 54) = 16.51, p = .0399] (Fig. 10C). There were significant differences in anxiety-like behavior as indicated by center time between feeding conditions and consumption history groups.

3.3 Morris Water Maze: Cognition

3.3.1 MWM Task (Week 3)

Measure of latencies and path lengths to reach the platform during all phases of the MWM assessed cognitive behavior. Performance was assessed by measuring swim speed, thigmotaxis and floating. Each cognitive and performance measure was analyzed using a two-way group x day repeated measures ANOVA during the hidden and visible platform phase. During hidden platform acquisition, both diet groups showed significant reduction in latencies and path lengths over days [main effect of day, F(6, 234) = 10.57, p<.0001, for latencies; F(6, 54) = 21.3, p<0.0001 for path lengths]. However, there were no significant main or interactive effects of diet (Fig. 11A, Fig. 11B). Furthermore, both groups showed there was a reduction in thigmotaxis behavior [main effect of day F(6, 54) = 4.443, p = .0010]. There was no significant difference in swimming speed over days or between diet groups (Fig. 11C). There was no significant main effect or interactive effects on diet on thigmotaxis and floating (Fig. 11D, Fig. 11E). In summary, both feeding groups showed acquisition to the hidden platform across days, but no difference between feeding conditions. There was also increased thigmotactic behavior, which can affect cognitive behavior, and interpretation of cognitive behavior may be uninterruptable.

During the visible platform test, both groups showed significant reduction in latencies and path lengths over days [main effect of day, F(1, 39) = 67.62, p<0.0001, for latencies; F (1, 9) = 25.2, p = .0007 for path lengths] (Fig. 11A, Fig. 11C). However, the low-fat diet group showed significant deficits in the latency to the visible platform[main effect of diet, F(1, 39) = 12.71, p =

.0010] (Fig. 11A). However, there were no group differences in path lengths (Fig. 11B). There was a significant group difference in thigmotaxis behavior (Fig. 11D). There were no significant main effect differences in swimming speed, or floating over days or any group differences (Fig. 11C, Fig. 11E). The significantly slower latencies of the low-fat group compared to the high-fat diet group was associated with greater thigmotactic behavior in the low-fat diet group, suggesting low-fat diet group could have significantly slower latencies due to increase thigmotactic behavior, when translated from the hidden to the visible platform training.

During the probe trial, an unpaired t-test was conducted between feeding conditions. There was no preference for location, and there was no difference in performance between diets (Fig. 12A). The low-fat diet group spent more time in thigmotaxis than the high-fat diet group $t(18) = 2.2$, $p = .0411$ (Fig. 10D). There was no difference in thigmotaxis, path length, floating or speed between feeding conditions (Fig. 12B, Fig. 12C, Fig. 12E, Fig. 12F). This suggests that during the probe trial the low-fat diet group were exhibiting anxiety-like behavior indicated by the significant increase in thigmotactic behavior compared to the high-fat diet group.

3.3.2 MWM Task (Week 8)

Measuring latencies and path lengths during all phases of the MWM assessed cognitive behavior. Performance was assessed by measuring speed, thigmotaxis and floating. Each measure was assessed using two-way repeated measures ANOVA during the hidden and visible platform phase. During acquisition, all groups showed significant reduction in latencies and path lengths over days [main effect of day, $F(6, 234) = 10.57$, $p<.0001$, for latencies; $F(6, 54) = 21.3$, $p<.0001$ for path lengths]. However, the low-fat diet group showed significant acquisition deficits as indicated by longer average latencies [main effect of diet, $F(1, 39) = 25.04$, $p<0.0001$] and path length average [main effect by diet, $F(1, 9) = 8.46$, $p<0.0174$] to the hidden platform, compared to the high-fat diet group (Fig. 13A, Fig. 13B). Furthermore, there was a significant reduction in swim speed over days [main effect by day, $F(6, 54) = 4.193$, $p = .0016$], and an increased floating across days [main effect of day, $F(6, 54) = 4.328$, $p = .0012$]. There was a decrease in thigmotaxis behavior over days [main effect of day $F(6, 54) = 11.14$, $p<0.0001$] (Fig. 13C, 13D, Fig. 13E). The low-fat diet group had higher percentage of thigmotaxis behavior [main effect of diet, $F(1, 9) = .9.847$, $p = 0.0120$] than the high-fat diet group (Fig. 13D). There were no significant group differences in floating and speed (Fig, 13C, Fig, 13E). In summary, the low-fat diet group had

poorer acquisition as indicated by slower latencies and longer path lengths, and this could be due to poor performance issues such as increase thigmotactic behavior and increase floating.

During the visible platform test, both groups showed significant reduction in latencies and path lengths over days [main effect of day, $F(1, 39) = 43.08$, $p<0.0001$, for latencies; $F(1, 9) = 27$,$p = .0006$ for path lengths]. However, the low-fat diet group showed significant deficits in finding the visible platform as indicated by longer average latencies [main effect of diet, $F(1, 39) = 11.02$, $p = .0020$]. There were no group differences in path lengths, indicating that the low-fat mice and high-fat mice did not differ in this measure of swimming performance (Fig. 13A, Fig. 11B). There were no significant main effect differences in swimming speed, thigmotaxis, or floating over days (Fig. 13C, 13D, 13E). However, the low-fat diet group had a higher percentage of thigmotaxis behavior [main effect by diet, $F(1, 9) = 13.6$, $p = .0050$] compared to the high-fat diet group in the visible platform test (Fig. 13D). In summary, the low-fat diet group's cognitive performance may have been associated with increased thigmotactic behavior.

During the probe trial, there was a preference for location [main effect of location, $F(1, 36) = 21.17$, $p<0001$], however the low-fat diet group and the high-fat diet group did not differ in preference for the location during the probe trial. Using the Sidak's multiple comparisons test to analyze individual groups, the low-fat diet mice spent more time in the target region compared to non-target region $t(9) = 3.771$, $p = .0262$. The high-fat diet also mice spent more time in the target region compared to the non-target region $t(9) = 4.232$, $p = .0131$ (Fig. 14A). There were no differences in path lengths, thigmotaxis time or path scores, floating or speed between feeding conditions (Fig. 14B, Fig. 14C, Fig. 14D, Fig. 14E, Fig. 14F). This indicates that the high-fat diet group had better acquisition during the hidden platform task compared to the low-fat diet group.

3.3.3 MWM Task (Week 13)

Measuring latencies and path lengths during all phases of the MWM assessed cognitive behavior. Performance was assessed by measuring speed, thigmotaxis and floating. Each measure was assessed using two-way repeated measures ANOVA during the hidden and visible platform phase. All groups showed significant reduction in latencies and path lengths over days [main effect of day, $F(6, 234) = 10.49$, $p<0.0001$, for latencies; $F(6, 54) = 14.76$, $p<0.0001$ for path lengths]. The low-fat diet group had deficits in acquisition as indicated by higher latencies [main effect by diet, $F(1, 39) = 16.33$, $p<0.0002$]. In contrast, there was no group difference in path lengths (Fig.

15A, Fig. 15B). There an increase in thigmotaxis behavior and floating across days [main effect of day $F(6, 54) = 2.962$, $p = .0142$ for thigmotaxis; $F(6, 54) = 5.800$, $p = .0001$for floating], but there were no significant group differences in thigmotaxis and floating (Fig. 15D, Fig. 15E). There was no significant difference in swimming speed over days or group differences (Fig. 15C). In summary, the low-fat diet group had longer latencies in finding the hidden platform compared to high-fat diet mice. The performance effects such as increased thigmotactic behavior and floating across days may have confounded the low-fat diet's deficit in latencies, an effect was not seen in the path length measure.

During the visible platform test, both groups showed a significant reduction in latencies and path lengths over days [main effect of day, $F(1, 39) = 29.93$, $p<0.0001$, for latencies; $F(1, 9) = 22.9$, $p<0.0010$ for path lengths]. However, the low-fat diet group showed significant longer latencies to find the visible platform [main effect of diet, $F(1, 39) = 6.652$, $p = .0138$]. There were no group differences in path lengths (Fig. 15A, Fig. 15B). There was not a significant difference in the percentage of thigmotaxis behavior across days; however, the low-fat diet mice had a significantly higher percentage of thigmotaxis behavior [main effect by diet, $F(1, 9) = 8.905$, $p = .0153$] compared to the high-fat diet group in the visible platform test (Fig. 15D). There was a not significant increase in speed or floating across days or between feeding conditions (Fig. 15B, Fig. 15E). In summary, the low-fat diet's cognitive behavior could be affected by increased anxiety as indicated by increased thigmotactic behavior.

During the probe trial, there was a preference for location [main effect by location, $F(1, 36) = 7.782$, $p = .0084$]. The low-fat diet group had a significantly lower preference for the location [main effect of diet, $F(1, 36) = 8.625$, $p = .0058$] compared to the high-fat diet group. Using the Sidak's multiple comparisons test to analyze individual groups, the low-fat diet mice spent less time in the target region compared to high-fat diet mice $t(9) = 3.689$, $p = .0296$. The high-fat diet mice spent more time in the target region compared to the non-target region $t(9) = 3.806$, $p = .0248$ (Fig. 16A). The low-fat diet group spent a significantly higher percentage showing thigmotaxis behavior and time floating compared to the high-fat diet group [$t(18) = 2.21$,$p = .0403$, for thigmotaxis; $t(18) = 2.152$, $p = .0452$ for floating] (Fig. 16C, Fig. 16E). There was no difference in path lengths, thigmotaxis path, and speed between feeding conditions (Fig. 16B, Fig. 16D, Fig. 16F). In summary, the low-fat diet group showed decreased cognitive performance

which could be due to confounding performance issues, such as increased floating and thigmotactic behavior.

3.3.4 MWM Hidden & Visual Platform (Across Group Comparison)

A two-way repeated measures ANOVA was conducted to compare the low-fat group across all consumption histories. There was a significant reduction in latencies and path lengths over days during the hidden platform test [main effect by day, $F(6, 819) = 6.916$, $p<0.0001$ for latencies; $F(6, 189) = 13.57$, $p<0.0001$ for path lengths] (Fig. 17A, Fig. 17C). There was a significant group difference in latencies between consumption histories [main effect by consumption history, $F(2, 819) = 14.81$, $p<0.0001$] (Fig. 17A). This indicates that cognitive performance was different from each other at each time point in the low-fat diet group.

A two-way repeated measures ANOVA was conducted to compare the low-fat group across all consumption histories. There was a significant reduction in latencies and path length over days for the visible platform test [main effect by day, $F(1, 234) = 40.04$, $p<0.0001$ for latencies; $F(1, 54) = 25.97$, $p<0.0001$ for path lengths] (Fig. 17A, Fig. 17C). There was a significant group difference in latencies between consumption histories [main effect by consumption history, $F(2, 234) = 7.25$, $p = 0.0009$] (Fig. 17A). This indicates that cognitive performance was different from each other at each time point in the low-fat diet group.

A two-way repeated measures ANOVA was conducted to compare the high-fat group across all consumption histories. There was a significant reduction in latencies and path lengths over days during the hidden platform test [main effect by day, $F(6, 819) = 22.14$, $p<0.0001$ for latencies; $F(6, 189) = 17.15$, $p<0.001$ for path lengths] (Fig. 17B, Fig. 17D). There was a significant group difference in latencies between consumption histories [main effect by consumption history, $F(2, 819) = 22.39$, $p<0.0001$] (Fig. 17B). This indicates that cognitive performance was different from each other at each time point in the high-fat diet group.

A two-way repeated measures ANOVA was conducted to compare the high-fat group across all consumption histories. There was a significant reduction in latencies and path lengths over days for the visible platform test [main effect by day, $F(1, 234) = 29.82$, $p<0.0001$ for latencies; $F(1,54) = 28.24$, $p<0.001$] (Fig. 17B, Fig. 17D). There was a significant group difference between consumption histories [main effect by consumption history, $F(2, 234) = 7.25$, $p = 0.0009$]

(Fig. 17B). This indicates that cognitive performance was different from each other at each time point in the high-fat diet group.

3.3.5 MWM Probe Trial (Across Group Comparison)

A preference score was conducted during the probe trial. There was a significant difference in preference for the target region between consumption histories [main effect by consumption history, $F(2, 54) = 5.825$, $p = 0.0051$]. There was not a significant difference in preference for the target region between feeding conditions [main effect by feeding condition, $F(1, 54) = 2.286$, $p = 0.1364$] (Fig. 17E). This indicates that acquisition to the platform differed in preference between consumption history groups, but did not differ between feeding conditions.

3.4 Forced Swim Test

3.4.1 Immobility measure

An unpaired t-test was conducted to measure immobility between the feeding conditions. On average, groups with 3 weeks of consumption history, the low-fat diet group spent less time immobile compared to the high-fat diet group $t(18) = 2.261$, $p = .0364$ (Fig. 18A). On average groups with 8 weeks of consumption history, there was no significant difference in time spent immobile between the low-fat diet group and the high-fat diet group (Fig. 18B). On average, groups with 13 weeks of consumption history, there was no significant difference in time spent immobile between the low-fat diet group and the high-fat diet group (Fig. 18C). This indicates that only at 3 week there was a significant difference between feeding conditions, and no differences at the 8 week and 13 week group.

An unpaired t-test was conducted to measure immobility between consumption history groups. There was no difference in immobility time across consumption histories or between feeding conditions (Fig. 19A, Fig. 19B). This indicates that there was no difference in immobility time between consumption history groups.

3.5 Correlational Data

3.5.1 Weights

In the 3-week group, there was a negative correlation between weight and total distance in the low-fat diet group ($r(8) = -.776$, $p = .008$) (Table 1). In the 8-week group, there was a negative correlation between weight and entry total in the low-fat diet group ($r(8) = -.746$, $p = .013$ (Table 2). There were no significant correlations between body weight and behavioral tasks measured in the 13-week group (Table 3). This indicates that in the low-fat diet group, as increased weight occurred, there was a decrease in general locomotor activity from the EPM and OF test.

3.6 Weights

3.6.1 Body weight

An unpaired t-test was conducted to measure difference in weight between the feeding conditions. In the 3-week group, the high-fat diet group was significantly higher in weight compared to the low-fat diet group on Day 11 $t(9) = 7.302$, $p<.0001$ (Fig. 19A). In the 8-week group, the high-fat group was significantly higher in weight compared to the low-fat diet group on Day 1 $t(9) = 8.787$, $p<.0001$, and Day 12 $t(9) = 8.787$, $p<.0001$ (Fig. 19A, Fig. 19B). In the 13-week group, the high-fat diet group had a significantly higher weight than the low-fat diet group on Day 1 $t(9) = 8.686$, $p<.0001$, Day 3 $t(9) = 8.686$, $p<.0001$, and Day 9 $t(9) = 8.686$, $p<.0001$. This indicates that the high-fat diet group has been statistically higher in weight compared to the low-fat diet group in all consumption history groups.

CHAPTER 4 DISCUSSION

The first aim of this study was to assess the cognitive and behavioral consequences of various histories of high-fat diet consumption. I hypothesized that increased high-fat consumption would increase cognitive impairment and behavioral dysregulation, and that these impairments would worsen over weeks of high-fat diet consumption. My second aim was to investigate cholesterol levels in the brain as a consequence of a high-fat diet consumption, and if cholesterol accumulation in the brain was due to *de novo* synthesis. Analysis of cholesterol in the brain is still a work in progress in Dr. Elmendorf's lab. I hypothesized that brain cholesterol levels will increase with longer consumption durations in vulnerable areas such as the hippocampus, cerebral cortices and cerebellum.

4.1 Main Findings

Consumption of the experimental specialty diets produced a number of significant behavioral effects. These significant effects began to emerge after only 3 weeks of low-and high-fat feeding. During the acquisition phase of the MWM task, there was a significant reduction in latency and path lengths to the hidden platform across days, indicating the mice were learning the location of the hidden platform. However, low-fat and high-fat fed mice did not differ in these parameters. There was a significant difference in feeding condition during the MWM visible platform task, with the low-fat diet group displaying a significantly higher latencies to find the platform, thigmotactic behavior was also higher in the low-fat diet group. There was no difference in path lengths between feeding conditions. These collective results suggest that differences seen in low-fat latencies were likely due to thigmotactic behavior and not cognitive impairment. In terms of behavioral dysregulation, the high-fat diet group had higher time spent immobile, indicating they are expressing depression-like symptoms to a greater degree than the low-fat fed mice.

After 8 weeks of high-fat feeding, there was a reduction in locomotor activity in the OF. In the MWM, all measures exhibited changes across days; i.e., reduction in latencies and path lengths for cognitive assessment, and an increase in speed, thigmotaxis behavior, and floating for performance measures. Between feeding conditions, the low-fat diet group actually exhibited

greater deficits as indicated by higher latencies, path lengths. This pattern was similar in the visible platform test for latencies. Similar to that observed after 3 weeks of specialty diet consumption, the low-fat diet group had significantly higher thigmotactic behavior in both the visible and hidden platform task. Performance measures such as thigmotactic behavior may confound cognitive measures since latencies and path lengths rely on the mouse to explore the pool and reach the platform. Since the low-fat diet mouse is exhibiting anxiety-like behavior, they are swimming just in the outer regions of the pool, which looks like cognitive impairment due to higher latencies and path lengths. This makes cognitive performance measures for latency and path lengths uninterruptable. During the probe trial there was a significant preference for the target region compared to the non-target group in both the low-fat diet group and the high-fat diet groups, but there was no significant difference between feeding conditions.

After 13 weeks of high- or low-fat feeding, the main findings in OF showed that the low-fat diet group traveled further than the high-fat diet group, indicating they had more general locomotor activity. Furthermore, low-fat fed mice spent more time in, and traveled more in the center compared to the high-fat diet group, suggesting that the high-fat diet group could be expressing anxiety-like symptoms (thigmotaxis). In the MWM task, there was an overall reduction in latencies, and path lengths (cognitive measures), and an increase in speed, thigmotaxis behavior, and floating in performance measures across days. The low-fat diet group had exhibited higher latencies and thigmotaxis behavior in both the hidden and visible platform test. During the probe trial, the low-fat diet group also had increased floating and thigmotaxis behavior, suggesting possible fatigue or anxiety could be affecting their performance. There was also a preference for the target region, with the high-fat diet group exhibiting higher preference score.

4.2 EPM

The EPM task is a staple tool for assessing anxiety-like behavior in rodents. Yet, published data assessing anxiety-like behavior after high-fat consumption is limited. One study assesses such anxiety-like behavior using the OF and Zero Maze Test apparatuses (Gainey et al., 2016). They found elevated anxiety-like symptoms after 6 weeks of diet consumption. However, there were no differences between the high- and low-fat feeding conditions. We found a significant decrease in time and entries into the open arms of the EMP with increased consumption history. However, this may not be due to high-fat consumption per se, but instead to isolation since we single house our

6N mice. We single housed our mice for such studies because social hierarchy in rodents shows the subordinate rodent will eat less food than the dominant one (Davis, 1953). Therefore, this may affect physiological changes seen with high-fat consumption, and subsequence cognitive and behavioral performance. Several studies have shown that single housing can reduce the number of entries and time spent in the EPM open arms (Hunt & Hambley, 2006; Walf & Frye, 2007; Zhu et al., 2006). Therefore, in sum, our data likely demonstrate the negative effects of isolation instead of high-fat consumption.

4.3 Open Field Test

The OF is another assessment tool for assessing anxiety-like symptoms, as well as general locomotor activity. At 13 weeks, our data reflected reduced time and distance spent in the center (thigmotaxis) in the high-fat diet group, which is nearly double the time course found from the OF study done by Gainey et al. (2016). This suggests that there could be strain differences in performance since Gainey et al. (2016) used 6J mice. Isolation effects may also have been present in this behavioral measure too as our results showed a decrease in locomotor activity and time spent in the center. Similar to the EPM, single housing mice for a long duration will reduce general locomotor activity and time spent in the center (Hunt & Hambley, 2006; Walf & Frye, 2007; Zhu et al., 2006). Thus, single housing would appear to be an important issue to consider for future studies as it is a confounding variable that limits our interpretation of a high-fat consumption effects on anxiety.

4.4 MWM

The MWM is a common assessment task for investigating the relationship between cognitive impairment and a high-fat consumption. There are numerous articles reporting that a high-fat diet is associated with a cognitive impairment in this task, but a majority of these studies were performed with rats. Limited mouse model data show deficits from high-fat diets in the range of 60%. We assessed cognitive impairment at fat percentages at 10% and 45%, which are much lower than previous studies (Arnold et al., 2015; Gainey et al., 2016; Kleine et al., 2016). In our study, cognitive assessment (latencies and path lengths) could not be interpreted due to performance issues, including floating and thigmotactic behavior with increased days. These performance issues could be a result of performance fatigue, anxiety or increased stress due to

long-term single housing. The increase floating and thigmotactic behavior was highest near the last few days of the 9-day behavioral experiment, providing evidence for performance fatigue and anxiety behavior. Also, the effects appeared to worsen with greater durations of consumption.

The low-fat diet group exhibited more anxiety-like symptoms compared to the high-fat diet group as indicated by increased thigmotactic behavior. This ultimately led to an increased latencies and path lengths during cognitive assessment of behavioral testing since the mice spent more time in outer areas of the pool vs searching for the platform, therefore making assessment uninterpretable. This was in opposition to our hypothesis which was the LF diet group would exhibit lower negative behavioral affect compared to the high-fat diet group. Assessment of another cognitive behavioral task that will lead to less negative behavioral affect is recommended to properly assess cognitive symptoms. Therefore, In sum, this experiment was ultimately unable to assess high-fat diet induced cognitive impairment due to this performance issues.

4.5 Forced Swim Test

The FS test is a common measure of depressive-like behavior. There is little data addressing the potential relationship between a high-fat diet and depressive-like behavior. The current project showed that there was only a difference in FS immobility between the low-fat and high-fat diet group at week 3. This difference was not seen in week 8 and 13, which is in the opposite direction of my hypothesis. However, because this experiment took place immediately after the MWM task, it may have been possible that carryover effects of floating seen in last days of MWM influence the FS behavior. In other words, the floating behavior that developed in the MWM task may have ended up manifesting again in the FS task. Furthermore, the mice may have developed a conditioned response to the researcher, learning that they would be rescued from the water after a certain amount of time, again influencing willingness to simply float. Therefore, the behavior despair that we were attempting to measure may have been compromised by the previous MWM testing. Finally, according to Petit-Demouliere, Chenu, & Bourin, (2005), single housed mice tend to exhibit greater immobility compared to group house mice. All in all, the order of behavioral testing, and our decision to single house the mice may have confounded our FS test data.

4.6 Weights

In all groups, there was a consistent pattern of the high-fat diet groups weighing statistically more than the low-fat diet group. This is a significant factor to acknowledge since body weight could affect performance in any of the behavioral studies. There was a negative correlation in the low-fat diet group, indicating as weight increased, there was a decrease in general locomotor activity at week 3 and week 8. Interestingly, this was not true for the high-fat diet group at any consumption history time point. The higher body weight may have been protective from the cold temperature in the Morris Water Maze due to insulation. All in all, the increased body weight difference in the high-fat diet group is an important factor to consider when evaluating the behavioral tests.

4.7 Brain Cholesterol

This study will examine brain cholesterol levels in the cerebral cortices, hippocampi and cerebellum. I hypothesize that vulnerable brain regions such as the cerebral cortices and hippocampi will have elevated brain cholesterol levels; whereas, the cerebellum will have no changes in cholesterol levels. Brain regions' cholesterol levels will be determined via between-groups analysis among feeding conditions and consumption history groups. There will also be a Pearson's correlation to see if there are any correlations between cholesterol levels and behavioral tasks. All in all, cholesterol level analysis is an important part of this project since it bridges the underlying physiological mechanism with the cognitive and behavioral performance. Furthermore, this data will allow us to see the progression of cholesterol levels with increased consumption duration.

4.8 Future Research

This project has given greater insight into the behavior consequences of high-fat consumption. Due to the limited assessment of a high-fat diet mouse model, standardization of specific aspects in the design can be beneficial to understanding the consequences of the specialty diet consumption. For example, our standardizing the percentage of saturated fat to be reflective of Western society was aimed to provide us insight into relevant negative consequences of overeating as seen in our society. Unfortunately, there have only been assessments in the upper

region of 60% (Arnold et al., 2015; Gainey et al., 2016; Kleine et al., 2016). Our project utilized a low-fat diet (10%) and a high-fat diet (45%), which is akin to that eaten in more metabolically healthy and non-healthy individuals, respectively. An unexpected result was the low-fat diet group's performance in the MWM, in which they performed worse than the high-fat diet group. Certain controls such as the inclusion of a standard mouse chow (4.5% crude fat) group could give us insight into what behavior looks like in 6N mice in the absence of any diet manipulation. This could tell us if the low-fat diet group is considered a form of "high-fat" diet and is equally affecting performance as the high-fat (45%) diet group. Another control group that could be beneficial is a group reflective of a normal Western diet (33%) (National Center for Health Statistics, 2017). Another aspect of the current study that was not standardized was age group; a majority of high-fat diet studies have investigated older populations, as they tend to be vulnerable to manipulation of diet. Age is an important component since is it a confounding variable when assessing cognitive performance, so standardizing a mouse model reflective of a normal adult population is essential. Biological sex is another factor to consider as behavioral measurements such as the FS test indicate that females have shorter immobility time (Petit-Demouliere, Chenu, & Bourin, 2005).

Due to performance issue such as fatigue and anxiety in the MWM task, we were unable to clearly interpret the cognitive aspects of the behavior. Utilization of a behavior with a task that do not require multiple days or placing them in a stressful environment such in a pool of water could help alleviate performance fatigue. An alternative cognitive task that has been used to assess the relationship between high-fat diet and cognitive impairment is the NOR task. This is a compound behavioral task that is able to assess multiple brain regions and does not require any physical stamina (Cordner & Tamashiro, 2015). In conclusion, a standardized mouse model is essential for determining the behavioral consequences of a high-fat consumption.

4.9 Conclusion

This study allowed us to analyze the gaps in the literature about the effects due to chronic consumption of a high-fat diet. The most robust data came from analyzing the consumption histories and the progression of cognitive and behavioral dysfunction. The 8 week group appeared to exhibit the most anxiety-like behavior in the high-fat group in the EMP and OF, and the low-fat diet group showed increased thigmotactic behavior in the MWM at this time point. The symptoms were less detrimental at 13 weeks, indicating some form of adaptation. We also were able to see

the progression of isolation effects in the EMP and OF test due to single housing the mice. So, future research should weigh the risks and benefits of isolation effects and dominance that may affect food consumption. Another key finding in this study was in anxiety measurements in EPM, OF and MWM. Anxiety was a major confound in the MWM that made cognitive performance uninterruptable. All in all, this study allowed us to get insight into various factors and fill the gaps in the literature of the effects of chronic consumption of a high-fat diet.

FIGURES

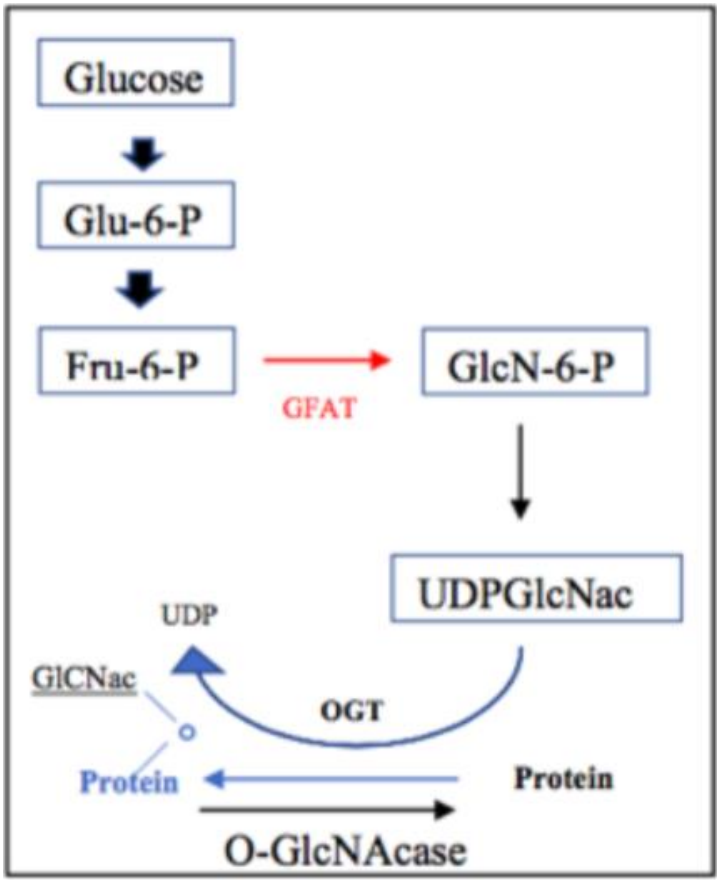

Figure 1. Proposed HBP pathway to insulin resistance

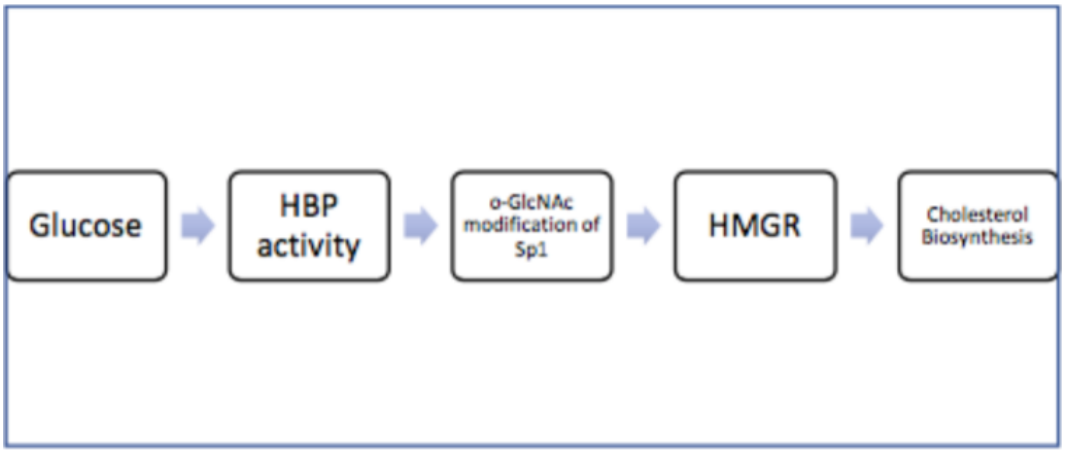

Figure 2. Pathway to cholesterol biosynthesis

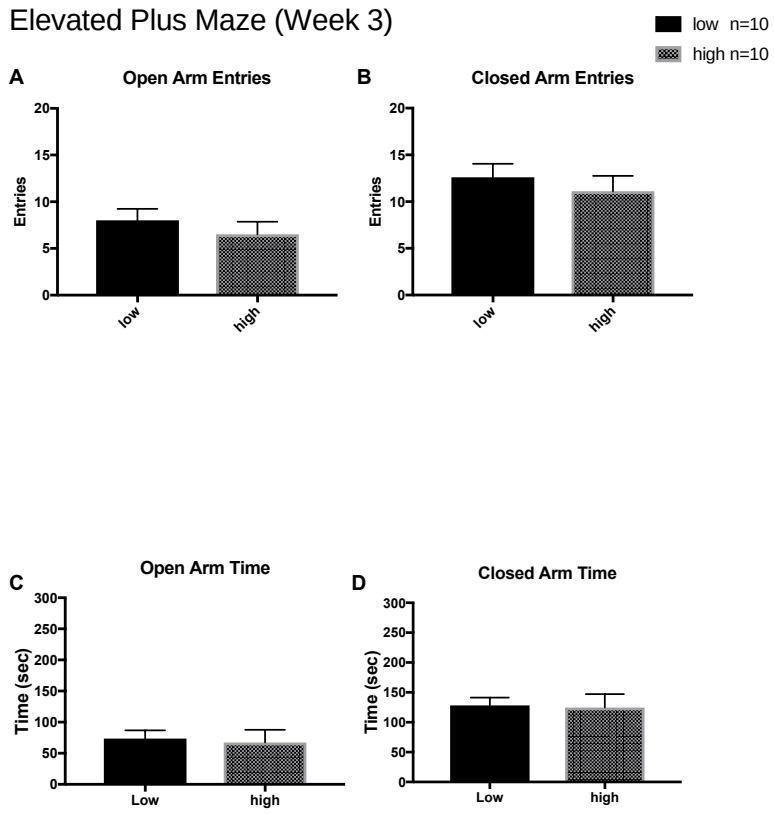

Figure 3. Elevated Plus Maze (Week 3)

A comparison of EPM entries and time in the open and closed arms between the low-fat diet group and high-fat diet group was assessed with the 3 weeks of consumption history group.

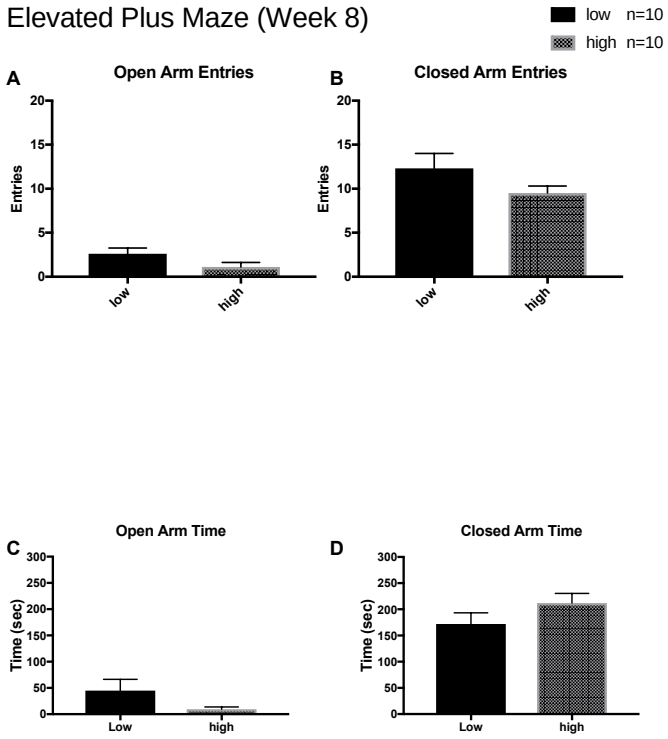

Figure 4. Elevated Plus Maze (Week 8)

A comparison of EPM entries and time in the open and closed arms between the low-fat diet group and high-fat diet group was assessed with the 8 weeks of consumption history group.

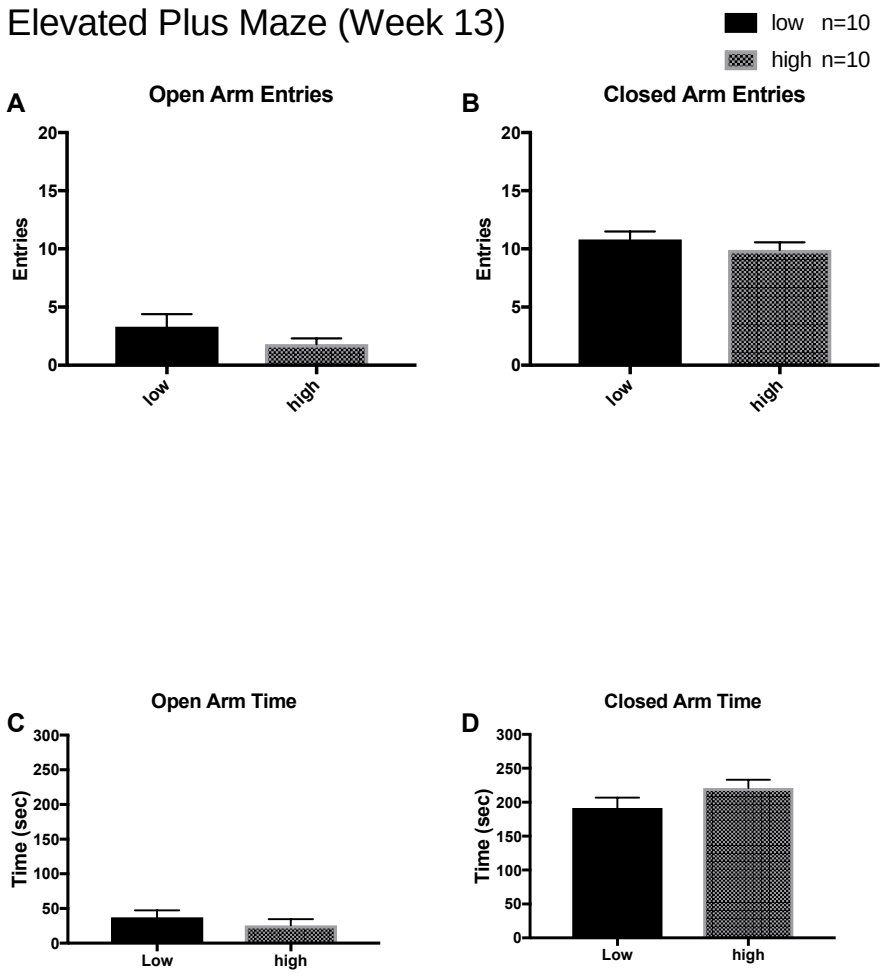

Figure 5. Elevated Plus Maze (Week 13)

A comparison of EPM entries and time in the open and closed arms between the low-fat diet group and high-fat diet group was assessed with the 13 weeks of consumption history group.

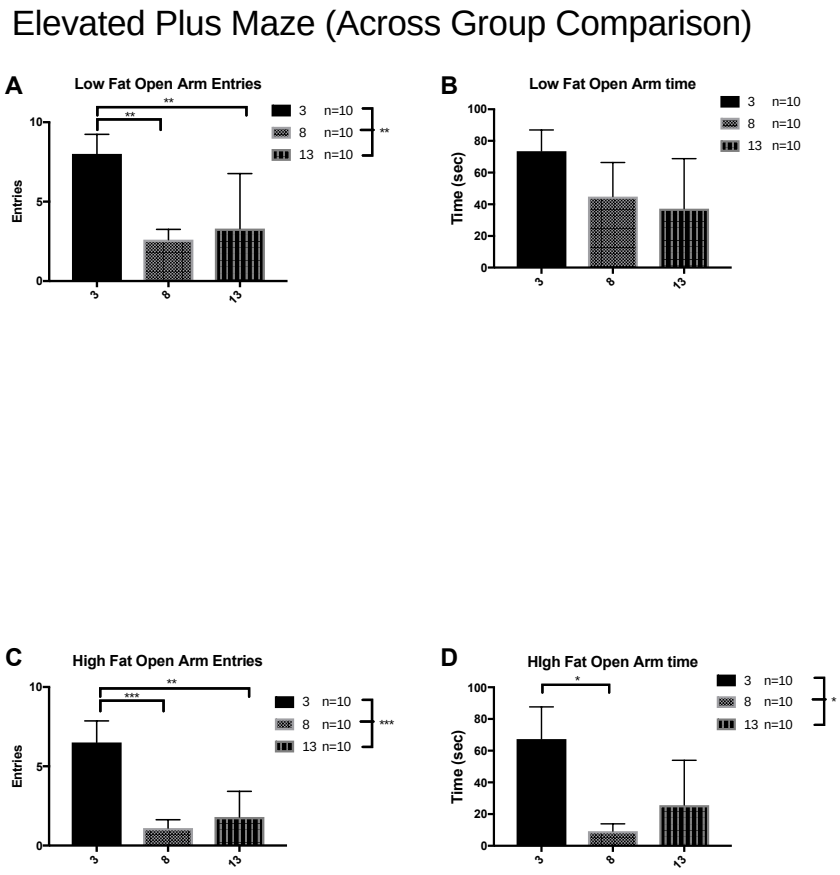

Figure 6. Elevated Plus Maze (Across Group Comparison)

A comparison of EPM entries and time in the open arm was assessed across all consumption history groups.

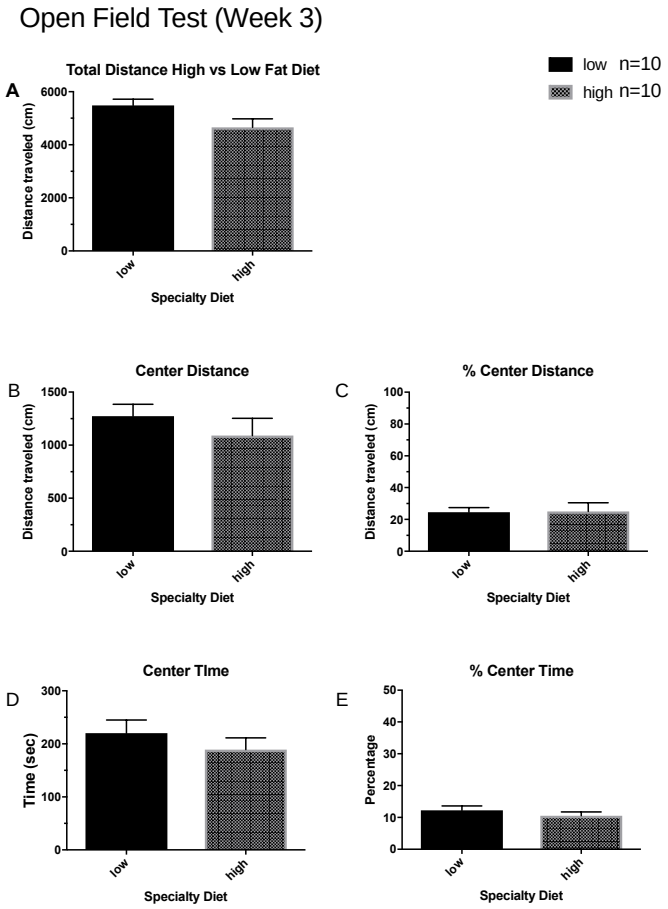

Figure 7. Open Field Test (Week 3)

In the OF, a comparison of total distance, center distance, percentage distance spent in center, center time and percentage time spent in center were assessed between the low-fat diet and high-fat diet group with a 3-week consumption history.

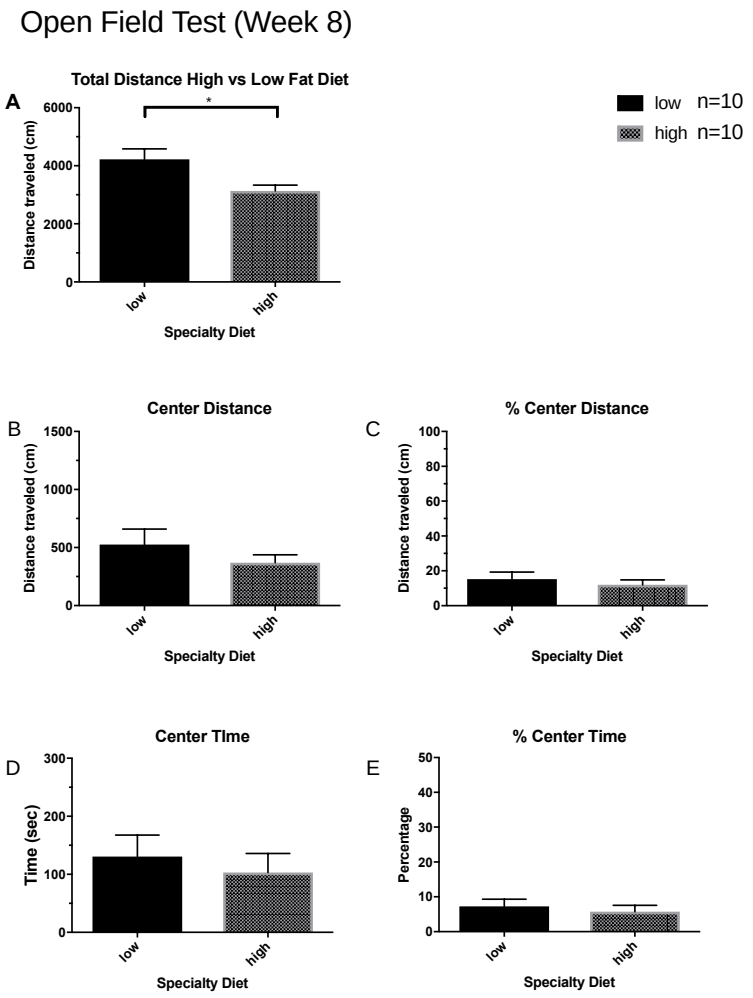

Figure 8. Open Field Test (Week 8)

In the OF, a comparison of total distance, center distance, percentage distance spent in center, center time and percentage time spent in center was assessed between the low-fat diet and high-fat diet group with an 8-week consumption history.

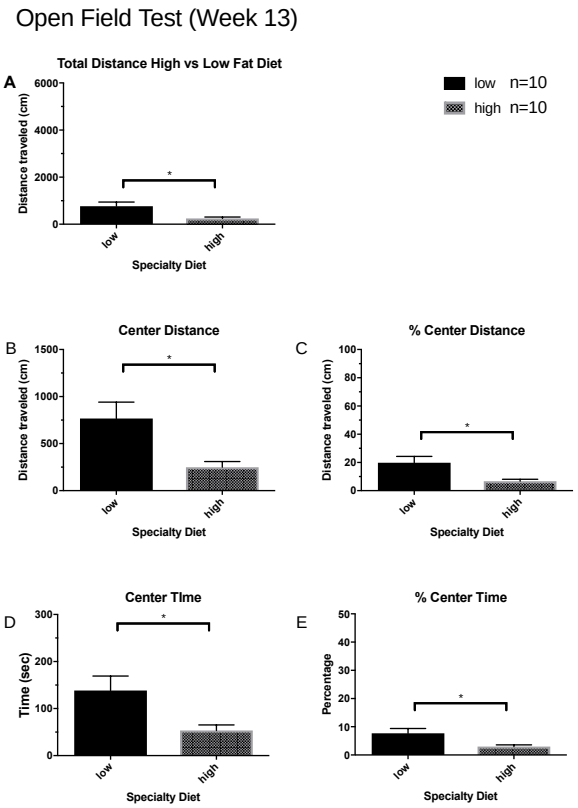

Figure 9. Open Field Test (Week 13)

In the OF, a comparison of total distance, center distance, percentage distance spent in center, center time and percentage time spent in center was assessed between the low-fat diet group and high-fat diet group with a 13-week consumption history.

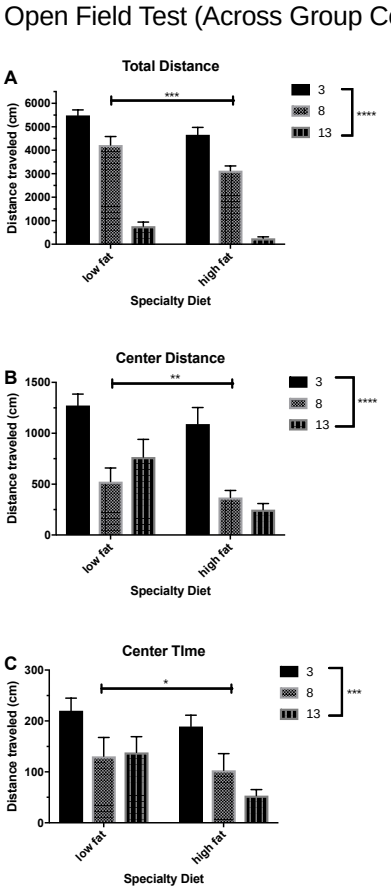

Figure 10. Open Field Test (Across Group Comparison)

In the OF, a comparison of total distance, center distance and center time was assessed across consumption history groups.

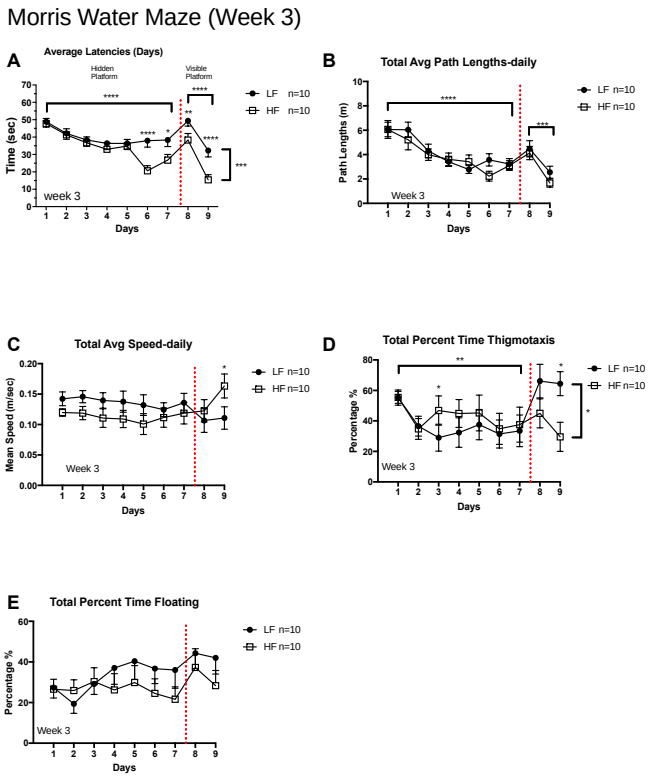

Figure 11. Morris Water Maze (Week 3)

During the Hidden and Visual Platform phase of the MWM, a comparison of lantecy, speed, path lengths, thigmotaxis, and floating were assessed between the low-fat diet and high-fat diet group in the 3 week group.

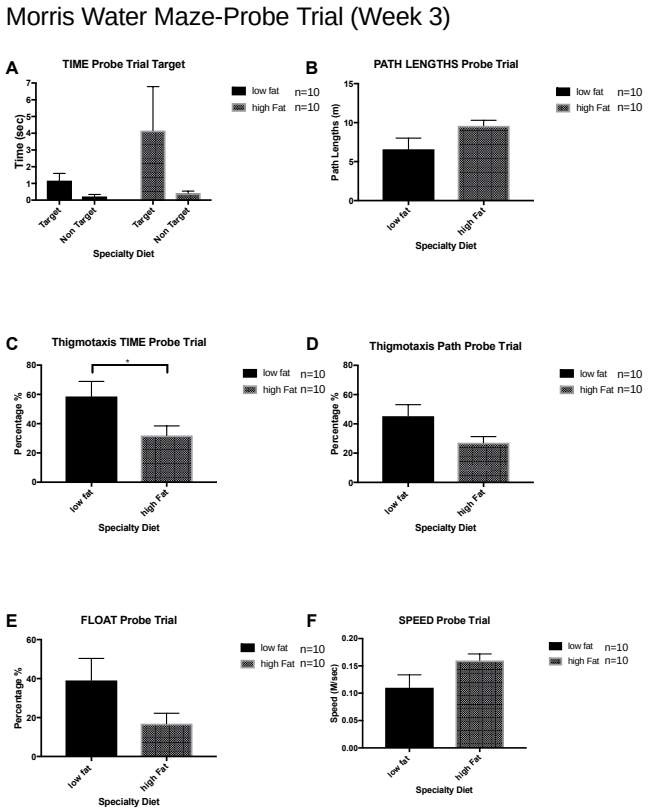

Figure 12. Morris Water Maze-Probe Trial (Week 3)

During the Probe trial of the MWM, a comparison of time (preference in target region), path lengths, thigmotaxis time, thigmotaxis path lengths, floating, and speed were assessed between the low-fat diet and high-fat diet group in the 3 week group.

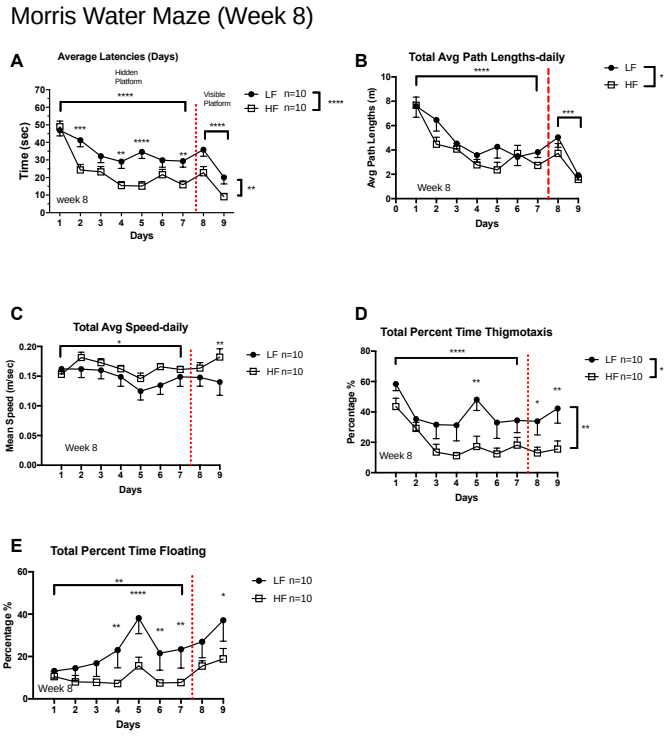

Figure 13. Morris Water Maze (Week 8)

During the Hidden and Visual Platform of the MWM, a comparison of lantecy, speed, path lengths, thigmotaxis, and floating were assessed between the low-fat diet and high-fat diet group in the 8 week group.

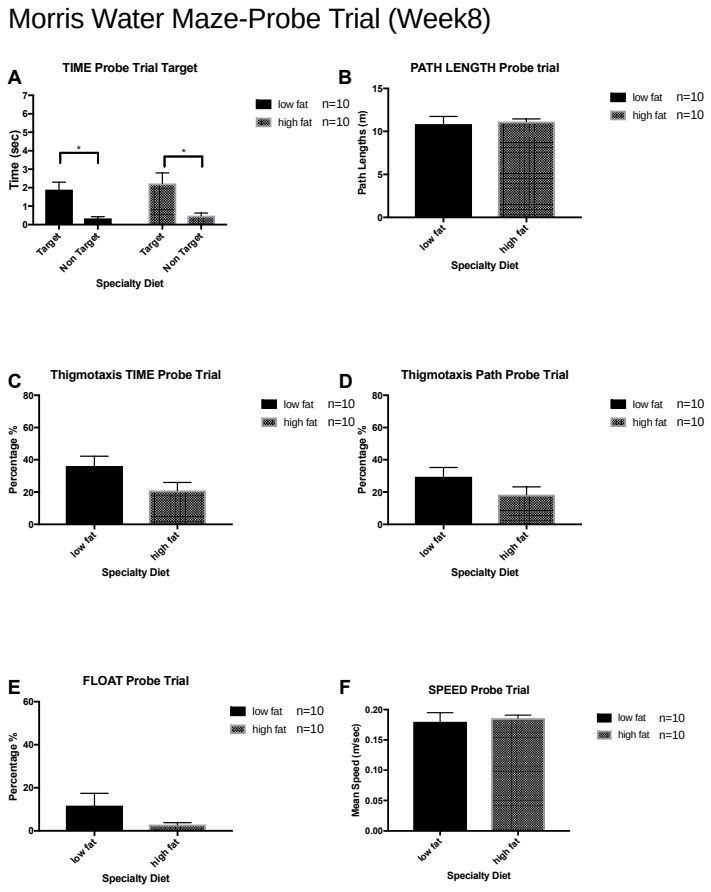

Figure 14. Morris Water Maze-Probe Trial (Week 8)

During the Probe Trial of the MWM, a comparison of time (preference in target region), path lengths, thigmotaxis time, thigmotaxis path lengths, floating, and speed were assessed between the low-fat diet and high-fat diet group in the 8 week group

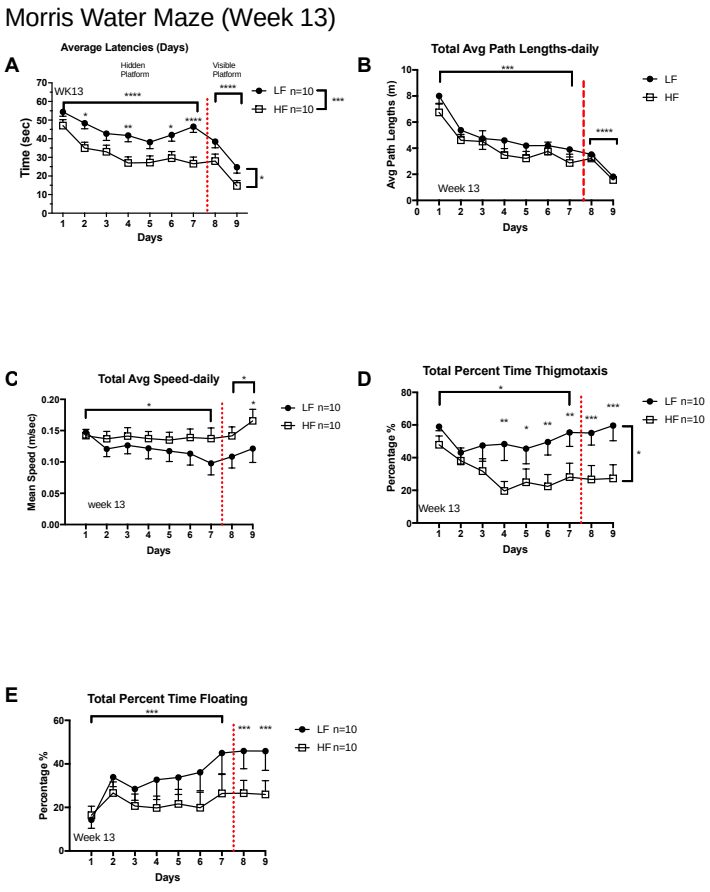

Figure 15. Morris Water Maze (Week 13)

During the Hidden and Visual Platform of the MWM, a comparison of lantecy, speed, path lengths, thigmotaxis, and floating were assessed between the low-fat diet and high-fat diet group in the 13 week group.

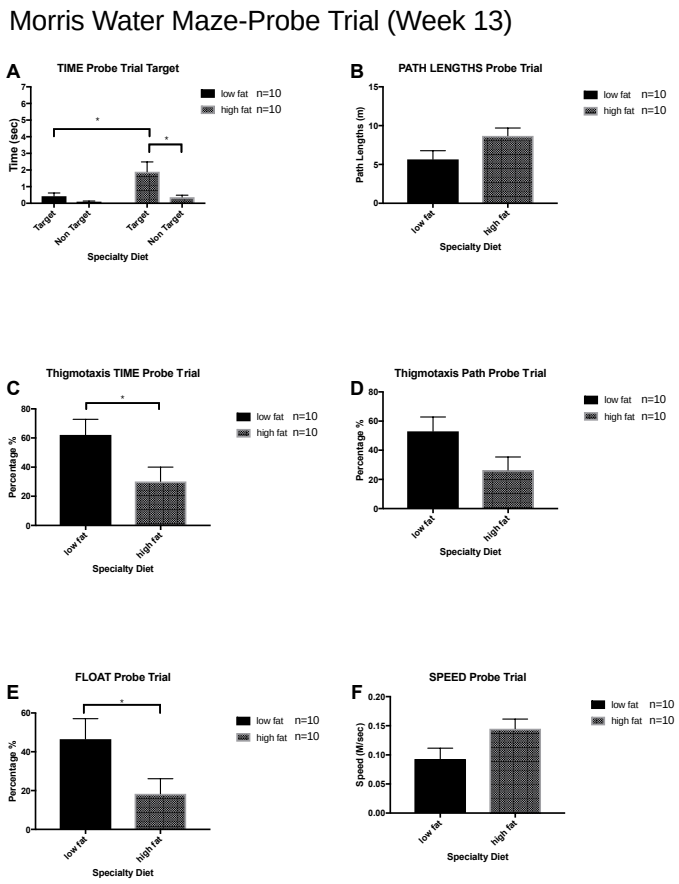

Figure 16. Morris Water Maze-Probe Trial (Week 13)

During the Probe Trial of the MWM, a comparison of time (preference in target region), path lengths, thigmotaxis time, thigmotaxis path lengths, floating, and speed were assessed between the low-fat diet and high-fat diet group in the 13 week group.

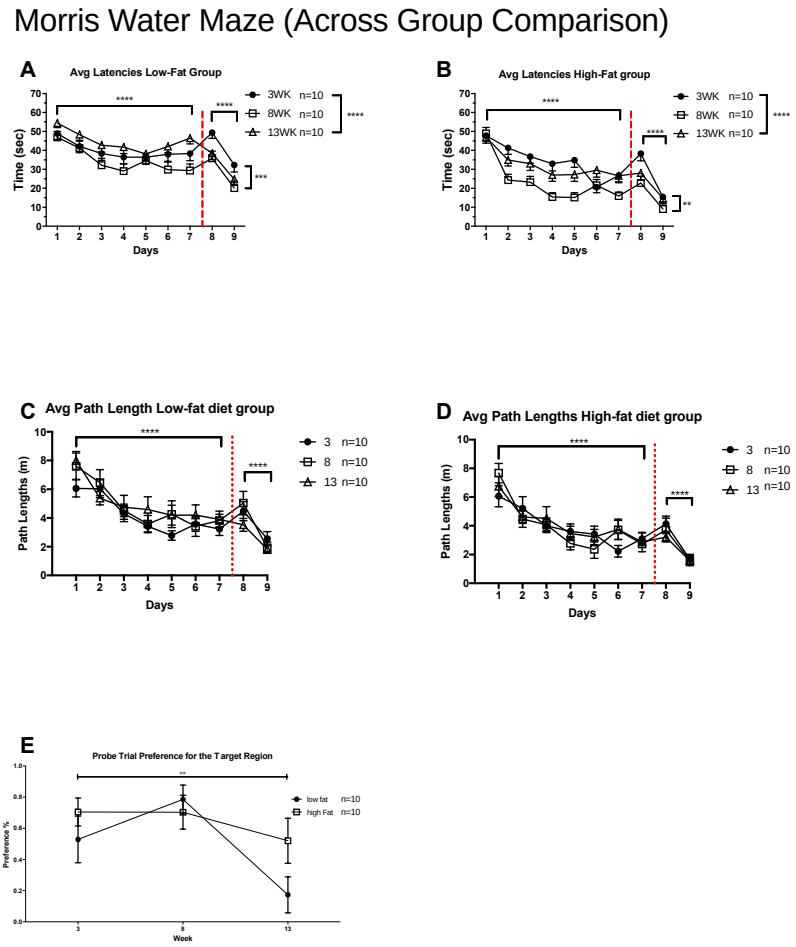

Figure 17. Morris Water Maze (Across Group Comparison)

A comparison of latencies were assess between consumption history groups during the hidden and visual platform test from the MWM. Preference scores for target region was measured during the probe trial of the MWM.

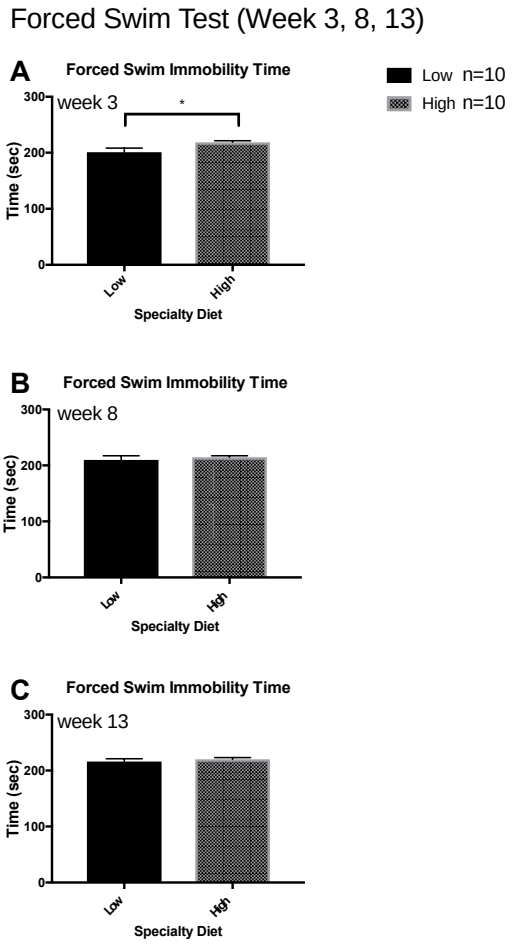

Figure 18. Forced Swim Test (Week 3, 8, 13)

In all consumption history groups, comparison of immobility time between the low-fat diet group and high-fat diet group were assess during the FS.

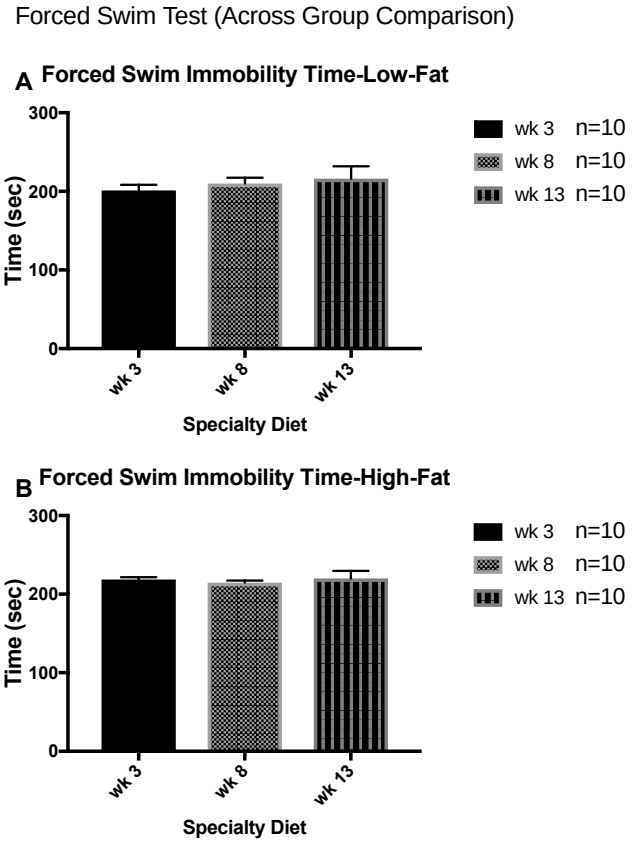

Figure 19. Forced Swim Test (Across Group Comparison)

Comparison of immobility time across consumption history groups was assessed in both the low-fat diet and the high-fat diet group during the FS.

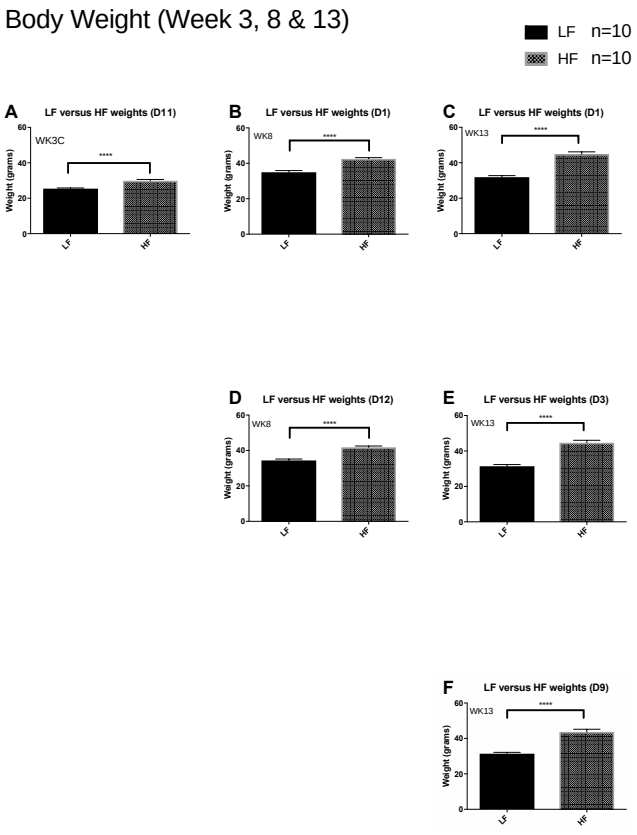

Figure 20. Body Weight (Week 3, 8 & 13)

Body weight of low-fat and high-fat diet mice during the behavioral experiment phase

TABLES

Table 1. Week 3 Correlations: Weight & Behavioral Tasks

Week 3 Correlations: Weight & behavioral tasks

		EPM Ent_CA	EPM Ent_OA	EPMEnt_Total	EPM Ent_CA_Pct	EPM Ent_OA_Pct	EPM Time_CA	EPM Time_OA	OF TotDist	OF CtrDist	OF CtrDist_Pct
Weight 3Wk LF	Pearson Correlation	0.039	-0.110	-0.058	0.109	-0.110	-0.207	0.040	**-.776****	-0.502	-0.300
	Sig. (2-tailed)	0.915	0.762	0.873	0.764	0.763	0.567	0.913	0.008	0.139	0.400
	N	10	10	10	10	10	10	10	10	10	10
Weight 3WK HF	Pearson Correlation	-0.505	0.211	-0.393	-0.423	0.423	-0.394	0.324	-0.517	-0.165	-0.257
	Sig. (2-tailed)	0.136	0.558	0.261	0.223	0.223	0.260	0.361	0.126	0.648	0.474
	N	10	10	10	10	10	10	10	10	10	10
		OF CtrTime	OF CtrTime_Pct	MWM time	MWM dist	PRB TIMETarget	MWMVis time	MWMVis dist	MWM SPD	MWMVis SPD	MWM THG
Weight 3Wk LF	Pearson Correlation	0.094	0.094	-0.139	0.489	0.069	-0.231	-0.086	-0.428	0.307	0.588
	Sig. (2-tailed)	0.797	0.797	0.703	0.151	0.850	0.521	0.813	0.217	0.388	0.074
	N	10	10	10	10	10	10	10	10	10	10
Weight 3WK HF	Pearson Correlation	-0.058	-0.058	0.322	0.534	0.521	0.300	0.352	-0.159	-0.328	0.483
	Sig. (2-tailed)	0.873	0.873	0.364	0.112	0.122	0.400	0.319	0.661	0.355	0.157
	N		10	10	10	10	10	10	10	10	10
		MWMVis THG	MWM FLT	MWMVis FLT	PRB SPD	PRB FLT	PRB THGTime	PRB THGPTH	PRB TimeNonTarget	PRB Dist	FS Immob
Weight 3Wk LF	Pearson Correlation	-0.401	-0.315	-0.424	-0.061	0.109	0.068	-0.002	0.120	-0.059	-0.329
	Sig. (2-tailed)	0.251	0.376	0.222	0.866	0.764	0.852	0.995	0.741	0.870	0.354
	N	10	10	10	10	10	10	10	10	10	10
Weight 3WK HF	Pearson Correlation	0.215	0.471	0.242	-0.411	0.419	0.025	0.095	0.359	-0.402	0.006
	Sig. (2-tailed)	0.550	0.170	0.500	0.238	0.229	0.945	0.794	0.308	0.250	0.986
	N	10	10	10	10	10	10	10	10	10	10

Table 2. Week 8 Correlations: Weight & Behavioral Tasks

Week 8 Correlations: Weight & behavioral tasks											
		EPM Ent_CA	EPM Ent_OA	EPMEnt_Total	EPM Ent_CA_Pct	EPM Ent_OA_Pct	EPM Time_CA	EPM Time_OA	OF TotDist	OF CtrDist	OF CtrDist_Pct
Weight 8WK LF	Pearson Correlation	-0.630	-0.562	**-0.746***	-0.127	0.126	-0.322	0.256	-0.509	-0.278	-0.184
	Sig. (2-tailed)	0.051	0.091	0.013	0.727	0.728	0.365	0.475	0.133	0.436	0.612
	N	10	10	10	10	10	10	10	10	10	10
Weight 8Wk HF	Pearson Correlation	-0.365	0.372	-0.089	-0.361	0.361	-0.355	0.439	0.248	0.106	0.117
	Sig. (2-tailed)	0.299	0.289	0.807	0.306	0.306	0.315	0.204	0.489	0.771	0.747
	N	10	10	10	10	10	10	10	10	10	10
		OF CtrTime	OF CtrTime_Pct	MWM time	MWM dist	PRB TIMETarget	MWMVis time	MWMVis dist	MWM SPD	MWMVis SPD	MWM THG
Weight 8WK LF	Pearson Correlation	-0.353	-0.353	-0.161	-0.016	0.088	-0.058	0.377	0.154	0.419	-0.124
	Sig. (2-tailed)	0.317	0.317	0.658	0.965	0.809	0.873	0.283	0.671	0.228	0.733
	N	10	10	10	10	10	10	10	10	10	10
Weight 8Wk HF	Pearson Correlation	0.237	0.237	0.096	0.077	-0.023	-0.267	-0.343	-0.098	-0.213	-0.485
	Sig. (2-tailed)	0.510	0.510	0.792	0.832	0.951	0.456	0.331	0.789	0.556	0.155
	N	10	10	10	10	10	10	10	10	10	10
		MWMVis THG	MWM FLT	MWMVis FLT	PRB SPD	PRB FLT	PRB THGTime	PRB THGPTH	PRB TimeNonTarget	PRB Dist	FS Immob
Weight 8WK LF	Pearson Correlation	-0.210	-0.175	-0.361	-0.012	-0.130	-0.113	-0.126	-0.155	0.031	0.276
	Sig. (2-tailed)	0.561	0.628	0.305	0.974	0.720	0.755	0.729	0.670	0.933	0.439
	N	10	10	10	10	10	10	10	10	10	10
Weight 8Wk HF	Pearson Correlation	-0.247	-0.340	0.188	-0.237	0.395	-0.075	-0.224	-0.102	-0.258	-0.328
	Sig. (2-tailed)	0.491	0.336	0.602	0.510	0.258	0.837	0.534	0.778	0.471	0.355
	N	10	10	10	10	10	10	10	10	10	10

Table 3. Week 13 Correlations: Weight & Behavioral Tasks

Week 13 Correlations: Weight & behavioral tasks											
		EPM Ent_CA	EPM Ent_OA	EPMEnt_Total	EPM Ent_CA_Pct	EPM Ent_OA_Pct	EPM Time_CA	EPM Time_OA	OF TotDist	OF CtrDist	OF CtrDist_Pct
Weight 13WK LF	Pearson Correlation	0.112	-0.583	-0.448	0.603	-0.603	0.321	-0.455	-0.517	-0.594	-0.629
	Sig. (2-tailed)	0.759	0.077	0.194	0.065	0.065	0.366	0.186	0.126	0.070	0.051
	N	10	10	10	10	10	10	10	10	10	10
Weight 13WK HF	Pearson Correlation	0.401	-0.065	0.254	0.168	-0.168	0.205	-0.221	0.422	0.299	0.407
	Sig. (2-tailed)	0.251	0.859	0.479	0.642	0.642	0.571	0.539	0.224	0.401	0.244
	N	10	10	10	10	10	10	10	10	10	10
		OF CtrTime	OF CtrTime_Pct	MWM time	MWM dist	PRB TIMETarget	MWMVis time	MWMVis dist	MWM SPD	MWMVis SPD	MWM THG
Weight 13WK LF	Pearson Correlation	-.677*	-.677*	-0.133	0.060	0.236	-0.183	-0.122	0.203	0.163	-0.036
	Sig. (2-tailed)	0.032	0.032	0.714	0.869	0.511	0.612	0.736	0.574	0.652	0.921
	N	10	10	10	10	10	10	10	10	10	10
Weight 13WK HF	Pearson Correlation	0.341	0.341	0.392	-0.023	-0.179	0.125	-0.053	-0.452	-0.489	0.534
	Sig. (2-tailed)	0.335	0.335	0.263	0.950	0.621	0.731	0.885	0.190	0.151	0.112
	N	10	10	10	10	10	10	10	10	10	10
		MWMVis THG	MWM FLT	MWMVis FLT	PRB SPD	PRB FLT	PRB THGTime	PRB THGPTH	PRB TimeNonTarget	PRB Dist	FS Immob
Weight 13WK LF	Pearson Correlation	-0.303	-0.067	-0.125	0.147	-0.030	-0.177	0.032	0.245	0.130	0.455
	Sig. (2-tailed)	0.395	0.854	0.731	0.685	0.934	0.625	0.930	0.495	0.720	0.187
	N	10	10	10	10	10	10	10	10	10	10
Weight 13WK HF	Pearson Correlation	0.231	0.485	0.494	-0.476	0.424	0.413	0.370	0.252	-0.446	-0.391
	Sig. (2-tailed)	0.521	0.156	0.147	0.165	0.223	0.236	0.293	0.483	0.196	0.264
	N	10	10	10	10	10	10	10	10	10	10

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