Does maternal obesity affect hippocampal-dependent spatial learning and memory retention in offspring?

by
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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Health Studies & Gerontology

Waterloo, Ontario, Canada, 2015

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

With a rise in the prevalence of obesity worldwide, behavioural, as well as physiological changes after consumption of a high-fat diet are becoming increasingly recognized. In addition to increased risk of chronic and degenerative diseases, obesity has also been linked with cognitive impairment, particularly in hippocampal-dependent behaviours such as spatial learning and memory retention. Maternal obesity is also becoming an issue across the globe, with an alarmingly high percentage of women of reproductive age who are either overweight, or obese. Past studies have not only revealed that early-life nutrition can impact brain development and subsequent behaviours across the lifespan, but also that exposure to a high-fat diet in utero may be particularly detrimental to susceptible structures such as the hippocampus. The present study hoped to elucidate the effects of a high-fat diet (45% kcal from fat) on spatial learning and memory retention in female Sprague-Dawley rats. Further, we examined whether maternal obesity could have intergenerational effects on spatial learning and memory retention in offspring. Although high-fat diet consumption was sufficient to induce obesity in the female animals, these dams did not demonstrate impaired spatial learning or memory retention in the Morris water maze task. Interestingly, in adolescence, male, but not female offspring of the obese dams were impaired in their performance on the Morris water maze task. However, the difference normalized by adulthood. Future research should aim to examine why females appear to be resilient to diet-induced cognitive impairment.

Acknowledgements

First and foremost, I would like to sincerely thank my faculty supervisor and committee chair, Dr. John G. Mielke, for his patience and guidance throughout my Master of Science experience. His persistence and passion for research is displayed every day, and he has been a constant support for all of his students (even when experiments do not turn out as expected). I have learned so much from Dr. Mielke over the last two years, both in class and in the lab. Without his direction, this thesis project would not have been completed.

I would also like to acknowledge my committee members Dr. Elham Satvat and Dr. Diane Williams, for their expertise and ability to make me think "outside-of-the-box" throughout my graduate training. This thesis document is a reflection of their feedback and support, for which I am very grateful.

A very special thank you goes to my lab partners: Isabelle Messa, Derrick Yeung, Jonathan Thacker, Shelley Ahad, Tony Li, and Erika Lui. Their company has made early mornings, weekends and holidays in the lab slightly less painful. My thesis would not be complete without their perseverance, feedback and dedication to this research project.

I would also like to thank all of the staff at the Central Animal Facility for their assistance and expertise. I thoroughly enjoyed my time working in the facility and will never be able to thank them enough for their guidance.

Finally, I would like to thank my family and friends for their support throughout my post-secondary studies. I would not be the person I am today without your influence.

This research project was supported by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC) awarded to J.G. Mielke.

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List of Abbreviations

ANOVA = analysis of variance

AUC = area under curve

BMI = body mass index

BW = body weight

CD = control diet

CORT = corticosterone

DOHaD = developmental origins of health and disease

EPM = elevated plus maze

HFD = high-fat diet

HPA = hypothalamic-pituitary-adrenal (axis)

mCD = maternal control diet

mHFD = maternal high-fat diet

MWM = Morris water maze

OGTT = oral glucose tolerance test

PFC = pre-frontal cortex

PND = post-natal day

SD = Sprague Dawley

SEM = standard error of the mean

SFA = saturated fatty acid

1.0 Overview

Although the ability to survive on large, sparse meals for extended periods of time was once considered evolutionarily favourable, individuals in many modern societies are increasingly at risk of developing degenerative disorders due to increased access to foods high in saturated fats and refined sugars (Beilharz, Maniam, & Morris, 2014; Marrisal-Arvy et al., 2014). In Canada alone, approximately one quarter of the population is obese (National Center for Health Statistics, 2011), and estimates suggest that more than $4.3 billion dollars are spent per year on costs directly and indirectly related to obesity (Public Health Agency of Canada, 2012).

Obesity is most commonly described as a body mass index (BMI) of 30 kg/m^2 or more, while individuals who score within 25.0 – 29.9 kg/m^2 are considered overweight (Allison et al., 2008). However, it does not account for general body composition or location of store body fat, suggesting that the BMI, although commonly used in clinical settings, may not be the most accurate measure of obesity (Frankenfield, Rowe, Cooney, Smith, & Becker, 2001). This important point could explain why not all individuals classified as obese using the BMI will experience poor health; body fat stored viscerally is most predictive of poor health (Arnold et al., 2014).

Due to issues such as underreporting and ethical limitations that exist when trying to explore the underlying mechanisms, much of what we know about the effects of obesity on physiology has come from animal models. A diet consisting of approximately 30% energy (kcal) from fat is adequate to induce an obese phenotype in animals (Hariri & Thibault, 2010), and although the diets chosen across studies vary in their composition of fat content (30-78%), a positive relationship has been found between the fat content of the diet and weight gain, suggesting that increased energy consumed from fat may be proportional to body fat composition (Hariri & Thibault, 2010).

As the obesity epidemic cannot be easily explained by genetic determinants, or by the abundance of high-fat and sugary foods available in the environment alone, it has been suggested that the true root of this health concern is an imbalance between food consumption and the expenditure of this energy. When intake exceeds output for an extended period of time, adipose tissue and gut microbiota trigger a cascade of changes resulting in a low level of inflammation in the body (Boitard et al., 2014; Hariri & Thibault, 2010). Further, the development of an obese state can occur very differently in male versus female individuals, influencing risk of associated chronic and metabolic diseases (Palmer & Clegg, 2015). For example, in the male body, fat stores are found primarily in the abdominal, or visceral area, while fat is usually stored subcutaneously in a gluteal-femoral pattern in premenopausal women. Although subcutaneous body fat is predictive of lower risk of cardiovascular disease and other obesity-related health outcomes, visceral fat, which surrounds primary organs can be particularly detrimental as adipocytes secrete proinflammatory markers once they expand to a certain degree, leading to problems such as insulin resistance over time (Palmer & Clegg, 2015).

Chronic consumption of a high-fat diet can lead to the metabolic syndrome (Arnold et al., 2014; Kanoski & Davidson, 2011), as well as increased risk of type 2 diabetes mellitus, cardiovascular disease (Anderson et al., 2013; Kanoski & Davidson, 2011; Lindqvist et al., 2006), as well as some cancers (e.g., primarily thyroid, uterine and breast in women, colorectoral and kidney in men; Hariri & Thibault, 2010). Quite unexpectedly, studies have also reported increased risk of mental illness such as depression, anxiety, schizophrenia, and an increased prevalence of Alzheimer's disease in individuals exhibiting symptoms of the metabolic syndrome (Anderson et al., 2013).

To further propagate the issue, the obese phenotype also stimulates a cascade of changes in neurochemical signalling which can modify hypothalamic regulation of food consumption (Stachowiak et al., 2013), as well as behaviours dependent on other susceptible structures in the brain, such as the hippocampus (Kanoski & Davidson, 2011; Sharma & Fulton, 2013). As a result, there is a general consensus in the literature that two domains are primarily affected by diet-induced obesity: the cascade of physiological changes that lead to increased risk of chronic and metabolic dysfunction and cognitive performance. More recently, researchers have also recognized that maternal obesity may have transgenerational effects, influencing offspring development and behaviour across the lifespan (Bilbo & Tsang, 2010; Howie et al., 2013). It can be argued that further study is required to extend our current understanding of the neurological and behavioural changes following chronic consumption of a high-fat diet, and any sex differences, and transgenerational effects that may be present.

2.0 Diet-induced Cognitive Impairment

Epidemiologists and nutrition-focused scientists are becoming increasingly mindful that diet can influence brain and behaviour, while brain and behaviour can also affect diet. For example, mild cognitive deficits have been reported in individuals newly diagnosed with type 2 diabetes mellitus (Soares et al., 2013), and impaired insulin sensitivity has also been associated with cognitive decline related to the effects of aging (McNay et al., 2010). It has been suggested that as individuals become obese, the accumulation of adipocytes leads to a proinflammatory state through the secretion of cytokines and an increase of macrophages, influencing insulin sensitivity (Lee et al., 2011), and leptin signaling (Lumeng & Sailtiel, 2014). Beyond the detrimental effects inflammation can have on visceral organs, the permeability of the blood brain barrier is also compromised after consumption of high-energy diets (Kanoski et al., 2010; Tucsek et al., 2014). The change in permeability allows inflammatory markers such as cytokines to pass through to the central nervous system, causing a proinflammatory cascade in the brain (Lee et al., 2011; McNay et al., 2010) and interfering with brain function and associated behaviour.

While polyunsaturated fats are generally considered to be beneficial for cognitive functioning throughout the lifespan, a population-based prospective study by Morris et al. (2003) found that the proportion of saturated fatty acids (SFAs) was associated with risk for Alzheimer's disease and other forms of cognitive impairment after 4- and 6 years of consumption. Further, the negative effects of a diet high in SFAs appear to selectively damage certain domains of learning and memory, such as prospective, episodic and delayed verbal memory in humans, while poor performance on tasks of hippocampal-dependent spatial learning and memory are most often observed in rodent models (Kanoski & Davidson, 2011). For example, a longitudinal study by Whitmer et al. (2005) found that overweight middle-aged adults were more likely to be diagnosed with dementia compared to their normal weight counterparts,

and this risk was further enhanced for individuals who were characterized as obese. The effects have also been noted elsewhere as an obese phenotype in midlife is suggested to increase susceptibility for dementia and Alzheimer's Disease in both women and men in North America (Hwang et al., 2010; Martin et al., 2011). In a study by Arnold et al. (2014) using 8 week old male C57BL/6J mice found that a 60% kcal HFD for 17 days, or a 45% kcal from fat diet for 8 weeks, was able to induce hippocampal-dependent memory impairments in the T-maze compared to their control diet counterparts. The HFD fed mice also demonstrated abnormal dendritic growth in the CA3 region of the hippocampus (Arnold et al., 2014).

2.1 Impairments in hippocampal-dependent spatial learning and memory following a high-fat diet

The hippocampus, which is a limbic structure located bilaterally in the medial temporal lobes of both the human and rat brain, is most widely recognized for its role in a wide range of learning and memory processes (Neves et al., 2012). Research has demonstrated that the hippocampus has a multitude of connections with its surrounding structures (e.g., prefrontal cortex, amydgala, subiculum, hypothalamus), suggesting that any morphological, or functional changes in this region may have implications for a range of behaviours including: episodic memory, working memory, cognitive flexibility, emotional regulation, stress reactivity, and eating behaviours, in addition to hippocampal-dependent behaviours like spatial learning and memory retention (Lucassen et al., 2013; Neves, Cooke, & Bliss, 2008).

In both human and animal models, research has demonstrated that the hippocampus is a structure that is particularly vulnerable to insult (Hsu & Kanoski, 2014; Kanoski & Davidson, 2011; Neves et al., 2012). For example, the hippocampus is often the first to be affected in neurodegenerative diseases, such as Alzheimer's disease (Hsu & Kanoski, 2014), and is a prime

target of both structural and functional modification following exposure to environmental toxins (Hsu & Kanoski, 2014), ischaemic injury (Gee et al., 2006; Nikoneko, Radenovic, Andjus, & Skibo, 2009), as well as metabolic disruption resulting from a diet high in saturated fats (Kanoski & Davidson, 2011; Neves et al., 2012). As proper functioning in the hippocampus has implications for many behavioural and self-regulating processes, the reasons and mechanisms underlying its vulnerability to exogenous insult has been studied for many years.

The hippocampus develops mostly between gestational day 18 and post-natal week three in rats, making it particularly sensitive to early-life experience and highly modifiable by undesirable circumstances (Lucassen et al., 2013). For example, nutrition in early-life may influence hippocampal neurogenesis, dendritic branching, synaptic density and the size of granule cells in the dentate gyrus, changes that together may influence performance on hippocampal-dependent tasks. Most notably, impairments in neurogenesis and long-term potentiation have been associated with impairments in spatial orientation tasks, declarative memory and pattern separation (Lucassen et al., 2013). Further, the metabolic demands of the large pyramidal cells present in the CA regions of the hippocampus could put the structure at risk of insult when changes in mitochondrial energy production or oxidative phosphorylation occur (Hsu & Kanoski, 2014).

Although animals may consume a Western type diet, or high-fat diet (HFD), for 30 days and not exhibit any deficits in non-spatial reference memory, spatial reference memory can be selectively affected even after consuming an obesogenic diet for only 72 hours (Hsu & Kanoski, 2014). Beilharz et al. (2014) illustrated that even short-term exposure to a HFD was sufficient to impair hippocampal-dependent memory in a group of male Sprague-Dawley rats using a place recognition task. With a tendency as a species to demonstrate interest in novelty, the animals

exposed to the HFD were less likely to recognize a novel location, while no diet-induced behavioural differences were found when a novel object (not hippocampal-dependent) was placed into a familiar location. In addition, Pistell et al. (2010) used a common measure of spatial ability, the Stone T-maze, to demonstrate that consumption of a HFD (60% kcal from fat) could impair memory performance in male C57BL/6 mice relative to their control group. These results were further corroborated by Arnold et al. (2014), who also reported impairments in the T-maze task when testing male mice fed a 60% HFD for 17 days, or 45% HFD for 8 weeks from young adulthood. These animals exhibited poorer performance in the alternation task in comparison to their matched controls, regardless of the concentration of saturated fat in the diet (Arnold et al., 2014).

In addition, a study by Valladolid-Acebes et al. (2013) found five week old male mice subjected to a high-fat diet (45% kcal from fat) for eight weeks were impaired in a novel location recognition task, while the same dietary protocol did not cause any impairments when the diet was introduced at eight weeks of age. The experiment was designed to ensure the cognitive impairments observed were not a result of excess energy consumption, therefore food intake was restricted for an additional five weeks post-HFD consumption to confirm deficits (Valladolid-Acebes et al., 2013). Other cognitive deficits, such as relational learning flexibility (Boitard, 2012), contextual fear conditioning (Hwang et al., 2010) and conditioned place preference (Privitera, 2011), have also been observed following chronic consumption of a HFD suggesting that networks associated with the hippocampus are likely to also be affected.

One of the most well-established behavioural techniques employed to measure hippocampal-dependent spatial learning and memory in rodent models is the Morris Water Maze (MWM; Vorhees & Williams, 2006). By making use of distal reference cues, rodents learn to

navigate through a tank of water with a platform hidden just below the surface. As long as the platform is not moved throughout training, this paradigm can be used as a test of hippocampal-dependent spatial learning with latency to platform (measured in seconds) as the dependent measure. At the end of the training period (usually 3-4 days), the submerged platform is removed, and the rodents are allowed to perform a 'free swim' for one minute; referred to as the "probe test". During this final testing day, investigators record time spent in the quadrant where the platform used to be (i.e., the 'correct' or 'target' quadrant), as well as average distance from the previous platform location (Maei et al., 2009; Vorhees & Williams, 2006). As high-energy feeding studies have found marked impairments on spatial learning as measured by latency to find platform (Kuang et al., 2014), and less time spent in the correct quadrant during the probe test of the MWM (Soares et al., 2013), it was presumed that similar impairments should result from diet-induced obesity.

Some studies, such as Mielke et al. (2006), have found no impairments in MWM performance even after male C57BL/6 mice consumed an *ad libitum* HFD for up to 10 months, while Boitard et al. (2014) were able to induce long-term spatial reference memory impairments on the task after only 1-2 months of consumption using male Wistar rats. Possibly, certain periods of development might be more susceptible to the effects of a HFD, for the rodents used in the study by Boitard et al. (2014) were three weeks old at the start of the diet, while an adult cohort of rats tested in the same study were not impaired in their performance on the probe portion of the MWM task. The conflicting nature of existing literature suggests there may be critical-periods of development where animals may be more susceptible to diet-induced impairments in hippocampal function. Although it is suggested that behavioural deficits may stem from low-grade inflammation (Erion et al., 2014; Pistell et al., 2010), impairments in

insulin and leptin signalling, as well as modifications to neurotrophin release and synaptic plasticity, the exact mechanisms triggering the behavioural changes following diet-induced obesity are still unclear.

Further complicating the issue, it is known that neuronal connectivity is inherently different in female and male brains (Ingalhalikar et al., 2014; Miller & Halpern, 2014). While women have a tendency towards more interhemispheric connectivity, as well as an advantage when it comes to verbally mediated memory and social cognition, men tend to be more intrahemispherically connected, with a tendency towards better motor coordination, spatial orientation and mathematical reasoning (Miller & Halpern, 2014; Wojniusz et al., 2013), as well as physical aggression (Ingalhalikar et al., 2014). Studies using rodent models have discovered that while male animals are more resilient than female animals to the effects of chronic stress on depression and anxiety-like behaviours, female rodents tend to be more resilient on tasks of object and place recognition (Russo et al., 2012). Given clear differences in the structure and function of the male and female brain, it is possible that the effects of a chronic HFD also present differently.

3.0 Maternal Obesity

As the rise in obesity has become a public health concern worldwide, it is important to also consider the effects this trend might have on future generations. In 2012, approximately 45% of women over 18 years old in Canada were considered to be overweight, if not obese (Statistics Canada, 2013), and recent reports note that women of reproductive age are becoming increasingly overweight in developed countries worldwide (Howie, Sloboda, Reynolds, & Vickers, 2013; Rodriguez et al., 2012; Tozuka et al., 2010). Obese women of childbearing age are at increased risk of gestational complications, such as prolonged labor and delivery (Shaw, Rasmussen, & Myers, 1997), gestational diabetes and impaired glucose tolerance, maternal hypertension, preeclampsia, and neonatal death (White et al., 2009), in addition to increased risk of post-partum depression (Hanson, 2012). Further, these pregnancy-related complications may affect their offspring by altering their developmental trajectory and possibly increasing risk for disease later in life.

3.1 Developmental programming

The notion of developmental programming stems from the developmental origins of health and disease theory (DOHaD), which is often traced back to work conducted by Barker and colleagues. In the late 1980s, the researchers used birth and death records to reveal an interesting relationship between the geographical location of infant mortality and mortality from certain diseases later in life (Barker & Osmond, 1986; Barker, Osmond, Golding, Kuh, & Wadsworth, 1989). More specifically, the researchers found that in the least affluent areas of England and Wales, poor nutrition in early life was associated with risk of ischaemic heart disease in later adulthood (Barker & Osmond, 1986). For decades, birth size has also been correlated with mortality from chronic disease in later life, and has been associated with risk of symptoms of the

metabolic syndrome, most notably insulin resistance and glucose intolerance, as well as dyslipidemia across the lifespan (Gluckman & Hanson, 2004).

According to the DOHaD theory, offspring development can be modified or programmed by early-life experiences (in utero, infancy and early childhood). Due to the extensive length of the maternal-offspring interaction (both pre- and post-natal), this critical span of development has been studied for decades (Champagne, 2008). Notably, exposure to external insult during the prenatal period seems to be more impactful on development and behaviour than stressors in adulthood (Champagne, 2008; Boitard et al., 2014). Although prospective cohort studies in humans do not exist due to time and cost, animal research has provided a means to model this phenomenon (Langley-Evans, Bellinger, & McMullen, 2005). For example, animal models of gestational growth restriction, as well as studies which modify macronutrient and micronutrient intake during gestation, have consistently been shown to influence the maternal-fetal endocrine exchange and led to issues such as increased adiposity, insulin resistance, glucose intolerance, and hypertension (Gluckman & Hanson, 2004; Langley-Evans et al., 2005). For example, White and colleagues (2009) found that male offspring born to Long-Evans rats C57BL/6L mice fed a HFD for four weeks before breeding, and throughout pregnancy and lactation, weighed more and had heavier retroperitoneal fat pads than offspring born to CD dams. These differences were further exaggerated if the HFD offspring continued to consume the HFD post-weaning, and this has also been found in a more recent study (Yokomizo et al., 2004).

The early post-natal period also seems to be unique in a way where risk of chronic and degenerative disease in later life can be determined by the cells and tissues developing at the time of experience (Harris & Seckl, 2011). For example, Heidbreder et al. (2000) found that rearing rats in isolation can increase motor activity, decrease performance on tasks of attention

and impair neurotransmission in the amygdala and pre-frontal cortex (PFC). In addition, Purcell et al. (2011) found brief exposure to restraint stress during the first week of life is sufficient to lead to impaired glucose tolerance and increased weight gain by the point of weaning at 21 days old. Further, Hertzman (1999) noted that appropriate handling of rats during critical periods of hypothalamic-pituitary-adrenal (HPA) axis development can be beneficial for stress reactivity. As overexposure to glucocorticoids during a stressful situation can be detrimental to processes such as neurogenesis and neuronal viability in the hippocampus, handling can prime the system to deal with stressful experiences later on in the lifespan. For example, handled rats tend to have better stress reactivity and lower CORT levels compared to non-handled rats when tested in the MWM in old age (Hertzman, 1999); whereas an influx of glucocorticoids (i.e., stress hormones) experienced by non-handled rats has been shown to inhibit synaptic plasticity in the hippocampus (Schmidt et al., 2013), as well as the hypothalamus and pre-frontal cortex (both of which are connected to the hippocampus), leading to changes in function and behaviour.

As another type of early-life adversity, maternal obesity impacts multiple aspects of development in the offspring, influencing not only appetite regulation, but also cognitive and mood-related behaviours. Notably, most research that currently exists to explain the effect of maternal obesity on offspring development is sex-specific, with a primary focus on male offspring.

3.2 The effects of maternal obesity on offspring health

A history of maternal obesity puts children at risk for increased body fat percentage, BMI, cardiovascular disease, and insulin resistance (Bilbo & Tsang, 2010; Howie et al., 2013). Some articles have also reported a decrease in energy expenditure over a 24 hour period, which can be exacerbated by changes in genotype and hormonal signalling (Ainge, Thompson, Ozanne,

& Rooney, 2011). Offspring born to obese mothers tend to have higher levels of triglycerides circulating throughout the body (Chang, Gaysinskaya, Karatayev, & Leibowitz, 2008), and maternal hyperglycemia and hyperinsulinemia put offspring at higher risk for obesity and higher concentrations of leptin in plasma when compared with those born to normal weight mothers (Ainge et al., 2011; Bilbo & Tsang, 2010; Tozuka et al., 2010). For example, using a rodent model, Sun et al. (2012) found that changes in plasma leptin concentrations may occur as early as post-natal day (PND) 7 in pups born to a dam fed a high-fat diet. An increased predisposition to hyperphagia may be one factor leading to increased adiposity and higher circulating leptin levels found in these offspring (Ainge et al., 2011). In addition to a predisposition to an obese phenotype, consumption of a HFD throughout pregnancy may lead to increased risk of sedentary behaviour and vascular dysfunction in the offspring (Elahi et al., 2009).

Beyond its peripheral effects, maternal obesity has also been seen to impact development of certain structures in the brain, which may influence behaviour. For example, research has demonstrated that gestational diabetes, hyperinsulinemia, and excessive sweet food consumption were associated with increased susceptibility to neural tube defects in offspring (Kuang et al., 2014). Therefore, properly monitoring blood glucose throughout pregnancy may reduce risk of congenital irregularities.

Behavioural impairments resulting from maternal obesity may develop through the impact of excessive energy intake on neuronal viability in utero (Kuang et al., 2014); affecting structural and functional organization in the hypothalamus and hippocampus, as well as related structures, and modifying dopaminergic, serotonergic and opioidergic signalling pathways (Rodriguez et al., 2012). Maternal obesity has been noted to increase offspring appetite through its impact on the hypothalamic feeding pathways and the upregulation of orexigenic

Neuropeptide-Y (Chen, Simar, & Morris, 2009; Stachowiak et al., 2013; Tozuka et al., 2010). Further, studies using rodents and non-human primate models have demonstrated that maternal high-fat feeding might have implications for reward-related behaviours, risk of mental illness, and stress reactivity, in addition to cognitive performance (Sullivan, Nousen, & Chamlou, 2014).

3.3 Maternal obesity and hippocampal-dependent behaviour in the offspring

Evidence from both human and animal models suggests that, in addition to peripheral and metabolic disruption, maternal obesity increases the likelihood of cognitive impairment in the offspring. For example, Rodriguez et al. (2012) note that children in a prospective study of maternal obesity were more likely to have problems with emotional regulation and inattention at five-years of age, in comparison to children of normal weight mothers. Further, animal studies have demonstrated that hippocampal-dependent behaviours such as spatial learning and memory retention, and anxiety appear to be most affected by maternal obesity.

To study the effects of maternal obesity, Peleg-Raibstein et al. (2012) fed C57BL/6L mice a 60% HFD or CD beginning at 12 weeks of age for three weeks before pregnancy, and throughout gestation and lactation. Using food neophobia (i.e., likelihood of trying a novel food), and the elevated plus maze (EPM), which is a behavioural measure where anxiety is measured by time spent in the open arms of the maze (indicative of less anxiety), in comparison to the time spent in the closed arms of the maze (indicative of anxiety-like behaviours), the authors were able to demonstrate that maternal consumption of a HFD was sufficient to enhance anxiety-like behaviours in the offspring as measured by time spent in the open arm of the EPM and delayed consumption of novel foods. Further, the authors found that maternal HFD increased GABAergic and serotonergic receptor expression in the ventral hippocampus, and nearly doubled the expression of BDNF in the dorsal hippocampus.

Another study of maternal HFD in Sprague-Dawley rats found elevated serum levels of pro-inflammatory cytokines (IL-6) and microglial activation in the hippocampus accompanied by increased anxiety in the EPM; however they also found enhanced performance in the MWM after gestational exposure to a HFD, with male rats spending more time in the target quadrant, and making more platform crossings than those who consumed a low-fat diet (Bilbo & Tsang, 2010). In contrast to the aforementioned studies, Rodriguez et al. (2012) did not find any diet-induced differences in anxiety as measured by the EPM in male offspring Wistar rats, but found reduced anxiety in the maternal HFD offspring exposed only in gestation when scored in an open field test against control offspring.

Looking specifically at spatial memory, Tozuka et al. (2010) performed an interesting experiment where they tested spatial learning and memory retention in male offspring born to female mice fed a HFD for six weeks before breeding. At four and ten weeks of age, the maternal HFD offspring exhibited impaired dendritic growth, and a longer latency to find the escape platform in the Barnes maze, however no differences were found for spatial memory retention (Tozuka et al., 2010).

Further, White et al. (2009) reported that maternal consumption of a 60% HFD for four weeks before breeding, and throughout gestation and lactation was sufficient to impair memory retention in male offspring, as shown across the later training days of the MWM. However, these differences were only found when the diet was consistently consumed across the lifespan, and no other behavioural differences were found despite microglial activation, inflammation (as indicated by circulating IL-6 levels), and increased fat pad weight in the maternal HFD group (White et al., 2009).

Finally, a study by Page et al. (2014) using adult Sprague-Dawley rats fed a 45% HFD, or 10% CD for one month, found that male offspring born to the HFD dams demonstrated altered performance in the MWM at PND 110, as measured by increased latency to find the platform, increased distance traveled, and faster swim speed. Further, the control animals spent more time in the correct reference quadrant during the probe task than the animals exposed to a HFD, regardless of whether the HFD exposure took place during gestation and lactation, only post-weaning, or pre- and post-weaning (Page et al., 2014).

4.0 Study Rationale

The main purpose of the present study was to explore the effects of a maternal high-fat diet on metabolic function and spatial learning and memory performance in both female and male offspring.

4.1 Experimental questions

1. Will consumption of a high-fat diet for ten weeks induce an obese phenotype and spatial learning and memory impairments in adult, female Sprague-Dawley rats?

2. a) Will offspring born to mothers fed a HFD, prior to and during gestation and lactation, exhibit altered metabolic function, in comparison to offspring born to CD mothers?

b) Will any observable differences be dependent on the sex of offspring?

3. a) Will offspring born to mothers fed a high-fat diet demonstrate impairments in spatial learning and memory?

b) Will any observable differences be dependent on the sex of offspring?

4.2 Hypotheses

Based on existing literature, deficits in metabolic function and hippocampal-dependent behaviours were expected to be found in the maternal generation consuming the high-fat diet. Further, it was expected that any cognitive impairment induced by the high-fat diet consumed by the maternal generation would be observable in the offspring by way of developmental programming. Further, as female animals tend to be less susceptible to impairments in spatial learning post-adversity (Russo et al., 2012), it was hypothesized that male offspring would be more affected by exposure to maternal obesity.

5.0 Methods

5.1 Experimental animals: Maternal generation

All experiments with animals received ethical clearance through the University of Waterloo Animal Care Committee, in accordance with the guidelines for use of experimental animals as mandated by the Canadian Council on Animal Care. Please see *Figure 1* for a detailed outline of all feeding and experimental protocols.

Sixty female, non-sibling, Sprague-Dawley rats were delivered on post-natal day (PND 21) and housed in polypropylene cages with wood chip bedding, PVC tubes and shredded paper towel for enrichment. The rats were fed a standard Harlan Teklad rodent diet for one week while being acclimatized to their environment. At PND 28, the animals were randomly assigned to either a high-fat (45% kcal from saturated fat), or control diet (CD; 10% kcal from fat; *see* Table 1 for the complete composition of each diet), with *ad libitum* access to their respective diets and water for ten weeks. The animals were identified using non-toxic markers, and group housed (three animals per cage) in a room with a constant temperature of 23°C and a 12 hour light/dark cycle. Body weight (g) was measured weekly throughout the feeding period. Each week, food hoppers were weighed and topped up to 500 g.

In order to determine the maternal generation's ability to handle a glucose load, oral glucose tolerance tests (OGTTs) were performed on a subset of the animals after four and eight weeks on their respective diets. Prior to being gavaged with a 50% glucose solution (4 g/kg), all animals were fasted for 10-12 hours with access to water only. At baseline, as well as 30, 60, 90, and 120 minutes after administration of the glucose bolus, blood glucose levels were recorded by tail vein poke using a standard glucose meter. Results were calculated using area under the curve (AUC) to compare blood glucose over time between the two dietary conditions.

5.1.1 Breeding protocol

After behavioural testing at 10 weeks on the diet, one female from each cage was selected to breed with a sexually naive, adult Sprague-Dawley male. All male breeders were maintained on a standard rat chow diet prior to breeding. After one week of breeding, dams were presumed to be pregnant and were individually housed, with continued access to their respective diets throughout breeding, gestation and lactation. As humans do not consume a HFD throughout only pregnancy or lactation, feeding a HFD pre-pregnancy in addition to gestation makes for a more ecologically relevant model of studying maternal obesity (Elahi et al., 2009).

Reproductive success was similar across maternal groups, and all dams from each diet condition delivered litters that were of sufficient size to allow litter size and sex distribution among offspring to be standardized in order to control for access to maternal resources. On PND 1, all litters were sexed (see protocol in Rodriguez et al., 2012) and culled to eight pups (four male and four female). Offspring body weights were recorded and averaged by sex each week after birth until weaning at PND 21 (See *Table 3*).

5.2 Behavioural testing: Morris water maze task

All animals were handled for three minutes/day, for three days prior to the first day of testing. All behavioural testing took place between 9:00 a.m. and noon. During experiments, animals were individually housed in clear polypropylene cages, and were returned to their group-housed environments at the end of testing each day. After each swim in the pool, the rats were towel dried and heating pads were placed beneath their cages to keep them warm during testing. All behavioural assessment was alternated by diet and/or sex condition, and all experiments were monitored and recorded using Noldus Ethovision XT v8.5 video tracking system (The Netherlands).

5.2.1 Maternal generation: spatial learning

To evaluate whether chronic consumption of a high-fat diet influenced spatial learning and memory retention in female Sprague-Dawley rats, CD and HFD animals (N = 15 each group) were assessed after ten weeks on their respective diets (PND 98), using an adapted version (Maei et al., 2009; White et al., 2009; Vorhees & Williams, 2006) of the water maze task originally developed by Morris et al. (1982).

Animals were trained to navigate through a water tank (1.52 m in diameter) and find a hidden platform (18.2 cm in diameter) submerged approximately 3 cm below the water. Black non-toxic paint was added to make the water opaque, and water temperature was always maintained at 23°C ± 1°C. The water tank was divided into virtual quadrants using Ethovision XT v8.5 software, and distal visual cues were located on nearby walls in the south, north, east and west quadrants.

Throughout spatial learning training, the platform was always located in the southwest (SW) quadrant (Maei et al., 2009; Vorhees & Williams, 2006), and animals were randomly assigned to one of four starting locations each day for each trial (NW, N, E, SE). The starting locations were selected for their relatively even distance from the platform (Vorhees & Williams, 2006).

Spatial learning training consisted of three days of testing, with four trials each day. The inter-trial interval was approximately five minutes for each rat. On the first day of training, rats were placed on the platform for 15 s to acclimatize to the pool environment before starting their first trial. In each trial, the rats were allowed to swim until they found the platform, or were guided to the platform after 60 s. Each rat was then allowed 15 s on the platform before being

returned to a heated cage until the next trial. Latency (s) and distance traveled (cm) were recorded throughout training.

5.2.2 Maternal generation: reference memory

To assess reference memory, a probe test was used 24 hours after the last training trial (Kuang et al., 2014; Vorhees & Williams, 2006). The submerged platform was removed and rats were placed in the water tank facing the north wall for a single 60 s free-swim. Throughout the probe test, time spent in the target quadrant (SW), time spent in the opposite quadrant, and average distance (cm) to previous location of the platform (Maei et al., 2009) were recorded for later analysis using the Noldus software.

5.3 Offspring generation

5.3.1 Experimental protocol

At the point of weaning, male and female siblings were group housed by sex (3-4 animals per cage) in the same room conditions as described for the maternal generation. All offspring were raised in similar environments and received the CD, regardless of the maternal diet. Each rat was marked for identification using a non-toxic marker, and body and food weights were recorded weekly until PND 60. Behavioural testing was performed in late adolescence (PND 40-48), and again in middle adulthood (PND 90-98). Around PND 120, all offspring were sacrificed for further analyses. Animals were placed in an induction chamber and were anesthetized with a 60% CO_2 gas before being decapitated. Two hour fasting blood glucose was measured using trunk blood and a standard glucose meter, and organ weights (liver, spleen, adrenal glands, and retroperitoneal fat pads) were also recorded.

5.3.2 Behavioural testing

To observe the effects of maternal obesity on hippocampal-dependent spatial learning and reference memory across the lifespan, all pups were assessed using the MWM task in late adolescence (PND 40-48) and young adulthood (PND 90-98; Gray, Chaouloff, & Hill, 2015). The same protocol used with the maternal generation was employed when testing the offspring in adolescence. However, when testing the animals the second time (PND 90-98), only one training day of four trials and one probe day 24 hours post-training were used as the animals remembered the platform location after the first training trial.

5.4 Data analysis

Outliers were removed from further analyses using Grubb's test. All dependent measures for the maternal generation were analyzed through unpaired, two-tailed Student's *t*-tests, as well as two-way analyses of variance (ANOVAs) in GraphPad Prism 6. Offspring data were stratified by sex and analyzed using two-way repeated measures ANOVAs with Tukey's post-hoc analyses where necessary, as well as unpaired Student's *t*-tests. Power calculations were performed to ensure a sufficient sample size was available to detect any differences present.

6.0 Results

6.1 Maternal generation: Biometric data

Although there were no initial baseline differences (*see Figure 2A*) in body weight (CD: 71.00 g ± 1.68 g; HFD: 72.27 g ± 1.66 g; t (58) = 0.053; p = 0.59), the HFD female animals weighed significantly more than those in the CD group after consuming the HFD for ten weeks (CD: 263.1 g ± 3.33 g; HFD: 272.9 g ± 2.99 g; t(58) = 2.18; p = 0.03, *Figure 2B*). The HFD dams also consumed significantly more energy (kcal) in total than the CD dams over the ten week period (CD: 990.3 kcal ± 42.16 kcal; HFD: 1122 kcal ± 25.63 kcal; t(18) = 2.67; p = 0.016, *Figure 4b*).

No significant differences in area under the curve (AUC) for ability to handle a glucose load were found after one month on the HFD, however, the female animals were trending toward impaired glucose tolerance after two months of HFD consumption (CD: 233.9 ± 15.6; HFD: 286.4 ± 21.8; t (34) = 1.96; p =0.058, *Figure 5a*). As a result, a two-way repeated measures ANOVA was conducted with the month two data, revealing an interaction between diet and time (F (4, 132) = 2.73, p = 0.032). Follow-up analyses using the Bonferroni post-hoc test revealed a significant difference at 30 minutes post administration of glucose bolus ($p < 0.05$).

No significant differences were found between the HFD and CD females in regards to their terminal spleen, liver and adrenal gland weight (Table 2). However, the HFD group had significantly heavier retroperitoneal fat pads than the CD animals at sacrifice (%BW: CD, 0.42 ± 0.024; HFD, 0.52 ± 0.031; t (28) = 2.63; p = 0.014; *Figure 6*).

6.2 Maternal generation: Morris water maze test

To ensure there were no differences in swim speed between the two diet conditions, an unpaired Student's t-test was conducted to compare swim speed during the very first trial of

training. No significant differences were detected (CD: 31.71 cm/s ± 0.76 cm/s; HFD: 31.77 cm/s ± 0.66 cm/s; t (28) = 0.0631; p = 0.95), confirming that any performance deficits observed on the task were due to cognitive impairment (*Figure 7*).

6.2.1 Spatial learning

Using a two-way repeated measures analysis of variance (ANOVA; diet condition x training day), no diet differences were detected between the CD and HFD animals in their ability to acquire spatial information as measured by latency to find the platform throughout water maze training (F (1, 28) = 0.33, p = 0.86), although a main effect of time (training day) was revealed confirming that both groups were able to learn across training days, regardless of diet condition (F (2, 56) = 40.07, p < 0.001, *Figure 8*). Further, no differences were found between diet conditions when distance traveled (cm) across training days was assessed using a two-way repeated measures ANOVA, (F (1, 28) = 0.39, p = 0.53), although a main effect of time was found once again (F (2, 56) = 0.32.57, p < 0.001, *Figure 9*).

6.2.2 Spatial memory retention

When spatial memory retention was tested, no diet effects were observed between the two groups for average distance (cm; average proximity), to the previous platform location during the free swim probe test (CD: 44.94 cm ± 2.00 cm; HFD: 44.95 cm ± 1.94; t (28) = 0.003; p = 0.99; Figure 10a). Further, no effect of diet was found when percent of time spent in the target quadrant was compared to time spent in the opposite quadrant across the diet conditions (F (1, 56) = 0.61, p = 0.44). Although time spent in the correct quadrant is the most popular dependent measure to examine the MWM probe test, average distance from the center of the previous platform location is said to be most sensitive for detecting treatment group differences (Maei et al., 2009).

6.3 Offspring generation: Biometric data

6.3.1 Breeding data

Using *t*-tests, litter size, sex distribution and offspring body weight on PND 1 and 7 were analyzed across the two diet conditions. Although litter size was not significantly different between the HFD and CD dams, there was a significant effect on sex distribution, with more female pups born to the HFD dams than the CD dams (CD: 6.00 ± 0.91; HFD: 10.40 ± 1.33; t (7) = 2.58; p = 0.04; Table 3). Notably, two of the five HFD dams delivered three or more stillborn pups, and one female HFD pup was euthanized by CO2 on PND 12 as she was emaciated and no longer receiving sufficient maternal care.

6.3.2 Body weight and food consumption

Overall, female offspring body weight on PND 1 was significantly higher for the maternal CD group (CD: 7.50 g ± 0.51 g; HFD: 6.20 g ± 0.20 g; t (7) = 2.54; p = 0.04), although these differences normalized by PND 7 (CD: 21.59 g ± 0.52 g; HFD: 20.24 g ± 1.00 g; t (7) = 1.27; p = 0.24; *Table 3*). Male offspring did not differ significantly in body weight on either PND 1 (CD: 7.65 g ± 0.45 g; HFD: 6.73 g ± 0.06 g; t (7) = 1.99; p = 0.09), or PND 7 (CD: 21.73 g ± 0.65 g; HFD: 21.25 g ± 0.65 g; t (7) = 0.51; p = 0.62).

As well, maternal diet did not appear to influence terminal body weight for either male offspring (CD: 473.20 g ± 10.30 g; HFD: 468.00 g ± 8.79 g; t (18) = 0.38; p = 0.71; *Figure 11b*), or female offspring (CD: 294.00 g ± 12.67 g; HFD: 281.10 g ± 3.67 g; t (18) = 0.98; p = 0.34; *Figure 12b*). Further, the offspring generation did not differ in their consumption of the CD (in both grams and energy) when measured across five weeks (PND 21-56; *Figures 13 and 14*).

Maternal diet did not significantly influence organ weights at sacrifice (spleen, liver, adrenal glands, and retroperitoneal fat pads) for male (*Table 4*), or female *(Table 5)* offspring. Further, no significant differences were found when terminal blood glucose was measured for

either male (CD: 6.61 ± 0.14; HFD: 6.98 ± 0.14; t (18) = 0.83, p = 0.41; *Figure 15a*), or female offspring (CD: 7.18 ± 0.14; HFD: 7.81 ± 0.38; t (18) = 1.61, p = 0.12; *Figure 16a*).

6.4 Offspring generation: Morris water maze test

6.4.1 Adolescence (PND 40-48)

Swim speed was examined for the first trial of testing to ensure the offspring groups did not differ in their mobility while performing in the MWM. No significant differences were found between the maternal diet conditions when examining speed (cm/s) in adolescence (male CD offspring: 27.52 cm/s ± 1.17 cm/s; male HFD offspring: 24.83 cm/s ± 1.02 cm/s; $t(8) = 1.73$; p = 0.12; female CD offspring: 29.46 cm/s ± 0.81 cm/s; female HFD offspring: 29.84 cm/s ± 0.75 cm/s; $t(8) = 0.34$; p = 0.74; *Figure 16*).

6.4.1.1 Spatial learning

Data were separated by sex and two-way repeated-measures ANOVAs were conducted to test for an interaction between diet condition and spatial learning as measured by average latency to reach the platform across the three training days.

When the average latency of male offspring was measured in adolescence (PND 40-48), no main effect of diet (F (1, 4) = 4.82, p = 0.093; *Figure 17*) was found, however an effect of training day (F (2, 8) = 26.42, $p < 0.001$) on spatial learning was observed. Using a Student's t-test, an effect of maternal diet condition was found for the third day of training (CD: 16.39 ± 2.05; HFD: 29.87 ± 4.53; t (8) = 2.71, p = 0.02). Further, analyzing distance traveled (cm) over training days in adolescence, a main effect of time was once again revealed (F (2, 16) = 20.27, $p < 0.001$), while the effect of maternal diet was trending toward significance (F (1, 8) = 4.72, p = 0.06; *Figure 18*).

For the female offspring, no effect of diet of maternal diet was found (F (1,8) = 1.48; p = 0.25) on ability to acquire spatial information, however a main effect was found to confirm that these rats learned across training days (F (2,16) = 23.66*; p < 0.001; Figure 19*). Looking at distance traveled (cm) over the three training days, a main effect of time on ability to acquire spatial information (F (1, 8) = 1.48; p = 0.25), and no effect of maternal diet (F (1,8) = 0.47*; p = 0.51; Figure 20*), were once again observed.

6.4.1.2 Spatial memory retention

Next, short-term spatial memory retention over a 24 hour period was examined using the probe test of the MWM. In comparison to the male offspring born to HFD dams, those born to CD dams were more accurately able to recall where the platform was previously located during the free swim probe test, as there were significant differences in average distance from the platform location (CD: 49.10 cm ± 1.51 cm; HFD: 60.44 cm ± 2.56 cm; t (8) = 3.82, p = 0.005, d = 2.70, r = 0.80; *Figure 21A*). Further, we found there to be an interaction when percent of time spent in the target versus opposite quadrants during the probe test was compared across maternal diet conditions (F (1, 16) = 5.09, p = 0.03). A multiple comparisons test revealed that male offspring born to the CD dams spent more time in the correct quadrant, while those exposed to the maternal HFD condition spent more time in the opposite quadrant (*Figure 21B*).

No differences were found in memory retention for female offspring exposed to a maternal HFD when average distance to previous platform location (CD: 51.97 cm ± 1.42 cm; HFD: 55.68 cm ± 1.91 cm; t (8) = 1.55, p = 0.16; *Figure 22A*), or percent time spent in the target quadrant versus opposite quadrant (F (1, 16) = 1.64, p = 0.21; *Figure 22B*).

6.4.2 Adulthood (PND 90-94)

Swim speed was also recorded for the first training trial in adulthood. No significant differences were found between the maternal diet conditions when examining speed (cm/s) in adulthood (male CD offspring: 24.59 cm/s ± 0.70 cm/s; male HFD offspring: 25.08 cm/s ± 0.53 cm/s; $t(8) = 0.56$; $p = 0.59$; female CD offspring: 25.81 cm/s ± 0.47 cm/s; female HFD offspring: 26.76 cm/s ± 0.88 cm/s; $t(8) = 0.94$; $p = 0.37$; *Figure 23*).

6.4.2.1 Spatial learning and memory retention

Comparing latency to find the platform for the first training trial in adulthood, male offspring born to a HFD dam were no different in their latency to find the platform (CD: 15.95 s ± 1.29 s; HFD: 19.05 s ± 5.42 s; t (7) = 0.49; $p = 0.63$), or distance traveled (CD: 308.7 cm ± 22.65 cm; HFD: 403.6 cm ± 120.1 cm; t (7) = 0.69; $p = 0.51$; *Figure 24*) relative to male offspring born to a CD dam. However, a significant difference was found on trial 2 using a Student's unpaired t-test for both latency to find the platform (CD: 12.44 s ± 2.86 s; HFD: 24.64 s ± 2.18 s; t (8) = 3.40; $p < 0.001$, $d = 2.40$, $r = 0.77$), and distance traveled (CD: 238.1cm ± 80.42 cm; HFD: 495.4 cm ± 58.34 cm; t (6) = 2.59; $p = 0.04$, $d = 2.11$, $r = 0.73$).

Upon analyzing female offspring performance in the MWM in adulthood, latency to find the platform (CD: 21.07 s ± 2.47 s; HFD: 22.56 s ± 5.04 s; t (8) = 0.27; $p = 0.79$) and distance traveled (CD: 411.6 cm ± 76.12 cm; HFD: 517.2 cm ± 156.3 cm; t *(8)* = 0.61; $p = 0.56$; *Figure 25*) were not found to be affected by maternal diet.

Spatial memory retention was once again examined weeks after initial training (PND 48 vs. PND 94) and occurred 24 hours after the first training day in adulthood (further training days were not completed as the animals appeared to recall their MWM experience from adolescence). Examining male offspring data, no significant differences were found in average proximity to the

previous platform location during the probe test (CD: 49.03 cm ± 2.96 cm; HFD: 42.72 cm ± 1.30 cm; t (8) = 1.95, p = 0.08; *Figure 26A*), while our second dependent variable revealed that male offspring born to the CD dams spent more time in the correct quadrant than the male offspring exposed to maternal HFD in utero and throughout lactation (F (1, 16) = 100.8, p < 0.001***;*** *Figure 26B*).

Similarly for female offspring, no differences were found when average distance to previous platform location (CD: 48.11 cm ± 2.61 cm; HFD: 43.53 cm ± 0.71 cm; t (8) = 1.69, p = 0.13) was examined. However, the maternal CD female offspring spent more time in the target quadrant than the HFD exposed female animals (F (1, 16) = 104.7, p < 0.001; *Figure 27B*).

7.0 Discussion

Previous research suggests that consumption of a HFD leads to an obese phenotype and hippocampal-dependent cognitive impairment. However, most of these studies have focused on male rodents (Arnold et al., 2014; Beilharz et al., 2014; Boitard et al., 2014; Hsu & Kanoski, 2014; Mielke et al., 2006). Further, researchers have recognized that the timing of exposure may be relevant when determining the long-term effects. For example, Boitard et al. (2014) and Valladolid-Acebes et al. (2013) have demonstrated that a chronic HFD fed to male adult rats does not cause any spatial memory impairments, but that impairments become visible when the rodents are exposed to the diet earlier in life. As mentioned, women are increasingly becoming obese leading to a range of negative health concerns, but the presence of an obese state during pregnancy has also been noted to influence developing offspring. Unfortunately, due to a lack of focus in the literature, it is largely unknown how chronic consumption of a HFD may affect female animals, as well as their offspring.

Aiming to fill these gaps in the literature, I examined female Sprague-Dawley rats to see if an obese phenotype and a resultant impairment in spatial memory could be induced by chronic consumption of a HFD starting in the juvenile period. Based on differences in body weight, retroperitoneal fat pads, and glucose tolerance, chronic consumption of a HFD beginning prior to adulthood appeared sufficient to induce an obese phenotype in female rodents. In addition to Warneke et al. (2014) who demonstrated an effect of a Western diet on glucose regulation in adulthood (*see* Ainge et al., 2011 for a review of impaired glycemic control in maternal and offspring generations), Hwang et al. (2010) found that after 8-11 months of HFD feeding, male mice developed symptoms of the metabolic syndrome (i.e., hyperglycemia, hyperinsulinemia, hypercholesterolemia, and hyperleptinemia), while female animals did not demonstrate

hyperglycemia, and had lower grades of hypercholesterolemia and hyperinsulinemia. Similarly, our CD and HFD animals were not different in their basal glucose levels although the HFD group did demonstrate impaired glucose tolerance, as well as increased weight gain after ten weeks of consumption.

Although there were demonstrable biometric changes after the ten week feeding protocol, the regimen was not sufficient to induce any spatial learning, or memory retention deficits. As power calculations revealed that the behavioural analyses were sufficiently powered to detect an effect, a type II error is not suspected. Therefore, the finding suggests that female rats may be less susceptible to the effects of a HFD, as previous work with male rats and mice have found that both longer term (Arnold et al., 2014; Valladolid-Acebes et al., 2013), and shorter (Beilharz et al., 2014; Boitard et al., 2014) term high-fat feeding regimens were able to induce spatial learning and memory impairments.

7.1 Consumption of a HFD and reproductive success

In addition to the metabolic changes demonstrated after ten weeks on a HFD, we also found interesting results after examining data collected from our breeding protocol. In our study, three out of five HFD dams gave birth to two or more stillborn pups. Past research has shown that, in addition to factors such as maternal age, previous caesarean delivery and congenital abnormalities, maternal obesity and gestational diabetes can contribute to the risk of stillbirth (Frias et al., 2011; Starikov et al., 2015); notably, obese women displayed a 2-5 fold increased risk of stillbirth in comparison with normal weight women (Yao et al., 2014). Examining reproductive success in a large sample of female Sprague-Dawley rats, Shaw et al. (1997) found that 70% of dams consuming a HFD from PND 27-65 lost one or more pup, in comparison to only 33% of dams consuming a CD. Further, the surviving pups of the HFD dams grew less well

during the first ten days of life (Shaw et al., 1997). As glucose concentrations increase in the blood, an abundance of reactive oxygen species can lead to cell and tissue damage, impairing placental development (Starikov et al., 2015), as well as decreasing vasodilation in the uterus (Frias et al., 2011; Gluckman & Hanson, 2004).

We found that the HFD dams gave birth to more female pups on average, and that these female offspring weighed significantly less on PND 1 than the female offspring born to the CD dams. Although reduced birth size does not always result in negative health outcomes, any modification to the developmental trajectory could put individuals at risk of catch up growth in environments abundant in high-fat and energy-dense foods (Boersma & Tamashiro, 2015; Gluckman & Hansson, 2004; Williams et al., 2013).

Previous studies have found a range of results when investigating the effects of maternal obesity on reproductive markers, such as litter size, sex distribution, and birth weight. While some reports suggest HFD dams may give birth to smaller pups on average (Ainge et al., 2011; Williams et al., 2013), others have found similar weight offspring between control and HFD dams (Stachowiak et al., 2013). It has also been noted in the literature that excess exposure to insulin, glucose, amino acids and lipids can lead to mitogenesis of adipocytes, inducing increased fat accumulation in the developing fetus (Starikov et al., 2015). Further, under optimal conditions pregnant female animals are said to be more likely to give birth to male offspring as these offspring are more likely to be successful breeders, yet, others have found conflicting results (Alexenko et al., 2007; Grant & Chamley, 2010). For example, Alexenko et al. (2007) found that mice fed a diet very high in saturated fats (54% kcal from lard) gave birth to more male offspring (60%) than did CD dams. According to Grant and Chamley (2010), pregnant animals may be more likely to deliver male offspring when exposed to chronic stress, or poor

environmental conditions, due to an influx of testosterone in peripheral tissues as a result of stress reactivity. Although these are in contrast to our finding of more female offspring being delivered by HFD dams, the type of species (i.e., mice vs. rats) might be influential in the effect observed.

7.2 The effects of maternal obesity on offspring development and cognition

Due to the heterogeneity of experimental protocols often used in the literature, which include length of feeding and composition of diet, as well as age, strain and sex of animals exposed, a consensus regarding the effects of a maternal HFD on markers of metabolic and brain development, as well as cognitive impairment in the offspring, does not exist (Ainge et al., 2011).

Previous studies have noted that prenatally stressed rats are at increased risk of affective disorders (Watson et al., 1999), altered stress reactivity through modifications to the HPA axis (Boersma & Tamashiro, 2015), and altered metabolic function. Further, exposure to maternal stress during the first week of pregnancy can alter body weight and food consumption patterns in later development (Boersma & Tamashiro, 2015). For example, in one study by Boersma and Tamashiro (2015), offspring tended to be hyperphagic when consuming a standard chow diet, but surprisingly gained less weight when consuming a HFD. However, it should be noted that within each population of certain strains of animals (e.g., Sprague-Dawley rats) lies a subset of vulnerable, as well as resistant animals (Boersma & Tamashiro, 2015; Levin et al., 1997). Therefore, it is possible that exposure to different types of stressors may impact different strains of animals differently depending on their genetic background and age of exposure (Boersma & Tamashiro, 2015).

In addition to *in utero* effects of stress, exposure to postnatal maternal behaviours, such as consumption of a HFD throughout lactation, has been demonstrated to also impact development of the offspring (Niculescu & Lupu, 2009). Typically, humans do not consume a HFD throughout only pregnancy or lactation, therefore feeding a HFD pre-pregnancy in addition to gestation makes for a more ecologically relevant model of studying maternal obesity (Elahi et al., 2009). In our model of maternal obesity, dams consumed their respective diets (HFD or CD) for ten weeks prior to gestation, as well as throughout gestation and lactation until offspring were weaned at PND 21. Through the analysis of body weights, food consumption, retroperitoneal fat pad weight, terminal glucose tolerance, and other terminal measures of organ weights, we found that maternal obesity did not impair the metabolic profile of the offspring.

In contrast to our results, Page et al. (2014) found that Sprague-Dawley rats exposed to a 45% kcal HFD for one month before pregnancy delivered male offspring with metabolic impairments demonstrable into adulthood. Around PND 110, male offspring born to obese dams weighed more, had higher blood glucose concentrations, higher concentrations of CORT, and larger retroperitoneal fat pads, independent of whether they were also exposed to a HFD throughout postnatal development (Page et al., 2014). Further, a systematic review by Ainge et al. (2011) noted earlier studies finding that male offspring born to HFD dams exhibited impaired tolerance for glucose at weaning after being exposed throughout gestation and lactation only. Therefore, it is unclear why we were unable to detect any statistically significant differences in metabolic function in the offspring exposed to a HFD during gestation and lactation.

Spatial learning and memory retention in offspring of HFD female rats have been less well studied than their metabolic profile, but some researchers have found that male offspring born to HFD fed, or obese mothers exhibit increased latency and longer path length in the

training, or learning, portion of the Morris water maze (Page et al., 2014), while others have only demonstrated impaired spatial memory retention on the probe part of the task (White et al., 2009). In the present study, exposure to a HFD throughout gestation and lactation was found to impair spatial learning, as measured by latency to find platform (s) and distance traveled (cm), in only male adolescent offspring. Further, the male offspring demonstrated less accurate memory retention in the probe portion of the water maze task, as their average distance from the previous platform location was significantly different from that of the CD offspring, and the HFD offspring spent less time in the target, or correct quadrant than the CD offspring. When spatial learning and memory retention were measured again in adulthood, the previously apparent differences in latency had normalized, although the CD exposed offspring continued to spend more time in the correct quadrant than the HFD exposed offspring. Further, although no significant differences were found when the female offspring were tested in adolescence, the CD exposed offspring were more accurate than the HFD exposed female offspring in their memory for the platform location as per their time spent in the target quadrant when tested in adulthood.

7.3 Possible mechanisms underlying diet-induced cognitive impairment

7.3.1 Insulin

Insulin has a primary role of glucoregulation in the body, stimulating the absorption of glucose into adipose tissue, skeletal muscle and the liver and also works to inhibit processes, such as gluconeogenesis in the liver, as well as lipolysis and proteolysis in muscle and adipose tissues (Martyn et al., 2008). Insulin resistance is characterized by a decreased response to insulin in the blood due to changes at the level of signal transduction, despite excess plasma levels which attempt to compensate for decreased sensitivity (Lee et al., 2011; Martyn et al.,

2008). Diagnosed in nearly all individuals who become obese (Martyn et al., 2008), it may be one factor mitigating cognitive disruption in these individuals.

Research suggests that impaired insulin signalling in the hippocampus may contribute to the cognitive deficits observed after consumption of a high-fat, or high-sucrose diet (Anderson et al., 2013; Arnold et al., 2014; Mielke et al., 2005; Soares et al., 2013). Greenwood et al. (2003) noted impairments in delayed, but not immediate, verbal memory, following poor post-prandial glycemic control, and another study reported decreased hippocampal volume in individuals diagnosed with type 2 diabetes (Gold et al., 2007). Stranahan & Mattson (2011) also mentioned that cognitive functioning is correlated with glycemic control in non-diabetic individuals, supporting a broad metabolic-neurocognitive relationship, even outside of obesity research. As mentioned earlier, individuals diagnosed with diabetes are also at increased risk of Alzheimer's disease, and poor glycemic control can exacerbate existing dementia (Stranahan & Mattson, 2011).

Research using animal models has also supported a role for insulin in cognition. For example, Mielke et al. (2005) demonstrated impaired insulin signalling pathways and decreased hippocampal synaptic plasticity in hamsters fed a high fructose diet. Additionally, Pathan et al. (2008) showed that administering an insulin sensitizer (rosiglitazone) was sufficient to restore Morris water maze performance following the consumption of a high-fat diet. Other animal studies have also found that diet-induced diabetes was able to impair synaptic transmission throughout the downregulation of insulin receptor substrate 2 (IRS-2), which appears to be important for long-term potentiation and memory formation, although the exact role of IRS-2 is not entirely understood (Martin et al., 2012). However, Ross et al. (2012) did not find impaired

insulin signalling when male rats were fed a high-fructose diet, despite deficits in hippocampal-dependent memory in the MWM.

7.3.2 Leptin

The main role of leptin rests in its ability to alter the hypothalamic feeding pathway to increase energy expenditure and reduce appetite, however leptin receptors are also expressed in the cerebellum, amygdala, brainstem and hippocampus (White et al., 2009). As a hormone, leptin acts as an afferent signal of adiposity to the brain, increasing in concentration relative to obesity in humans (Hariri & Thibault, 2010). Increases in leptin concentration can also be found in healthy males after consumption of a high-fat meal (Hariri & Thibault, 2010). However, despite the increased levels seen in obese individuals, the hypothalamus eventually becomes resistant and is no longer able to receive messages of satiety to support the control of appetite (White et al., 2009).

Similarly, the hippocampus, which relies on leptin for the maintenance of synaptic plasticity and memory formation, can develop leptin resistance (Grillo et al., 2011; Hwang et al., 2010). An important role for leptin in learning and memory has been shown by studies revealing that rodents without functional leptin receptors were unable to properly learn hippocampal-dependent memory tasks, such as the MWM (Stranahan & Mattson, 2011). Consumption of a HFD for 8 weeks has also been shown to sufficiently impair downstream leptin signaling in 5 week old male C57BL/6L mice, in addition to hippocampal-dependent memory (Valladolid-Acebes et al., 2013). Leptin also influences synaptic function in the hippocampus. Noted by Irving and Harvey (2014), exogenous administration of leptin to the rat hippocampus can enhance long-term synaptic transmission.

Although it was not directly examined for the offspring generation in the present study, leptin resistance could provide some explanation for the sex differences in cognitive impairment seen between male and female offspring of HFD dams. For example, Deroo and Korach (2006) noted in their review that estrogen may inhibit lipolysis. Although excess storage of fat in adipocytes may cause inflammation leading to cognitive-impairment, estrogen can alter adipocyte levels, indirectly altering leptin signaling. This might explain why female offspring in the present study did not demonstrate impaired spatial learning or memory retention in adolescence, while the male offspring were impaired.

7.3.3 Neurogenesis

Despite the initial controversy over the adult brain's ability to generate new neurons, it is now accepted that certain regions of the brain, such as the hypothalamus and dentate gyrus of the hippocampus, do continue to proliferate new neurons throughout the lifespan (Boitard, 2012; Jackson-Guilford et al., 2000; Lindqvist et al., 2006). Hippocampal neurogenesis, which is impaired after chronic consumption of a HFD, supports cognitive flexibility in rodents and declarative memory in humans; both of which have been found to be impaired after diet-induced metabolic dysfunction (Boitard, 2012; Jackson-Guilford et al., 2000; Lindqvist et al., 2006). Researchers have suggested that increased concentrations of corticosterone and neuroinflammation after chronic consumption of a HFD might impair hippocampal neurogenesis (Boitard, 2012; Lindqvist et al., 2006; Park et al., 2010; Pistell et al., 2010). Inflammation in the brain is inversely associated with brain-derived neurotrophic factor (Pistell et al., 2010), and while the exact mechanisms are still unclear, proinflammatory cytokines such as IL-6 and TNF-α may inhibit neurogenesis through the production of reactive oxygen species, leading to cell damage (Pervaiz & Hoffman-Goetz, 2011).

7.3.3.1 Brain-derived neurotrophic factor (BDNF)

A neurotrophin responsible for the growth, maintenance, and survival of many types of neurons, brain-derived neurotrophic factor (BDNF) is found throughout the hippocampus and thought to influence both synaptic plasticity and neurogenesis in this structure (Kanoski & Davidson, 2011). After chronic consumption of an obesogenic diet, research has demonstrated that BDNF levels, as well as synaptic plasticity and hippocampal-dependent memory, are impaired in the rat hippocampus (Molteni et al., 2002; Stranahan et al., 2008). However, in contrast, Ross et al. (2012) found that impairments seen in MWM performance of male rats fed a high-fructose diet were not associated with BDNF levels.

7.4 Possible explanations for a sex-stratified effect of a HFD on the brain

As early as 1948, a report on a birth cohort from the United Kingdom revealed that low birth weight and increased weight gain was able to influence age of menarche (Sloboda et al., 2009). As seen with cases of early life stress in humans, female animal offspring who experience poor maternal-child interaction (including parental support and bonding, increased stress reactivity, as well as exposure to maternal HFD) are at increased risk of early pubertal maturation and an altered stress response as measured through CORT and ACTH levels (Connor et al., 2012). Developmental programming of such a phenotype is likely to be evolutionarily adaptive as pubertal aging in females ensures early reproductive success in animals otherwise predisposed to adverse development (Connor et al., 2012; Sloboda et al., 2009). Although we did not directly measure CORT or pubertal maturation in early adolescence, it is possible that the female offspring in the present study also experienced early pubertal aging due to HFD exposure in utero and during lactation.

In rat models, experience of early life adversity through malnourishment, or exposure to excess glucocorticoids can lead to increased risk of obesity, insulin resistance, hyperphagia, a

preference for fatty foods, and reduced energy expenditure through unwillingness to exercise (Sloboda et al., 2009). Using Wistar rats, Connor et al. (2012) demonstrated that consumption of a HFD during pregnancy and lactation only was sufficient to induce obesity, hyperinsulinemia and hyperleptinemia in the offspring, and decrease maternal behaviours, such as licking and grooming, throughout early development. Despite standardization of litter size and sex distribution to account for maternal resources, it is possible that exposure to the HFD impaired endocrine changes in the pregnant dams that help to enhance natural maternal behaviour. Observed in our study, Connor et al. (2012) also noted that HFD pups tend to be smaller at birth and exhibit catch up growth by weaning.

Since the 1940s, it has been known that women are at increased risk of diabetes and cardiovascular disease at menopause, and some of these physiological changes can be improved through exogenous administration of estrogen-like compounds (Gitlow & Kurschner, 1943; Meyer, Clegg, Prossnitz, & Barton, 2011). Newer research has found that estradiol, which can fluctuate throughout the lifespan, can be produced in the brain, and may act as a transcriptional factor (slowly impacting long-term development), or may bind to membrane receptors for a fast, direct action (Luine, 2014; Wei et al., 2014). A study by Wei et al. (2014) found that when Sprague-Dawley rats were exposed to chronic stress, female animals were resilient to structural changes in the hippocampus, such as shrinkage of apical dendrites in the CA3 region, as well as impaired dendritic branching in the second and third layer of the medial prefrontal cortex, while male animals demonstrated such effects. Interestingly, when the female animals were prepubescent or ovariectomized, they were no longer resilient to these structural changes and were comparable to their male counterparts (Wei et al., 2014). The authors suggested that estradiol produced in the female brain during the reproductive years might mediate the inhibition

of corticosterone on the synapse by upregulating serotonin levels in the synaptic cleft when exposed to subchronic stress (Wei et al., 2014).

A study by Connor and colleagues (2012) noted that exposure to a HFD during gestation and lactation alone was able to induce early pubertal maturation as indicated by progesterone and androstenedione levels by PND 35 in 40% of male offspring, and 60% of female offspring, even if the animals consumed a standard chow diet post-weaning (Connor et al., 2012). Interestingly, if female offspring continued to consume the HFD post-weaning, nearly 80% went through pubertal changes by PND 32 (Connor et al., 2012). Similar results were also found in an earlier study by Sloboda et al. (2009) that did not test for reproductive success directly, but found increased progesterone levels and ovarian function indicative of early pubertal maturation in offspring of HFD dams consuming a 45% kcal from fat diet either pre-pregnancy, or during pregnancy and lactation. Although the neuroprotective effects of estradiol on diet-induced obesity in animal models are not well understood, it is known that estrogen can block signal transduction which would normally lead to proinflammatory cytokine production (Vegeto et al., 2008). As mentioned previously, estradiol is also said to prevent the inhibition of CORT on the synapse by upregulating 5HT in the synaptic cleft under conditions of subchronic stress (Wei et al., 2014), and more recent work by Yokomizo et al. (2014) has also recognized a protective role of estradiol on pancreatic β-cells, which could explain why female animals may have reduced risk of insulin deficiency and glucose intolerance (*see* summary schematic in *Figure 28*). As we did not test for the association between estrogen levels and cognition directly, it remains unclear whether the female offspring were resilient to impairment due to the neuroprotective effects of estrogen, or if some other mechanism was at play. As it is known that obesity and consumption

of a HFD impact male and female adult animals differently, premature reproductive development in the female offspring might be one explanatory factor in this dimorphism.

7.5 Future Directions

As chronic consumption of a HFD and diet-induced obesity can have a profound impact on the individual, it is crucial to continue examining the effects chronic consumption of a HFD and diet-induced obesity can have on the brain. The notion that estrogen can mediate inflammation in the brain caused by diet-induced obesity is interesting, and should continue to be examined for future interventions in a population that is becoming increasingly obese. As few researchers in the past have studied these effects in female models, and the present study did not directly test for pubertal changes due to a HFD, this should continue to be examined in future studies so sex differences after consumption of a HFD can be further understood. In addition, it is important for future generations that we continue to examine the transgenerational effects of maternal obesity on the brain and behaviour. Although we found differences in spatial learning and memory retention in the male adolescent offspring, the underlying mechanisms are still unknown. Further, we are unsure whether there would have been structural changes in the hippocampus of adult offspring, despite finding no behavioural impairment in MWM performance.

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Appendix A: Tables

	CD		HFD	
Composition	***%gram***	***%kcal***	***%gram***	***%kcal***
Protein	19.2	20	24	20
Carbohydrate	67.3	70	41	35
Fat	4.3	10	24	45
Total	-	100	-	100
kcal/gram	3.85	-	4.73	-
Ingredients	gram	Kcal	gram	Kcal
Casein, 30 Mesh	200	800	200	800
Corn Starch	550	2200	72.8	291
Maltodextrin 10	150	600	100	400
Lard	20	180	177.5	1598
Sucrose	0	0	172.8	691
Cellulose	50	0	50	0
Soybean Oil	25	225	25	225
L-Cystine	3	12	3	12
Mineral Mix	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H2O	16.5	0	16.5	0
Vitamin Mix	10	40	10	40
Choline Bitartrate	2	0	2	0
Red Food Dye	0.025	0	0.05	0
Blue Food Dye	0.025	0	0	0
Total	1055.05	4057	858.15	4057

Table 1. Composition of Control and High-fat Diets. CD = Control Diet, HFD = High-fat Diet.

	CD	HFD	*P*-value	*t*-value	*df*
Spleen (%BW)	0.26 ± 0.0093	0.24 ± 0.0038	0.13	1.56	28
Liver (%BW)	3.00 ± 0.071	3.01 ± 0.036	0.90	0.13	28
Adrenal Glands (%BW)	0.024 ± 0.00043	0.024 ± 0.0006	0.72	0.37	28
Retro-Peritoneal Fat Pads (%BW)	0.42 ± 0.024	0.53 ± 0.031	0.01	2.63	28

Table 2. Terminal Biometrics from Maternal Generation. Data are presented as mean ± S.E.M. *N* = 15. BW = Body Weight, CD = Control Diet, HFD = High-fat Diet.

	mCD	mHFD	*P*-value	*t*-value	*Df*
Total Litter Size	12.00 ± 1.05	14.25 ± 0.48	0.12	1.78	7
Average Number of Male Offspring/Litter	6.50 ± 1.32	6.60 ± 1.66	0.97	0.05	7
Average Number of Female Offspring/Litter	6.00 ± 0.91	10.40 ± 1.33	0.04	2.58	7
Average Male Offspring Body Weight on PND 1	7.65 ± 0.45	6.73 ± 0.06	0.09	1.99	7
Average Female Offspring Body Weight on PND 1	7.50 ± 0.51	6.20 ± 0.20	0.04	2.54	7
Average Male Offspring Body Weight on PND 7	21.73 ± 0.65	21.25 ± 0.65	0.62	0.51	7
Average Female Offspring Body Weight on PND 7	21.59 ± 0.52	20.24 ± 1.00	0.24	1.27	7

Table 3. Litter Size and Offspring Body Weight. Data are presented as mean ± S.E.M. *N* = 5 litters per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet, PND = Post-natal Day.

	mCD	mHFD	*P*-value	*t*-value	*Df*
Spleen (%BW)	0.16 ± 0.0043	0.17 ± 0.011	0.93	0.086	8
Liver (%BW)	3.17 ± 0.081	3.14 ± 0.16	0.85	0.19	8
Adrenal Glands (%BW)	0.021 ± 0.0091	0.021 ± 0.0096	0.95	0.065	8
Retro-Peritoneal Fat Pads (%BW)	0.55 ± 0.011	0.60 ± 0.076	0.51	0.70	8

Table 4. Terminal Biometric Data from Male Offspring. Data are presented as mean ± S.E.M. *N* = 10. BW = Body weight, mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

	mCD	mHFD	*P*-value	*t*-value	*Df*
Spleen (%BW)	0.21 ± 0.0069	0.22 ± 0.012	0.78	0.29	8
Liver (%BW)	2.90 ± 0.077	3.03 ± 0.092	0.32	0.11	8
Adrenal Glands (%BW)	0.022 ± 0.0011	0.022 ± 0.00095	0.90	0.13	8
Retro-Peritoneal Fat Pads (%BW)	0.37 ± 0.047	0.42 ± 0.061	0.51	0.70	8

Table 5. Terminal Biometrics Data from Female Offspring. Data are presented as mean ± S.E.M. *N* = 10. BW = Body Weight, mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

Appendix B: Figures

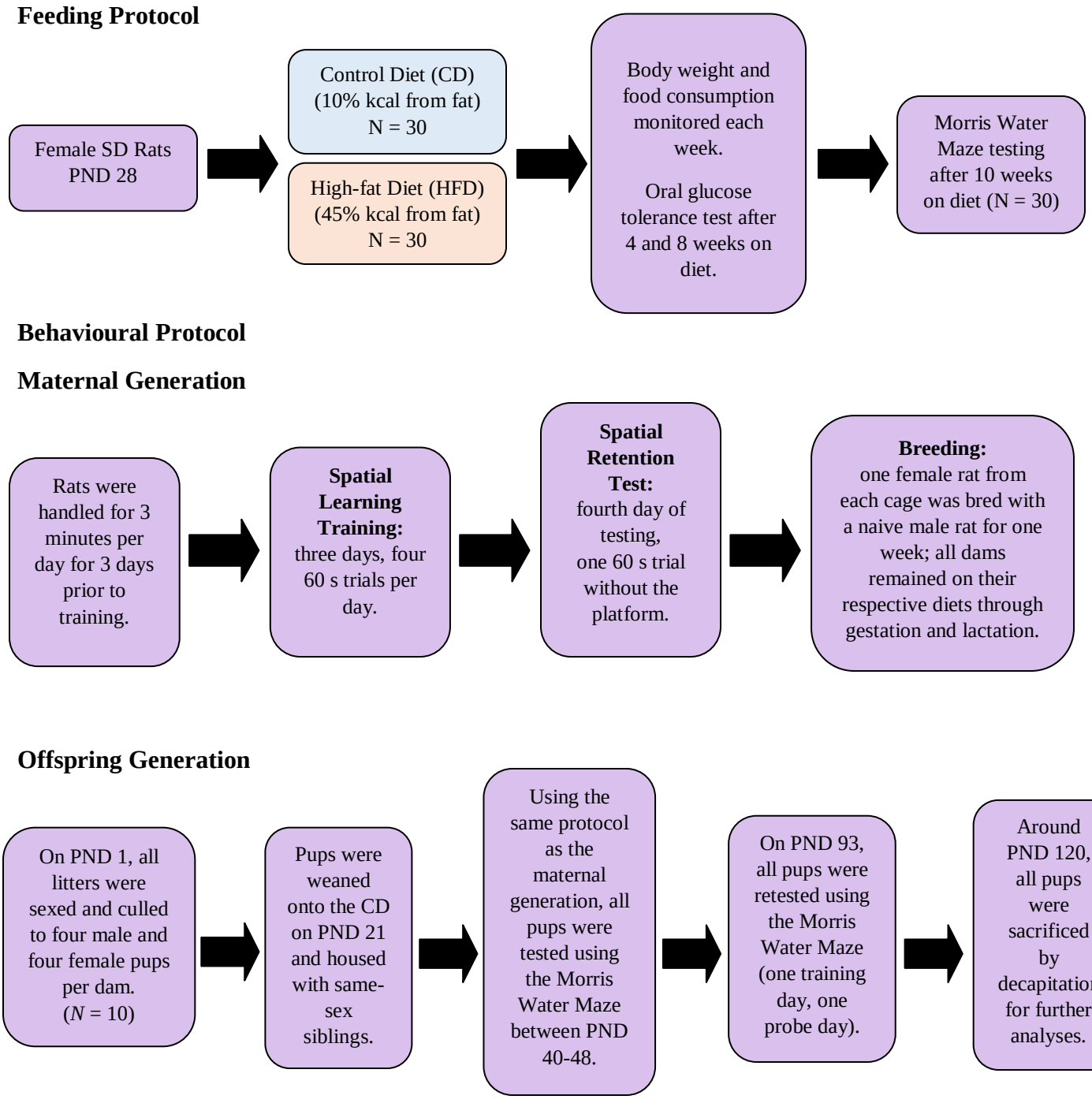


Figure 1. Experimental outline for both maternal and offspring generations. SD = Sprague-Dawley, CD = Control Diet, HFD = High-fat Diet, PND = Post-natal Day.

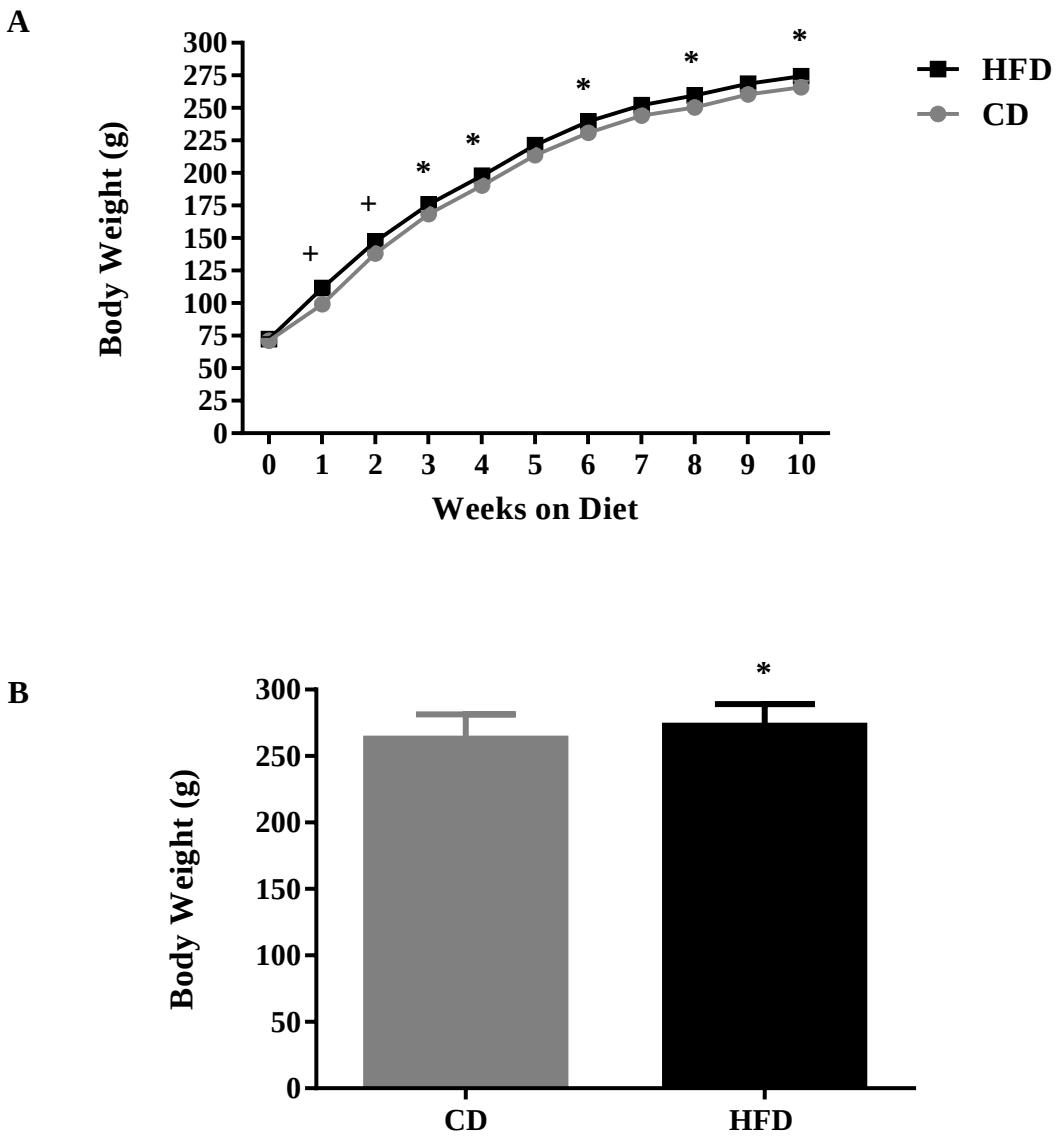


Figure 2. Maternal body weight throughout the feeding period (A), and after ten weeks (B). Data are presented as the mean ± S.E.M. *$p < 0.05$; + $p < 0.01$ using unpaired Student's *t*-tests; $N = 30$ for each diet condition. CD = Control Diet, HFD = High-fat Diet.

A

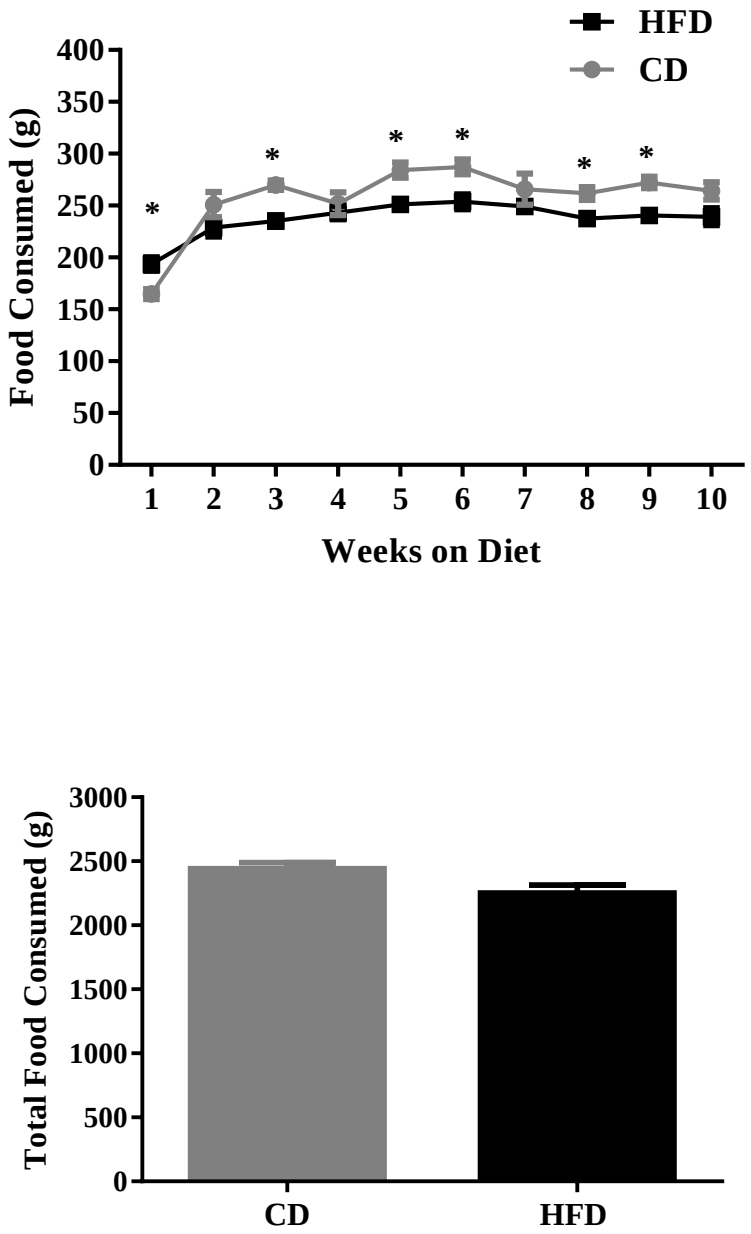
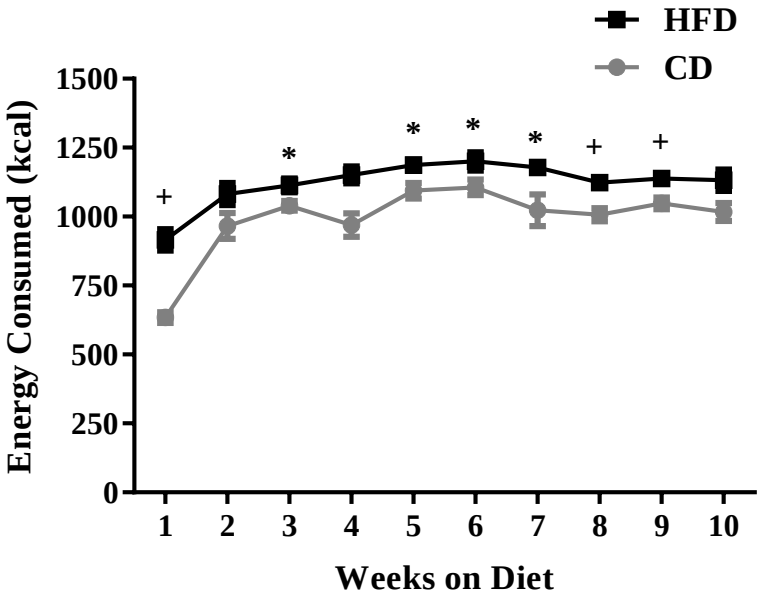


Figure 3. Maternal food consumption as total mass of food ingested per cage of three animals throughout the feeding period per diet condition (A), and as a grand total over the ten week period (B). Data are presented as the mean ± S.E.M. *$p < 0.01$ using unpaired Student's *t*-tests; *N* = 10 cages per diet condition. CD = Control Diet, HFD = High-fat Diet.

A



B

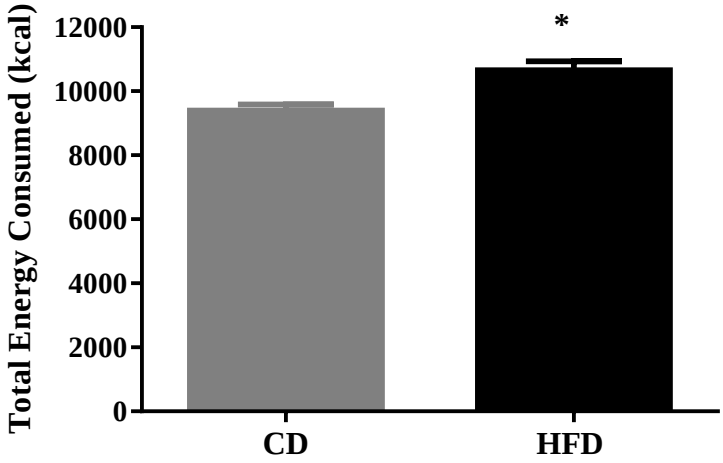


Figure 4. Maternal food consumption as energy (kcal) ingested per cage of three animals throughout the ten week period per diet condition (A), and as a grand total over the ten week period (B). Data are presented as the mean ± S.E.M. *$p < 0.05$, +$p < 0.01$ using unpaired Student's *t*-tests; $N = 10$ cages per diet condition. CD = Control Diet, HFD = High-fat Diet.

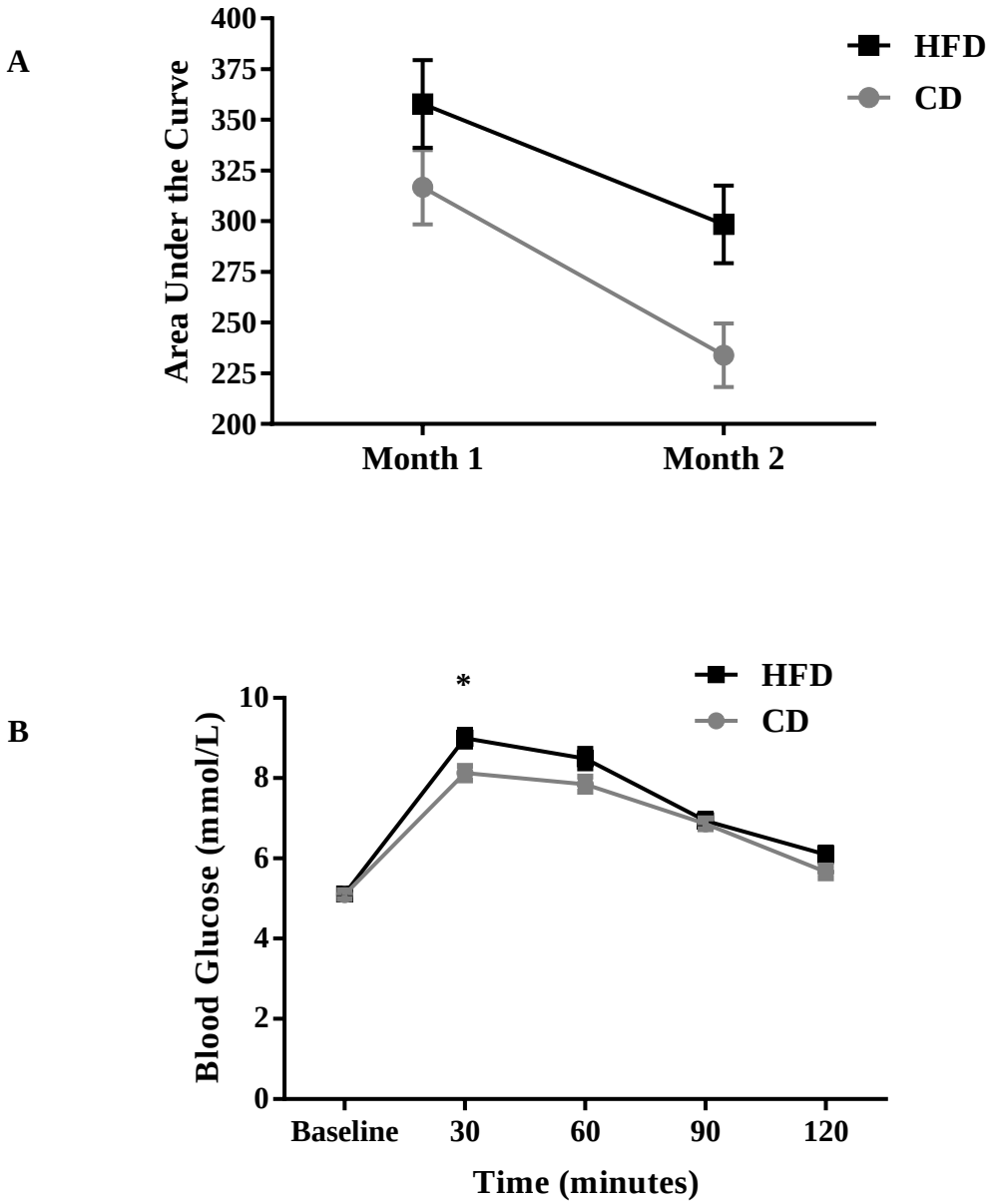


Figure 5. Maternal oral glucose tolerance presented as area under the curve (A) after one and two months consuming the High-fat Diet (HFD), or Control Diet (CD) diet. A two-way ANOVA was also conducted to examine the interaction between diet and time on oral glucose tolerance (B). Data are presented as the mean ± S.E.M. $N = 18$ per diet condition. *$p < 0.05$.

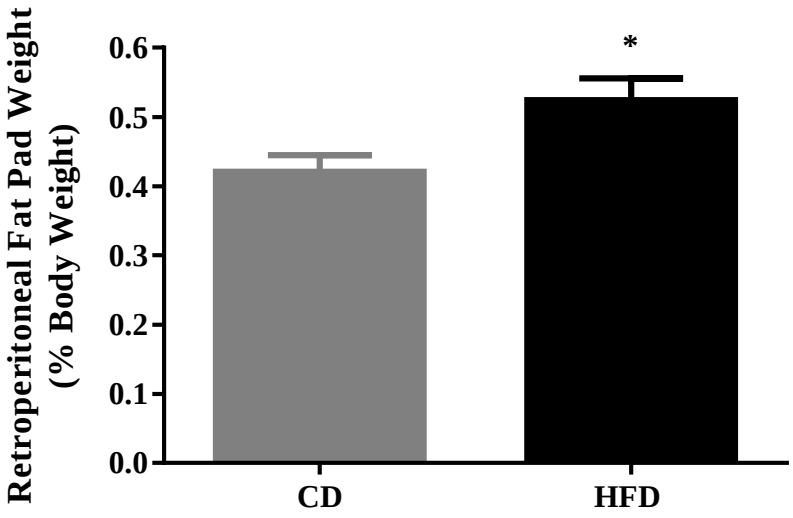


Figure 6. Maternal retroperitoneal fat pad mass. Data are presented as the mean ± S.E.M. *$p < 0.05$; $N = 15$ per group. CD = Control Diet, HFD = High-fat Diet.

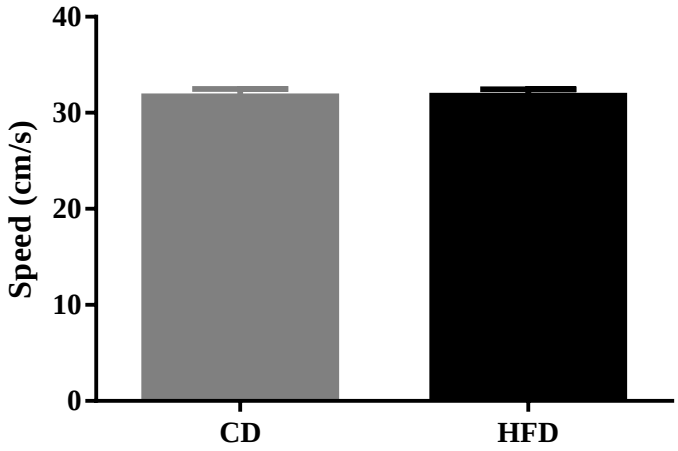


Figure 7. Average maternal swim speed, as shown in seconds. Data are presented as the mean ± S.E.M. $N = 15$ per group. CD = Control Diet, HFD = High-fat Diet.

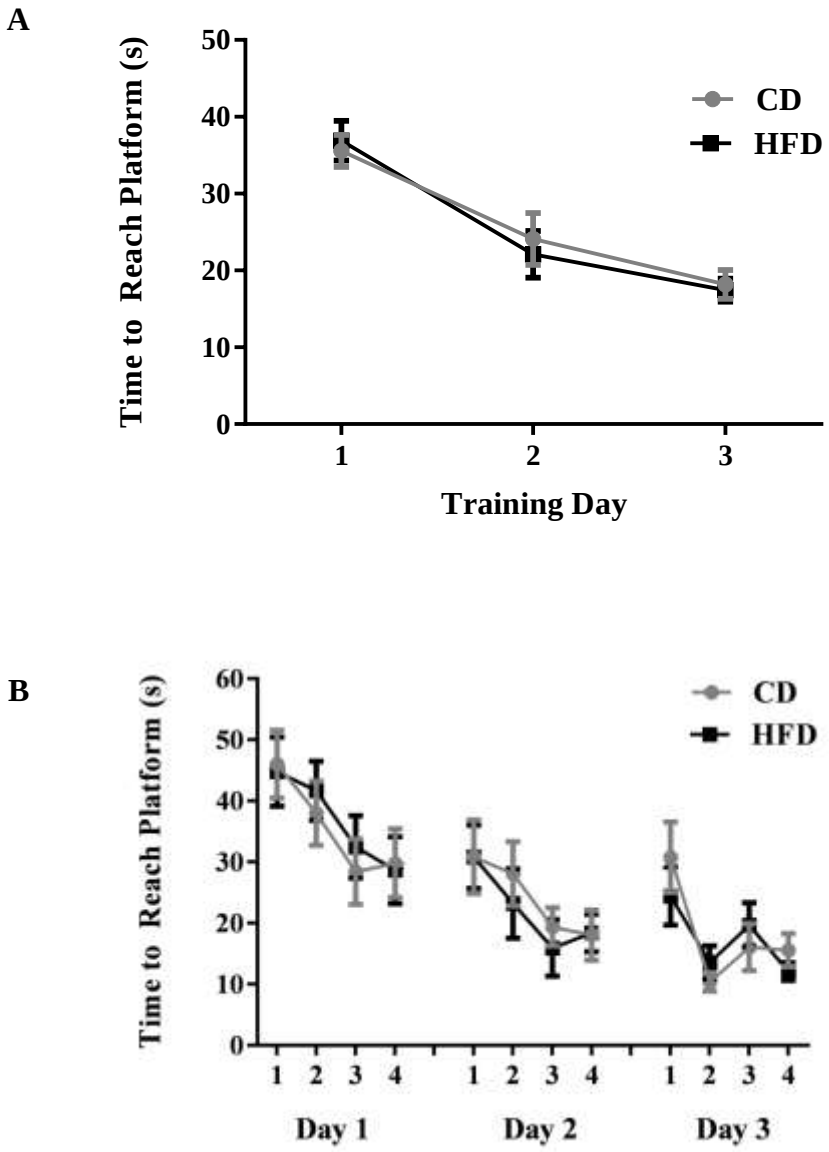
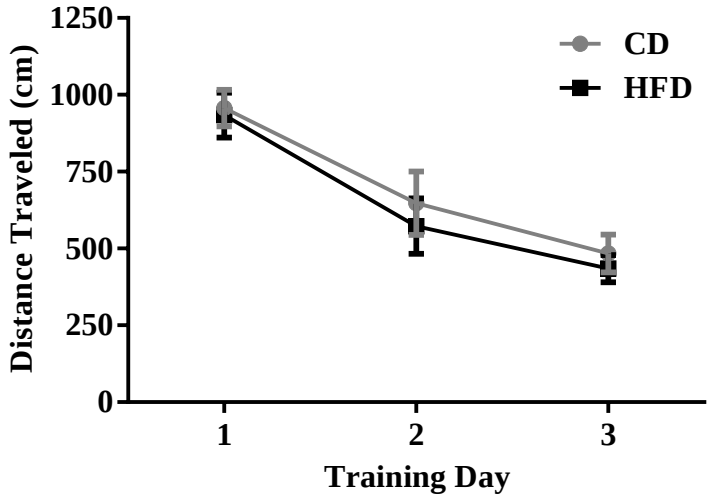


Figure 8. Spatial learning for maternal generation, as measured by latency in seconds, across training days (A), and displayed within each training day (B). Data are presented as the mean ± S.E.M. *N* = 15 per group. CD = Control Diet, HFD = High-fat Diet.

A



B

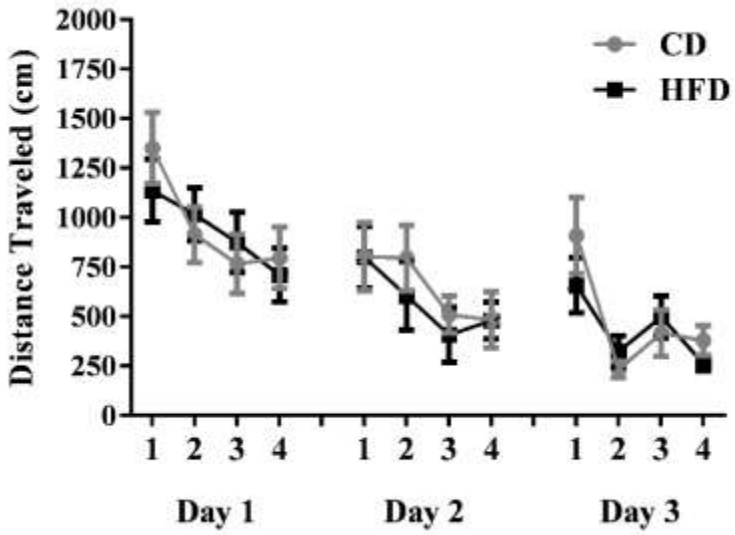


Figure 9. Spatial learning for maternal generation, as measured by distance traveled (cm), across training days (A), and displayed within each training day (B). Data are presented as the mean ± S.E.M. *N* = 15 per group. CD = Control Diet, HFD = High-fat Diet.

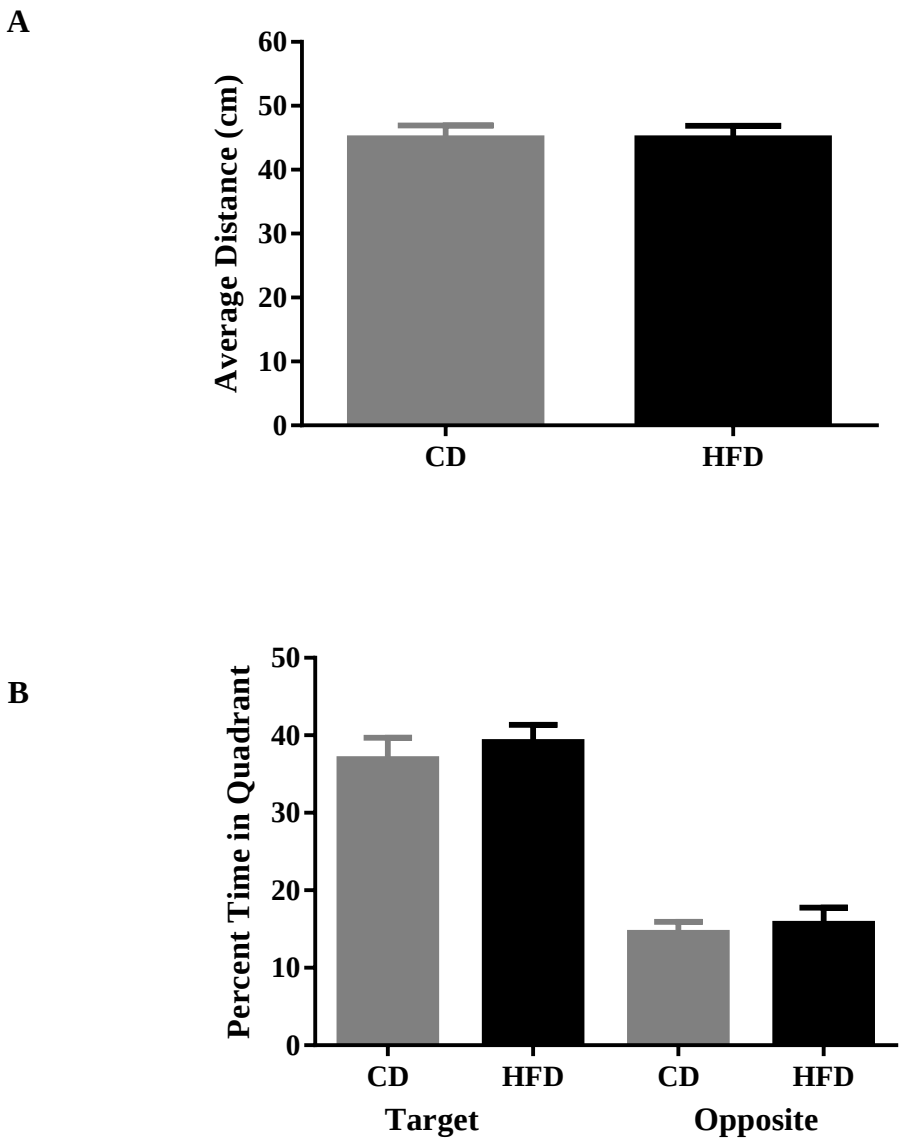
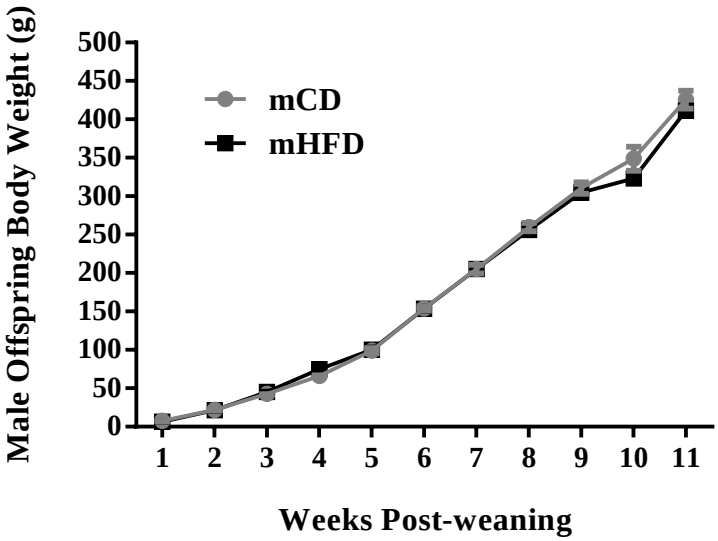


Figure 10. Maternal spatial reference memory, as measured by average distance to the previous platform location (cm) and percent time spent in the target quadrant (SW) and opposite quadrant (NE). Data are presented as the mean ± S.E.M. *N* = 15 per group. CD = Control Diet, HFD = High-fat Diet.

A



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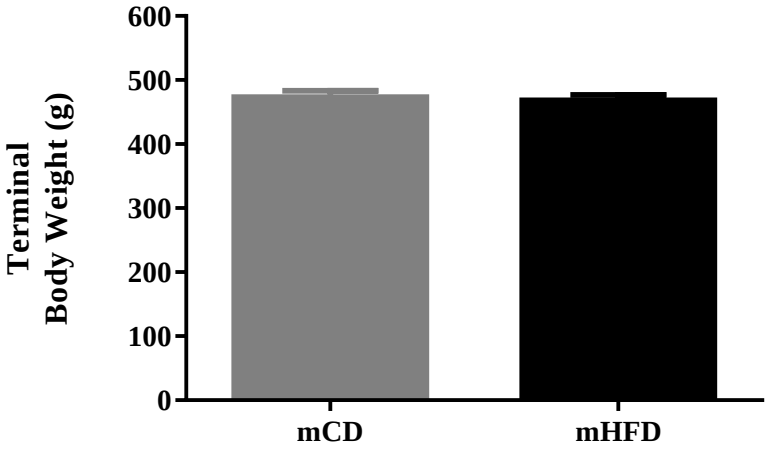


Figure 11. Body weight (g) for male offspring across 11 weeks from PND 1-PND 77 (A), and final body weight at sacrifice (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

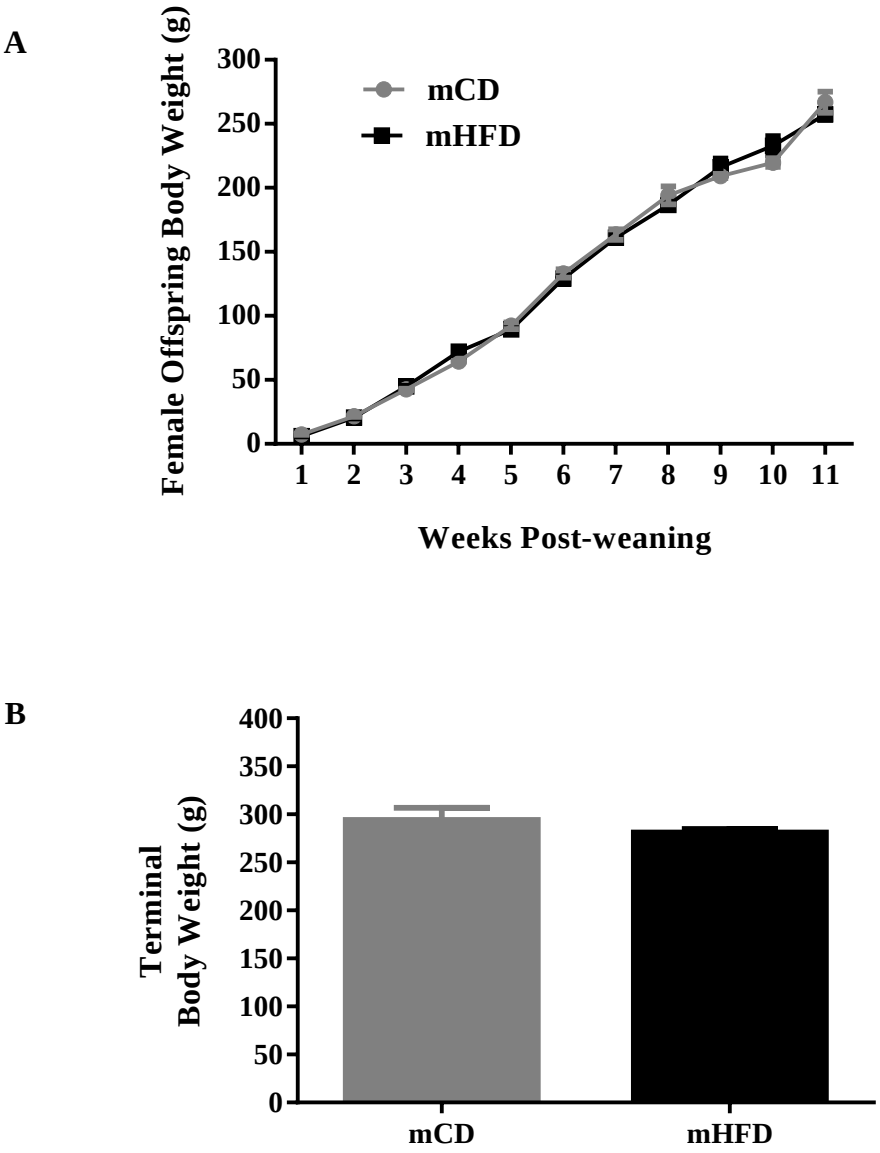


Figure 12. Body weight (g) for female offspring across 11 weeks from PND 1-PND 77 (A), and final body weight at sacrifice (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

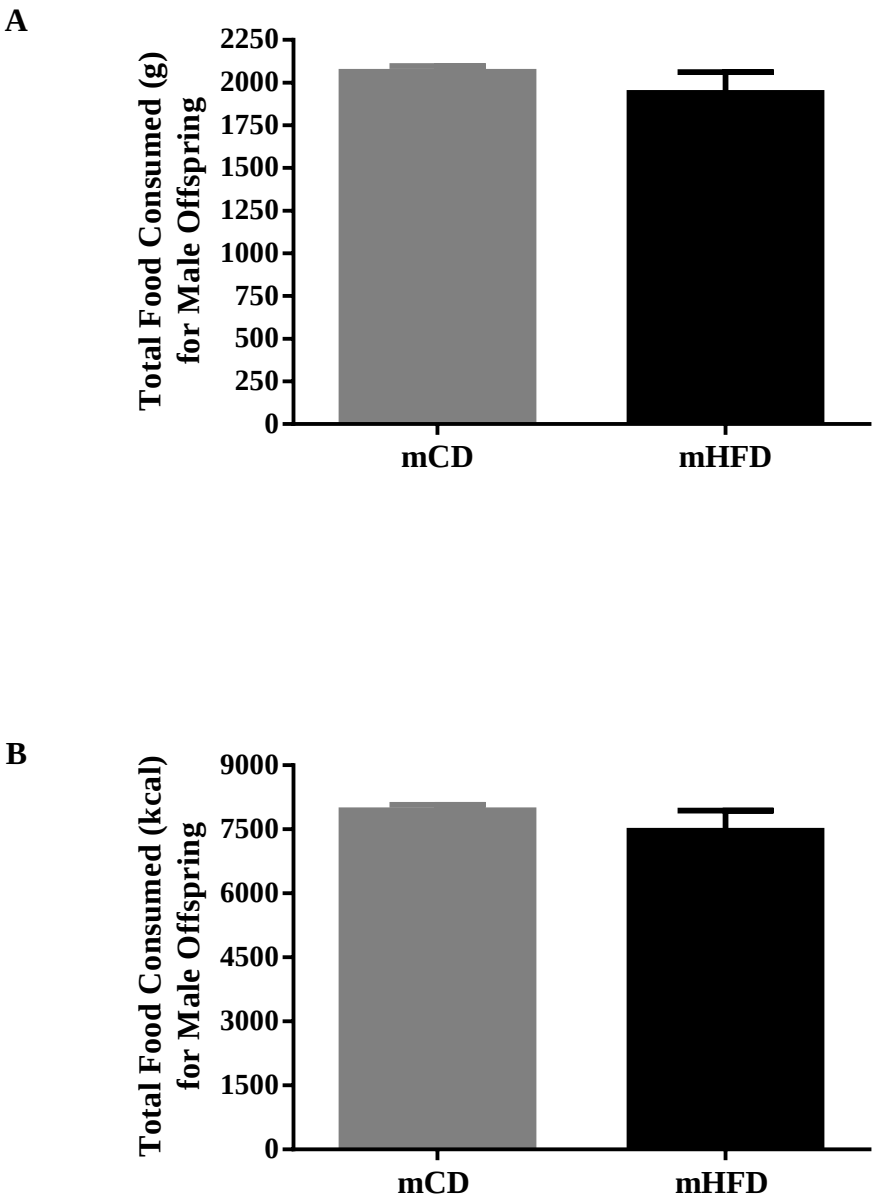


Figure 13. Total food consumption as measured in both grams (A), and energy ingested (B) per cage of three-four animals over a five week period post-weaning (PND 21-56) for male offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

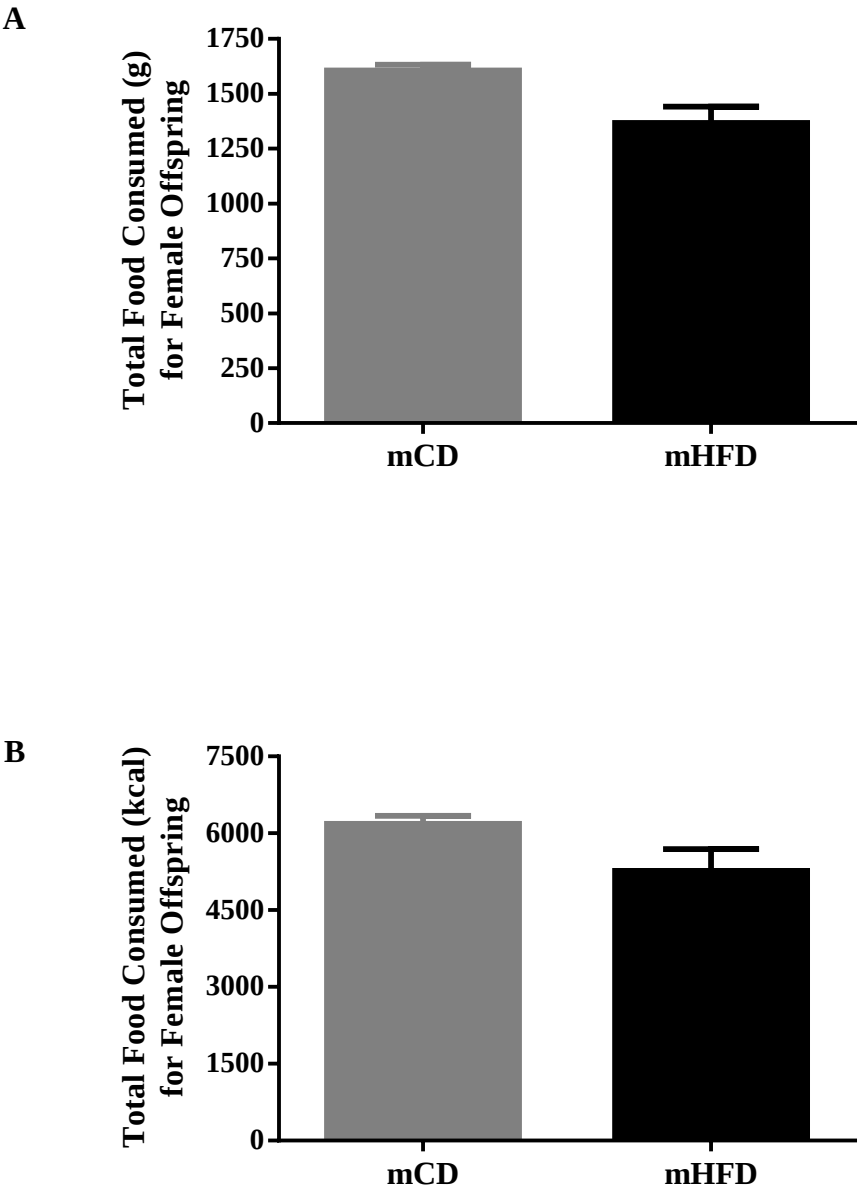
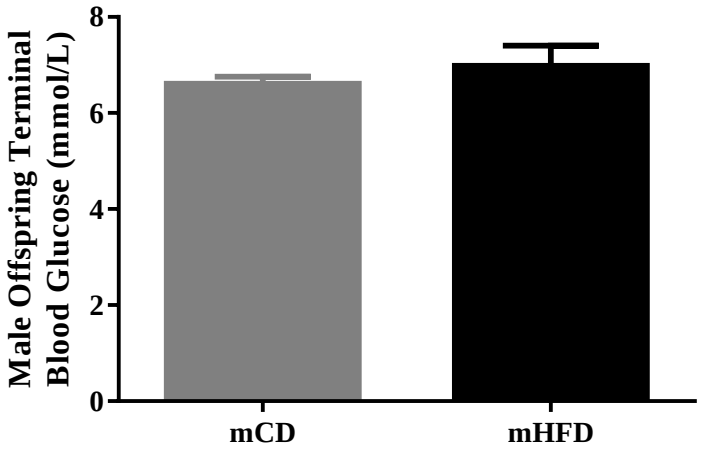


Figure 14. Total food consumption as measured in both grams (A), and energy ingested (B) per cage of three-four animals over a five week period (PND 21-56) post-weaning for female offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

A



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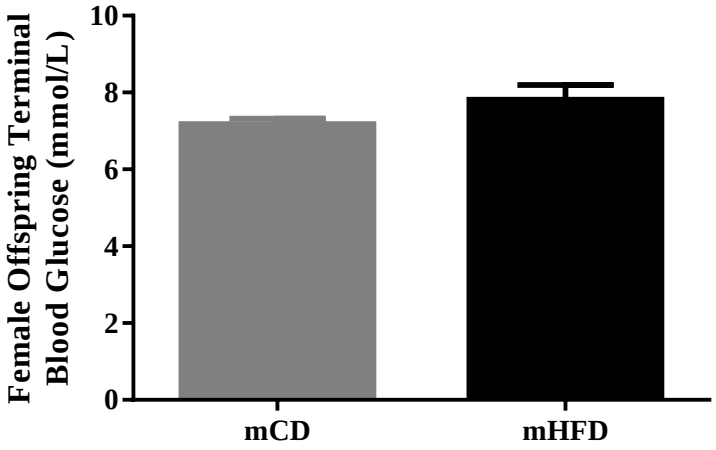


Figure 15. Terminal blood glucose levels as measured at sacrifice using a glucose monitoring system for male (A) and female (B) offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

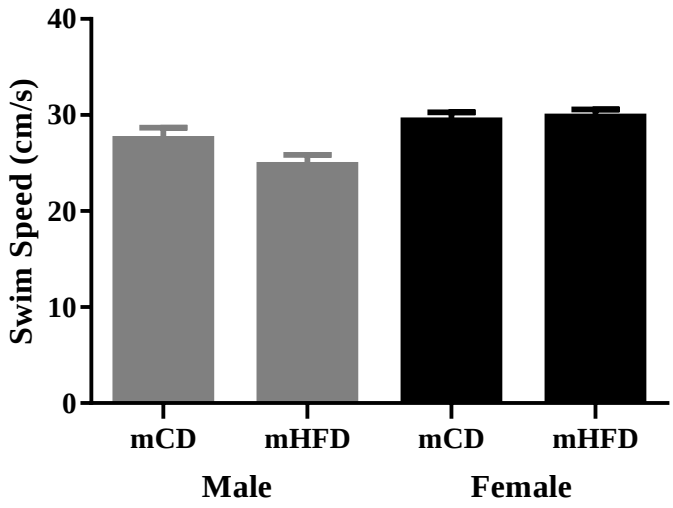


Figure 16. To ensure there were no initial differences in swim speed, or mobility when testing began in adolescence, swim speed (cm/s) was compared for the first training trial. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

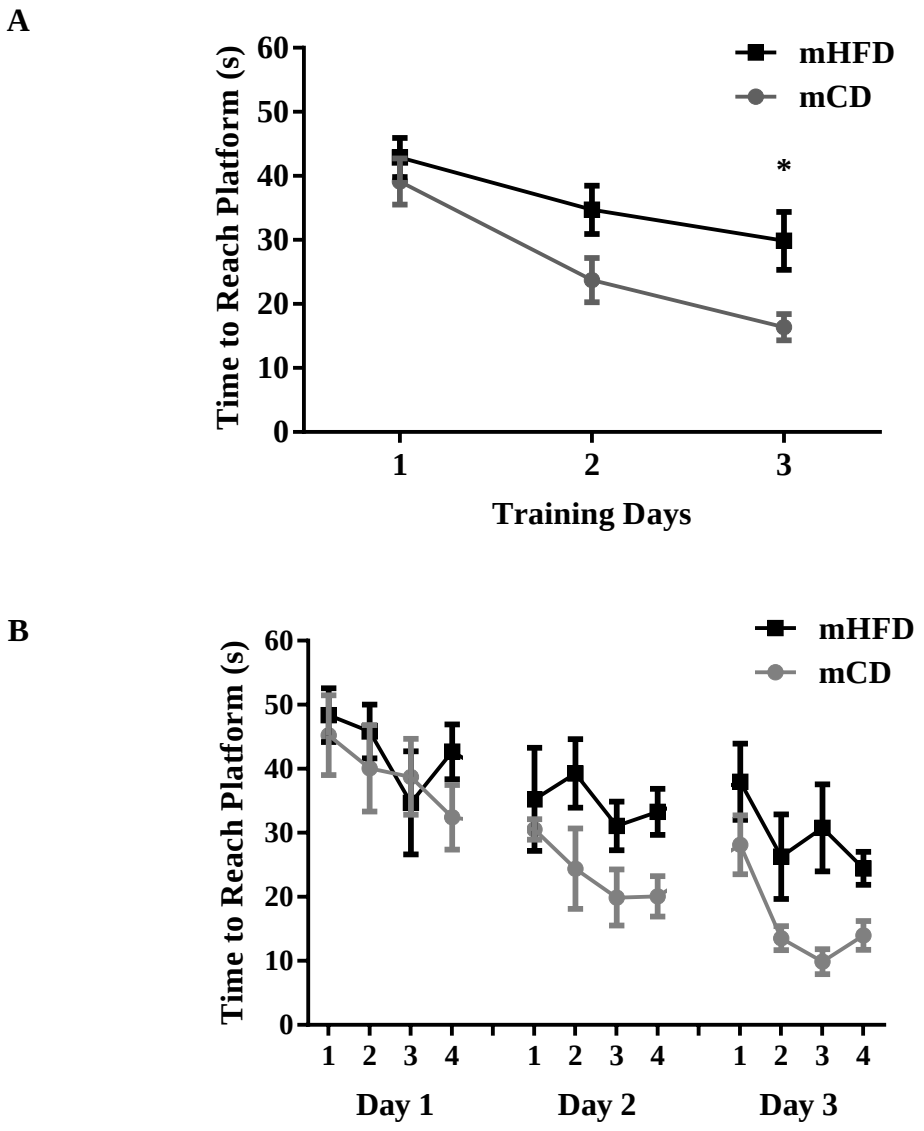


Figure 17. Hippocampal-dependent spatial learning for male offspring in adolescence, as measured by latency to reach platform (in seconds) across three training days in the water maze (A), and within each training day (B). mCD male offspring were faster to learn the platform location than the mHFD male offspring. Data are presented as the mean ± S.E.M. *$p < 0.05$, using a two-way repeated measures ANOVA; $N = 10$ per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

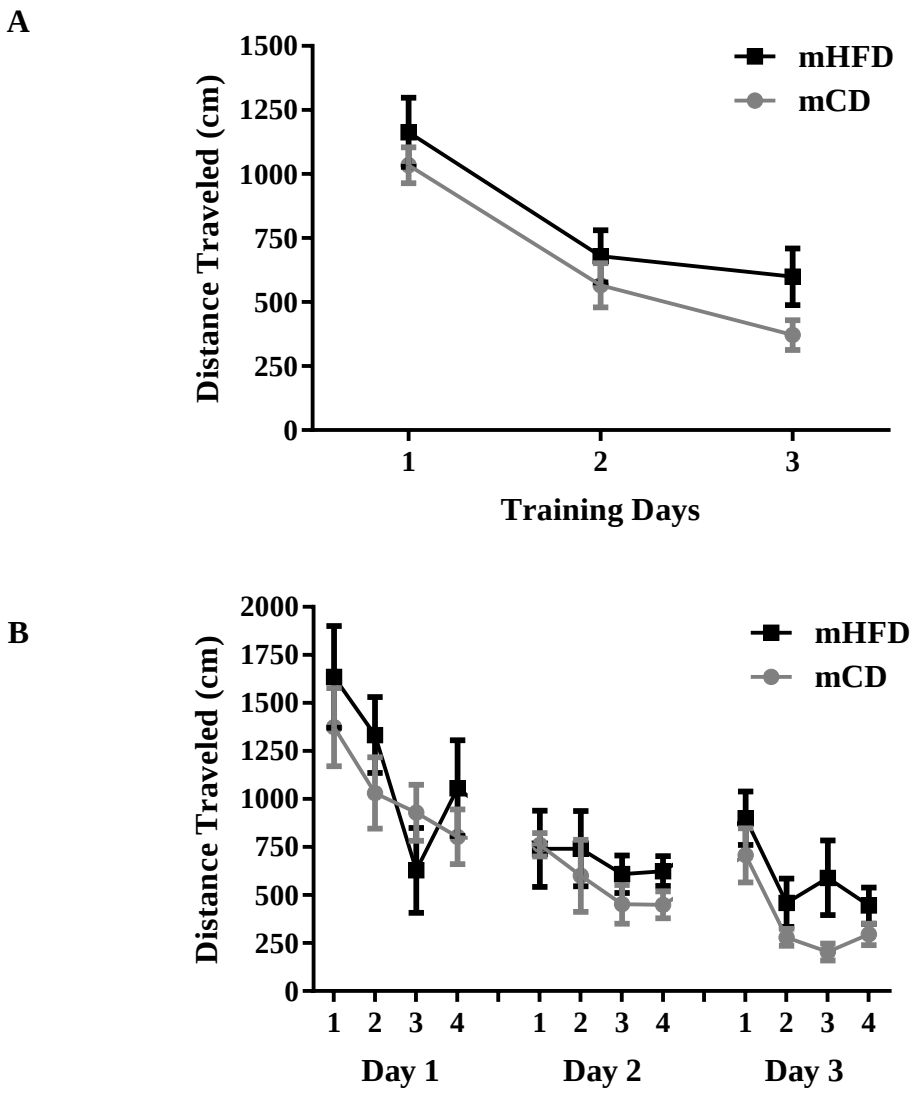


Figure 18. Hippocampal-dependent spatial learning for male offspring in adolescence, as measured by distance traveled to find hidden platform (cm), across three training days in the water maze (A), and within each training day (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

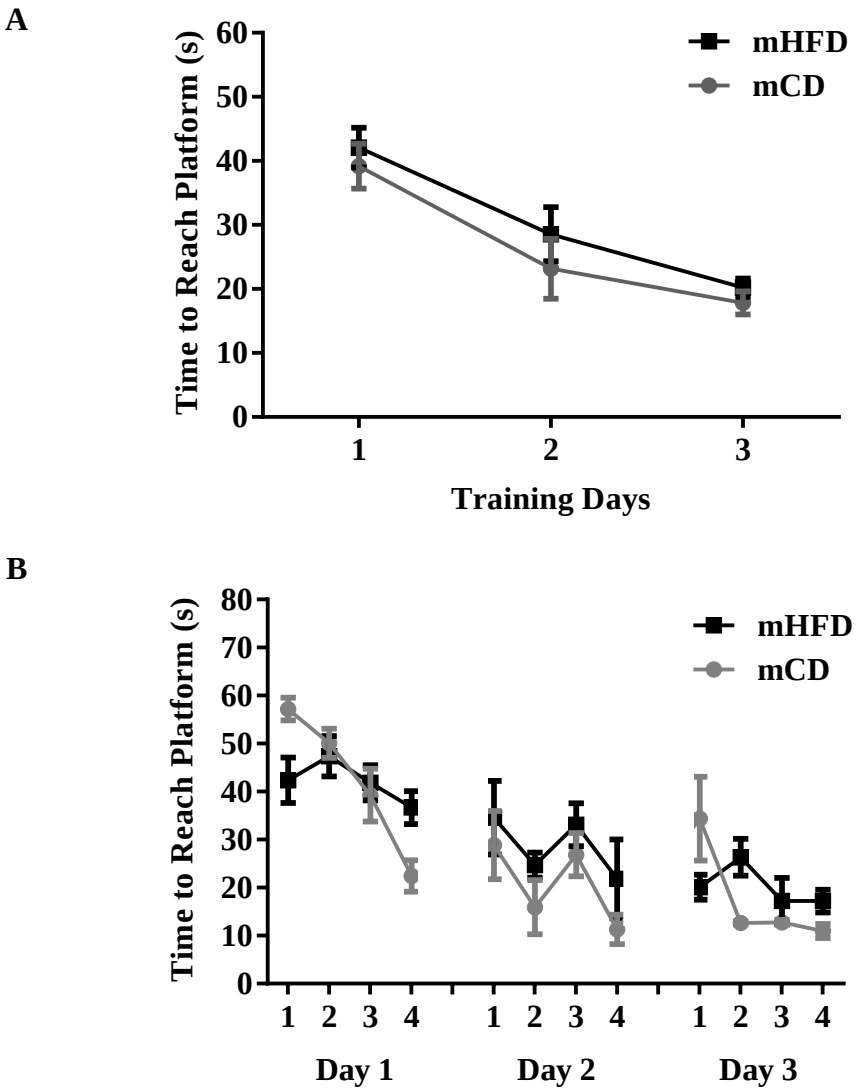


Figure 19. Hippocampal-dependent spatial learning for female offspring in adolescence, as measured by latency to find hidden platform (in seconds), across three training days in the water maze (A), and within each training day (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

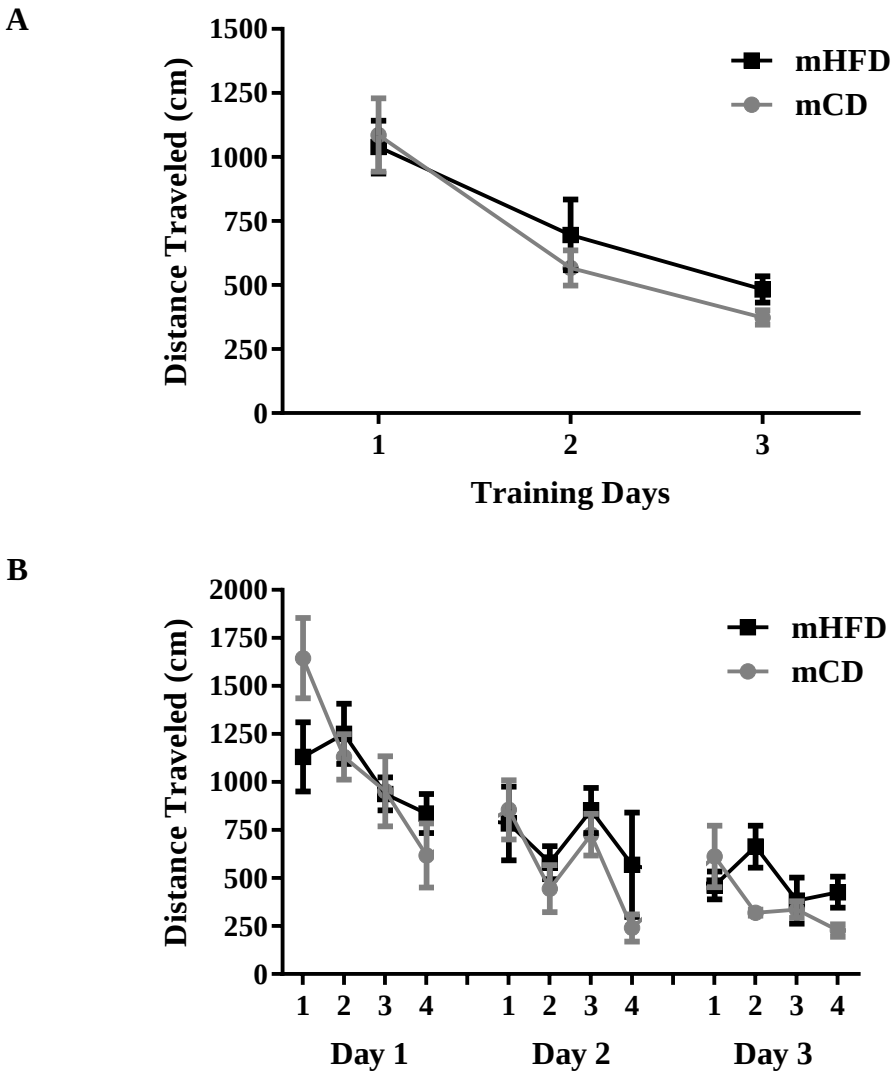


Figure 20. Hippocampal-dependent spatial learning for female offspring in adolescence, as measured by distance traveled to find hidden platform (cm), across three training days in the water maze (A), and within each training day (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

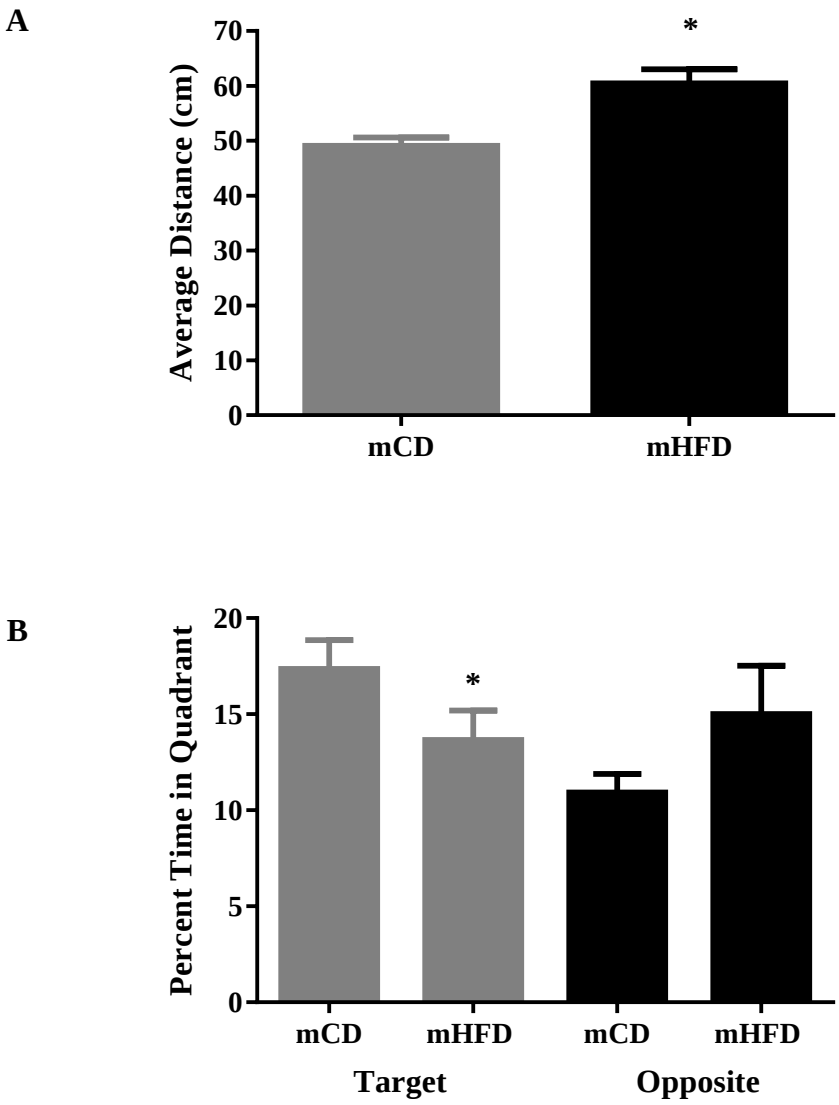


Figure 21. Spatial memory retention as measured by average proximity to previous platform location (A), and percent of time spent in the target and opposite quadrants during the probe task in adolescence for male offspring. Data are presented as the mean ± S.E.M. *$p < 0.05$, using an unpaired Student's *t*-test; $N = 10$ per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

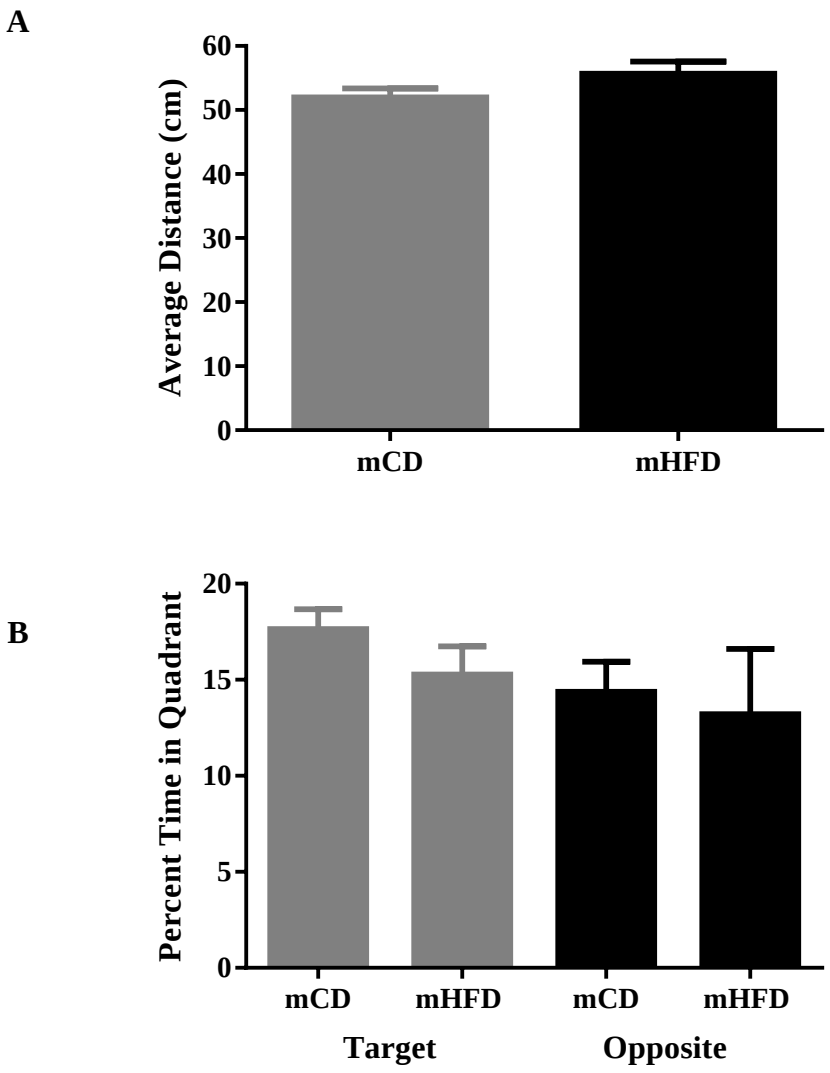


Figure 22. Spatial memory retention as measured by average proximity to previous platform location (A), and percent of time spent in the target and opposite quadrants during the probe task in adolescence for female offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

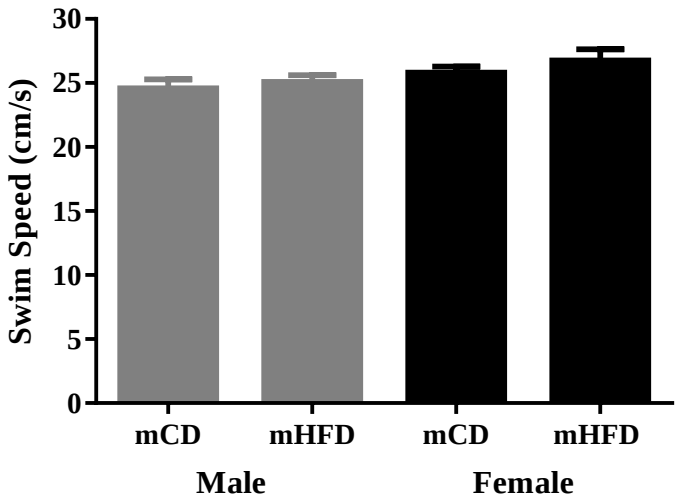


Figure 23. Swim speed (cm/s) was measured for the first trial in adulthood to ensure there were no initial differences in mobility for either male, or female offspring. Data are presented as the mean ± S.E.M. N = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

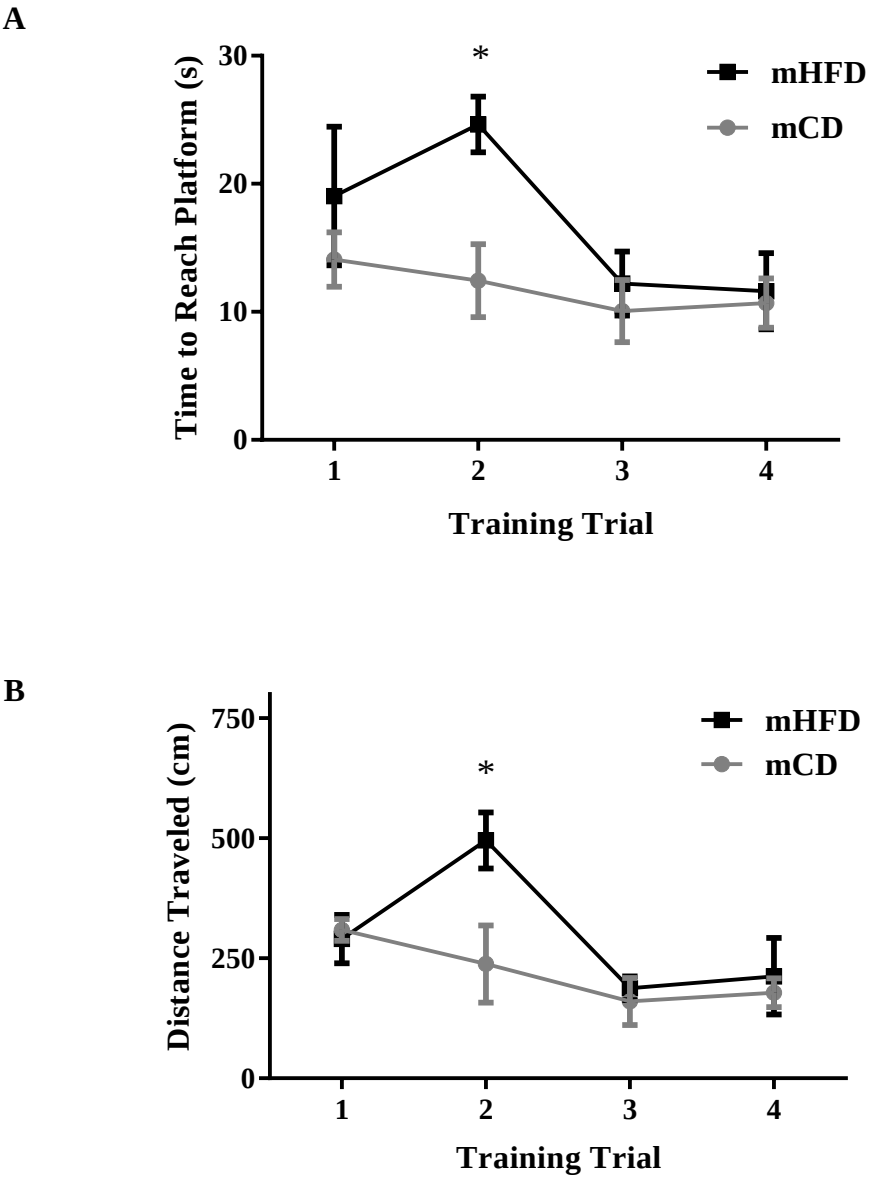


Figure 24. Hippocampal-dependent spatial learning on training day one in adulthood for male offspring, as measured by latency to reach platform (A) and distance traveled (B). Data are presented as the mean ± S.E.M. *$p < 0.05$, using an unpaired Student's *t*-test; $N = 10$ per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

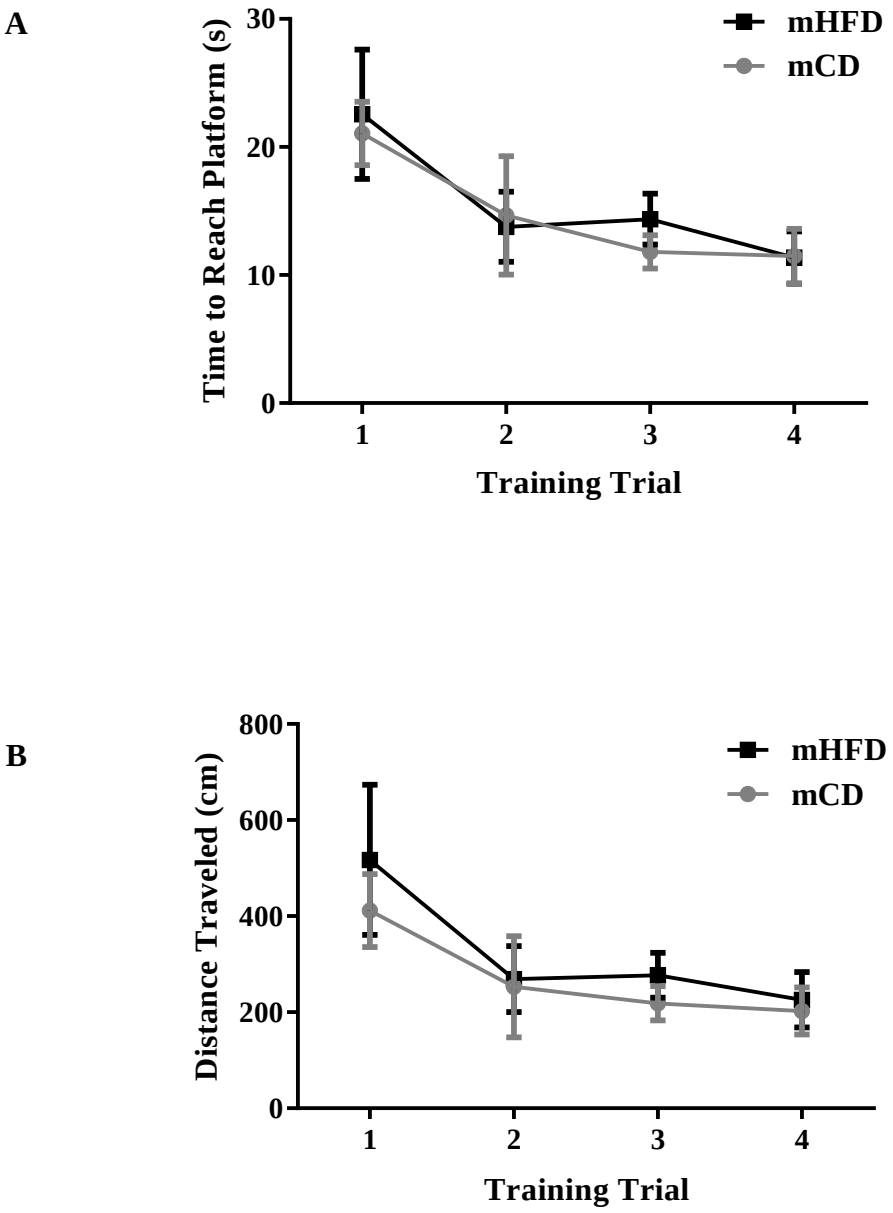


Figure 25. Hippocampal-dependent spatial learning on training day one in adulthood for female offspring, as measured by latency to reach platform (A) and distance traveled (B). Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

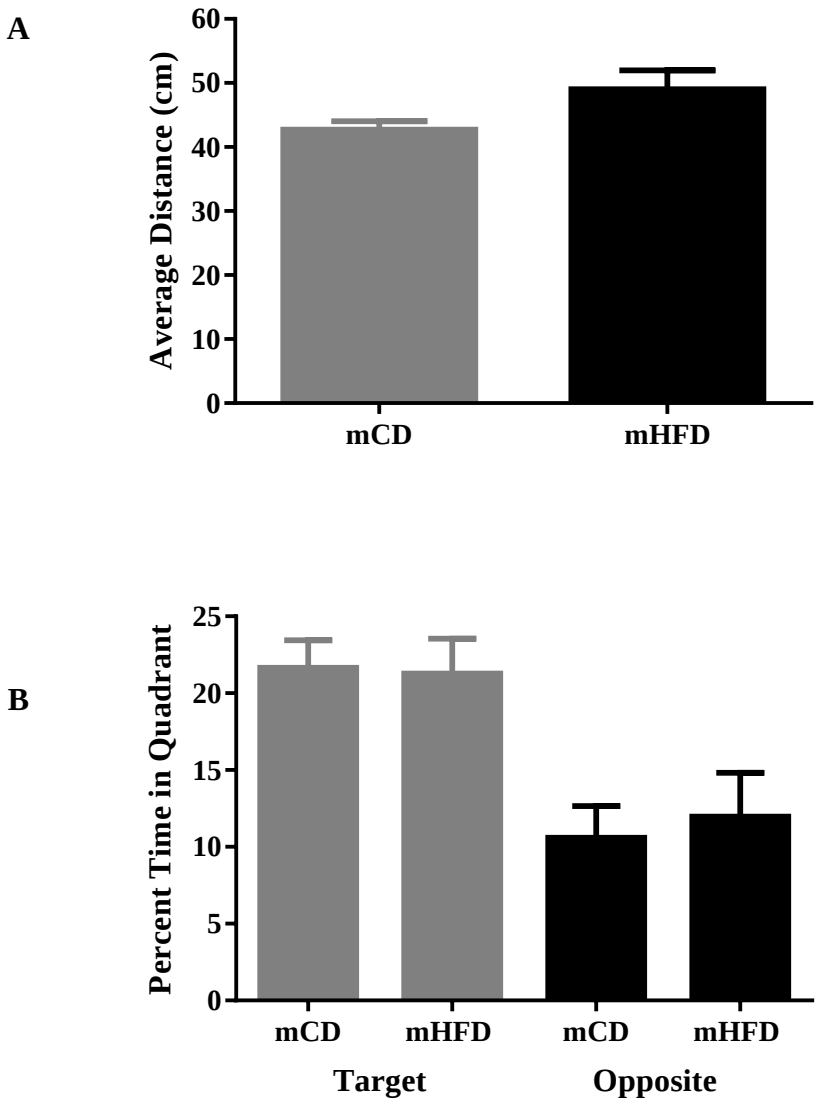
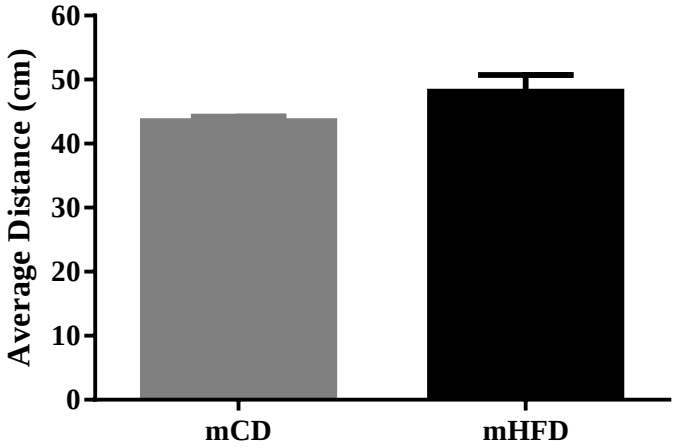


Figure 26. Spatial memory retention as measured by average proximity to previous platform location (A), and percent of time spent in the target and opposite quadrants during the probe task in adulthood for male offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

A



B

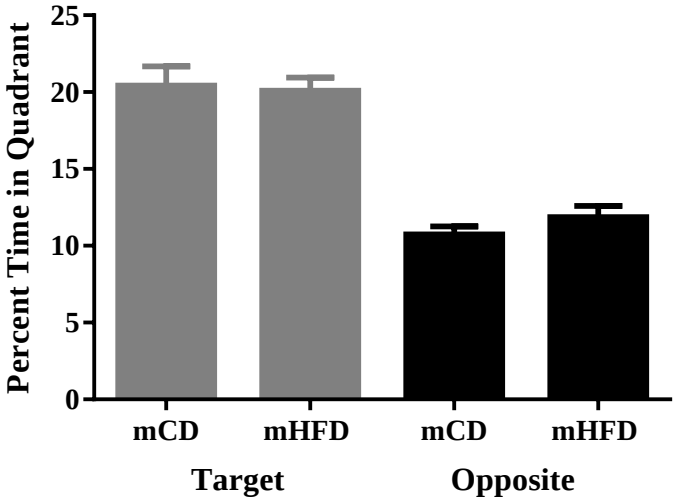
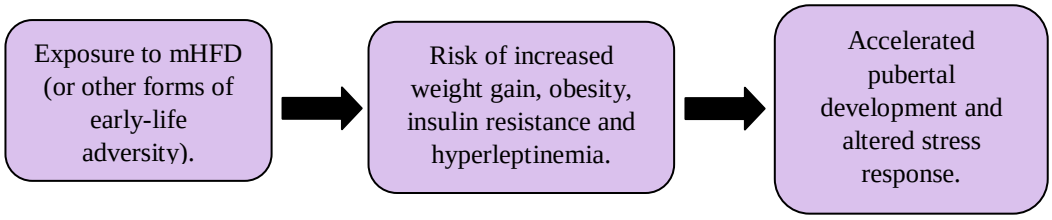


Figure 27. Spatial memory retention as measured by average proximity to previous platform location (A), and percent of time spent in the target and opposite quadrants during the probe task in adulthood for female offspring. Data are presented as the mean ± S.E.M. *N* = 10 per diet condition. mCD = Maternal Control Diet, mHFD = Maternal High-fat Diet.

Maternal obesity influences offspring development:



Possible reasons pubescent female animals may be resilient to the developmental and cognitive effects of a high-fat diet compared to male offspring:

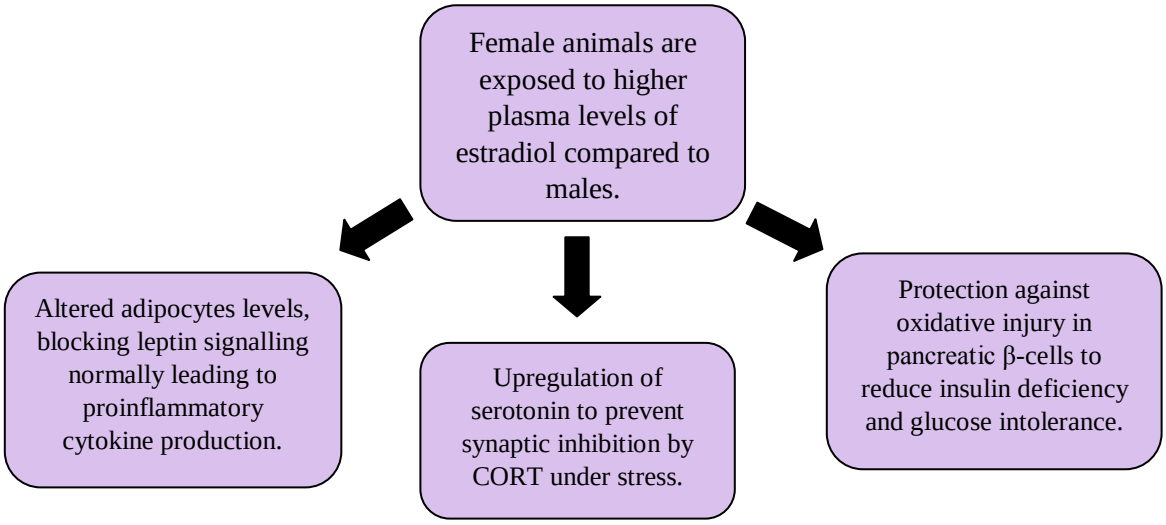


Figure 28. A summary schematic outlining the possible reasons for a sex-stratified effect of a maternal high-fat diet on metabolic and cognitive development in offspring. mHFD = Maternal High-fat Diet, CORT = corticosterone.