What are the Effects of Maternal Obesity on Synaptic Function in the Maternal and Offspring Hippocampus?

by

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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

Obesity is a global epidemic that is associated with several adverse health consequences. In addition, there is also a growing prevalence of obesity in pregnancy. Maternal obesity places the fetus in an abnormal *in utero* condition that can produce alterations in development leading to permanent programming of physiological systems. Obesity is also associated with cognitive dysfunction, which calls for investigations into its effects on the hippocampus, a brain area involved in learning and memory. Long-term potentiation (LTP), a neurophysiological correlate for learning and memory, can be examined in hippocampal slices. This study aimed to fill in the gap in literature regarding the effect of obesity on hippocampal synaptic plasticity in female rats, and maternal obesity effects on offspring hippocampal synaptic plasticity. Female Sprague-Dawley rats were fed either a control diet (CD), or a high-fat diet (HFD; 40% of calories from saturated fat) for 16 weeks. Impaired glucose tolerance and greater retroperitoneal fat pad weight indicated an obese phenotype in HFD rats; as well, the modified diet led to impaired LTP: CD rats had 10% more potentiation in amplitude, and 11% more potentiation in slope than HFD rats. Offspring were weaned onto control diet at post-natal day 21. Reduced success rates for achieving LTP, and lowered magnitudes of mean LTP in the offspring, strongly suggest that maternal obesity may have compromised hippocampal synaptic plasticity, and warrants further study.

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List of Abbreviations

ACE	=	angiotensin converting enzyme
ACSF	=	artificial cerebrospinal fluid
AF	=	atrial fibrillation
AMPA	=	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
ARC	=	arcuate nucleus
BMI	=	body mass index
CA1	=	Cornu Ammonis 1
CA3	=	Cornu Ammonis 3
CD	=	control-diet
CHD	=	coronary heart disease
CRP	=	C-reactive protein
DG	=	dentate gyrus
DIO	=	diet-induced obesity
DOHaD	=	developmental origins of health and disease
EC	=	entorhinal cortex
fEPSP	=	field excitatory post-synaptic potential
GDM	=	gestational diabetes mellitus
HFD	=	high-fat diet
IL-6	=	interleukin 6
IUGR	=	intrauterine growth retardation
LTP	=	long-term potentiation
MCP-1	=	monocyte chemoattractant protein 1
NMDA	=	*N*-methyl-D-aspartate
OGTT	=	oral glucose tolerance test
PND	=	post-natal day
POMC	=	pro-opiomelanocortin
PVN	=	paraventricular nucleus
RAAS	=	renin angiotensin aldosterone system
T2DM	=	type 2 diabetes mellitus
TNF-α	=	tumor necrosis factor α
VGCC	=	voltage-gated calcium channels
WC	=	waist circumference
WHO	=	World Health Organization
WHR	=	waist-to-hip ratio

1.0 Introduction

Obesity, the excess accumulation of adipose tissue, is becoming a great concern (WHO 2003). With a steady increase in prevalence that extends across the world, the World Health Organization refers to obesity as a "global epidemic" (WHO 2003). Obese individuals have a poorer quality of life and shorter life expectancies (Fontaine 2003). As well, obesity contributes to significantly increased mortality risk, with a number of co-morbidities such as heart disease, diabetes mellitus type 2, osteoarthritis, hypertension, coronary artery disease, obstructive sleep apnea, and many forms of cancer (Lopez-Jimenez 2010, Kulie et al 2011, Mamun et al 2011).

In parallel with the trend of obesity, there has also been a steady increase in the prevalence of obese women of reproductive age (Kulie et al 2011). Evidence has also accumulated regarding the large prevalence of obesity in pregnancy. Importantly, studies have reported that pregnant women who are overweight or obese have an increased risk of gestational diabetes, hypertensive disorders (including pre-eclampsia), thromboembolic events, respiratory complications, prolonged delivery, congenital anomalies (such as spina bifida and omphalocele), macrosomia, and higher rates of caesarean sections (Dixit 2008, Yogev 2009, Kulie et al 2011, Mamun et al., 2011, Afifi 2011). In addition, maternal obesity may have further long-term consequences for the fetus (Ramachenderan et al., 2008).

There are critical periods during the prenatal and early postnatal developmental stages that determine the future health of the fetus (Solomons 2009). During these critical periods the fetus is highly influenced by the maternal environment, and important physiological changes in fetal development can occur (Vickers 2011). There is a general developmental programming thesis that states there may be adaptations in the fetus from sensing changes in the maternal environment, which lead the fetus to be adaptively programmed to respond to what would be

expected in postnatal life (Solomons 2009). A fundamental part of the *in utero* environment is maternal nutrition (Redmer 2004). Many studies have been devoted to investigating the effects of under-nutrition during gestation. However, with the escalating prevalence of obesity and obesity in pregnancy, there is a rising interest in the potentially harmful programming effects of over-nutrition during gestation. Extensive research has provided evidence that maternal obesity increases the risk that offspring may become obese and develop other components of the metabolic syndrome, like diabetes and insulin resistance (Vickers 2011). Not surprisingly, maternal obesity also appears to have programming effects on the brain (Undurti 2010).

There have been reports that maternal obesity is associated with offspring memory and cognitive function (Scholtz 2009). Since the hippocampus thought to play an important role in learning and memory (Kanoski 2011), focus has been placed on this structure. Indeed, further studies have shown offspring of obese dams to have alterations in their hippocampal organization and neurogenesis (Tozuka et al 2009). However, these structural changes do not necessarily denote a functional change, and the effect of maternal obesity on offspring hippocampal function has not been explored.

2.0 Literature Review

2.1 Obesity

2.1.1 Definition

Obesity is often defined as simply a condition of adipose tissue accumulation to the extent that health may be impaired (Saravanakumar 2006). Diagnosis of obesity is indirectly measured via body mass index (BMI). Also known as Quetelet's Index, BMI is calculated as weight divided by height squared (kg/m^2) (WHO 1995). BMI acts as a surrogate marker for adiposity since it is based only on weight and height, and body composition is not taken into account. Although BMI calculation is argued to be closely correlated to the actual amounts of fatty tissue otherwise derived from more complex methods (Maennig et al 2008). On the contrary, BMI as a measure for body fat is sometimes thought to be inadequate because it fails to differentiate fat and nonfat mass like bone and muscle (Rothman 2008). Obesity as a risk factor predominantly concerns abdominal obesity, which can be attributable to the visceral adipose tissue, where better measures of its accumulation can be calculated with waist circumference (WC) and waist-to-hip ratio (WHR) (de Koning 2007). However, WHR can theoretically be the same between a non-obese and an obese individual (Caan 1994). Magnetic resonance imaging and computed tomography are the most accurate anthropometric measurements for assessing abdominal fat, but are impractical for routine clinical use (NIH 1998). In addition, there are difficulties in obtaining accurate and consistent WC and WHR measures (de Koning 2007), making BMI a better measure to be globally used to provide internationally comparable results. The classification according to BMI places a BMI of $\geq$25 as overweight, and a BMI of $\geq$30 as obese.

2.1.2 Epidemiology

Worldwide obesity has more than doubled since the 1980s, and it is currently the most common metabolic disease (Yu 2008, Kulie et al 2011). The alarming increase in obesity prevalence worldwide has led the World Health Organization to consider it one of the most serious global health problems of the 21st century (Gunstar et al 2008). The WHO estimated that in 2008, 1.5 billion adults were overweight – over 500 million of which were obese (Kulie et al 2011). The overwhelming increase in obesity prevalence is apparent through statistics gathered from several parts of the world (Gaziano 2010). The prevalence of obesity has been steadily rising in developing countries, and began to affect many impoverished nations during the last half century (Popkin 1998). According to the WHO Global Database on BMI, the percentage of obese adults rose from 7.3% in 1985 to 23.1% in 2000 (WHO 2003). In the period from 1995 to 2000, the number of obese people rose by 50% (Maennig 2008). In some areas, such as parts of North America, Eastern Europe, the United Kingdom, the Middle East, and China, obesity rates have risen three-fold, or more, in the last 100 years (WHO 2003). An estimated 1 in 5 adults in China are obese or overweight (Wu 2006).

The relationship between BMI and mortality was considered to be a U-shaped or J-shaped curve (WHO 2005). After adjusting for confounders like pre-existing disease, smoking, and stable weight maintenance, the association is more linear, with increased mortality at high BMI (>29-30) (Adami 2008). The shape highlights the complex association of mortality at BMI extremes. In Western Europe, obesity accounts for an estimated 200,000 deaths each year (Maennig 2008). In the United States, an estimated 300,000 people die annually as a consequence of obesity (Flegal 2005). South Asian countries are facing a rapid increase in obesity-related non-communicable disease (Misra 2011). The most recognizable diseases – type

2 diabetes mellitus (T2DM), hypertension, coronary heart disease (CHD), and dyslipidemia – can arise from physiological changes as a result of obesity.

2.1.3 Physiological changes leading to disease

A. Respiratory changes

Obese individuals have increased oxygen consumption and carbon dioxide production. Obesity has a direct effect on respiratory well-being because it increases the mechanical work needed for breathing. The increased amount of adipose tissue around the rib cage and in the visceral cavity shifts the inflationary and deflationary pressures, resulting in a reduction in total lung capacity and under-ventilation of lower zones of the lungs (Salome 2009). Increasing BMI is also associated with a reduction in forced expiratory volume, forced vital capacity, functional residual capacity, and expiratory reserve volume (Poulain 2006).

Even with no previous respiratory illness, obese people are at an increased risk of respiratory system related difficulties, such as breathlessness and bronchoconstriction. The reduced maximal inspiratory pressure seen in obese subjects compared to subjects with normal body weight indicates that respiratory muscle strength is compromised in obesity (Poulain 2006). Other concerns regarding the mechanical effects of obesity involve possible contributions to airway dysfunction that potentially could induce or worsen asthma (Salome 2009). Obese individuals have reduced tidal lung expansion, which compromises dilating forces in airway maintenance, leading to increased airway responsiveness via greater contractile responses of airway smooth muscle (Poulain 2006).

The increased airway closure seen in obese individuals leads to increased gas trapping and unequal ventilation distribution, potentially leading to mild hypoxemia, dyspnea, and chronic obstructive pulmonary disease (Salome 2009). According to Sin and colleagues (2002), there is a

clear association between dyspnea and obesity. Obesity stiffens the respiratory system due to a combination of effects on the lung and chest wall compliance, which may be a result of mechanical effects of fat on the diaphragm, increased pulmonary blood volume, closure of dependent airways, or increased alveolar surface tensions (Poulain 2006, Salome 2009). Reductions in chest wall compliance and respiratory muscle strength create an imbalance between the demand on respiratory muscles and their capacity to generate tension, forming the perception of increased breathing effort (Poulain 2006).

Obesity is a well-recognized risk factor for obstructive sleep apnea. Approximately 70% of people with obstructive sleep apnea are obese; about 40% of obese individuals have obstructive sleep apnea (Resta 2001). Obstructive sleep apnea is characterized by intermittent upper airway obstruction - airways are predisposed to repetitive closures during sleep (Poulain 2006). With obesity, there is increased adipose tissue deposition in the pharyngeal musculature, and, in combination with reduced total lung capacity, upper airway competence is reduced and the risk for collapsed lungs is increased (Poulain 2006).

The obesity hypoventilation syndrome has similar symptoms to obstructive sleep apnea, however it also involves daytime hypercapnia that is accompanied by compensated respiratory acidosis and hypoxemia (Olsen 2005). Prevalence of obesity hypoventilation syndrome is unknown, but a recent study on hospitalized obese patients (BMI $\geq$35) found 31% to have daytime hypercapnia unexplained from other disorders (Nowbar 2004). Although most patients with obesity hypoventilation syndrome have obstructive sleep apnea, some patients do not, suggesting that obesity alone can lead to chronic hypoventilation (Poulain 2006). Increased mechanical load on the respiratory system resulting in respiratory muscle fatigue is suspected to contribute to the pathogenesis of obesity hypoventilation syndrome (Poulain 2006).

B Cardiovascular changes

The association between obesity and different forms of cardiovascular disease (CVD) is well recognized. A prospective study of over a million US adults followed for 14 years reported a strong association between obesity and an increased risk of all-cause and cardiovascular mortality (Calle 1999). The study also associated CHD mortality risk with increasing BMI, stating a twofold to threefold greater risk in individuals with BMI ≥35. Indeed, more than two-thirds of patients with CHD are overweight or obese, and being obese doubles the risk for heart failure compared to those with normal BMI of <25 (Lopez-Jimenez 2011). A meta-analysis that involved more than 258,000 subjects reported a progressive increase in CVD risk with increasing waist circumference and waist-to-hip ratios, stating that every 1 cm increase in waist circumference was associated with a 2% increased relative risk of a cardiovascular event (DeKoning 2007). The mechanisms through which obesity increases risks of CVD are characterized by physiological changes related to the cardiovascular system.

Increased body fat content and BMI has been associated with endothelial dysfunction, which induces chemotaxis of adhesion molecules, differentiation of monocytes into macrophages, platelet aggregation, and decreased nitric oxide bioavailability that promotes thrombosis (Lopez-Jimenez 2011). Obesity is also positively associated with elevated C-reactive protein (CRP); CRP is associated with increased risk for cerebrovascular disease, peripheral arterial disease, myocardial infarction, and CHD death (Kuller 1996). Although still unclear, it is speculated that obesity leads to elevated CRP through increased cytokine levels found in adipose tissue that stimulates CRP production in the liver (Lopez-Jimenez 2011).

Structural changes of the heart have been observed in obese individuals. Obesity is associated with several compensatory cardiovascular alterations mostly as a result of increased

demands from an excessive body mass and hyperdynamic circulation. Obesity is associated with increased myocardial fibrosis (seen to develop through fat infiltration into the myocardium) and left ventricular hypertrophy (Zalesin 2008, Lopez-Jimenez 2011). The pathologic change of left ventricular hypertrophy is suggested to stem from increases in blood volume, cardiac output, stroke volume and filling pressures in obese individuals (Zalesin 2008). Obesity is also associated with both diastolic and systolic heart failure (Kenchaiah 2002). The pathologic changes previously mentioned may culminate with impairments in diastolic relaxation and induce diastolic dysfunction (Zalesin 2008). In age-adjusted regression models, an increase in BMI of 1.25 in women and 1.70 in men was associated with a 1 mm Hg increase in systolic blood pressure (Engeli 2002).

When excessive body fat accumulates, visceral storage sites fill to capacity, resulting in the release of triglycerides and free fatty acids into circulation that then accumulate within the myocardium; the accumulation of lipids in the muscle and vasculature tends to increase with the extent of adiposity (Malavazos 2007). Furthermore, there is also an abnormal aggregation of apicardial and pericardial adipose tissue (Zalesin 2008). These accumulated stores of fat secrete hormones, cytokines, and proteins that expose the myocardium to inflammation and may intensify the progression of atherosclerosis (Iacobellis 2005). There is also lipid metabolism by-product accumulation in the myocardium, leading to lipotoxicity and activates signaling cascades that induce cell death and contribute to left ventricular remodeling and diastolic dysfunction (McGravrock 2006). The association between obesity and CHD is partially mediated by accelerated coronary atherosclerosis, which obesity potentiates via increased fat stores, as well as intravascular volume and vascular wall stress (Lopez-Jimenez 2011). The prothrombotic state

that obese individuals are in also contributes to the onset of acute coronary events (Scarabin 1996).

Various studies suggest that obesity favors the appearance of ventricular arrhythmias and atrial fibrillation (AF). Obese individuals have increased electrical irritability that may trigger the onset of ventricular arrhythmias, evidenced through electrophysiological studies (Lopez-Jimenez 2011). A 2008 meta-analysis showed that obese individuals have a 50% higher risk of AF, and the risk continues to increase with increasing BMI (Wanahita 2008). Evidence has emerged that left atrial diameter enlargement is the strongest echocardiographic predictor of AF and has been directly correlated with increasing BMI levels (Wang 2004). Atrial enlargement may also be in part due to the influence of lipotoxicity (McGrarock 2006).

C Endocrine changes

Adipose tissue is a powerful endocrine organ. Adipose tissue is predominantly adipocytes surrounded by highly innervated and vascularized loose connective tissue (Ahima 2006). Consequently, obese individuals experience notable endocrine changes compared to normal weight individuals. For example, the increased fat mass in obesity puts individuals in an inflammatory state. Adipose tissue endothelium in obesity is populated by inflammatory cells that include increased activated macrophages (leading to increases in tumor necrosis factor-α TNF-α, interleukin-6 IL-6, and other cytokines) and monocytes (due to increased monocyte chemoattractant protein 1, MCP-1, and other chemokines) (Ahima 2006). The factors secreted by adipose tissue are collectively referred to as adipokines (Dahlman 2007).

The best characterized obesity hormone is leptin, which is found in the blood circulation in proportion to fat mass, and acts primarily in the central nervous system to inhibit food intake and promote energy expenditure, but has additional peripheral effects as well (Dahlman 2007,

Bouret 2010). Obesity is associated with increased leptin production and plasma leptin concentration, and leads to leptin insensitivity (Constidine 1996). Leptin crosses the blood brain barrier, controlling specific neuronal groups to increase anorectic peptides, stimulate thermogenesis, and reduce intracellular lipid levels in skeletal muscle, liver, and pancreatic β cells (Ahima 2006, Konstantinos 2009).

Adipose tissue, particularly mature adipocytes, secretes high levels of amylin (Bigal 2007). As a potent, long-lasting vasoactive peptide, amylin is a speculated mechanism in the association of obesity and chronic migraines via its suggested pro-nociceptive function in primary sensory neurons (Bigal 2007).

Adiponectin is an important adipokine that is exclusively expressed in differentiated adipocytes and has endocrine effects in the liver, muscle, and vasculature (Bigal 2007). Adiponectin suppresses hepatic gluconeogenesis, glucose uptake in skeletal muscle, and fatty acid oxidation (Konstantinos 2009). Adiponectin is reduced in obesity (Kadowaki 2005), and adiponectin receptors are downregulated in obese individuals (Dahlman 2007). Adiponectin increases insulin sensitivity, hence, there is insulin resistance seen in obese individuals (Undurti 2010). Enhanced adipose tissue expression of proinflammatory mediators (TNF-α, IL-6, MCP-1) from obesity may also contribute to insulin resistance (Dahlman 2007, Konstantinos 2009).

Insulin regulates the uptake, oxidation, and storage of fuel (Kahn 2000). Insulin resistance is a condition in which higher than normal insulin concentrations are required to achieve a normal metabolic response (Kahn 2000). Obesity is associated with elevated basal plasma insulin levels and resistance to the metabolic effects of insulin (Lichtenstein 2000). Insulin resistance is an important predictor of T2DM and CVD (Konstantinos 2009). Various studies show strong associations between obesity and insulin resistance. Obesity is associated

with impaired glucose tolerance and insensitivity to the blood glucose lowering effect of insulin (Lichtenstein 2000). Differential fat distribution also affects insulin sensitivity. The increased amount of visceral adipose tissue, often predominant in obese individuals, is composed of large adipoctyes that are more metabolically active than cells of subcutaneous adipose tissue. The result is increased secretion of adipokines, as well as less sensitivity to the anti-lipolytic effects of insulin (Konstantinos 2009).

Insulin sensitivity is strongly associated with hepatocellular lipid content (Stefan 2008). Non-alcoholic fatty liver disease is present in about 30% of the general US population, and up to 75% of obese Americans (Konstantinos 2009). Fat accumulation in the liver impairs hepatic insulin signaling, which increases hepatic gluconeogenesis that is normally suppressed by insulin (Konstantinos 2009). Fatty liver also secretes more fetuin-A, a factor that inhibits insulin receptors in the liver as well as skeletal muscles, thus contributing to insulin resistance (Konstantinos 2009).

Another endocrine change observed in obese individuals involves the renin angiotensin aldosterone system (RAAS). The association of obesity with hypertension is related to changes in RAAS. A study of 449 obese individuals from Jamaica showed significantly higher levels of serum angiotensin converting enzyme (ACE) and circulating angiotensinogen (Cooper 1997). A study by Engeli and colleagues (2005) reported that obese women had higher circulating angiotensinogen, renin, aldosterone, and ACE levels than non-obese women. Subsequent weight reduction by 5% reduced plasma angiotensinogen by 27%, renin by 43%, aldosterone by 31%, and ACE activity by 12%. Obese individuals are also seen with higher blood pressures since aldosterone increases blood pressure (acting on mineralcorticoid and glucocorticoid receptors in brain, heart, kidney, and vasculature) (Rahmouni 2005).

D Renal System

Obesity can cause structural and functional changes in the renal system. Most obese subjects exhibit the same pattern of glomerular hemodynamics as patients with reduced renal mass – preglomerular vasodilation, increased glomerular filtration rate and filtration fraction (Praga 2010).

Obesity increases tubular sodium reabsorption and shifts pressure natriuresis toward higher blood pressures. The underlying mechanism is speculated to be the accumulation of adipose tissue around the kidney that results in medullary compression (Kurukulasuriya 2008). The association of obesity with afferent renal artery vasodilation and increased glomerular filtration rate is considered compensatory for maintaining sodium balance and help overcome the increased tubular sodium reabsorption (Kurukulasuriya 2008). Unfortunately, obese patients experiencing chronic renal vasodilation also see increased hydrostatic pressures and wall stress in the glomerularus, increasing the risk for glomerulosclerosis and loss of nephron function (Kurukulasuriya 2008). In addition to hyperfiltration, proteinuria and secondary glomerulosclerosis are now recognized as complications of severe obesity (Praga 2010). Mechanisms of renal damage among obese individuals develop from hemodynamic, metabolic, and inflammatory disorders that are side effects of obesity (Stolic 2010). Consequently, obesity is an increasingly frequent cause of end-stage renal disease (Hall 1998).

E Brain-related changes

Although there is a large array of studies regarding obesity and its effects on various organ systems, the effects of obesity on the central nervous system are not understood nearly as well. High BMI in middle age is reported to be associated with higher dementia risk (Kivipelto 2005). Whitmer and colleagues (2005) found that obese people had a 74% greater risk of

dementia compared to normal weight individuals at mid-life. Although some studies that do explore this relationship have reported obesity in the elderly to be related to greater dementia risk, the findings are conflicting as a result of incorrect BMI measurements. Taking this into account, Fitzpatrick and associates (2009) also found higher BMI at ages 70, 75, and 79 years predicted dementia (Gustafson 2003). Independent of age, there are reports for gross reductions in brain volume (Gunstad 2008), and prefrontal structural abnormalities (Pannacciulli 2006) and baseline metabolic activity (Volkow 2009) in obese individuals. Neurocognitive functioning tests, further supported by evidence from neuroimaging studies, have shown frontal-subcortical dysfunction with diminished executive functioning, and reductions in complex attention and speed processing in obese men and women (Boeka 2009, Fergenbaum 2009).

As previously mentioned, obesity leads to altered circulating leptin levels. Studies have shown that diet-induced obese (DIO) rats show reduced leptin sensitivity even before they develop obesity, and this reduction in leptin sensitivity alters the architecture of the hypothalamic neurocircuitry, such as the inability for leptin to promote neurite outgrowth from neurons in the arcuate nucleus (Bouret 2010). This is important since the anorectic effects of leptin are largely a result of projections of the arcuate nucleus to the paraventricular nucleus in the hypothalamus. Other DIO rat studies have shown abnormal dendrite morphology in the hypothalamus, but have not concluded these structural changes to be a result of reduced leptin sensitivity (Bouret 2010).

Most studies have focused on gray matter, and not disruptions to white matter. Since white matter pathways play a role in neural transmission speed and information processing, they may contribute to cognitive impairments. Unfortunately, work on the effects of obesity on white matter is limited. Jagust and colleagues (2005), in the same study where they saw increased waist-to-hip ratio to be negatively correlated to hippocampal volume, also saw it to be positively

correlated to white matter hyperintensities. White matter hyperintensities are patchy white matter areas and smooth periventricular areas of high signal intensity on brain images (Fazekas 1993). They are associated with cerebrovascular risk factors and cognitive decline, and believed to be involved in ischemia, hypoperfusion, blood-brain barrier leakage, and neurodegeneration (Longstretch 1996, Fazekas 1993, Jeerakathill 2004).

Normal aging processes may possibly interact with the processes linking obesity to some of the observed brain-related changes in previous studies (Stanek 2011). Indeed, most studies regarding obesity and its effects – not only on brain-related changes, but also other systems (like cardiovascular) – have targeted populations in middle age and beyond. There is a call for more research into other subpopulations of obese individuals, and this is becoming exceedingly more important due to the growing rates of obesity.

2.2 Maternal Obesity

2.2.1 Definition and Epidemiology

Consistent with the larger population trend, the prevalence of obesity is increasing rapidly among women of reproductive age worldwide. According to Statistics Canada, the prevalence of obesity in Canadian women has increased 8% between the late 1980s and 2010 (Shields 2011). Other surveys found that one in three Australian women are either overweight or obese, and 44% of American women are overweight or obese (Mamun et al 2011). In the United States, >50% of non-pregnant women of reproductive age (20-39) were overweight or obese, and >30% of girls (12-19) were at risk of being overweight or obese (Ogden 2006). A Department of Health survey in England reported that 32% of women aged 35-64 were overweight, and 21% were obese (Dixit 2008, Afifi 2011). The WHO has estimated that as many as 60% of South

African women may be overweight or obese (Balkau 2007). Collectively, these statistics indicate that many babies were, and will be born from overweight and obese mothers.

2.2.2 Physiological Changes Associated with Maternal Obesity

Obese women are at a greater risk of infertility than normal-weight women, and are at greater risk of developing oligo-amenorrhea and polycystic ovarian syndrome (Dixit 2008). Women who are exposed to maternal obesity during pregnancy are also subject to additional complications for both the mother and fetus. Maternal, and fetal morbidity risk are increased with maternal obesity (Yu 2008). Maternal obesity is associated with higher risk of hypertensive disorders and thrombo-embolic disease incidence (Dixit 2008). Studies have reported the incidence of hypertensive disorders during pregnancy was 28.8% in morbidly obese women compared to the 2.9% incidence in non-obese women (Dixit 2008).

Complications due to obesity, for the mother, include increased risks for obstructive sleep apnea, and postpartum hemorrhage (Dixit 2008). Obese women are also more likely to have pre-existing diabetes, and are also at increased risk of developing gestational diabetes mellitus (GDM) (Dixit 2008). An estimated 17% of obese women develop GDM, compared to the approximate 1-3% of non-obese women (Linne 2002). Furthermore, it has been reported that 15 – 60% of women who have GDM will develop T2DM 5-15 years after delivery (Kim 2002).

In women who do not have diabetes prior to their pregnancy, GDM first appears in the second half of gestation, when placentation and opening of uterine spiral arteries have been completed (Yu 2008). The majority of fetal and neonatal pathological conditions occurring as a result of GDM are a function of maternal glycemic control (Dixit 2008). Approximately 40-50% of glucose taken up by the placenta is subsequently transferred to the fetus (Yu 2008). In GDM women, there is hyperglycemia, hyperinsulinemia, and increased proinflammatory cytokines that

are also found in the fetal compartment (Dixit 2008). There are also morphological changes of the placenta in GDM women.

One feature often seen in the placenta with GDM is a thickening of the trophoblast basement membrane that is mainly is a result of increased amounts of collagen, which can compromise oxygen delivery to the fetus (Dixit 2008). The structural changes result in functional alterations of the placenta that affect the transport of glucose, amino acids, lipids, and other nutrients to the fetus (Yu 2008). The imbalance is further exacerbated with an increased fetal demand for oxygen due to hyperinsulinemia and hyperglycemia-induced stimulation of the fetal aerobic metabolism, which has been reported to increase fetal oxygen consumption by up to 30% (Dixit 2008, Desoye et al 2003). The combined effects of reduced oxygen supply from an altered placenta, and the increased fetal oxygen consumption, may lead to fetal hypoxia. Obesity during pregnancy may also involve further long-term complications for the fetus (Ramachenderan et al., 2008).

2.3 Developmental Origins of Health and Disease

Epidemiological and experimental studies have highlighted relationships between peri-conceptual, fetal, and early infant stages of life, and the subsequent development of adult disorders. Together, these relationships have helped to generate a theory referred to as the "developmental origins of adult health and disease" (DOHaD). A central tenet in DOHaD is programming, which is defined as "either the induction, detection, or impaired development of a permanent somatic structure of the 'setting' of a physiological system by an early stimulus or insult operating at a 'sensitive' period" (Lucus 1991).

The formation of the DOHaD hypothesis can be primarily attributed to the early work of David Barker and colleagues (1998). During the latter half of the 20th century, they postulated

that events in utero that reduce fetal growth permanently can alter both physiology and structure of the offspring to the extent that their risk of cardiovascular disease and diabetes is later increased. Adaptations may include resetting of set points of metabolic homeostasis and endocrine systems, and down-regulation of growth (Vickers 2011). Hence, DOHaD is a derivation of the fetal programming hypothesis initiated by Barker and colleagues. The paradigm of DOHaD is rooted in the process of developmental plasticity. A nutritional or environmental stimulus at a critical period of development leads to a permanent change in the offspring physiology (Vickers 2011).

While the physiological changes may be beneficial for in utero survival, it is thought that the changes to fetal tissue structure and function may be maladaptive in postnatal life (Vickers 2011). According to the Predictive Adaptive Responses hypothesis, proposed by Gluckman and colleagues (2005), upon exposure to stimuli in the in utero environment, the fetus will predict that the post-natal environment is also the same, and adapt physiologically based on this prediction. When the predictive adaptive response is correct, the fetus' phenotype into adulthood will be normal. However, when there is a mismatch between predicted and actual post-natal environment, risks to developing chronic diseases increase (Gluckman 2005).

Studies from the Dutch Hunger Winter of 1944-1945, a case of disparity where nutrition was plentiful following the famine, revealed the importance of the timing of the exposure as a major determinant in phenotypic outcomes. Ravell and colleagues (1976) found that women with famine exposure during early gestation gave birth to normal-sized infants that later developed adult hypertension and obesity; the reduction in maternal intake in late gestation was associated with increased risk factors for coronary heart disease like hypercholesterolemia, elevated blood pressure, and glucose intolerance compared to early or mid-gestation. Hulshoff and colleagues

(2000) also found prenatal exposure to the Dutch Hunger Winter to be associated with the twofold increase in schizophrenia incidence. Other studies correlate low birth weight and greater risk of diseases ranging from coronary heart disease, stroke, depression, T2DM, and osteoporosis (Tang 2007). The early studies have led to the development of specific research committed to uncovering the mechanisms that underlie DOHaD.

2.3.1 The Effects of maternal nutrition on offspring development

2.3.1.1 Maternal Undernutrition

Nutrient requirements increase during periods of growth and development, such as pregnancy. An adequate amount of nutrients is needed to support fetal growth and development, as well as maternal metabolism and specialized tissue development (i.e., placenta and mammary gland) (Picciano 2003). For example, micronutrient deficiency has been reported to cause defects of the CNS. Weight gain during pregnancy is expected, representing components of the fetus (including amniotic fluid and placenta), and maternal accretion of tissues (i.e., enlargement of uterus and mammary glands, enlargement of maternal stores in the form of adipose tissue, expansion of blood and extracellular fluid). Given the evidence that pre-pregnancy weight-for-height is a greater determinant of fetal growth beyond that of gestational weight gain, the recommendations for weight gain during pregnancy are individualized (Picciano 2003).

Extensive studies have been conducted on prenatal famine and the effects on adult health. Lumey and colleagues (2011) examined the health status of those with prenatal exposures to the Dutch Hunger Winter, and found associations with T2DM, increased blood pressure, altered lipid profiles, prevalence of mild to severe mental retardation, congenital nervous system anomalies, and increased mental disorders (e.g., schizophrenia, antisocial personality disorder, mood disorder). In terms of brain development, Hulshoff and colleagues (2000) found prenatal

exposure to the Dutch Hunger Winter was associated with the increased incidence of focal brain abnormalities and, specifically, white matter hyperintensities. Observations that intracranial volume decreased only in schizophrenia patients with prenatal famine exposure further suggests an association between prenatal undernutrition and brain development (Hulshoff et al., 2000).

Insufficient weight gain in the mother is associated with intrauterine growth retardation (IUGR). IUGR is associated with fetuses that are small for their gestational age, and greater perinatal mortality (Reeves 2008). In a longitudinal study, Eriksson and colleagues (2003) found that children born with low birth weight, and who have low body weight at age 1, display increased incident T2DM. This cohort also experienced "catch-up growth', resulting in elevated weight and BMI in later childhood, as well as an association with diabetes. Other human studies established important relationships between fetal growth restriction and placental size, which is inversely related to fetal size and positively related to adult hypertension, even though placental size gives only an indirect measure of placental function (Godfrey 2002). The placental role in regulating nutrient availability to the fetus deserves careful consideration, as other studies reveal that alterations in placental growth, vascular resistance and subsequent nutrient transmission to the fetus, have also been associated with development of cardiovascular disease in later life (Godfrey 2002).

Animal models have been used in DOHaD research and successfully replicated the epidemiological observations in human studies. Parallel to the findings from pregnancies in the Dutch famine, feeding rats a low protein diet during pregnancy raised systolic blood pressure in the offspring post weaning, and the magnitude of effect was greatest when the modified diet was consumed during the final week of gestation (Langley-Evans 1996). Specific nutrient imbalance in protein in pregnancies exposed to an undernourished environment has been theorized to be the

critical factor that determines cardiovascular outcome in the offspring. Indeed, glycine supplementation of a low protein diet was seen to prevent the increased postnatal systolic blood pressure, while methionine supplementation further impaired blood pressure (Langley-Evans 2000). In addition to revealing the importance of adequate nutrients, this study also alluded to the possibilities of excess nutrition and its effects on the offspring.

2.3.1.2 Maternal Overnutrition

Many studies have established an association between maternal obesity and fetal macrosomia (Yogev 2009, Drake 2010), and that maternal obesity constitutes a risk for childhood obesity (Sirimi 2010). Compromised prenatal nutrition, and its consequence in insulin resistance, can directly influence development, growth and differentiation of insulin sensitive tissues like the pancreas, liver, and skeletal muscles. Maternal hyperglycemia has been demonstrated to induce diabetes in offspring later in life.

Maternal obesity as a strong predictor of childhood obesity and metabolic syndrome in offspring has motivated studies investigating its transgenerational effects (Vickers 2011). The associations seen in humans have been reproduced in rodent models, which showed that offspring from obese dams become obese and have abnormal glucose metabolism (Guo 1995, Drake 2010). Both male and female macrosomic rat offspring show accelerated growth during the first 10 weeks of life, and by 10 weeks of age have higher plasma insulin and glucose concentrations post oral glucose challenge, and decreased peripheral insulin sensitivity (Gelardi 1991). The intergenerational programming hypothesis proposes that the stimulus the pregnant mother (F_0) is exposed to permanently program the F1 generation with altered metabolic function, and these symptoms of the F1 will affect the following F2 generation upon pregnancy (Drake 2010). Vickers and colleagues (2011) have shown that a moderate maternal high fat diet

results in male and female rat offspring obesity and hyperinsulinemia, independent of post-weaning diet. Given the extensive amount of physiological changes seen with obesity, as reviewed in the previous section, the increased risk of offspring obesity from maternal obesity will also substantially increase their risk for developing other chronic diseases as well.

2.3.2 Maternal Overnutrition effects on brain development - hypothalamus

Studies have suggested several mechanisms that may explain the programming effects of maternal obesity on offspring obesity risk, much of which surround changes in the hypothalamus, a region of the brain critical in feeding regulation and glucose homeostasis (Hovrath 2006). The hypothalamus integrates endocrine, metabolic, and neural signals to regulate energy homeostasis. Hypothalamic neurogenesis is influenced by maternal obesity, as offspring born to obese dams show increased proliferation of hypothalamic neuronal precursors that are ultimately orexigenic (appetite stimulating) neurons in the mature hypothalamus (Bouret 2010). Leptin, a metabolic hormone which is secreted by adipocytes into the bloodstream, is transported across the blood-brain barrier and act on pro-opiomelanocortin (POMC) neurons found in the arcuate nucleus (ARC) of the hypothalamus. Neurons of the ARC then project to the paraventricular nucleus (PVN) of the hypothalamus and inhibit feeding. Leptin normally acts as a neurotrophic agent that promotes ARC neural projections. Rodent studies have shown hyperleptinemia and diminished leptin sensitivity in rats born to obese dams, as well as affected hypothalamic neurogenesis that was seen in the attenuated development of neuronal projections from ARC to PVN (Nivoit 2009, Kirk 2009).

Proper neurodevelopment requires a neonatal leptin surge during the second postnatal week in rodents, which is also observed in humans (Walker, et al 2007). The observed morphological alterations in ARC connectivity in the hypothalamus in offspring of obese rats

were corrected with leptin treatment as neonates, but not with treatment in adulthood (Vickers 2005, Kirk 2009). The degree of leptin's actions have been demonstrated to differ between adults and neonates, establishing the critical period of hypothalamic organization in which leptin operates to be in the very early stages of life (Bouret 2010).

The hypothalamic changes observed from altered leptin levels are seen to have consequences on hypothalamic feed circuits (Bouret 2010). Increased leptin levels that accompany maternal obesity are considered to expose the fetus to an obesogenic intrauterine and postnatal environment (Bouret 2010). Increased leptin exposure results from increased transfer between the placenta and the fetus, and in milk fat post-natally, that may range from 9-22% (Bouret 2010). With the evidence gathered on fetal hypothalamic differences as a result of maternal obesity, there is reason to explore the effects of maternal obesity on the development of other fetal brain regions.

2.4 The hippocampus

2.4.1 Maternal Overnutrition

Compared to other brain regions, the hippocampus is preferentially susceptible to a variety of insults (e.g., environmental toxicants, cardiovascular and metabolic perturbations) (Walsh 1988). Maternal nutritional status through pregnancy has been associated with long-term consequences on memory, cognitive function, and brain senescence (Gordon 1997, Scholtz 2009). Since learning and memory consolidation is largely the role of the hippocampus, it is the appropriate brain region to focus upon.

Maternal obesity effects on leptin levels in the fetus have been well established (see section 2.2.2 C). Leptin receptors have been identified in the hippocampus as well, particularly in the dentate gyrus and CA1/CA3 regions (Fig 1; Wayner 2004, Harvey 2006). O'Malley and

colleagues (2007) have shown in neonatal hippocampal cell culture that leptin treatment increases motility and density of dendritic filopodia, and evidence suggests that prolonged exposure to leptin can promote the formation of new synaptic contacts. This demonstrates an extra-hypothalamic role of the observed neonatal leptin surge that can be potentially affected by maternal obesity.

Neural progenitor cells are located in the dentate gyrus of the hippocampus, and produce new neurons that can contribute to hippocampal-dependent cognitive functions like learning and memory (Shors 2001, Zhang 2008, Dupret 2008). Certain animal models have shown that maternal condition (such as stress and nutrient supply) can modulate hippocampal neurogenesis (Mirescu 2004, Wong-Goodrich 2008). Under stressful conditions, increased corticosterone levels in mother dams have been reported to inhibit hippocampal progenitor cell proliferation (Cameron 1994). Tozuka and colleagues (2009) have demonstrated in mice that offspring from obese dams have impaired hippocampal neurogenesis, independent from stress hormone-mediated cascades, but via increased lipid peroxidation. Hippocampal organization and neuronal development was observed to accompany reduced hippocampal growth in transplanted fetal hippocampal-intraocular grafts obtained from offspring of obese dams (Willis et al 2005, Freeman et al 2010). However, there is little evidence (from both human and animal studies) on the effects of maternal obesity on the brain function of the offspring.

2.4.2 Anatomy

The hippocampus is a complex bilateral structure that is part of the forebrain, and located in the medial temporal lobe. The hippocampal region consists of the dentate gyrus (DG, an input stage), the hippocampus proper (Cornu Ammonis CA3 and CA1 fields), and the enclosing cortical tissue (the entorhinal, perirhinal, and parahippocampal cortices). Classically described as

consisting of a tri-synaptic pathway, the hippocampus receives information from the entorhinal cortex, sending it through the dentate gyrus, to CA3, and then to CA1 (Fig 1). Fibres from the entorhinal cortex form synapses with granule cells in the molecular layer of the DG, and form bilateral connections with pyramidal neurons in CA1 (Granger et al 1996). Neurons from DG extend their axons along the mossy fibre pathway to pyramidal neurons in the CA3 stratum radiatum. These CA3 neurons then extend via Schaffer collaterals and form synapses with apical dendrites of pyramidal cells in CA1 (Granger et al 1996). The efferent projections from CA1 integrate the hippocampus through the subiculum and into a complex network, involving other cortical structures like the olfactory bulb, nucleus accumbuns, amygdala and hypothalamus (Granger et al 1996).

The DG and subiculum are considered a transition zone from the 6-layered surrounding cortices to the 3-layered structure of the hippocampus (Granger et al 1996). The deepest layer of the DG is the stratum moleculare, containing proximal dendrites that are the main synaptic junction for perforant path fibres from the entorhinal cortex, and sits directly above the hippocampal fissure separating the DG from the CA1. The layer below is the stratum granulosum, containing cell bodies of granule cells (the main excitatory neurons of the DG) that project to CA3. CA3 neurons have multiple destinations to send information – out of the hippocampus to other cortices (through the fornix), back to the DG, back towards themselves, or to CA1 (via Schaffer collaterals).

The CA fields also contain 3 distinct layers. The alveus, which is the most superficial layer, contains fibres of pyramidal cells. These fibres are collectively referred to as the fimbria, and are a source of output from the hippocampus. The stratum oriens layer contains basal dendrites of the excitatory pyramidal cells, as well as basket cells (inhibitory interneurons).

Inhibitory interneurons are believed to regulate activity levels in the hippocampus, and play an important role in the hippocampal system (Bauer 2002). This stratum oriens also contains fibres received from the contralateral hippocampus (Granger et al 1996). The stratum pyramidale mainly contains the cell bodies of pyramidal neurons, as well as some mossy fibre connections between DG and CA3, and interneurons in CA3. The stratum moleculare is divided into 3 sublayers – stratum lucidum, stratum radiatum, and stratum lacunosum (Granger et al 1996). The stratum moleculare is where Schaffer collaterals synapse in CA1 (Granger et al 1996). It has been speculated that the feed-forward pathway is important for establishing encoding of new memories, whereas the recurrent connectivity within CA3 represents a function for recalling previously stored memories (Bauer 2002).

2.4.3 Synaptic Plasticity

The ability to learn from past experiences is key to survival. Most eloquently said by Malenka (1995), "One of the most remarkable features of the mammalian central nervous system is its ability to store large amounts of information for periods approaching lifetime". Memory is arguably one of the most fundamental aspects of brain functioning, though over the course of history it has been primarily studied due to its absence. Supported by cases of human amnesia, medial temporal lobe structures, especially the hippocampal formation, are now established to be critical for learning and memory consolidation (Squire 1986, Tulving 1998). In the late 1800s, neuroanatomist Ramon y Cajal was the first to suggest that changes in connections between neurons may provide the foundation for learning and memory (Anderson et al., 2007). Subsequent postulations by Sherrington (1906) and Hebb (1949) suggested memories are formed through synaptic plasticity, as new information is represented as patterns of neuronal activity in the brain and results in modifications to synapses in the particular neuronal circuits

involved (Malenka 1995). Indeed, with the complex circuitry within the hippocampus, alterations among synapses would likely modify learning and memory processes. There has been evidence to support the significance of synaptic communication in learning and memory, which indicates certain structural and biochemical changes that need to occur with synaptic plasticity in the brain (Bauer 2002).

Calcium influx into post-synaptic neurons, through L-type voltage-gated calcium channels (VGCC) and excitatory amino-acid receptors, has been generally accepted as a mechanism that initiates synaptic plasticity (Nicoll 1995, Bauer 2002). The excitatory amino-acid receptors are primarily ionotropic glutamate receptors, with subtypes α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, *N*-methyl-D-aspartate (NMDA) receptors, and kainite receptors (Nusser 2000). AMPA and NMDA receptors are the subtypes that participate in synaptic plasticity (Pickard et al 2000). Elevation of intracellular calcium activates additional signaling pathways that contribute to synaptic plasticity; for example, phosphorylation of constitutively present protein kinases like calcium/calmodulin-dependent protein kinases (CaMK) and protein kinase C, whose phosphorylation results in increased synaptic efficacy (Tanaka 1994, Lisman 2002). Further changes may occur subsequently, such as activation of gene transcription and protein synthesis (such as brain derived neurotrophic factor, BDNF) that can also lead to structural changes in synapses (West 2002, Tanaka 2008). Another study suggested that the large calcium influx induces cytoskeletal changes of the neuron that include new dendritic spines through rapid actin polymerization (Lamprecht 2004).

2.4.4 Hippocampal slice models in research

Brain slices can be maintained on a porous membrane filter at an interface between a medium (typically artificial cerebrospinal fluid, ACSF) and a humidified atmosphere to closely resemble the *in vivo* environment (Stoppini 1991). Brain slices are often used as experimental models since tissue architecture of the brain regions that the slices originated from are preserved (Cho 2007). Notably, the trisynaptic circuitry of the hippocampus, is maintained in slice preparations, and is readily accessible for optical imaging or electrophysiological studies (Lein et al., 2011). The patterns of connections within the slice are minimally altered relative to the *in vivo* patterns. Also, brain slice models are useful since there is no need for laborious monitoring of multiple accompanying physiological parameters that typically follow *in vivo* manipulations (Cho 2007). Advances in multi-electrode array technology have allowed for the measurement of neural activity patterns among discrete locations within each slice (Lein et al., 2011). Similarly, the targeting of specific brain areas (that is with the nature of slice models) facilitates research to establish clear correlations between molecular changes and the particular physiologic or pathophysiologic context (Lein et al., 2011). Group studies that combine electrophysiology with behaviour studies show consistency in their findings (Cho 2007, Dawson 2005, Finley 2004) that further indicate slice models to make substantial contributions to research beyond the cellular level from which it represents.

2.4.5 Long Term Potentiation

Electrophysiological studies provide important clues regarding the nature and development of synaptic health and impairment. Long-term potentiation (LTP) is an artificially induced phenomenon demonstrating synaptic plasticity, and is widely used for investigating the cellular mechanisms behind memory. LTP is characterized by a stable increase of synaptic

response following a moderately high frequency of stimulation (usually 100 Hz) applied to a specific pathway in the hippocampus (Bliss 1973). After high frequency stimulation, the baseline stimulation that previously induced low activation, leads to a heightened response that can last up to weeks in intact animals (Bliss 1973). One characteristic of LTP that makes it an attractive model of memory is that the induction of LTP is selective, in that only inputs which were specifically stimulated display increased efficacy, much like memories that are formed for specific facts or events.

The LTP phenomenon is thought to be primarily related to postsynaptic events (explained by an increase in postsynaptic responsiveness to glutamate) (Sevens 1993, Nicoll 1995). NMDA and AMPA receptors are the main subtypes of glutamate receptors. Increased AMPA receptor function or number would increase the amplitude of the EPSPs, which is a hallmark of LTP (Pickard et al., 2000). NMDA receptors are permeable to Ca^{2+}, so their activation causes a significant, but transient increase in Ca^{2+} concentration in the postsynaptic spine (Pickard, et al., 2000), which is then thought to initiate second messenger cascades and ultimately engage cellular mechanisms underlying synaptic plasticity (as discussed in 2.4.2).

2.5 Animal models in research

The ultimate goal of experimental models using animals is to gain insight into the pathophysiology of disease in humans. Animal models are useful when studies are addressing particular research questions that cannot be conducted in humans due to ethical concerns and cannot be addressed by alternative methods that do not imply live animals (Farnaud 2009). Animal use in research, particularly rodents, provides the opportunity to study basic and clinical processes otherwise unfeasible with human samples. Suitable characteristics of rodents for research include the availability from many commercial or private sources, the costs to purchase

and maintain, genetic uniformity for control, well-defines physiologic parameters, and short lifespan that provide the opportunity to study long-term effects on health and well-being that may be extrapolated for the human condition.

To maximize relevance, the selected rodent model should relate as closely as possible to the human condition it is attempting to represent. In the case of obesity research, obese rodent models include diet-induced models, genetic models (e.g., Zucker (*fa/fa*) rat, *ob/*ob mouse, Agouti mouse), and pharmacologically induced models (e.g., gold-thioglucose mouse) (Buettner et al., 2007). Genetically modified rodent strains are useful for studying the underlying biological mechanisms and pathways of obesity. A diet-induced rodent model, like a high-fat diet (HFD), more accurately represents the genesis of obesity in humans – from environmental factors like the excessive intake of calories with a disproportionately higher fat composition of the diet (Li et al., 2007). It is important that not only does the phenotype, but also the pathogenesis, of the animal's condition resemble the human condition being examined (Buettner et al., 2007).

Normal rat diets have 3-9% of calories from fat, 72-82% of calories from carbohydrates, and 14-20% of calories from protein, and do not contain refined sugars or high levels of saturated fat (Harlan Teklad; Rothwell 1988, Tschop 2001). HFD models alter diets to be ≥20% calories from fat (mostly saturated fat), making trade-offs with calories from carbohydrates while maintaining typical calories from protein (Tschop 2001, Buettner et al., 2007). HFD fed rodents have been shown to develop insulin resistance and impaired glucose tolerance, indications that the progression to obesity in the rodent on a HFD fed diet properly mimics the obese phenotype seen in humans (Buettner et al., 2007). Adipokines (e.g., leptin, adiponectin) are systemic factors that are altered in HFD fed rats, and mirror that seen in human obesity (Buettner et al., 2007). 1998, Schrauwen 2004). In addition to increased adiposity, adipocyte changes seen with HFD-

induced rodents also resemble the pro-inflammatory state found with human obesity (see section 2.1.2 C, Li et al., 2008). Finally, HFD-fed rodents display hepatic steatosis, which resembles that observed in obese humans (Buettner et al., 2007, Konstantinos 2009). The similarities in physiological changes found in HFD-fed rodents and obese humans suggests that the HFD rodent model is a valid representation for the pathophysiological progression and condition of human obesity.

In pregnancy research, rodents are often selected as the animal model of use (Jawerbaum 2010). Ethically, in any animal model selected, the number of animals used should be as low as possible. Rodents are selected for their multiparity, human-like hemochorial placentation, and the short duration of their pregnancies (Jawerbaum 2010). The extensive knowledge of rodent embryonic development further grants rodent use in pregnancy research so that experimental results can contribute to a human situation with confidence (Jawerbaum 2010). For example, regional development of the rodent brain proceeds in days, versus weeks to months in humans, although progression through stages and patterns of neurogenesis (proliferation and migration of neurons) are relatively parallel (Rice 2000). Furthermore, with animal research, it is possible to select and evaluate the health in the population of females to be studied even before pregnancy (Nathanielsz 2006).

Maternal-induced impairments in fetal and neonatal development have both short- and long-term adverse effects (see section 2.3). Congenital malformations seen in neonatal rodents on experiments in teratogen research are consistent with that seen at the human level (Jawerbaum 2010). Consistency among human epidemiology and rodent studies on long-term effects (Jawerbaum 2010) only adds to the rationale of rodent use for scientific research to better understand mechanisms, outcomes, and potential interventions related to human pregnancy.

2.6 Summary

Obesity is now a global matter. Like the increasing drift in prevalence of obesity, there is also a rise in the prevalence of obese women of reproductive age. With the knowledge that the maternal environment can significantly influence the fetus to develop physiological changes that increase its risk for developing disease, there is a strong necessity to explore all aspects of this phenomenon.

Maternal obesity has effects on fetal brain development. Besides the endocrine-related hypothalamic area, these effects have not been significantly studied. The hippocampus is a brain structure easily subject to insult, and is critical for learning and memory consolidation. From previous findings in animal models, there is evidence that maternal obesity may affect the offspring hippocampus. Unfortunately, due to the novelty of this area, a clear consensus and magnitude of the effects are not established.

Functional synaptic connectivity in response to environmental cues form the basis for the neural substrate of learning and memory. LTP is an artificial model that has been repeatedly utilized to study synaptic plasticity, the cellular mechanism implicated behind learning and memory. The association between maternal obesity and altered cognition in offspring is suggestive that induction of LTP will also be altered in the offspring, however, this has not yet been explored.

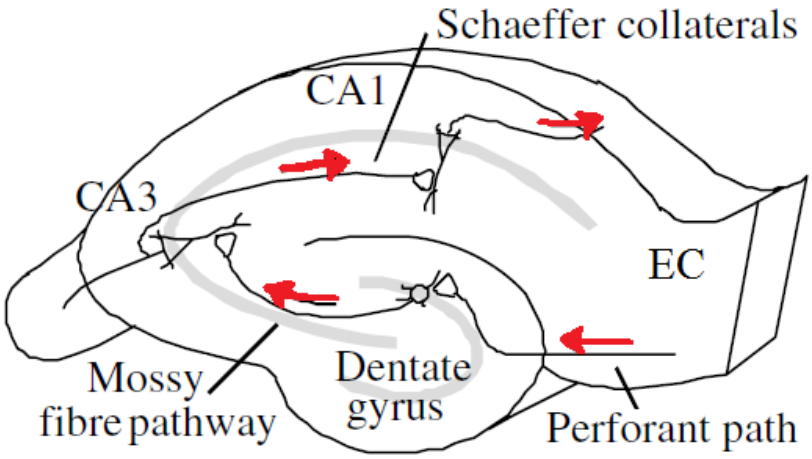


Figure 1 Cross-section of the Hippocampus. Red arrows indicate direction of activity. EC = entorhinal cortex. Modified from Nguyen PV (2006).

3.0 Materials and Methods

3.1 Animals Used

Please refer to Figure 2 for a schematic of the study.

Phase 1: Each week, for a total of 5 weeks, 6 female Sprague-Dawley rats were received as young adults and housed in polypropylene cages with woodchip bedding and stainless steel wire lids. Environmental conditions were controlled, and maintained at constant room temperature with a 12 hour light/dark cycle. Animals were initially weighed and separated into two groups (N = 3/group), so that each group had approximately the same average weight. The rats were fed Harlan Teklad standard rodent diet ad libitum with free access to water for 10 days, and were then switched, one group to a control diet (CD; 20% protein, 70% carbohydrate, 10% fat; Research Diets D12450B), and the other to a HFD (20% protein, 35% carbohydrate, 45% fat; Research Diets D12451) (Table 1). Ear notching was performed to identify each rat.

Phase 2: After 16 weeks, one female rat from each group (control diet-fed and high fat diet-fed) was bred with a normal weight Sprague-Dawley male rat.

Phase 3: During lactation, mothers were maintained on their respective diets, and, after weaning, all pups were fed the control diet. For each diet group, two rats of each sex were taken for data analysis: the first were pre-adolescent (PA; post-natal day 28-35) and the second were young adult (YA; post-natal day 55-70). All animal care procedures were approved by the University of Waterloo Animal Care Committee.

3.2 Data Collection

3.2.1 Phase 1

Food consumption per rat was measured every Monday, Wednesday, and Friday by weighing the amount of food per cage, subtracting it from the weight of food measured

previously, and dividing this amount by the number of animals per cage. The amount of food consumed was averaged to obtain weekly food consumption (g) and calculate caloric consumption (kcal). Body weights were measured twice a week. Bi-weekly fasting glucose measurements were taken using blood collected via the tail vein following a 12 h fast. An oral glucose tolerance test (OGTT) was performed once a month. Initial fasting blood glucose was measured, and then rats were gavaged with a 50% (w/v) glucose solution prepared that morning. Each rat was weighed and given an equivalent of 2 g glucose per kg body weight. Blood glucose measurements were taken at 30 min, 60 min, 90 min, 120 min, and 180 min post gavage.

The 2 animals of each set that were not bred were sacrificed to gather additional measurements of altered metabolism: body weight, fasting blood glucose, fasting serum insulin, serum leptin, retroperitoneal fat pad weight, adrenal gland weight, spleen weight, and liver weight. The various measurements were taken as indications for the progression of our rats into an obese phenotype (see section 2.5). Electrophysiological recordings were also performed on slices obtained from one of the sacrificed rats.

Serum insulin and leptin levels were obtained from blood collected on the day of sacrifice. Whole blood was collected and allowed to clot for 30 min at room temperature. Whole blood was then centrifuged at 2000 x g for 15 min, and the resulting supernatant was designated as serum. Serum samples were stored at -80ºC. The quantitative measurement of the hormones in serum was performed with either an insulin ELISA kit (EMD Millipore, USA), or leptin ELISA kit (EMD Millipore, USA) following the manufacturer's recommended protocols. Serum analyses were performed using a plate reader (SPECTRAmax Plus; Molecular Devices).

3.2.2 Phase 3

At each age (PA and YA), 2 pups from each set of animals were sacrificed, one of each sex. A preference was given for testing at the YA stage when there was an inadequate number of pups to allow testing at both ages. An OGGT was performed at the YA stage only. Body weight, fasting blood glucose, retroperitoneal fat pad weight, adrenal gland weight, spleen weight, and liver weight were taken on the day of sacrifice.

3.3 Electrophysiology

A Slice Preparation

Female rats from phase 1, and pups (postnatal day 28-35, and postnatal day 55-70), were sacrificed via decapitation after anesthesia with carbon dioxide, and the brains quickly removed and placed in chilled oxygenated (95% O_2 : 5% CO_2) artificial cerebrospinal fluid (ACSF; 4ºC; composition 127.0 mM NaCl, 2.0 mM KCl, 1.2 mM KH_2PO_4, 26.0 mM $NaHCO_3$, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 10.0 mM glucose; pH 7.4; osmolality 300-320 mOsm). The left hippocampus was extracted and cut with a McIlwain tissue chopper into 350 µm thick slices (Figure 3). Slices were then incubated on a microfilter and allowed to recover for a minimum of 1 hour in a chamber with warm ACSF (35ºC) and flowing carbogen (95% O_2 : 5% CO_2) prior to the start of experiments.

B Field Potential Recording

Slices from each animal were treated as replicates, and not as separate data points. Field excitatory postsynaptic potentials (fEPSPs) were recorded by placing the recovered hippocampal slice onto a 8 x 8 multi-electrode array probe (electrode size 50 x 50 µm, and interelectrode distance 100 µm). The fEPSPs were sampled using the MED64 system (Alpha MED Scientific Inc., Osaka). Slices were placed on the probe, immersed in warmed ACSF, and immobilized by

a mesh and a small anchor (Figure 4). The probe was connected to a perfusion system running at 1.6 mL ACSF/min.

After 20 minutes of stabilization, points for stimulation were selected. One stimulation point was placed on the Schaffer collaterals, and the other stimulation point was placed between CA1 and EC (used as a control pathway). The recording point was within the CA1 dendritic field.

An input-output curve was made to determine the test stimulation intensity needed to evoke a response with 30-50% of maximum fEPSP amplitude. To measure the input-output (IO) relationship, fEPSP amplitudes were recorded against increasing stimulation intensities at increments of 5 μA. Maximum fEPSP amplitudes were defined as those that occurred immediately before the generation of a population spike (when a group of neurons synchronously fire their action potentials), and ranged between 500-1000 μV in our slices. Test stimulation intensities generally ranged from 15-35 μA. To generate IO curves, fEPSP amplitudes for a slice were taken as a percentage of the maximum fEPSP recorded, and the normalized fEPSP amplitudes plotted against increasing stimulation intensities at steps of 5 μA.

Both amplitude and slope of the waveforms were recorded (Figure 5). Amplitude measurements were taken from peak-to-peak between cursor positions, and slope measurements were taken as 10-90% of slope values between cursor positions. A control period of baseline activity was recorded (test stimulation every minute) for a minimum of 20 minutes before a tetanus was applied to one stimulation pathway (high frequency stimulation, HFS, two 1 s trains at 100 Hz, 20 s apart). fEPSP recordings were continued for 30 minutes after HFS.

3.4 Analysis

Data acquisition and analysis were performed using MED64 Mobius software (Alpha MED Scientific Inc., Osaka). Data were normalized to the average pre-HFS slope, or amplitude, collected during the last 10 min of baseline recording. Post-HFS slope and amplitude means were determined by averaging the last 10 min of post-HFS recording. The data were plotted and analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Statistical analysis was performed in Prism using Student's unpaired t-test with Welch's correction, or two-way ANOVA. Linear regression analysis was performed on the slopes of the normalized IO curves. The Fischer's exact test was performed on the number of slices that passed or failed reaching an arbitrary potentiation threshold of 120% of baseline. Biometric data were normalized to 100 g body weight before being analyzed. P values less than 0.05 were considered to be statistically significant. All data were presented as mean ± SEM. Confidence intervals were also presented. Error bars presented were standard errors of the mean (group data).

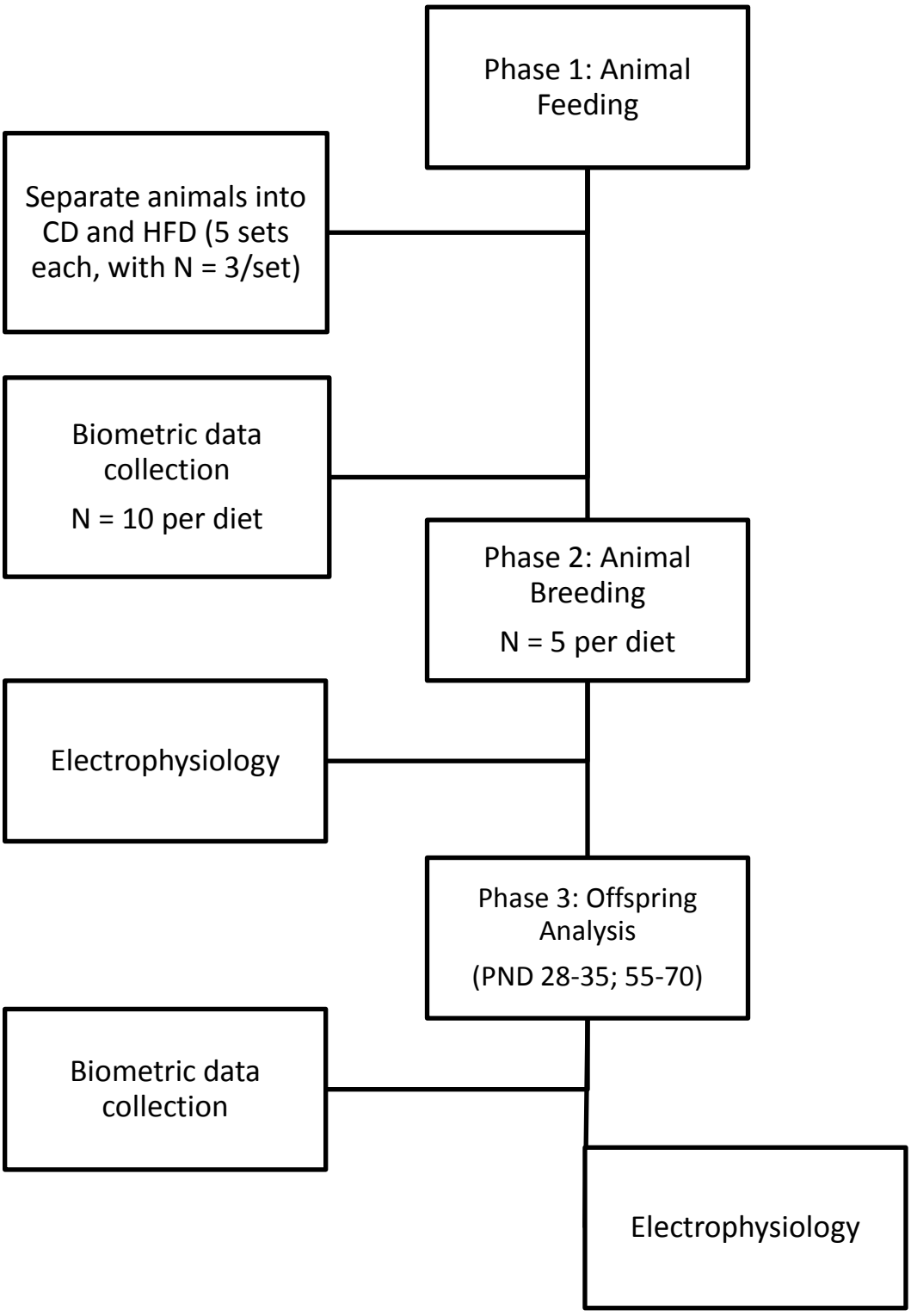


Figure 2 Schematic Timeline of the Study. CD: control diet. HFD: high-fat diet. PND: post-natal day.

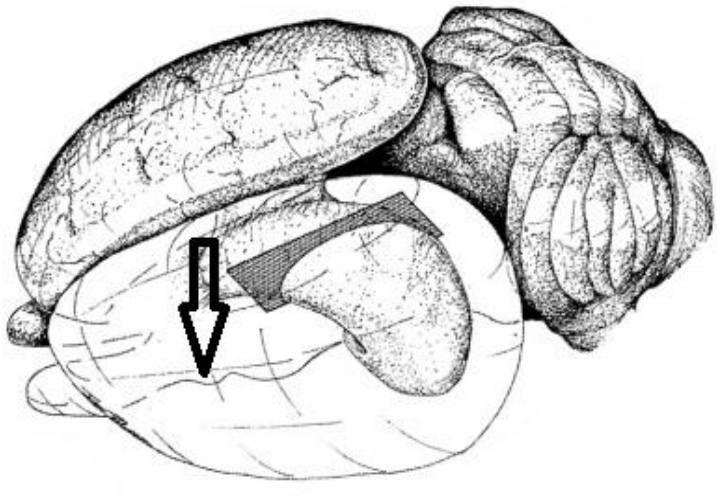


Figure 3 Position of Slicing of the Left Rat Hippocampus. Slices were taken from a septal to temporal orientation, as indicated by the arrow.
Modified from Cheung THC and Cardinal RN (2005).

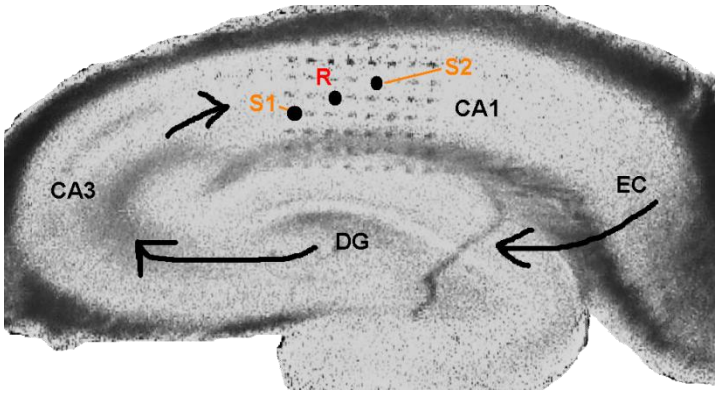


Figure 4 Brain Slice Position on Multi-electrode Array. DG: dentate gyrus, EC: entorhinal cortex, S1 and S2 are stimulation points, R: recording channel.

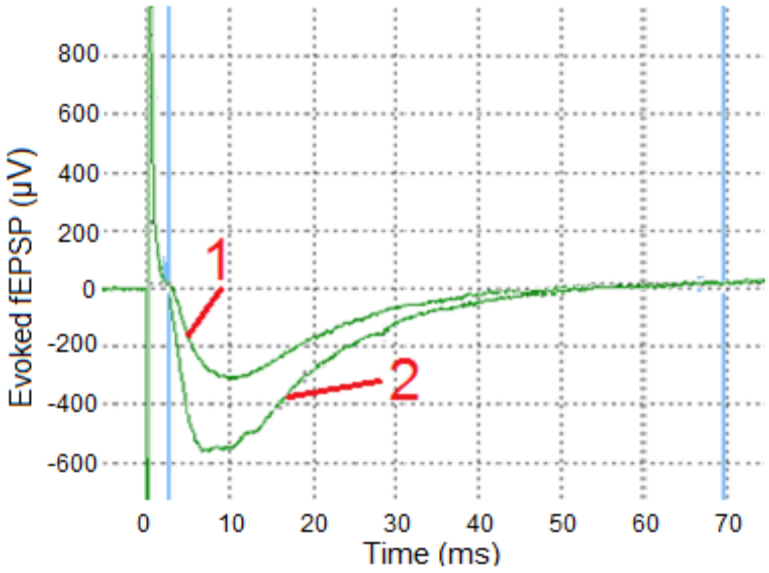


Figure 5 Example of Waveform Presentation. Waveforms are used to obtain amplitude and slope information. 1 is pre-HFS application; 2 is post-HFS application. The first vertical line indicates the stimulation artifact, while the remaining vertical lines are the measurement cursors.

Table 1 Complete Diet Breakdown.

	Control Diet (CD)		High Fat Diet (HFD)	
Ingredient	Amount (mg)	Calories (kcal)	Amount (mg)	Calories (kcal)
Casein, 80 Mesh	200	800	200	800
L-Cystine	3	12	3	12
Cornstarch	315	1260	72.8	291
Maltodextrin 10	35	140	100	400
Sucrose	350	1400	172.8	691
Cellulose	50	0	50	0
Soybean oil BW200	25	225	25	225
Lard	20	180	177.5	1598
Mineral Mix S10026	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 V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
FD&C Yellow Dye #5	0.05	0	0	0
FD&C Red Dye #40	0	0	0.05	0
Total	1055.05	4057	858.15	4057

4.0 Results

4.1 Phase 1 Results

Food Consumption

The animals were randomly divided into two groups (CD, HFD) and were given their respective diets for 16 weeks. Food consumption was measured for each set. During this time, the HFD animals began to consume less food (Figure 6). Total food consumption, after 16 weeks of feeding, showed that each dietary group was also significantly different ($p = 0.03$). HFD animals consumed, weekly, an average of 22.5 g less than CD animals ($p < 0.01$, Figure 7), or, an average of 2049 kcal more than CD animals ($p = 0.01$, Figure 8).

Biometric Data

There were no statistically significant differences in total body weight between treatment groups ($p = 0.83$, Figure 9), or total weight gained (as a percentage of initial body weight at week 0) between treatment groups ($p = 0.12$). Mean HFD animal BMI was $0.03 g/cm^2$ more than the mean BMI of CD animals ($p = 0.11$). Upon sacrifice, adrenal gland, spleen, liver, and retroperitoneal fat pad weights were obtained (Figure 10, Table 2). There was no statistically significant difference between treatment groups in regards to adrenal gland, spleen, or liver weight (Table 3). However, retroperitoneal fat pad weights were significantly larger in HFD animals, and averaged 0.18 g more than those from CD animals ($p < 0.01$).

Metabolic Data

Fasting blood glucose levels were compared using measurements obtained on the date of sacrifice. There was no statistically significant difference in fasting blood glucose levels between treatment groups ($p = 0.96$). Both CD and HFD animals had an average fasting blood glucose level of 6.4 mmol/L. An OGTT was performed every 4 weeks on each animal (Figure 11). The

AUC of OGTT (Figure 12) after 4 weeks had a mean difference of 62.88 ($p = 0.26$); OGTT after 8 weeks had a mean difference of 79.5 ($p = 0.030$); OGTT after 12 weeks had a mean difference of 109.9 ($p < 0.01$); OGTT after 16 weeks had a mean difference of 74.9 ($p = 0.018$).

Serum Analysis

Serum was evaluated for insulin and leptin concentrations (Figure 14). Data presented were concentrations (ng/mL) normalized to 100 g body weight of the specific rat. Values exceeding %CV range remained incorporated in the analyses in order to have enough data points for comparisons. Preliminary measurements showed HFD animals averaged greater serum insulin (0.07 ng/mL) and leptin (0.5 ng/mL) concentrations than CD animals, with no statistical significance.

Electrophysiology

Electrophysiological recordings were made using four hippocampal slices prepared from each animal; that is, $n = 4$ slices for each of $N = 5$ animals. Slices obtained from HFD animals required greater stimulation intensities to evoke a maximum response, but there was no significant difference between intensities ($p = 0.62$). Also, linear regression analysis found the differences between the slopes of the IO curves were not significant ($p = 0.63$; Figure 13). From the evoked fEPSPs, amplitude and slope were measured and compared between diet groups. The CD animals had an average of 9.75% more potentiation in amplitude (Figure 15, $p < 0.0001$) and an average of 11.26% more potentiation in slope (Figure 16, $p < 0.0001$) relative to the HFD animals. Representative waveforms recorded from slices obtained from CD and HFD animals were shown in Figure 17.

85% of slices prepared from CD animals met our amplitude threshold limit, compared to 60% of the slices prepared from HFD animals. 90% of slices prepared from CD animals met our

slope threshold limit, compared to 60% of the slices prepared from HFD animals. The number of slices that passed or failed reaching the threshold limit of 120% of baseline was significantly different between dietary groups (Table 4, $p = 0.0001$).

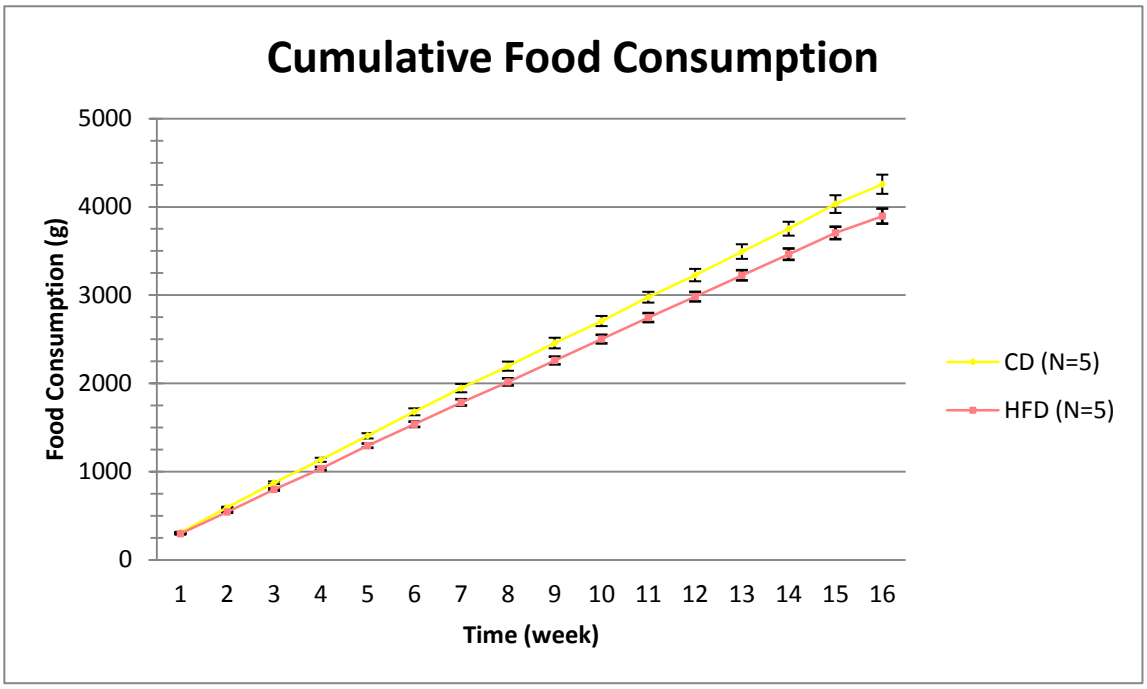


Figure 6 Mean cumulative food consumption per week (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet.

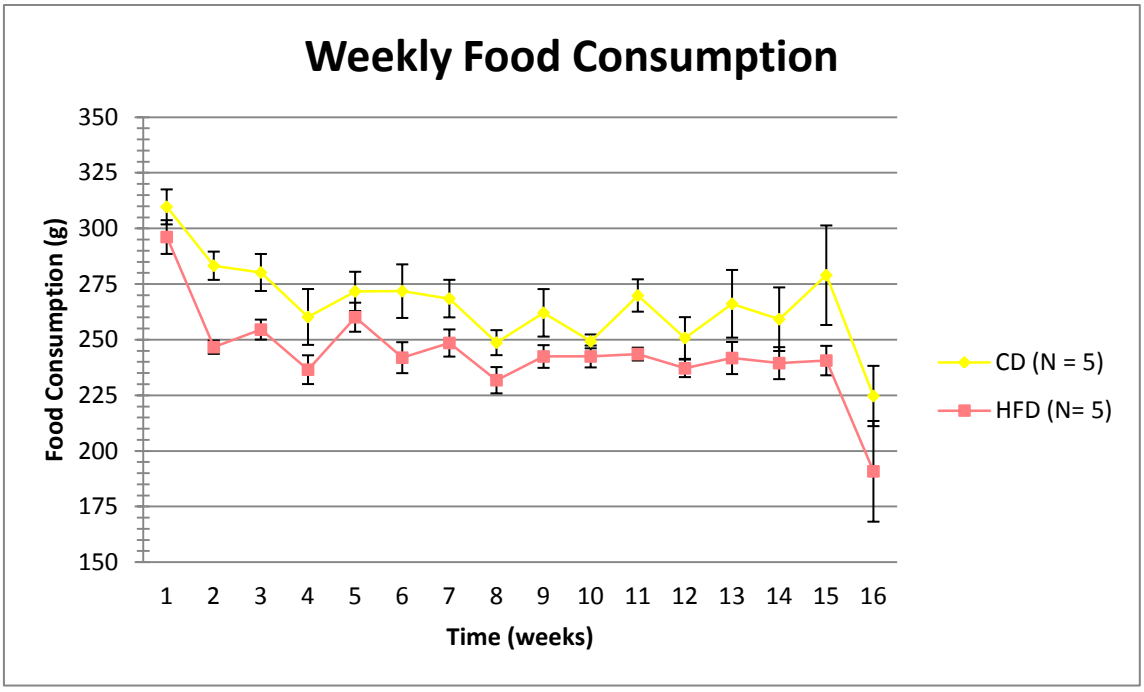


Figure 7 Mean food consumption per week (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet.

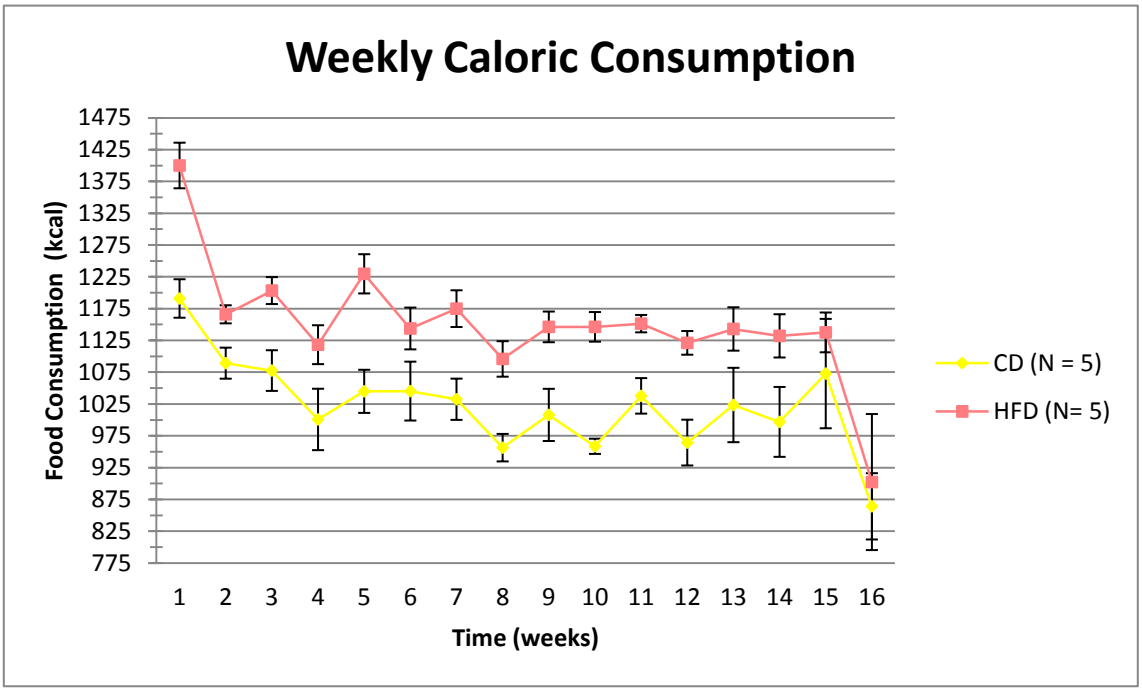


Figure 8 Mean caloric consumption per week (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet.

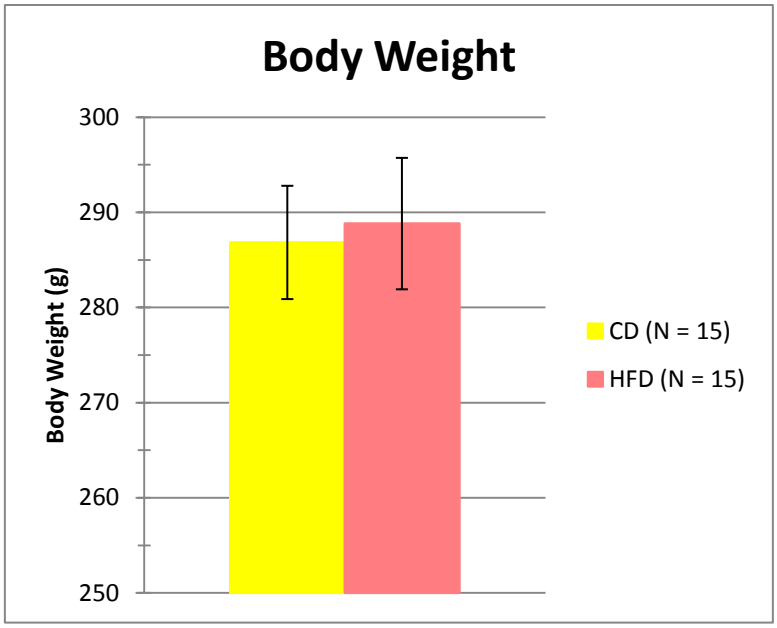


Figure 9 Mean Body Weight (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet.

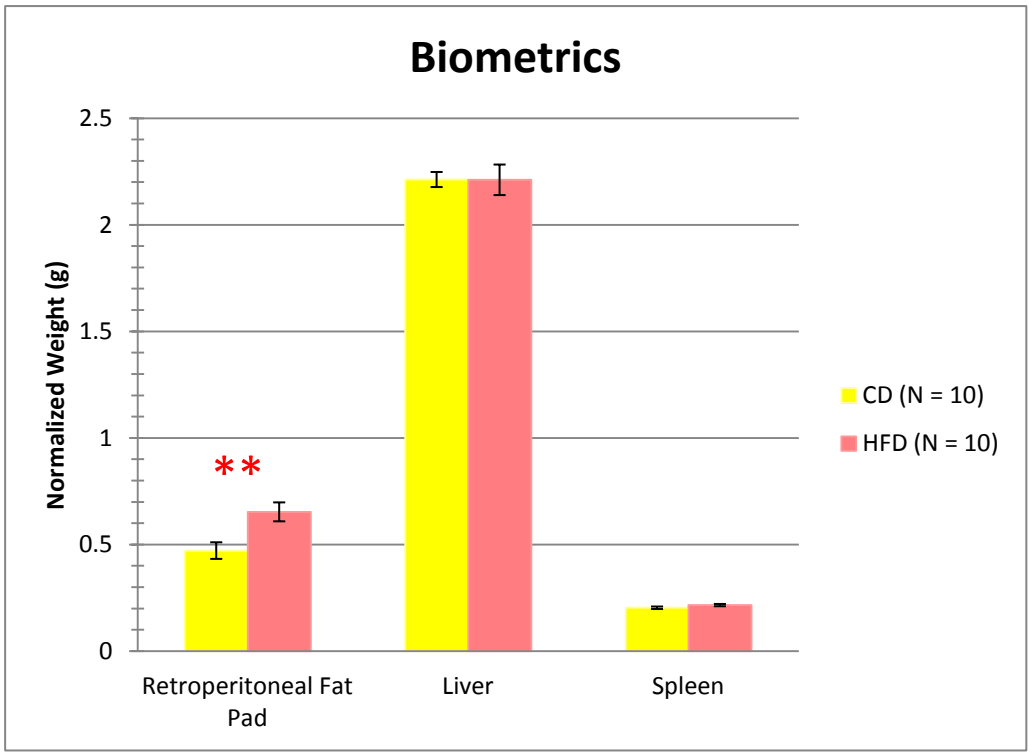


Figure 10 Mean Retroperitoneal Fat Pad Weight (+/-) SEM, Liver Weight (+/-) SEM, and Spleen Weight (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet. ** $p < 0.01$

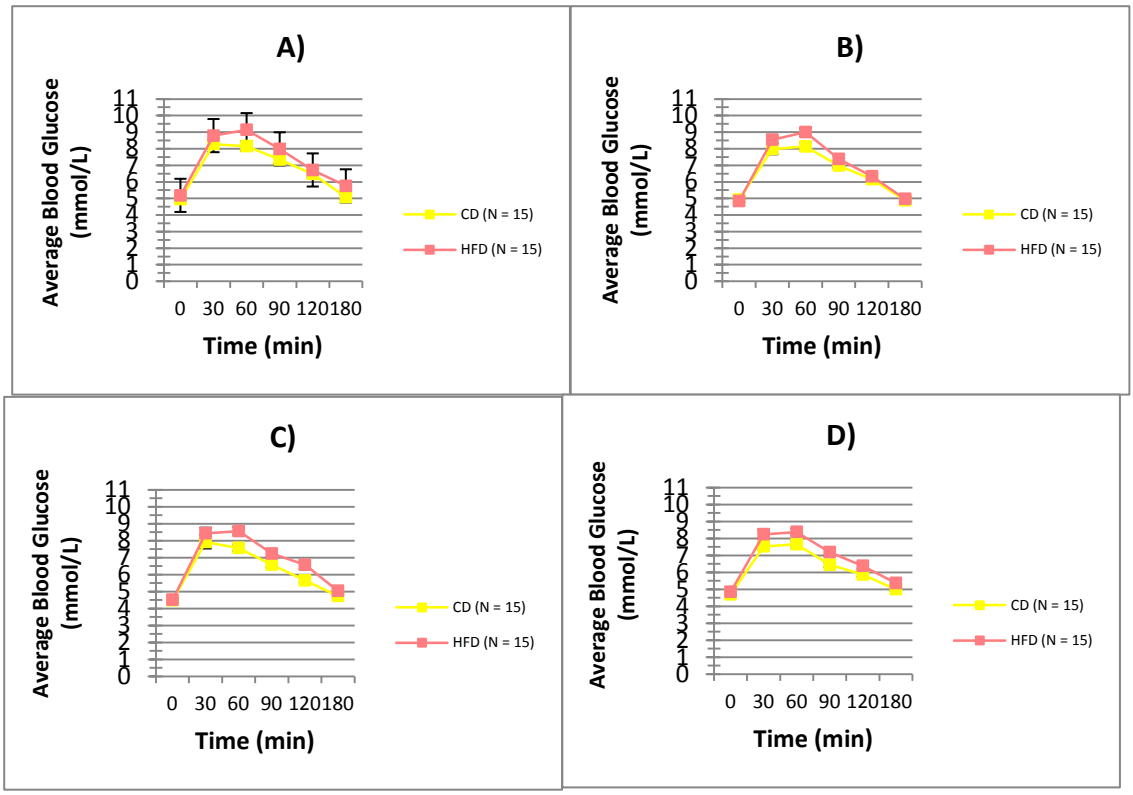


Figure 11 Mean Oral Glucose Tolerance Test values (+/-) SEM post glucose bolus administration comparing female Sprague-Dawley rats eating control or high-fat diet at A) 4 weeks; B) 8 weeks; C) 12 weeks; D) 16 weeks.

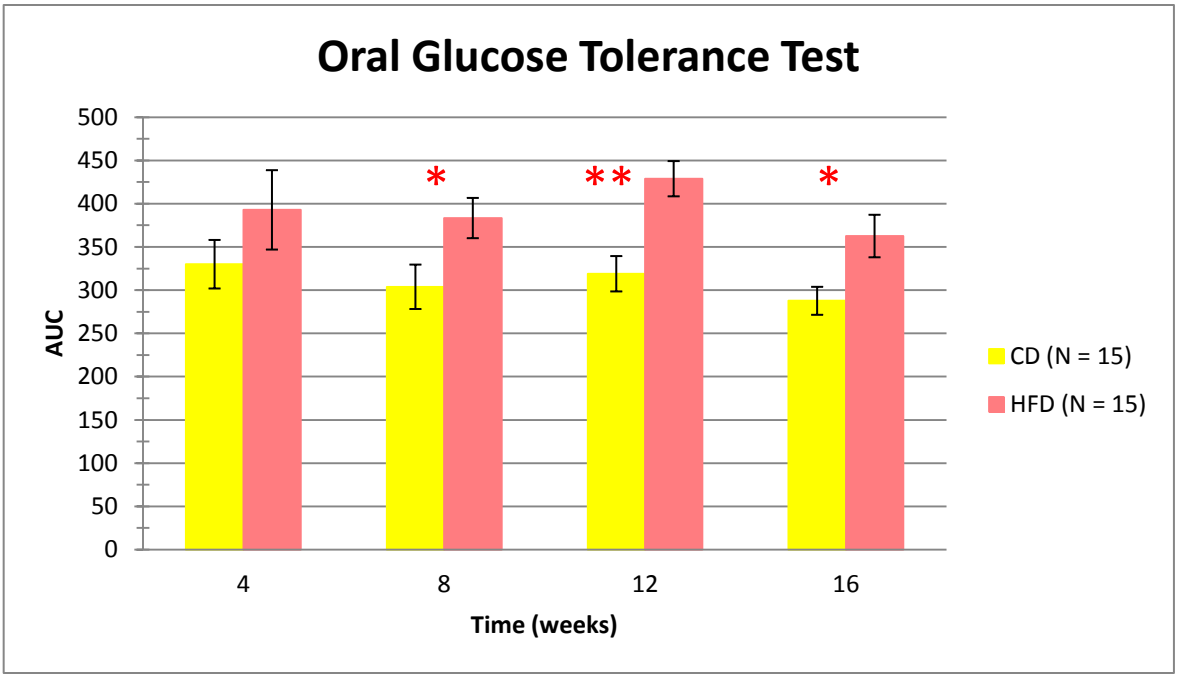


Figure 12 Mean Oral Glucose Tolerance Test values (+/-) SEM comparing female Sprague-Dawley rats eating control, or high-fat diet. * $p < 0.05$, ** $p < 0.01$.

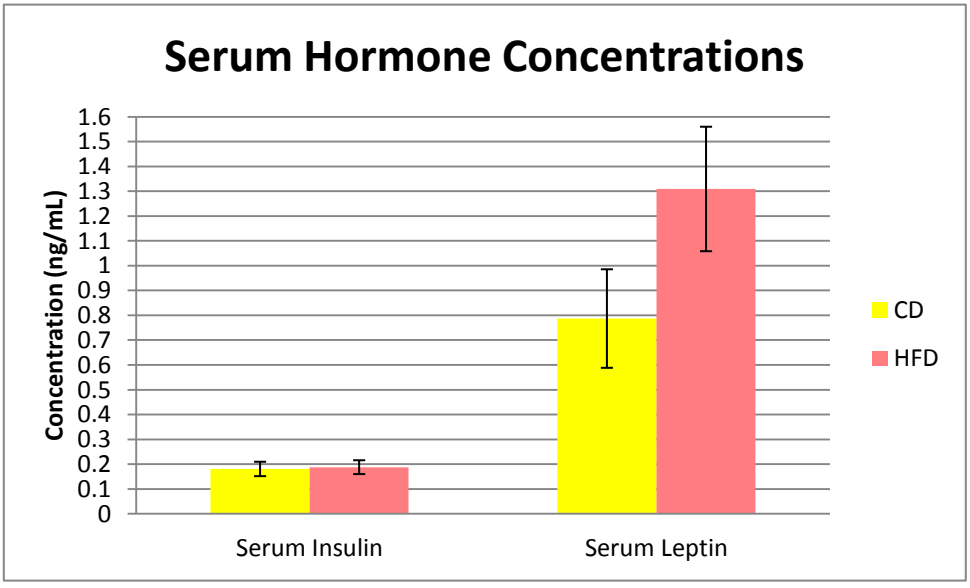


Figure 13 Mean Serum Concentrations of insulin and leptin, (+/-) SEM comparing female Sprague-Dawley rats eating control, or high-fat diet. Serum insulin from CD (N = 10) and HFD (N = 10). Serum leptin from CD (N = 5) and HFD (N = 5).

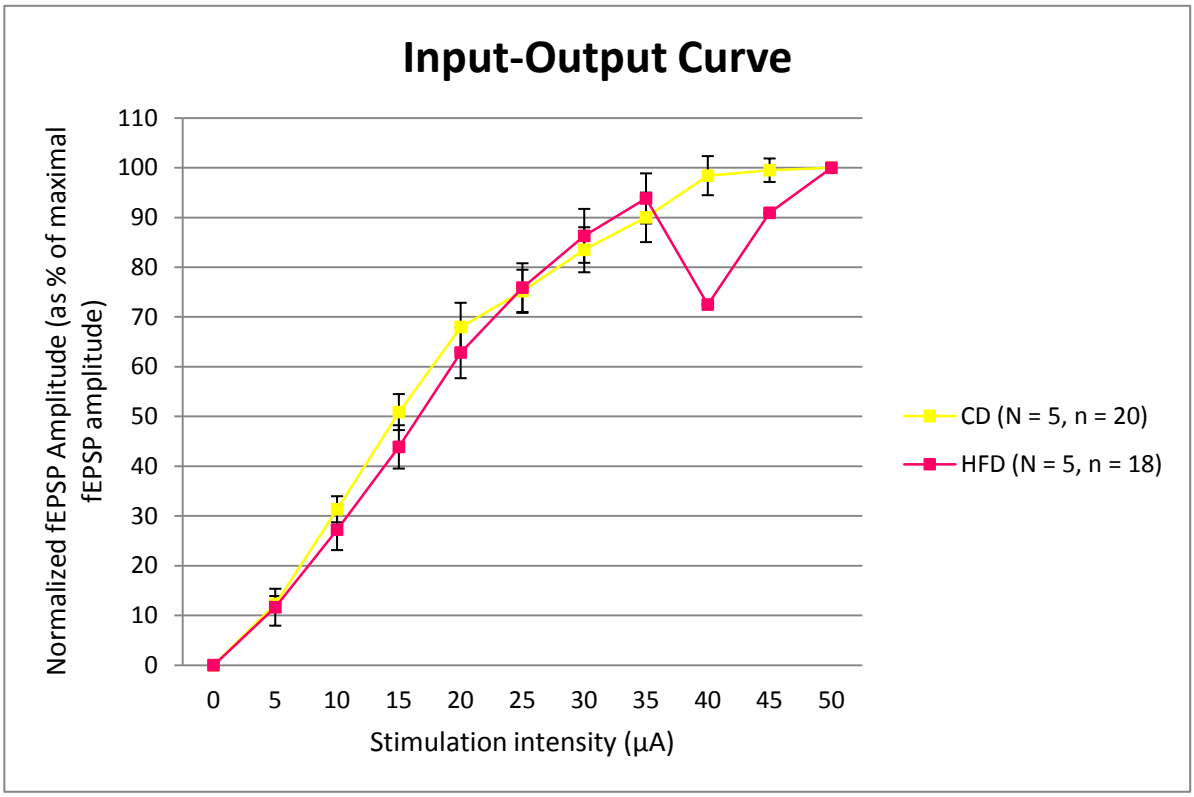
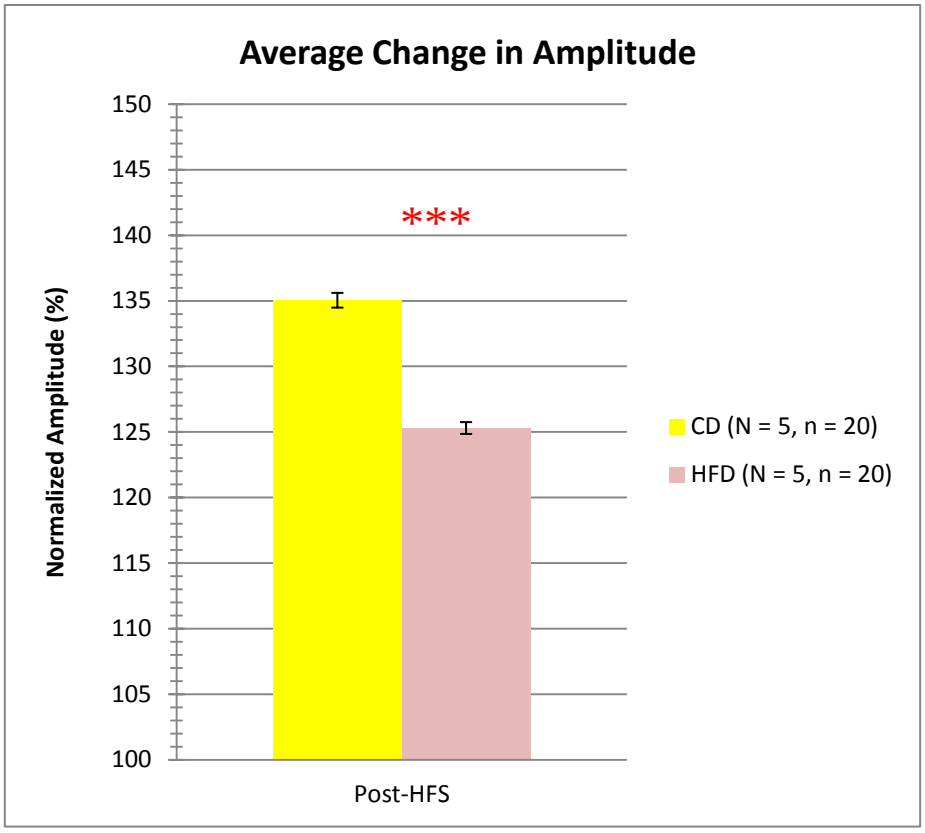


Figure 14 Input-output (IO) curves (+/-) SEM comparing female Sprague-Dawley rats eating control, or high-fat diet.



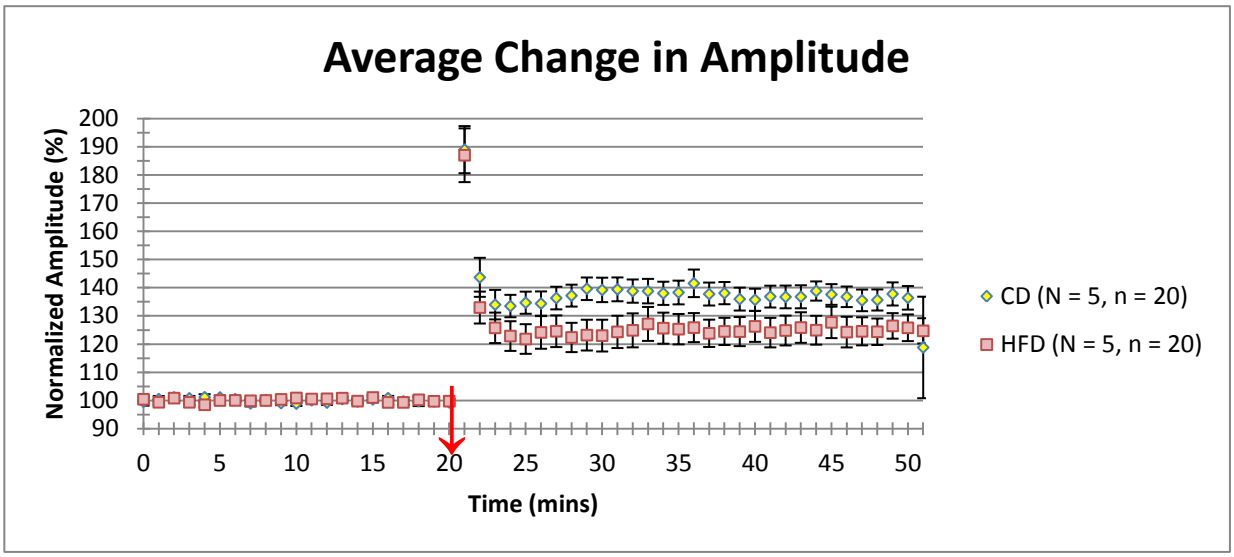
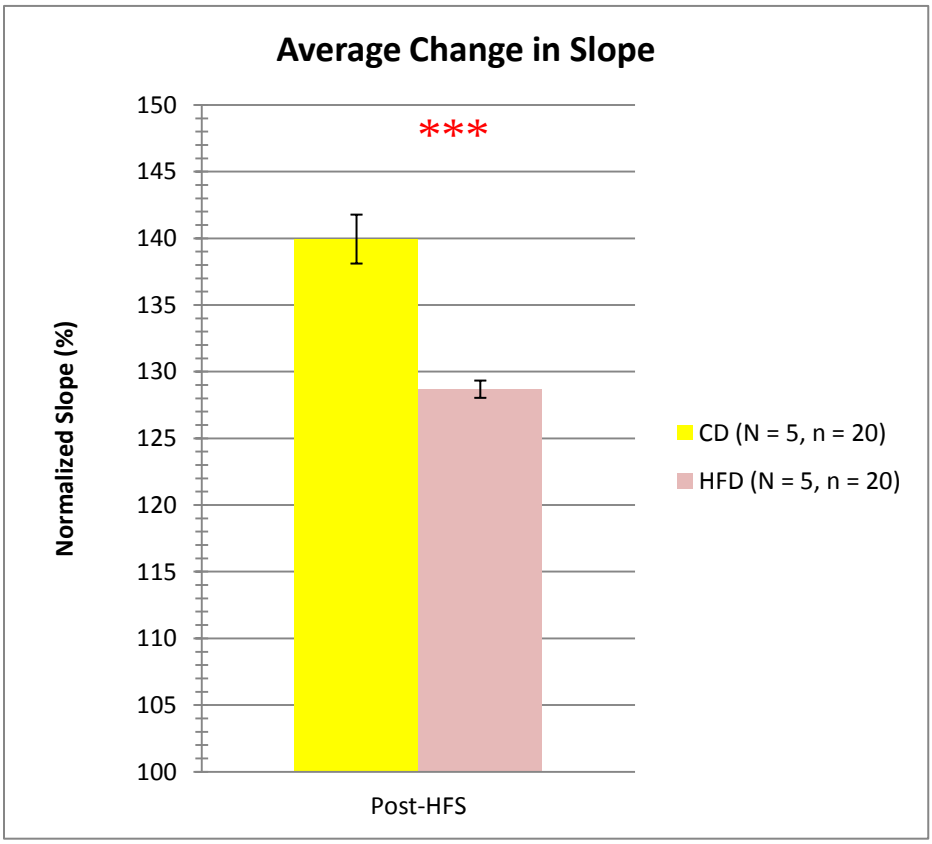


Figure 15 Mean change in amplitude of fEPSPs (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet (N = 5). Arrow indicates tetanus application. *** $p < 0.001$.



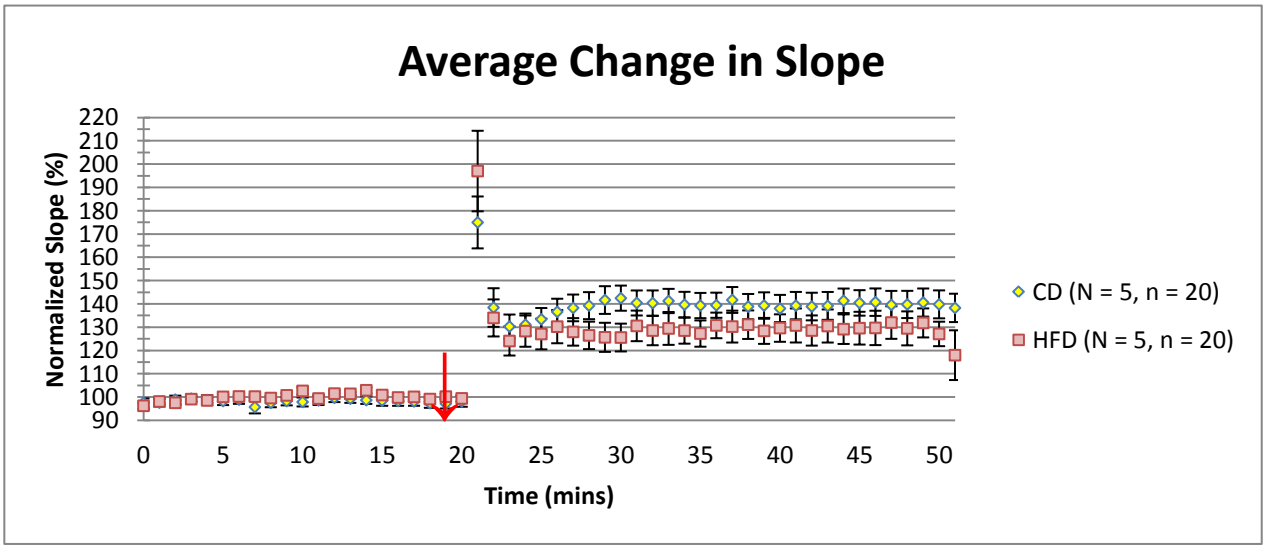


Figure 16 Mean change in slope of fEPSPs (+/-) SEM between female Sprague-Dawley rats eating control, or high-fat diet (N = 5). Arrow indicates tetanus application. *** $p < 0.001$

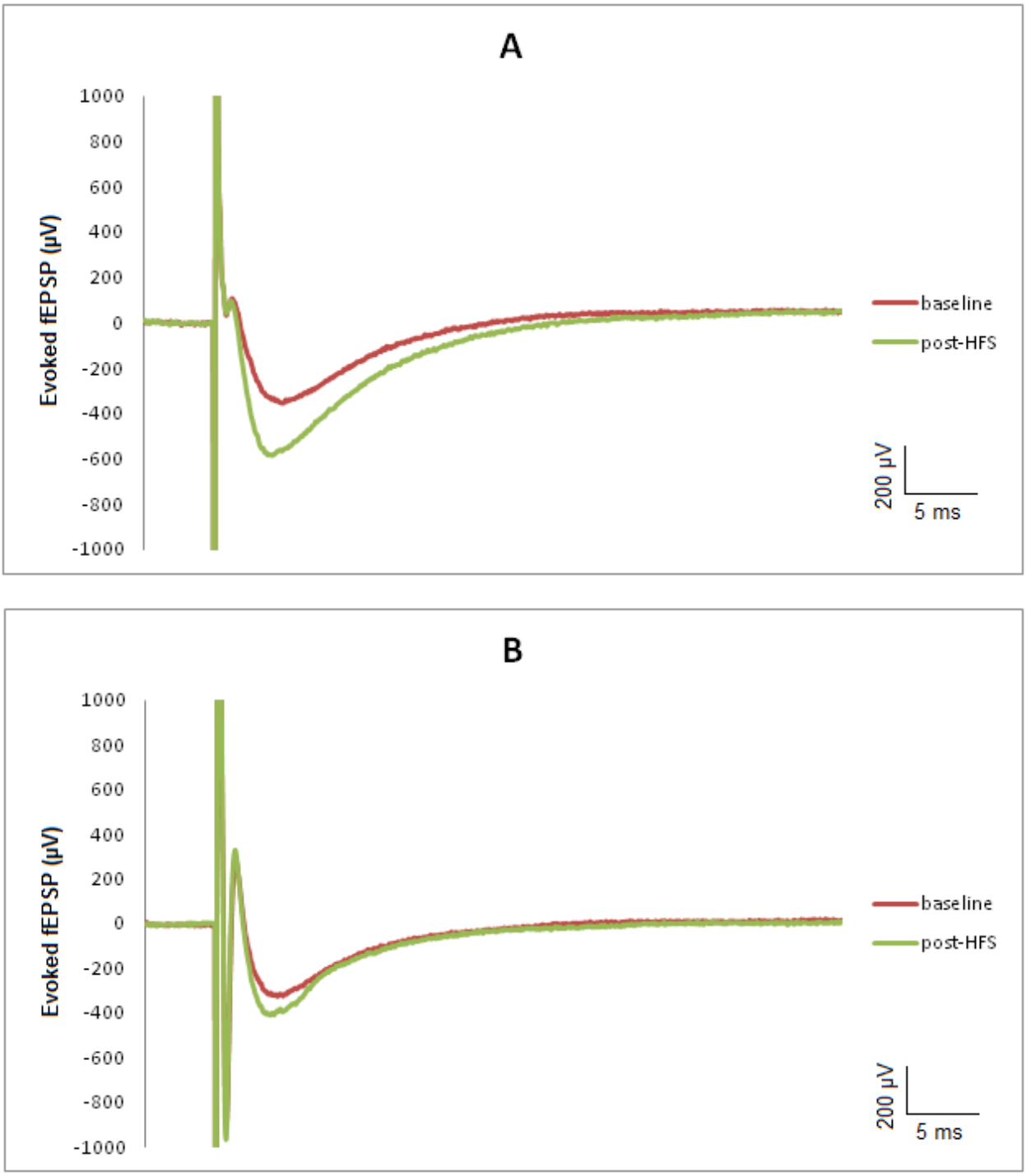


Figure 17 Superimposed fEPSPs before and after HFS (two trains of 100 Hz) from A) CD slices and B) HFD slices.

Table 2 Control and High-Fat Diet Group Characteristics

BIOMETRICS	CONTROL DIET		HIGH FAT DIET	
	Mean	Standard Error	Mean	Standard Error
total food consumption (g)	4255.00	108.70	3894.00	85.20
weekly food consumption (g)	265.94	4.69	243.39	5.10
weekly caloric consumption (kcal)	16362.00	417.90	18411.00	402.8
body weight (g)	286.80	5.95	288.80	6.91
total weight gain (g)	87.15	3.35	89.92	3.97
total weight gain (%)	38.30	1.23	42.29	2.16
rodent BMI (g/cm^2)	0.54	0.01	0.56	0.02
fasting blood glucose (mmol/L)	6.39	0.50	6.36	0.21
OGTT1 (AUC)	330.00	28.06	392.90	45.90
OGTT2 (AUC)	303.90	25.67	383.40	23.27
OGTT3 (AUC)	319.00	20.43	428.90	20.39
OGTT4 (AUC)	287.70	16.17	362.60	24.58
insulin levels (ng/mL)	0.18	0.03	0.19	0.03
leptin levels (ng/mL)	0.79	0.20	1.31	0.25
adrenal gland weight (g) *	0.02	0.00062	0.02	0.00062
retroperitoneal fat pad weight (g) *	0.47	0.03	0.65	0.04
liver weight (g) *	2.21	0.04	2.21	0.07
spleen weight (g) *	0.20	0.01	0.22	0.01
ELECTROPHYSIOLOGY				
stimulation intensity to reach maximum (μA)	30.75	1.93	32.5	2.87
slope of line of best-fit on input-output curve	2.11	0.21	1.55	0.27
normalized amplitude post-LTP (%)	135.00	1.84	125.3	0.35
normalized slope post-LTP (%)	140.00	0.30	128.70	1.26

*organ weights normalized to 100 g body weight.

Table35 Student Unpaired t-test Results (control vs. high fat diet), with Welch's correction

BIOMETRICS	Mean of differences	*p*-value	95% confidence interval
total food consumption (g)	360.6	0.03	34.06 to 687.2
weekly food consumption (g)	22.54	<0.01	8.40 to 36.68
weekly caloric consumption (kcal)	-2049.00	0.01	-3422.00 to -676.50
body weight (g)	-1.98	0.83	-20.69 to 16.73
total weight gain (g)	-2.77	0.60	-13.43 to 7.886
total weight gain (%)	-3.98	0.12	-9.13 to 1.16
rodent BMI (g/cm^2)	-0.03	0.11	-0.07 to -0.01
fasting blood glucose (mmol/L)	0.03	0.96	-1.16 to 1.22
OGTT1 (AUC)	-62.88	0.26	-175.70 to 46.96
OGTT2 (AUC)	-79.50	0.03	-150.5 to -8.55
OGTT3 (AUC)	-109.90	<0.01	-169. 0 to -50.77
OGTT4 (AUC)	-74.90	0.01	-135.6 to -14.21
insulin levels (ng/mL)	-0.07	0.86	-0.09 to 0.08
leptin levels (ng/mL)	-0.52	0.14	-0.7222 to 1.267
adrenal gland weight (g)	-0.00096	0.29	-0.00281 to 0.00088
retroperitoneal fat pad weight (g)	-0.18	0.01	-0.31 to -0.06
liver weight (g)	0.00	0.99	-0.17 to 0.18
spleen weight (g)	-0.01	0.14	-0.03 to 0.00
ELECTROPHYSIOLOGY			
stimulation intensity to reach maximum (μA)	-1.75	0.62	-8.79 to 5.29
normalized Input-Output curve (%)	0.58	0.97	-28.10 to 29.27
normalized amplitude post-LTP (%)	9.75	<0.0001	5.82 to 13.67
normalized slope post-LTP (%)	11.26	<0.0001	8.53 to 13.98

a)

	PASS (%)	FAIL (%)
CD	85	15
HFD	60	40

b)

	PASS (%)	FAIL (%)
CD	90	10
HFD	60	40

Table 4 Contingency table of slices from CD vs. HFD treatment that meet 120% potentiation threshold. a) Percent of slices meeting amplitude threshold. b) Percent of slices meeting slope threshold.

4.2 Phase 3 Results

Litter Sizes

All CD dams produced sufficient offspring for both pre-adolescent, PA, and young adult, YA, pup examinations (PA: males N = 5, females N = 5; YA: males N = 5, females N = 5). A total of N = 10 pups born from HFD-dams was tested (PA: males N = 3, females N = 1; YA: males N = 3, females N = 3). The average CD litter size was 8 (Table 6). HFD dams had very small litter sizes (average litter size was 4, Table 5). An unpaired t-test was performed on the litter sizes and showed there was no significant difference across treatment groups ($p = 0.23$).

Organ and Tissue Measurements

A two-way ANOVA was performed on adrenal gland weight, liver weight, spleen weight, and retroperitoneal fat pad weight (Table 6). Weights were normalized to 100 g body weight before analysis. The two-way ANOVA on adrenal gland weight showed no main effect of the age factor ($p = 0.08$); no main effect of the diet factor ($p = 0.79$); and no interaction between age and diet factors ($p = 0.44$). A two-way ANOVA on liver weight showed a main effect of the age factor ($p = 0.04$); no main effect of the diet factor ($p = 0.95$); and no interaction between age and diet factors ($p = 0.23$). A two-way ANOVA on spleen weight showed no main effect of the diet factor ($p = 0.98$); no interaction between age and diet factors ($p = 0.64$); but showed a main age effect alone that was significant ($p = 0.01$). A two-way ANOVA on retroperitoneal fat pad weight showed a main effect of the age factor ($p < 0.01$); no main effect of the diet factor ($p = 0.18$); and no interaction between age and diet factors ($p = 0.38$). An unpaired t-test showed no significant differences in adrenal gland weight, liver weight, and spleen weight between groups, at both ages (Table 7). An unpaired t-test showed a statistical difference between retroperitoneal fat pad weights between PA pups ($p = 0.049$), but no difference at the YA age ($p = 0.83$).

Metabolic Data

Fasting blood glucose levels were compared using measurements obtained on the date of sacrifice. A two-way ANOVA on fasting blood glucose showed no main effect of the age factor ($p = 0.27$); no main effect of the diet factor ($p = 0.60$); and no interaction between age and diet factors ($p = 0.59$, Table 6). An unpaired t-test showed no significant difference in fasting blood glucose levels between groups of PA pups ($p = 0.72$), and no significant difference between groups of YA pups ($p = 0.69$). An unpaired t-test on the OGTT performed on YA pups showed that those from HFD dams did not significantly differ from pups born to CD dams ($p = 0.13$, Table 8).

Electrophysiological Data

Electrophysiological recordings were made from 73 slices obtained from pups born to CD dams, and 35 slices from pups born to HFD dams. Of the slices from pups born to CD dams, 35 slices were obtained at the PA age category ($N = 10$), and 38 slices were from the YA category ($N = 10$). Of the slices from pups born to HFD dams, 13 slices were obtained at the PA age category ($N = 4$), and 22 slices were from the YA category ($N = 6$). There was no significant difference in the IO curves between treatment groups (PA: $p = 0.96$, Figure 18; YA: $p = 0.93$, Figure 19). Linear regression analysis found that the differences between slopes of IO curves were not significant, at the PA stage ($p = 0.90$) and YA stage ($p = 0.28$). From the fEPSPs, amplitude and slope were measured and compared between treatment groups. Slices obtained from PA pups born to HFD dams required significantly greater stimulation intensities to evoke a maximum response ($p < 0.01$), but there was no significant difference at the YA age ($p = 0.41$). A two-way ANOVA was performed on normalized amplitude and normalized slope post-HFS. The two-way ANOVA (Table 6) on amplitude post-HFS showed no main effect of the age factor

($p = 0.95$); no main effect of the diet factor ($p = 0.42$); and no interaction between age and diet factors ($p = 0.99$). The two-way ANOVA on slope post-HFS showed no main effect of the age factor ($p = 0.64$); no main effect of the diet factor ($p = 0.37$); and no interaction between age and diet factors ($p = 0.94$). Unpaired t-tests were also conducted. PA pups born from HFD dams had an average of 7.5% less amplitude potentiation (Figure 20, $p < 0.05$), and an average of 4% more slope potentiation (Figure 21, $p < 0.05$) than pups born CD dams. Baseline values were not significantly different between groups at the YA age. YA pups born from HFD dams had an average of 30% less amplitude potentiation (Figure 22, $p < 0.05$) and an average of 24% less slope potentiation (Figure 23, $p < 0.05$) than pups born CD dams.

The Fischer's exact test was performed on the percent of slices that passed or failed reaching the threshold limit of 120% of baseline (Table 8). There was no significant relationship between percent of PA slices meeting amplitude threshold of pups born from HFD dams to those of pups born to CD dams (Table 8a, $p = 0.7161$). There was a significant relationship between percent of PA slices meeting slope threshold of pups born from HFD dams to those of pups born from CD dams (Table 8b, $p = 0.0008$). There was a significant relationship between percent of YA slices meeting amplitude threshold of pups born from HFD dams to those of pups born from CD dams (Table 8c, $p = 0.0010$). There was no significant relationship between percent of YA slices meeting slope threshold of pups born from HFD dams to those of pups born from CD dams (Table 8d, $p = 0.3523$).

Table 5 Litter ratios of female Sprague-Dawley rats eating control or high-fat diet.

	Dam	Male Pups	Female Pups	Total Pups	Average	SEM
CD	1	4	5	9		
	2	2	2	4		
	3	5	2	7		
	4	9	7	16		
	5	2	2	4		
					8	2.214
HFD	1	0	0	0		
	2	0	0	0		
	3	5	1	6		
	4	4	1	5		
	5	5	5	10		
					4.2	1.908

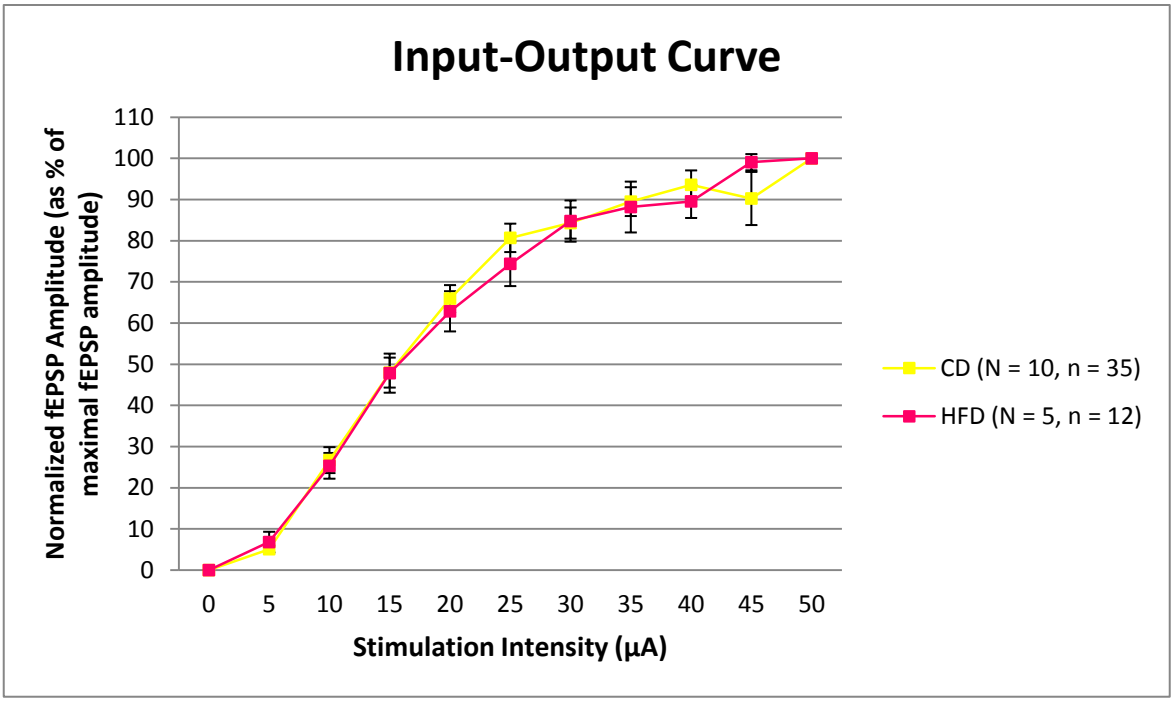


Figure 18 Input-output (IO) curves (+/-) SEM between pre-adolescent pups born to Sprague-Dawley dams eating control, or high-fat diet.

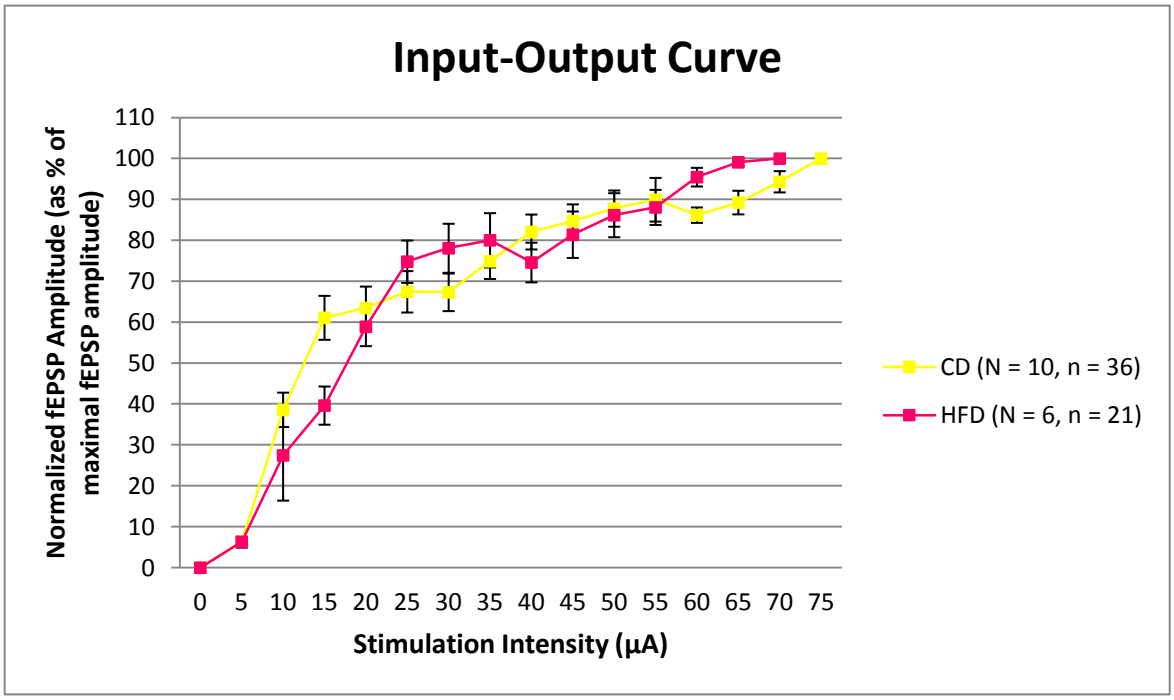
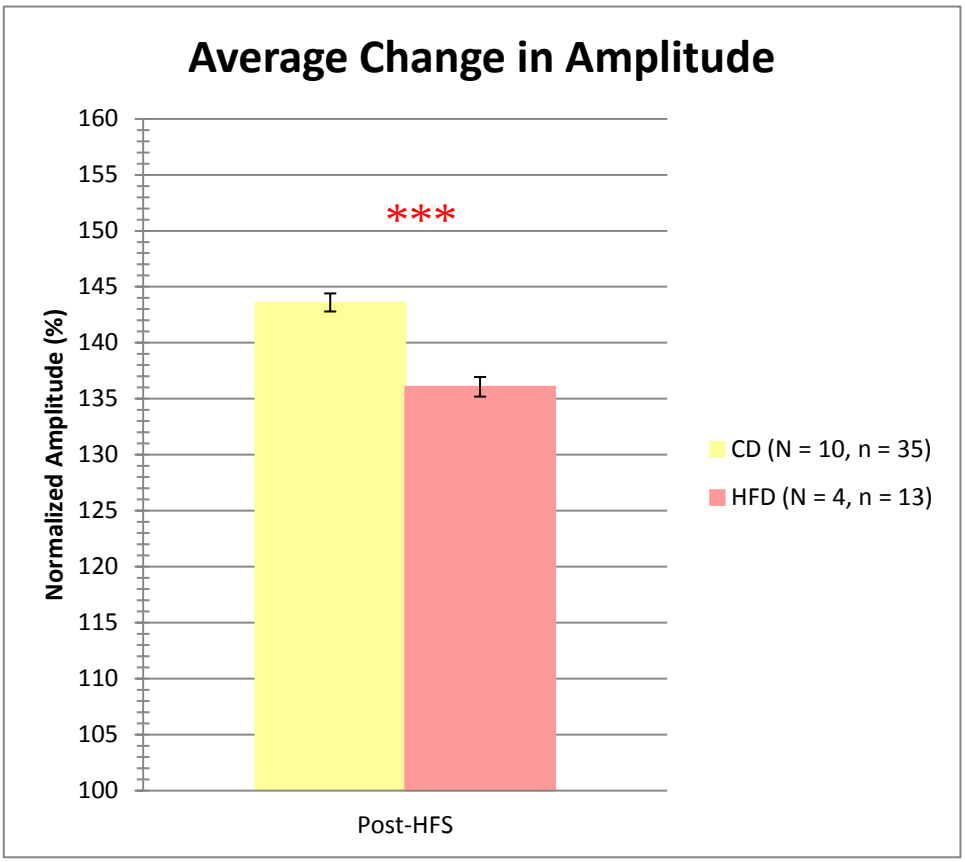


Figure 19 Input-output (IO) curves (+/-) SEM between young adult pups born to Sprague-Dawley dams eating control, or high-fat diet.



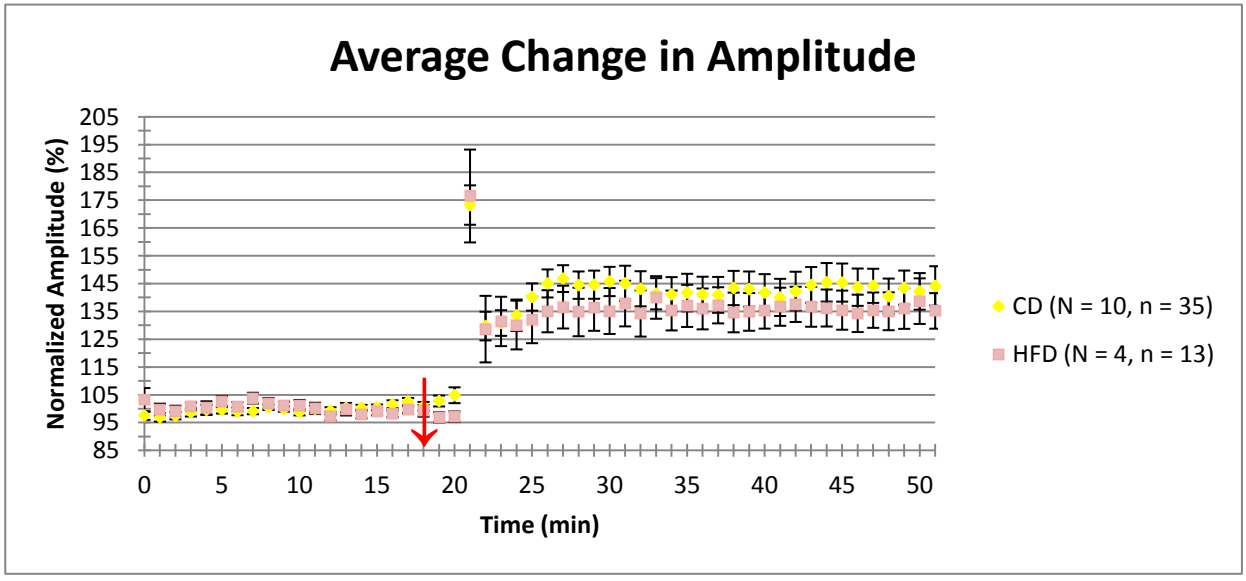
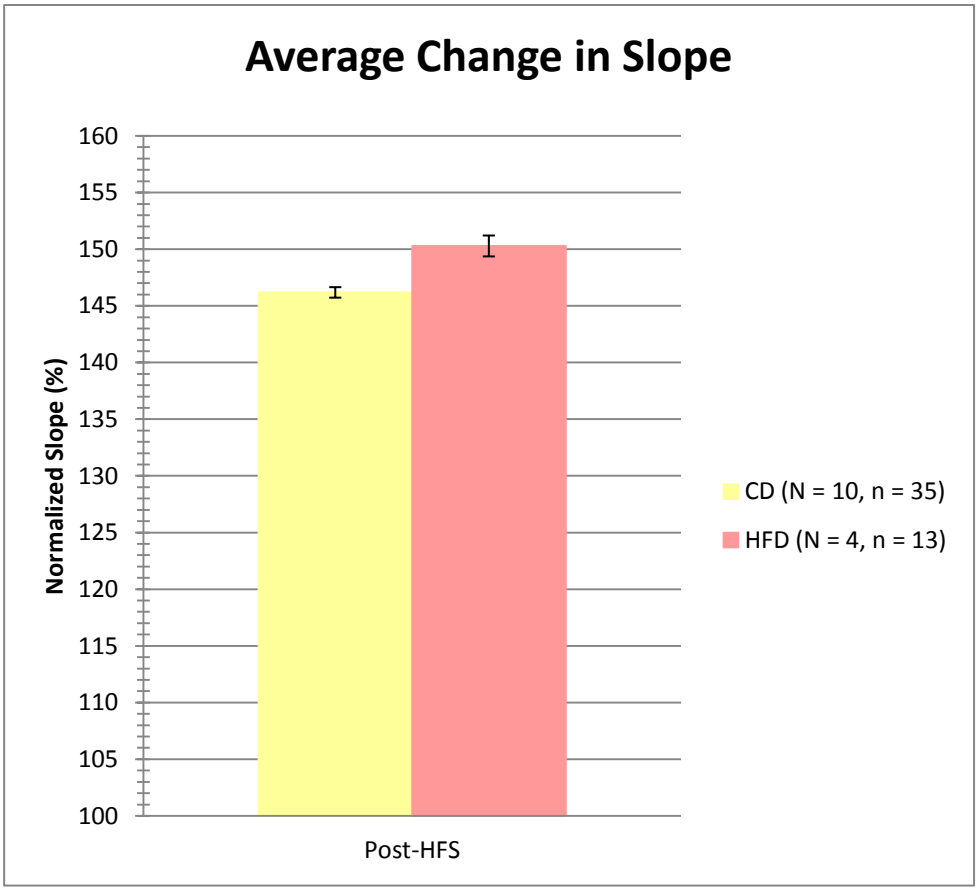


Figure 20 Mean change in amplitude of fEPSPs (+/-) SEM between pre-adolescent pups born to Sprague-Dawley dams eating control, or high-fat diet. Arrow indicates tetanus application. *** $p < 0.001$



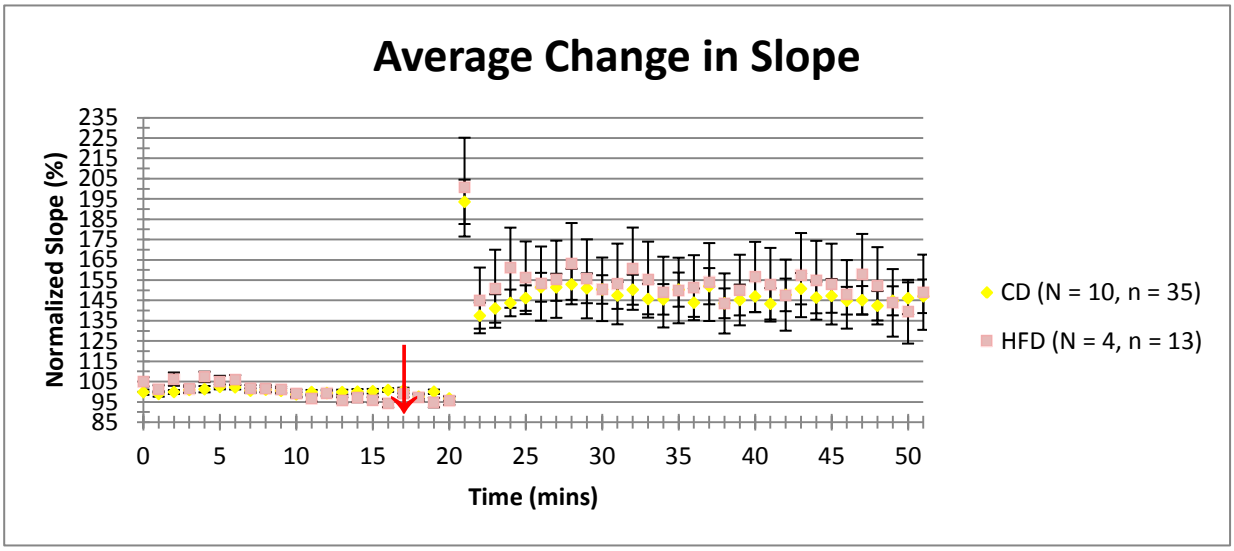
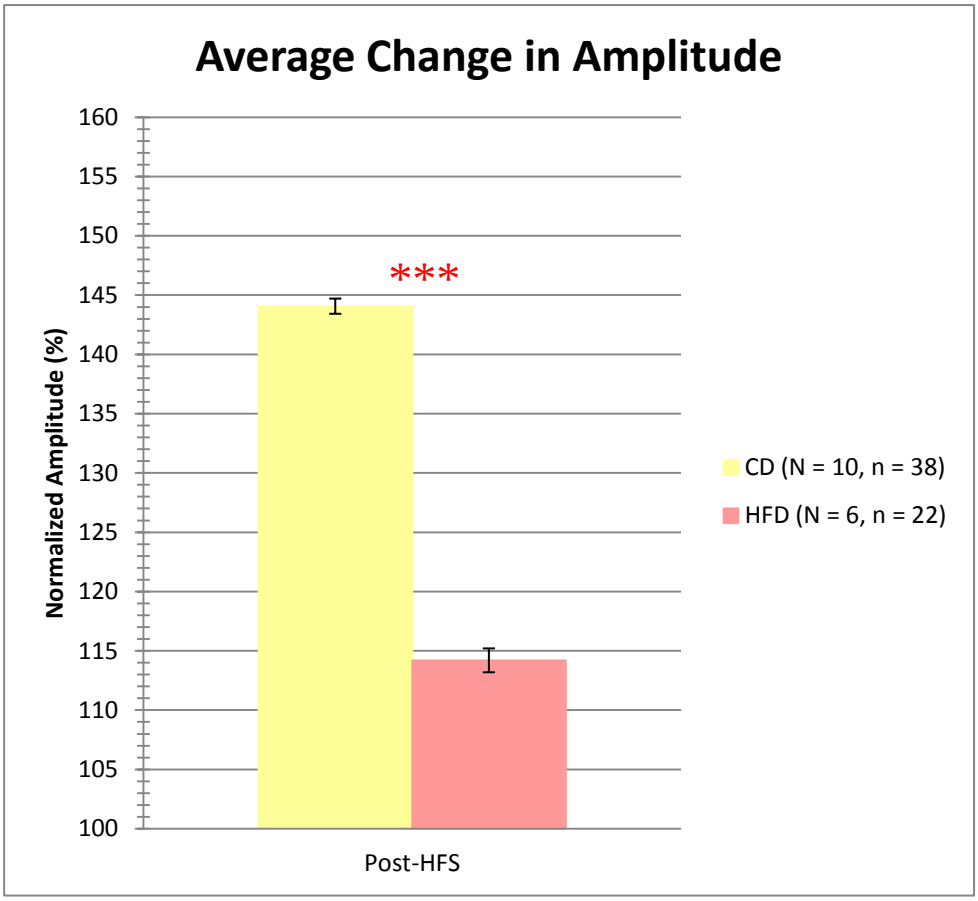


Figure 21 Mean change in slope of fEPSPs (+/-) SEM between pre-adolescent pups born to Sprague-Dawley dams eating control, or high-fat diet. Arrow indicates tetanus application.



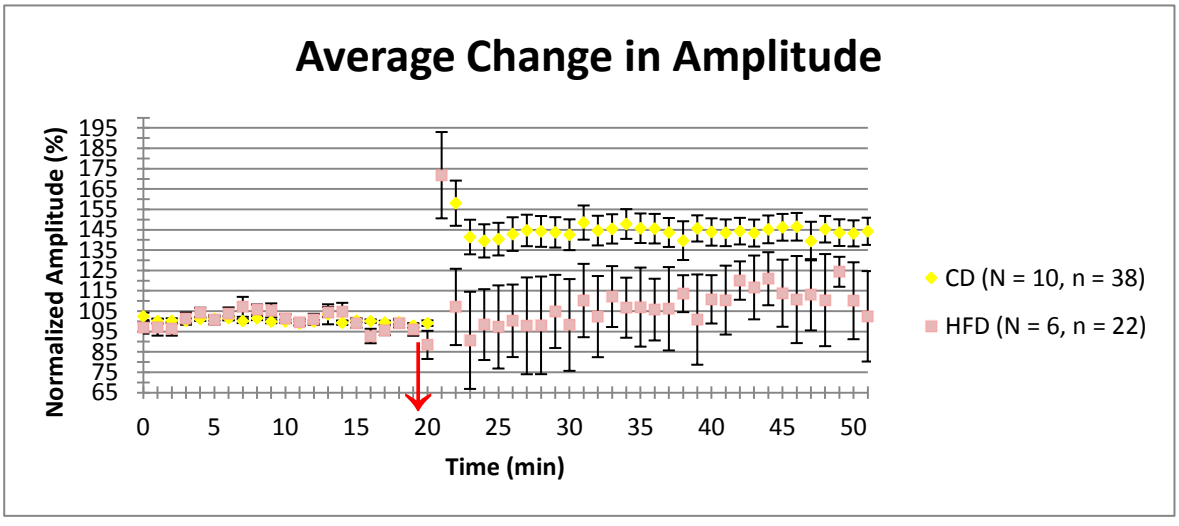
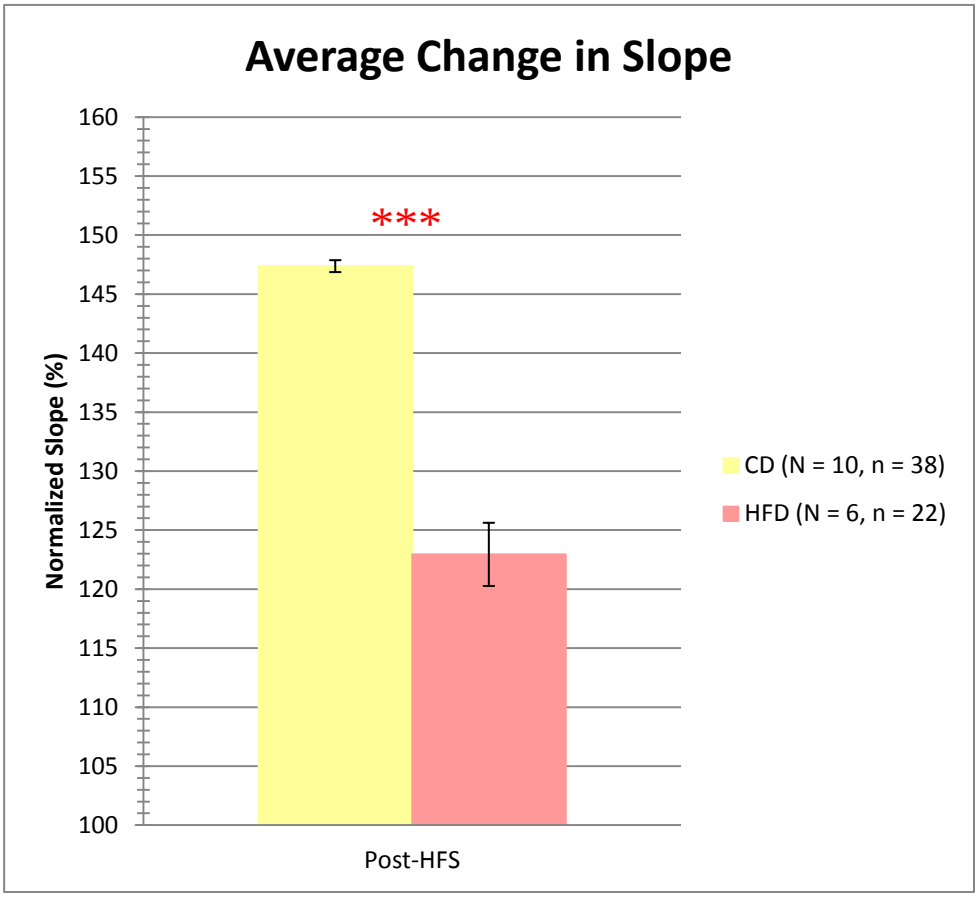


Figure 22 Mean change in amplitude of fEPSPs (+/-) SEM between young-adult pups born to Sprague-Dawley dams eating control, or high-fat diet. Arrow indicates tetanus application. *** $p < 0.001$



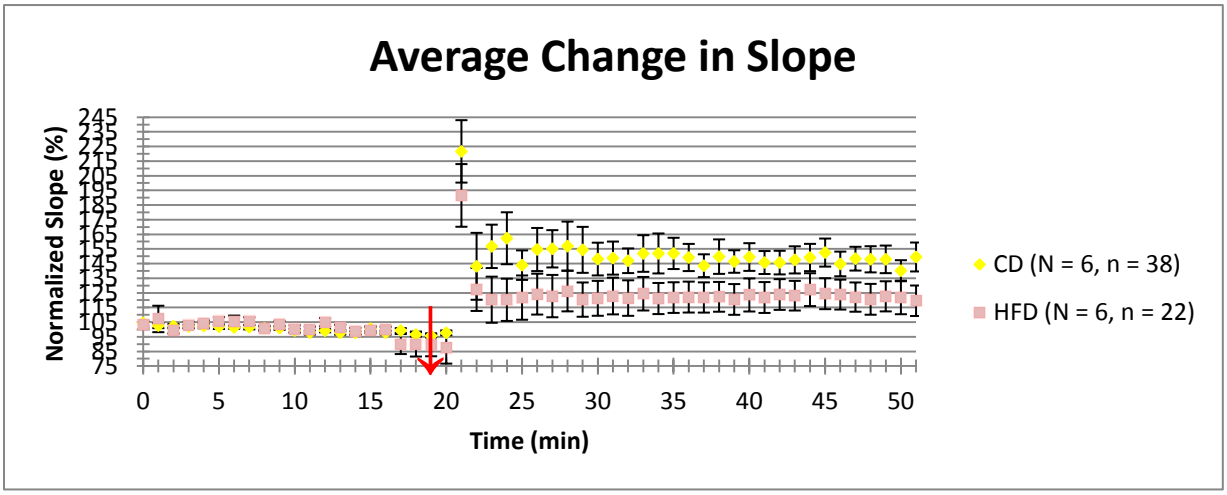


Figure 23 Mean change in slope of fEPSPs (+/-) SEM between young-adult pups born to Sprague-Dawley dams eating control, or high-fat diet. Arrow indicates tetanus application. *** $p < 0.001$

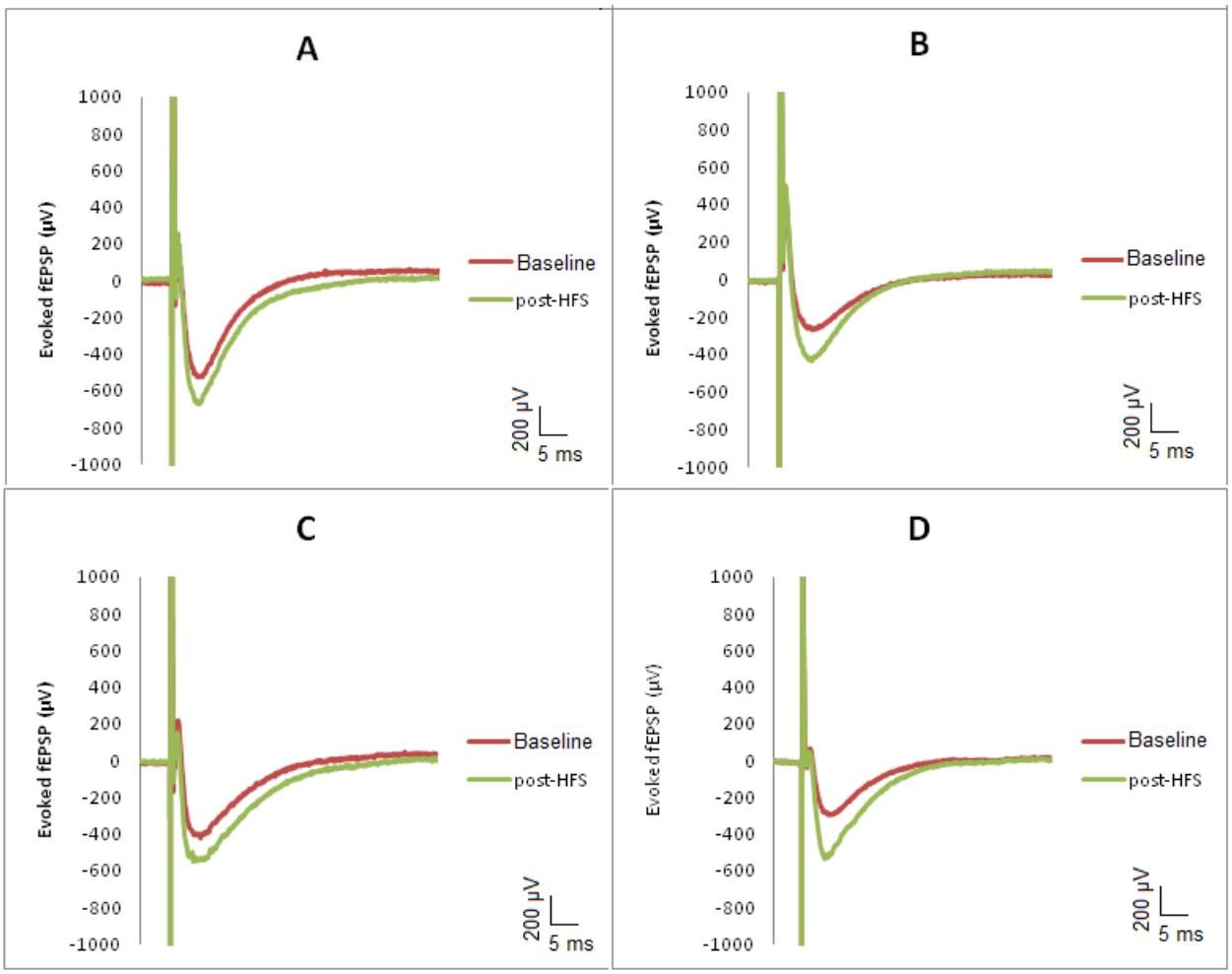


Figure 24 Superimposed fEPSPs before and after HFS (two 1 s trains of 100Hz stimulation) from A) PA-CD slices, B) YA-CD slices, C) PA-HFD slices, D) YA-HFD slices

Table 6 Pup Biometrics Summary - Two-way ANOVA Analysis

Measurement	ANOVA table					
		SS	DF	MS	F	P value
rodent BMI (g/cm^2)	Interaction	31.78×10^{-5}	1	0.0003178×10^{-5}	10.50×10^{-2}	0.76
	Age	24.58×10^{-3}	1	0.02458×10^{-5}	81.18×10^{-3}	0.05
	Diet	99.41×10^{-5}	1	0.0009941×10^{-5}	32.84×10^{-3}	0.60
	Residual	12.11×10^{-3}	4	0.003027×10^{-5}		
fasting blood glucose (mmol/L)	Interaction	18.60×10^{-2}	1	18.60×10^{-2}	34.43×10^{-2}	0.59
	Age	88.44×10^{-2}	1	0.8844×10^{-5}	16.37×10^{-3}	0.27
	Diet	17.40×10^{-2}	1	0.1740×10^{-5}	32.21×10^{-3}	0.60
	Residual	21.61×10^{-1}	4	0.5404×10^{-5}		
retroperitoneal fat pad weight (g)*	Interaction	10.20×10^{-4}	1	10.20×10^{-4}	95.35×10^{-3}	0.38
	Age	56.43×10^{-3}	1	56.43×10^{-3}	52.72×10^{-3}	<0.01
	Diet	28.19×10^{-4}	1	28.19×10^{-4}	26.34×10^{-3}	0.18
	Residual	42.81×10^{-4}	4	10.70×10^{-4}		
adrenal gland weight (g)*	Interaction	3.44×10^{-5}	1	34.40×10^{-6}	73.80×10^{-2}	0.44
	Age	25.44×10^{-5}	1	25.44×10^{-5}	54.59×10^{-3}	0.08
	Diet	39.59×10^{-7}	1	39.59×10^{-7}	84.95×10^{-3}	0.79
	Residual	18.64×10^{-5}	4	46.61×10^{-6}		
liver weight (g)*	Interaction	26.42×10^{-2}	1	26.42×10^{-2}	19.95×10^{-1}	0.23
	Age	11.96×10^{-6}	1	11.96×10^{-2}	90.29×10^{-3}	0.04
	Diet	59.88×10^{-5}	1	59.88×10^{-5}	45.21×10^{-4}	0.95
	Residual	52.98×10^{-2}	4	13.24×10^{-2}		
spleen weight (g)*	Interaction	40.35×10^{-5}	1	40.35×10^{-5}	26.54×10^{-2}	0.64
	Age	30.08×10^{-3}	1	30.08×10^{-3}	19.78×10^{-5}	0.01
	Diet	13.97×10^{-7}	1	13.97×10^{-7}	91.087×10^{-5}	0.98
	Residual	60.82×10^{-4}	4	15.21×10^{-4}		
normalized amplitude post-LTP (%)	Interaction	78.20×10^{-3}	1	78.20×10^{-3}	20.73×10^{-5}	0.99
	Age	12.87×10^{-6}	1	12.87×10^{-6}	34.11×10^{-4}	0.95
	Diet	25.52×10^{-4}	1	255.20	67.67×10^{-2}	0.42
	Residual	9052.00	23	377.20		
normalized slope post-LTP (%)	Interaction	2.942×10^{-5}	1	4.94	50.04×10^{-4}	0.94
	Age	222.0×10^{-5}	1	222.00	22.49×10^{-2}	0.64
	Diet	831.0×10^{-5}	1	831.00	84.16×10^{-2}	0.37
	Residual	22709 .00	23	987.30		

*organ weights normalized to 100 g body weight.

Table 7 Pup Biometrics - Student unpaired t-test results (control vs. high fat diet) with Welch's correction

	Pre-Adolescent			Young Adult		
BIOMETRICS	Mean of differences	*p*-value	95% confidence interval	Mean of differences	*p*-value	95% confidence interval
fasting blood glucose (mmol/L)	0.25	0.72	-1.25 to 1.74	-0.30	0.69	-1.95 to 1.35
OGTT (AUC)				-81.67	0.13	-195.20 to 31.90
retroperitoneal fat pad (g)*	-0.069	0.049	-0.1392 to 0.0012	-0.008	0.83	-0.089 to 0.073
adrenal gland weight (g)*	<0.01	0.053	<-0.01 to 0.02	<-0.01	0.35	-0.01 to <0.01
liver weight (g)*	0.28	0.17	-0.14 to 0.69	-0.061	0.70	-0.41 to 0.29
spleen weight (g)*	0.04	0.34	-0.05 to 0.12	-0.02	0.40	-0.08 to 0.04
ELECTROPHYSIOLOGY (%)						
stimulation intensity to reach maximum (μA)	-13.55	<0.01	-20.56 to -6.536	-4.21	0.41	-14.47 to 6.06
normalized Input-Output curve (%)	0.79	0.96	-28.41 to 30.00	-0.94	0.93	-22.50 to 20.63
normalized amplitude post-LTP	7.49	<0.0001	6.150 to 8.832	29.87	<0.0001	25.13 to 34.60
normalized slope post-LTP	-4.26	0.056	-8.67 to 0.14	24.43	<0.0001	21.78 to 27.07

*organ weights normalized to 100 g body weight.

a)

Slices	PASS (%)	FAIL (%)
CD	83	17
HFD	69	31

b)

Slices	PASS (%)	FAIL (%)
CD	77	23
HFD	46	54

c)

Slices	PASS (%)	FAIL (%)
CD	82	18
HFD	64	36

d)

Slices	PASS (%)	FAIL (%)
CD	63	37
HFD	50	50

Table 8 Contingency table of slices from CD vs. HFD treatment that meet 120% threshold. a) Percent of pre-adolescent pup slices meeting amplitude threshold. $p = 0.7161$ b) Percent of pre-adolescent pup slices meeting slope threshold. $p = 0.0008$ c) Percent of young adult pup slices meeting amplitude threshold. $p = 0.0010$ d) Percent of young adult pup slices meeting slope threshold. $p = 0.3523$

5.0 Discussion

To our knowledge, this is the first study to evaluate the impact of maternal obesity on synaptic function in the maternal and offspring hippocampus. We first sought to establish a diet regimen that would induce obesity in female Sprague-Dawley rats. Secondly, we aimed to determine the effects of obesity on the maternal generation's hippocampal synaptic function. Lastly, we wanted to investigate the effects of maternal obesity on offspring hippocampal synaptic function.

Obesity is a growing global health problem. Similarly, the increasing rate of maternal obesity is also a concern. Maternal obesity can result in negative outcomes for both the mother and the fetus, and, with advances in research reinforcing the significance of the prenatal environment, all aspects of maternal obesity need to be explored. The present study expands upon previous maternal obesity research in that it confirms obesity effects on cognition in the maternal generation, and fills in the gap between the observed structural changes (for example, hippocampal volume, neurogenesis, and organization of cells) and behavioral changes (such as decreased performance in spatial memory tests) in offspring from maternal obesity.

Although the quantity of obesity research is increasing, there is not yet a standardized HFD model for inducing obesity in an animal model (Buettner 2007). As well, many rodent studies using HFD treatment groups do not specifically aim to establish an obese phenotype, but simply assess the effect of the diet. As a result, the change in phenotype caused by our HFD protocol needed to be evaluated in order to investigate the consequences of obesity on synaptic function in the offspring.

The purpose of this animal study was to determine what effects maternal obesity may have on synaptic transmission, a measure of brain function. Specifically, we looked at the CA1 region of the hippocampus, an area with particularly robust synaptic plasticity. Synaptic

plasticity was evaluated using LTP since it is an established experimental model for examining the synaptic mechanisms behind learning and memory (see section 2.5).

Confirming an Obese Phenotype in the Maternal Generation

One of the typical barriers in most studies is translating results from a rodent model to humans. For instance, most humans do not consistently consume the same thing every day, and instead have a diet gathered from different sources (resulting in a variable diet composition). However, this inconsistent behavior cannot be accurately reproduced in animal models via a cafeteria-style diet (where the foods provided are from a variety of sources). Cafeteria-style diets also compromise the accuracy in food consumption calculations, and, consequently, using a defined diet was more appropriate for this study.

A significant positive relationship has been shown between the amount of dietary energy from fat and the proportion of the population that is overweight, and clinical studies show a positive relationship between level of dietary fat and body-weight gain, and reduction in dietary fat and weight loss (Hariri and Thibault 2010). In addition, human studies have shown that diets rich in saturated fat are more obesogenic than diets containing mono- or poly-unsaturated fatty acids (DeLany 2000, Piers 2003). Animal studies have also shown saturated fat to be more obesogenic (Yaqoob 1997, Ellis 2002). Fat content of diets used in DIO research vary within the literature, ranging from 13% to 85% (Hariri and Thibault 2010). To mimic the human condition and what has been referred to as the 'Western diet', several studies (including ours) use 40-45% calories from saturated fat, which is the proportion of fat that would best resemble that consumed in the 'Western diet' (Buettner 2007). Though diets of high-sucrose content also exist in DIO research, the higher palatability of the diet may affect food consumption and therefore could confound several of our measurements. Rats have a sensitivity for glucose polymers, and

are shown to exhibit a preference for saccharide solutions, such as those with maltose or sucrose, over water (Sclafani and Nissenbaum 1987).

In this study, a diet with 45% of calories from saturated fat administered to female Sprague-Dawley rats resulted in an obese phenotype. While we did not detect a statistically significant effect of the diet on adrenal gland, liver, or spleen weights, after 16 weeks, animals showed a trend toward greater total and percentage weight gained, and statistically significant differences in retroperitoneal fat pad weights and OGTT outcomes (at 8, 12, and 16 weeks). Cumulative food consumption data indicated that HFD animals increasingly ate less than CD animals as time passed. The differences in total weight gained, when paired with the significantly greater fat pad weights, indicate that our HFD animals had an altered fat to lean muscle mass ratio. Since the OGTT was performed as a means for measuring glucose metabolism, statistically significant differences in the results of the OGTT suggest that the HFD animals had compromised glucose metabolism compared to CD animals. Our study also detected a trend that seemed to suggest a difference in serum leptin concentration, with higher levels being observed in HFD animals. The alterations to body composition, and the metabolic changes, observed in our HFD animals strongly suggest that our treatment resulted in an obese phenotype.

To further confirm that an obese phenotype was established, other measures could also have been evaluated. For example, conducting measures on physical activity and the metabolic rates of our rats could reconcile the significantly greater caloric consumption in the HFD rats with the lack of difference in body weight. Also, future trials using the same treatment protocol could examine fat cell size. Adipocyte hypertrophy is seen with obesity (refer to 2.1.3 C). Triglyceride content could also be measured, since higher levels of triglycerides exist in the metabolic syndrome (Matyskova et al 2007, Yamato et al 2007). Measuring triglyceride levels in

our animals would also be desirable since Banks and colleagues have shown that triglycerides can impair the transport of leptin across the blood-brain barrier and may contribute, in part, to peripheral leptin resistance (Banks et al 2004). Because leptin may enhance cognition (Harvey 2007), and leptin-receptor deficient animals have impaired hippocampal LTP and poor spatial memory (Li et al 2002), triglycerides are particularly important to measure because they can prevent leptin from reaching brain areas important for learning and memory (i.e., hippocampus). Since obesity is also characterized as a systemic inflammatory condition, peripheral cytokines (from liver, fat, and serum) should also be measured (Bilbo and Tsang 2010).

Diet-Induced Changes in Synaptic Plasticity Among Female Rats

There are no studies investigating the effects of obesity on synaptic plasticity in female rat hippocampus. The majority of obesity research has focused on the hypothalamus, but more recent studies suggest that the complications of obesity may also affect the integrity of the hippocampus (Farr et al, 2008, Li et al 2002, Molteni et al 2002, Ogden et al 2006). For example, impaired hippocampal neurogenesis that resulted in a decreased number of newly generated cells in the DG, altered differentiation and proliferation of neural progenitor cells in the DG, and decreased apoptosis in specific hippocampal areas (Ammon's horn and fimbria). However, the structural changes found in these studies do not reveal the full relationship between obesity and memory. Epidemiological evidence has found associations between obesity and impaired cognitive performance and memory (refer to section 4.4.1). Consequently, there is a need to examine the effects of obesity on female rat hippocampal synaptic plasticity.

Input-output curves provide information about the basal synaptic transmission of the pyramidal cells, as they illustrate the relationship between the intensity of the stimulation delivered and the amplitude of the evoked fEPSP (Woolley et al 1997). Our observations of IO

curves showed that there was no difference in baseline synaptic transmission between diet groups. Results from electrophysiological recordings also showed that HFS was able to cause significantly less potentiation in both amplitude and slope of the fEPSP in HFD animals compared to CD animals. Specifically, HFD animals showed an average of 10% less potentiation in measured amplitude and 11% less potentiation in measured slope relative to fEPSPs of CD animals. The success rate of LTP induction was also compared across treatment groups. The success rates of slices reaching the pre-determined amplitude and slope threshold (i.e., at least a 20% increase relative to baseline) were also significantly different between CD and HFD animals. While 85% of slices from CD animals reached the amplitude threshold, only 60% of slices from HFD animals reached the threshold. In terms of slope, 90% of slices from CD animals reached the threshold, whereas only 60% of slices from HFD animals reached threshold. Taken together, the electrophysiological findings indicate that hippocampal synaptic plasticity was affected by our dietary treatment. Although there is no literature on DIO effects on the synaptic plasticity of the female rat hippocampus, the findings from our study agree with previous evidence that demonstrated HFD consumption and obesity can negatively affect learning and memory (refer to section 2.3.2b). In particular, Greenwood and Winocur (2005) fed male rats a diet of 40% calories from saturated fat for 3 months, and saw consistently lower performances on the three tests of learning and memory (of which, the water maze tested hippocampal-dependent spatial memory). Farr and colleagues (2008) found that their obese male mice (identified by having as 30% greater body weight relative to controls) performed more poorly than normal mice on hippocampal-dependent water maze and T maze tests, and also failed to maintain LTP.

Our study has demonstrated that HFD may compromise synaptic plasticity in female animals. As mentioned previously, additional measures such as triglyceride and cytokine levels need to be evaluated in order to reveal the possible mechanisms behind the observed electrophysiological phenomena in this study. While the exact mechanisms for the observed associations between obesity and cognition and memory deficit have not been defined, BDNF has been suggested. BDNF is abundant in the hippocampus and has a significant role in the survival, maintenance, and differentiation of neurons - it acts on certain neurons, helping support the survival of existing neurons and encouraging the growth and differentiation of new neurons and synapses (Tozuka et al 2010). BDNF expression is increased in the hippocampus of animals that learn a spatial memory task (Mizuno et al 2000), and animals with decreased BDNF levels show deficits in learning and memory (Linnarsson et al 1997). BDNF facilitates LTP induction by enhancing synaptic response to tetanus stimulation, most likely due to BDNF regulation of synaptic vesicle mobilization and docking (Jovanovic et al 2000, Rex et al 2006, Yano et al 2006). Notably, brain inflammation associated with HFD has been shown to decrease BDNF levels (Molteni et al 2002, Tozuka et al 2010). Therefore, future trials should investigate what role BDNF may have had in our study, as BDNF has been reported to be negatively regulated by cytokines, and, as a neurotrophin, has large potential to be involved in the observed altered synaptic function in this study (Poo 2001, Yamada and Nabeshima 2003, Martinowich et al 2007).

Long-term potentiation can be divided into an early phase (E-LTP, that lasts up to 2 hours) and later phase (L-LTP, that lasts 8 hours and up to days) (Lu et al 2008). The current set of experiments examined only E-LTP. As our pilot study established a difference in potentiation between HFD and CD animals, and among their respective offspring, it would be interesting to

investigate memory via induction of L-LTP. E-LTP and L-LTP involve different, although partially overlapping, biochemical pathways that lead to distinct changes at synapses (Lu et al 2008). Differences seen in L-LTP would imply changes at the transcription and de novo protein synthesis level. If future experiments are to examine the role of BDNF, L-LTP needs to be employed. Patterson and colleagues (1992) have shown that stimulation of Schaffer collaterals with HFS could increase BDNF mRNA in postsynaptic CA1 neurons. As mentioned, BDNF plays an important role in the proliferation, differentiation, and survival of neurons. Since BDNF levels can be measured using hippocampal homogenates, it would be interesting to determine whether HFD would decrease BDNF regulation, something which L-LTP could explore.

Transgenerational Effects of Maternal Obesity

There are no studies investigating the effects of maternal obesity on the synaptic plasticity of the offspring hippocampus. Experimental animal studies have demonstrated structural changes in the hippocampus of offspring from high-fat fed mothers (Niculescu 2009, Tozuka et al 2009, Park et al 2010), but, as previously explained, the structural changes are insufficient in signifying a change in synaptic function.

The third phase of our longitudinal study investigated the pup generation, and examined two factors – diet (pups born from HFD dams or CD dams) and age (pre-adolescent, PA, or young adult, YA). The uneven sample number of female offspring (too few from either diet group) prevented analysis at the factor level of sex; therefore, this factor was removed from the ANOVA analysis. Drake and Reynold (2010) have reviewed the evidence from several human and animal studies to show that maternal obesity is associated with programming obesity and metabolic risk in the offspring. Although most of our biometrics showed no interaction between diet and age factors, some p values approached statistical significance and suggested a trend,

which, given the small sample size of the HFD pups, warrants further study to determine whether the model can produce offspring with an obese phenotype.

Our data showed no interaction between diet and age in terms of normalized fEPSPs of post-HFS recordings. IO curve analyses further indicated that baseline synaptic transmission between pups was not different. At the PA stage, pups born from CD dams averaged 7% more potentiation in fEPSP amplitude than pups born from HFD dams. Diet-related differences in potentiation were much greater in the YA stage. Possibly, the impact of HFD on potentiation of fEPSP did not become apparent until a later age. These observations are consistent with other studies that identified age-dependent effects of exposures (e.g., nutrient deficiencies, toxin exposure), where older animals were more affected than younger animals (Queen et al 1993, Lu et al 2007). However, due to the small sample size of the current study, interpretations of the observed differences need to be made with caution. There were initially no reference values in the literature to help calculate a sufficient sample size. The large 95% confidence intervals of several of our measures was an indication that sample size was small, suggesting that the precision may be lacking in our observations. Moreover, using an average of the effect size and the standard deviations obtained from our pilot study, we were able to calculate that 22 animals would be needed in each group for phase 3 of our study (National Research Council Committee on Guidelines for the Use of Animals in Neuroscience and Behavioural Research, 2003). Consequently, the observations in this study do not permit conclusions regarding changes in fEPSP potentiation and age.

The potentiation success rate was also calculated for this phase of our study. The success rate of slices reaching the slope threshold was significantly different between slices from CD animals and HFD animals in the pre-adolescent stage, and the success rate of slices reaching

amplitude threshold was significantly different between slices from CD animals and HFD animals in the young adult stage. Given that our pilot study lacked a sufficient sample size, the significant differences in slope and amplitude success rates between age groups warrants further trials.

Future trials of this study should measure cytokine levels and microglial activation markers. Bilbo and Tsang (2010) found that offspring from dams fed a HFD (60% calories from saturated fat) showed increased peripheral cytokines and increased hippocampal IL-1b responses at birth and in adulthood, indicating that systemic inflammation had been programmed into the offspring. They argued that maternal HFD primed microglial activation, and associated hyperleptinemia programmed into the offspring further exacerbated the already sensitive immune response. In addition, White and colleagues (2009) have shown that offspring from HFD dams had increased IL-6 in the brain. The authors speculated that increased IL-6 in the immature brain of the offspring could lead to white matter damage, and resulted in their observed declines in water maze performance. Without the cytokine profiles of our animals, we cannot comment upon brain inflammation as a cause for the observed changes between HFD and CD animals. However, future trials should measure inflammation markers to evaluate their potential involvement in the deleterious effects of maternal HFD on hippocampal function in both maternal and offspring generations.

One of the aims of this study was to observe the effects of obesity on brain function across generations of animals. We hoped to extrapolate from our results, and apply our findings toward humans. It is possible to make inferences from our rodent research since all of the major brain structures in humans are also present in rodents and subserve approximately the same functions. Furthermore, the time-course of hippocampal development (specifically in the DG,

which forms prenatally and displays continued postnatal proliferation of granule cells) is the same in both rodents and humans (Rice 2000). The exposure to maternal obesity, therefore, is the same in both rodents and humans, throughout the stages of hippocampal development. For these reasons, using electrophysiological measures to illustrate the effects of our dietary treatment on rat hippocampus can be extrapolated from our study to that in humans.

Maternal Obesity and Offspring Synaptic Plasticity: strengths and limitations

The greatest strength of this study was that many variables, like housing environment and diet consumption, were well controlled between treatment groups. Many animal studies in the literature do not show CD composition, or use a non-purified chow for the CD, which could have confounding effects when making comparisons with the HFD (Ainge, et al 2010). Several publications have reported composition variability in non-purified diets, which could result in variable findings across studies (Newberne 1975, Schecter 1996, Thigpen et al 2004, Jensen and Ritskes-Hoitinga 2007). Notably, concentrations of essential ingredients can vary across different batches, as well as the presence of unintentional additives and biologically active components that can significantly influence animals consuming the diet. Variable protein, fat, carbohydrate, vitamin, and mineral compositions can have physiological effects that can confound the experimental results. As well, non-purified diets can contain bioactive compounds (such as soy isoflavones) that can significantly influence different endpoints, such as effects on the skeletal system, and sex organ weights (Jensen and Ritskes-Hoitinga 2007). Most relevant to this study is the estrogenic and antiestrogenic effects elicited by isoflavones (via the chemical structure similar to 17-β -estradiol). Estrogen receptors are found throughout the CNS, and activation of certain subtypes of estrogen receptors have been shown to modulate hippocampal synaptic

plasticity (Ooishi Y et al 2012). Since isoflavones can cross the blood brain barrier and affect the brain (Pilsakova 2010), it was important that our study used purified diets.

A great limitation of this study involved small litter size that resulted in inadequate numbers of offspring. The typical litter size of Sprague-Dawley rats is 10.6, with sex ratio being equal (Vanheest 1997). Given that obesity affects fertility (Dixit 2008), and that previous studies have indicated breeding difficulties (Niculescu 2009), the difficulty experienced in this pilot study, where our litter sizes ranged greatly, should have been expected.

Several improvements may be made regarding the breeding protocol, in order to increase litter size. As this was the first time animal breeding occurred within our lab, several recommendations from the animal care staff were received that may be employed for future trials. For example, males bred with female rats should be experienced, and the breeding phase held in a separate room to avoid any distress that may influence the success of breeding. Maternal cannibalism occurs in the first 24 hours after birth, and the stress-induced infanticide that may have occurred in our study could have been due to physical factors, such as excessive noise and too frequent cage cleaning or movement (Lane-Petter, et al 1968). The entire gestational period should also be held in a separate room to avoid stress on the dams, particularly considering that prenatal stress has been reported to affect LTP (Yang, et al 2006). Some reports indicate alterations in sex ratio from prenatal nutrition manipulation studies. Increasing saturated fat content in mice and polyunsaturated fatty acid composition in sheep have been shown to skew the sex ratio toward more male offspring (Alexenko et al 2007, Green et al 2008). Although reported in a limited number of studies, there tended to be a greater number of males per litter with our study as well. However, sample size and litter numbers are too small to make any real conclusions on sex ratios.

In future trials, it would be greatly beneficial to conduct this study with an inclusion of the additional sex factor. Estrany and colleagues (2011) have shown significant sex dimorphisms between male and female rats exposed to the same diet. The study investigated rats fed a high-fat diet (30% calories from saturated and unsaturated fat), and found increased triglycerol accumulation in male inguinal fat depots, but decreased accumulation in the same depot of female animals. Therefore, the factor of sex has probable interaction effects with our other two factors (diet and age), and it would be interesting to observe the extent of the sex dimorphism on the RT fat pad weights that would be measured.

General Conclusions

Obesity is a risk factor for many conditions, such as type 2 diabetes and CVD (Kulie et al., 2011). In addition, obesity is fast approaching tobacco use as a leading cause of preventable death (Mokdad 2000). In addition to the effects of obesity on overall health, obesity is affecting the economy as well. As rates of obesity and related co-morbidities increase, so do the associated health care and disability costs. In 1998 the estimated medical costs of obesity approached $78.5 billion in the US, and these costs were thought to have reached $147 billion in 2008 (Firkelstein 2009). It is estimated that the health care costs for overweight and obese individuals in the US are 37% higher compared to those of normal weight (Firkelstein 2009). Despite the fact that obesity is clearly damaging to our population, insurance companies and health care providers have not adjusted policies to consider obesity a health risk, like that of tobacco use and alcohol abuse (Bhattacharya and Sood, 2006). Therefore, the results from this study not only demonstrate the damaging effects of obesity, but may also help form new policies towards obesity awareness and prevention.

The results of the current study demonstrate that DIO can be accomplished in female rats fed a 45% kcal fat diet (with lard as the primary source of fat calories). Metabolic data obtained reveal changes in glucose tolerance in animals on HFD treatment as early as 8 weeks, and suggest that further testing be performed to determine whether an obese phenotype can be established with a shorter length of diet treatment. The results of this study supplement current literature on DIO animal models, which have not applied the current treatment regimen. The most common challenge in DIO research is diet composition (Kirk 2009, Ainge 2011). Among the studies that use a diet consisting of 45% calories from fat, some lack detail in describing the full diet composition as well as applying a consistent length of exposure to the diet (Shankar 2008, Howie 2009, Samprey 2011). The shorter duration of treatment suggested by our data provides an even more promising and efficient DIO model.

In our study, dams were kept on their diets throughout gestation, and pups continued on the respective treatment diets of their mothers until postnatal day 21. Similar to humans, it is known that maternal nutrition during pregnancy impacts neurodevelopment, and brain development continues postnatally, and is very sensitive to environmental influences in the neonatal period (Walker, et al 2007). Using our diet regimen, studies in the future should compare effects on dams that do not remain on their diets during gestation, and also on pups that do not continue on their mothers' respective diets. Walker and colleagues (2007) have shown that fat content in the maternal diet affects milk composition and subsequently induces significant increases in the plasma level of leptin in pups, which, in the neonatal period, has large influences on proper brain development (refer to section 4.3.2).

We have designed the current pilot study to mimic the human condition, which was best represented by having our groups remain on their respective maternal diets until weaning.

Studies have shown similarities in dietary patterns between children and their parents (Davison 2001, Vauthier et al 1996). Although the mother's obese status cannot be changed in time to prevent effects on the already developing fetus, it would be interesting to see how modifications to their diet during gestation and after parturition could affect the synaptic abilities of the offspring. Sun and colleagues (2012) showed significantly higher plasma leptin levels in pups (at PND 7, and PND 21) of dams fed a high-fat diet during lactation, and Ahima and colleagues (1999) have reported that decreased neonatal leptin levels can affect myelin proteins. Although electrophysiology was not conducted, the impact of leptin should be considered in affecting synaptic transmission since abnormal myelination will affect synaptic efficacy. Depending on the findings of the proposed future studies, targets of interventions could be directed at maternal nutritional knowledge and parental child feeding practices.

To our knowledge, no previous studies have performed electrophysiological recordings on pups born from either HFD dams or CD dams. The main goal of our study was to determine the effects of maternal obesity on offspring hippocampal function. By utilizing the LTP protocol, our data suggest that offspring from obese dams showed memory impairment through a lowered potentiation in their evoked responses. Our data showed pups from HFD dams achieving significantly less potentiation than pups from CD dams, which suggests possible learning and memory deficiencies. Extrapolating to the human condition, this would suggest greater attention be given to children born under maternal obesity. Indeed, in several longitudinal studies, maternal BMI and GDM status have suggested that, as Vickers and colleagues (2011) have replicated in rodents, maternal obesity increases the risk of childhood obesity (Whitaker 2004, Boney 2005, Reilly 2005). This suggests interventions be targeted to children at the pre-pubescent stage, where inequalities in learning abilities are less pronounced, but are projected to

exacerbate with age. Further investigations should also include brain-damaged pups (e.g., genetically modified strains, traumatic, or ischemic brain injury), so that the extent of impairment that we have detected electrophysiologically between our diets can be compared to the compromised potentiation of brain damaged pups. For example, Winocur and Greenwood (2005) saw that rats fed HFD were impaired on all task performances compared to CD rats, and the deficits were comparable to brain-damaged rats fed normal diets that were tested under the same conditions. There is a limited collection of literature on maternal obesity, and the results from comparisons with brain-damaged rats would not only contribute to maternal obesity research, but also have practical implications as well (i.e., provide a solid dissuasion from maternal obesity).

The data obtained from this study suggest that our high-fat diet is obesogenic and negatively affects maternal hippocampal synaptic plasticity, and that pups born from dams fed this diet likely have impaired hippocampal synaptic plasticity, as well. The results of this study complement epidemiological findings in humans, and the understanding of such developmental programming will allow for better pre-pregnancy advice and care to improve the health of children in the future. Evidence has supported the claims of maternal obesity and its influence on offspring risks of cardiovascular disease, diabetes, and obesity. Whether maternal obesity affects learning and memory at a pre-adolescent stage, young adult stage, or both, the novel knowledge from this study will allow for the development of better treatments suited towards the particular phenotype of the child.

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