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Maternal Lifelong Western Diet Consumption Impacts Placental and Brain Development in the Term Guinea Pig Fetus

Carlene H. Cihosky, *The University of Western Ontario*

Supervisor: Regnault, Timothy RH, *The University of Western Ontario*

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ABSTRACT

Both metabolic and cognitive dysfunction can originate from fetal reprogramming precipitating from adverse conditions experienced *in utero*. Of note is the western diet (WD), which is associated with maternal energy imbalances that may hinder fetal development through altered placental function. Brain-derived neurotrophic factor (BDNF), a growth factor that supports the placenta and developing brain, is responsive to such energy imbalances. This study sought to investigate the impact of lifelong maternal WD consumption on fetoplacental development, focusing on relations between placental changes, and fetal growth and neurodevelopment in a guinea pig model. Maternal WD consumption resulting in a lean metabolically unhealthy maternal phenotype was associated with lean fetal hepatic steatosis. Placentae of these fetuses were large yet inefficient and showed reduced BDNF expression. Similar reductions in BDNF were noted in fetal brains, coinciding with decreased cell density. Such cellular changes may convey long-term cognitive deficits, although their consequences remain unknown.

KEYWORDS

Western Diet, Placenta, Brain Development, Brain-derived Neurotrophic Factor (BDNF), Guinea Pig, Developmental Origins of Health and Disease (DOHaD)

LAY SUMMARY

Over the last century, rates of metabolic disease and mental illness have risen. While once thought to possess distinct pathologies, metabolic and cognitive dysfunction show commonalities in their etiology; both may originate from adversity experienced *in utero* through the process of fetal reprogramming. Adverse intrauterine conditions can lead to changes in the development of the placenta, a critical organ with diverse function that exists to support the developing fetus. Abnormal placental function translates to altered fetal development, predisposing the child to later disease. As such, optimizing the maternal environment to foster healthy placental development is critical to minimizing the child's risk of disease. While obesity has been identified as a risk factor to fetal development, it is difficult to distinguish between detriments caused by obesity, versus the lifestyle factors which precede its development. Of note is the Western Diet (WD); rich in saturated fat and added sugar, the WD is associated with the onset of metabolic and neurological dysfunction. Despite its prevalence, little is understood about the risks associated with habitual WD consumption before and during pregnancy. This study sought to investigate how lifelong maternal WD consumption would impact the development of the placenta and fetus, focusing on fetal growth and brain development in a guinea pig model. Both maternal and fetal populations exposed to the WD developed fatty livers yet maintained lean body compositions. The placentae of these animals were oversized, inefficient, and showed significant tissue damage. In addition, these placentae showed lower levels of a growth factor essential for placental and fetal brain development. Similar reductions in the growth factor were noted in the fetal brain of those born to WD-fed mothers, which coincided with reduced brain cell density. Taken together, habitual WD consumption before and during pregnancy may convey both metabolic and neurological complications to the fetus, possibly through alterations in growth factor production. The significance of these findings is magnified by their occurrence in a "lean" model, which emphasizes that evaluating lifestyle factors such as dietary patterns in parallel with markers of maternal metabolic health is potentially more pertinent to assessing pregnancy risk than BMI alone.

CO-AUTHORSHIP STATEMENT

The following individuals contributed to the contents of this thesis as follows:

Dr. TRH Regnault	Supervisor throughout research project, provided grant funding, edited manuscript, and provided advice and support
B Sutherland	Performed animal feeding and collections
Dr. L Friesen-Waldner	Performed MRI, ultrasounds, animal feeding and collections
Dr C M^{c}Kenzie	Provided expertise on magnetic resonance imaging
L Morris	Performed magnetic resonance imaging segmentations
Dr. P Kiser	Performed placental pathology scoring
K Nygard	Provided expertise in immunostaining, image acquisition, and analysis
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LIST OF ABBREVIATIONS

BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CA1	Cornu ammonis 1
CA3	Cornu ammonis 3
CC	Corpus callosum
CD	Control diet
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
EVT	Extravillous trophoblast
H&E	Hematoxylin and eosin
HPFF	Hepatic proton-density fat fraction
Iba1	Ionized calcium-binding adapter molecule 1
IDEAL	Iterative decomposition of water and fat with echo asymmetry and least-squares estimation
LDL	Low-density lipoprotein
LMM	Linear mixed model
M1	Pro-inflammatory microglia activation

M2	Anti-inflammatory microglia activation
MAPK	Mitogen-activated protein kinase
MRI	Magnetic Resonance Imaging
MUFA	Mono-unsaturated fatty acid
NAFLD	Non-alcoholic fatty liver disease
p75NTR	p75 neurotrophin receptor
PI3K	Phosphoinositide 3-kinase
PLCγ	Phospholipase C, gamma 1
proBDNF	Precusor of brain-derived neurotrophic factor
PUFA	Poly-unsaturated fatty acid
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SEM	Standard error of the mean
SFA	Saturated fatty acid
T	Thalamus
TAT	Total adipose tissue
TrkB	Tropomyosin receptor kinase B
VAT	Visceral adipose tissue
WD	Western diet

CHAPTER 1: LITERATURE SEARCH

1.1 Western Diet and Health Outcomes

1.1.1 Western Diet

A dietary pattern encompasses the quantity, variety, and combination of foods and beverages habitually consumed by an individual. Numerous dietary patterns exist around the world established based on cultural differences, socioeconomic status, resource availability, and ethical practices. These diets can vary greatly in their nutritional value and their impact on overall health. One such dietary pattern is the Western Diet (WD), a nutritionally poor, calorically dense diet that is characterized by high levels of saturated fats and added simple sugars. Following the Neolithic and Industrial revolutions, human nutrition shifted towards the consumption of refined foods with added sugars and domesticated meats with higher fat content, giving advent to the WD[1]. While this dietary pattern originated in North America, the increased affordability and accessibility of processed and pre-packaged foods, along with global economic growth, globalization, and urbanization, have led to its global adoption[2]. Unsurprisingly, the growing popularity of the WD has been paralleled by rising rates of obesity and metabolic disease[1].

Macronutrients (proteins, lipids, and carbohydrates) are required for the body to function, but increasing evidence suggests that not all sources of macronutrients are created equal. Saturated fatty acids (SFAs) such as lauric, myristic, palmitic, and stearic acids, are typically found in dairy products and fatty meats. SFAs are more calorically dense and are considered pro-inflammatory[3]. When consumed in excess (>10% of caloric intake), they are associated with a greater risk of atherosclerosis as they promote the production of low-density lipoprotein (LDL) cholesterol[3,4]. This contrasts monounsaturated fatty acids (MUFAs) like oleic acid and poly-unsaturated fatty acids (PUFAs) such as linoleic and α-linoleic acids, which have been found to lower one's risk of heart disease by the opposite effect[3]. Within the PUFA family, omega-3 fatty acids (α-linoleic, eicosapentaenoic acid, and docosahexaenoic acid) have been highlighted for their benefits to brain function, heart health, glucose tolerance, and skeletal muscle metabolism due to their anti-inflammatory and antioxidant properties[5]. Looking to the WD, which features an abundance of SFA with minimal MUFAs and PUFAs, it is likely that the relative ratios of these

fatty acids contribute to the development of many of the metabolic dysfunctions associated with the diet's habitual consumption[3].

The WD typically contains a low proportion of dietary fibre and few fruits and vegetables, which although a source of natural sugar also supply micronutrients and antioxidants[6]. Instead, carbohydrates in the WD are largely sourced from added simple sugars like high-fructose corn syrup, offering no additional nutritional benefit[7]. As with SFA, refined sugars when consumed in excess can lead to increased risk of metabolic conditions, including heart disease, non-alcoholic fatty liver disease (NAFLD), and type-2 diabetes, as well as cognitive disorders like dementia and depression[7–10]. Conversely, a diet high in fibre maintains colorectal health, promotes a healthy gut microbiome, lowers LDL cholesterol, and regulates blood sugar levels[11].

Considering the diet as a whole, the WD is associated with increased production of reactive oxygen species (ROS) and oxidative stress, the development of hyperinsulinemia and insulin resistance, low-grade inflammation, and abnormal activation of the sympathetic nervous system[1]. Increasing evidence suggests that the detrimental effects of the WD are related to its impact on the gut microbiome[12]. The WD promotes dysbiosis, a shift in the bacterial populations cultivating the colon such that there are greater proportions of "bad bacteria" (Ex. *Enterobacteriaceae*) relative to "good bacteria" (Ex. *Lactobacillus*)[12]. Good bacteria are classified as such due to their vital role in maintaining the integrity of the colon and in releasing anti-inflammatory cytokines[12]. Conversely, bad bacteria increase the permeability of the colon, promoting the passage of endotoxins into systemic circulation, and increasing the release of pro-inflammatory cytokines[12]. Through these changes, the habitual consumption of the WD promotes a multitude of metabolic diseases and is increasingly being considered as a proponent of disruptions across multiple organ systems, including the central nervous system[1].

1.1.2 Obesity and Metabolic Health

Obesity is a progressive chronic disease that is characterized by abnormal or excessive fat accumulation (BMI $\geq$30 kg/m^2). In 2016, The World Health Organization estimated that globally there were 2 billion adults (39%) who were overweight and 650 million (13%) who were classified as obese. If current trends continue, they predict that rates of obesity will nearly double

by 2025. These statistics are concerning because as a multi-system disease, obesity has a host of associated co-morbidities, including type-2 diabetes, dyslipidemia, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD)[13].

Generally speaking, obesity arises due to a chronic surplus of energy intake relative to energy expenditure, which ultimately promotes the storage of triglycerides as adipose tissue[1]. Beyond this simplified explanation is a complex energy balance equation that includes genetic and epigenetic factors, endocrine signalling, dietary patterns, lifestyle, and physical activity[1,13,14].

While obesity is a growing epidemic that can have severe complications, the obese classification alone does not preclude health. Obesity is commonly classified based on body mass index (BMI), which is calculated solely from weight and height. As such, BMI offers little information about an individual's insulin sensitivity, blood pressure, blood lipid profile, or systemic inflammation, which are among the factors that determine metabolic health[15]. It follows that the obese classification captures individuals of varying metabolic health, including those who carry no additional risk for metabolic disease termed the "metabolically healthy obese"[15–18]. Conversely, metabolic dysfunction may arise in the absence of obesity as a "lean metabolically unhealthy" phenotype[15–18]. While often out shadowed by discussions around obesity, this lean metabolic dysfunction conveys similar detriment to long-term health and describes a significant proportion of individuals, affecting approximately 20% of the population (estimated from the US)[18]. As a relatively novel area of study, the factors that favour the development of one metabolically unhealthy phenotype over another are not yet clear, although it is currently largely attributed to genetic factors[15].

Amongst both populations, NAFLD is a prominent feature of metabolic dysfunction and poses its own risks to long-term health. NAFLD is a broad term used to describe a spectrum of progressive conditions that results from fat deposition in the liver unrelated to alcohol or viral causes[19,20]. This spectrum begins with triglyceride accumulation in the absence of inflammation; but over time, the disease progresses into non-alcoholic steatohepatitis (NASH), which is characterized by hepatic inflammation[19]. With this inflammation comes increasing fibrosis that eventually culminates in cirrhosis and liver failure[19].

While obesity itself is a risk factor of this disease, NAFLD is increasingly being identified in individuals with a lean phenotype. Globally, around 40% of those with the disease

are considered non-obese, and almost 20% were deemed lean[21]. Unfortunately, a lean phenotype does not preclude an individual from liver damage as multiple studies have indicated that those with lean NAFLD are at higher risk of developing severe liver disease compared to their obese counterparts[22,23]. Outside of disease progression, lean NAFLD also communicates greater risk than metabolically healthy obesity for the development of type-2 diabetes[24]. This once again highlights the importance of adopting a holistic understanding of metabolic health that adequately identifies the risks incurred by lean metabolically unhealthy individuals.

Multiple risk factors for the development and progression of this disease have been identified; however, the exact etiology is not fully understood[20]. It is suspected that hyperinsulinemia and insulin resistance play a key role in its pathophysiology by increasing the release of free fatty acids from peripheral adipose stores and decreasing free fatty acid oxidation in skeletal muscle and liver[19,25]. The result is an imbalance in the supply and utilization of free fatty acids that not only promotes its accumulation in hepatocytes but also leads to the production of ROS and inflammation through mitochondria dysfunction[19].

As a major proponent of insulin resistance, WD consumption is strongly associated with NAFLD[19]. High-fructose corn syrup, for example, is a simple sugar abundantly found in the WD that is considered more lipogenic than other complex sugars and has been found to induce hepatic inflammation[26,27]. Prior to the onset of insulin resistance, the high glycemic index of the WD leads to rapid increases in insulin and postprandial blood glucose, which further promotes liver lipogenesis[25]. Moreover, hypertriglyceridemia is correlated with the development and severity of NAFLD independent of obesity and other metabolic conditions, emphasizing the important role of diet in disease progression[28,29].

In sum, metabolic dysfunction in all its forms represents a severe public health crisis. While the exact phenotype, lean or obese, may be largely dependent on uncontrollable factors like genetics, lifestyle factors such as habitual WD consumption still perpetuate the development of metabolic dysfunction regardless of body size.

1.1.3 Western Diet and the Brain

Obesity and metabolic disorders have long been considered independent of cognitive dysfunctions as they implicate different regions of the brain. The hypothalamus is well

established as a regulator of energy balance[30,31]. It follows that experimental manipulations of the hypothalamus in animal models have profound effects on eating behaviours and body mass, and in humans, energy imbalances have been associated with alterations in neurohormone signalling pathways within the hypothalamus[31]. On the other hand, cognitive dysfunction and mood disorders are thought to arise from alteration at the hippocampus, a brain region primarily responsible for learning and memory[32–34].

Increasing evidence posits that the WD is a common etiology of both metabolic and cognitive impairments and that the two may be intrinsically linked. The hippocampus is particularly sensitive to a variety of stressors including metabolic perturbations and energy imbalances[34,35]. In addition to metabolic diseases, the consumption of the WD has been strongly correlated with increased incidence of Alzheimer's disease and milder forms of cognitive impairment that involve hippocampal structures[36–39]. These impairments may be the consequence of WD-induced changes in glucoregulation, neurotrophin levels, neuroinflammation, and blood-brain barrier integrity that directly or indirectly induce hippocampal dysfunction[40,41].

High levels of saturated fats and refined sugars contribute to the development of insulin resistance and glucose intolerance[42]. Insulin and its receptor are abundantly expressed in the hippocampus and at optimal doses, insulin has been shown to enhance memory in rodents and humans[43,44]. Consequently, poor glycemic control and subsequent insulin resistance, independent of BMI, hypertension, and dyslipidemia, were previously linked to memory impairments and hippocampal atrophy in humans[45]. Healthy brain function and ongoing processes that mediate memory and learning at the hippocampus are similarly supported by other hormones and growth factors, the expression of which may be altered by energy-status[46,47]. Brain-derived neurotrophic factor (BDNF), for example, has been described as a mediator of WD-induced neurological changes because its expression in both human and animal models is largely impacted by changes in energy balance and more specifically, is reduced by WD consumption[47,48]. The effects of the WD on neurotrophic factor expression may also involve inflammation. SFA specifically are considered pro-inflammatory, and when consumed in high proportions, as is characteristic of the WD, have been associated with chronic low-grade inflammation[3]. Neurotrophic factors like BDNF help to mediate anti-inflammatory responses to acute inflammation that facilitate tissue remodelling following brain injury; however, chronic inflammation has been found to

downregulate neurotrophin expression, thus perpetuating continuous inflammation and hampering tissue repair[49,50]. In addition, disrupting the integrity of structures such as the blood-brain barrier (BBB) may also perpetuate neuroinflammation and cognitive impairment[51,52]. Specifically, the WD was found to reduce the expression of tight junction proteins, which would normally restrict the permeability of the BBB, in a rodent model[40]. A "leaky" BBB fails to prevent toxins and pathogens from entering the CNS, which may then disrupt the neural environment leading to poor cognitive outcomes[40].

In addition to memory and learning deficits, the hippocampus is also postulated to be involved in regulating eating behaviours, as experimentally induced hippocampal lesions result in obesity and hyperphagia[53,54]. The exact mechanism behind this effect has not been elucidated; however, it has been postulated that impairing hippocampal-dependent memory may interfere with the ability of satiety cues to suppress the memory of positive reinforcement achieved through eating, leading to poor appetite control and increased energy consumption[41].

Taken together, the WD and subsequent metabolic dysfunction are likely detrimental to adult brain health, disrupting sensitive structures like the hippocampus. These hippocampal disruptions likely result in both cognitive impairment and further metabolic dysfunction. Given the detriment noted in the adult brain, these findings also beg the question of whether maternal WD consumption and metabolic dysfunction may evoke similar detriments to fetal development.

1.2 Developmental Programming of Neurodevelopment

1.2.1 Fetal Programming

During critical periods of development, adverse events in the maternal environment can have a significant impact on the short and long-term health outcomes of the fetus[55,56]. Unfavourable conditions during pregnancy lead to developmental adaptations in the fetus that alter its structure, physiology, and metabolism, thus 'programming' susceptibility to later disease[57]. This is the foundation of what is now referred to as 'The Developmental Origins of Health and Disease' (DOHaD) hypothesis.

This theory came to fruition through multiple epidemiological observations, the first being from Dr. Anders Forsdahl in 1977 who reported a correlation between early-life poverty

and adulthood cardiovascular disease[58]. He speculated that nutritional deficits incurred during early childhood may result in permanent damage that predisposes the development of cardiovascular disease in adulthood[58]. These observations were then furthered in conjunction with epidemiologist Dr. David Barker and his colleagues who extended the findings to a fetal population, suggesting poor nutrient availability *in utero* leads to fetal adaptations that program the development of adult-onset non-communicable disease (cardiovascular disease, obesity, metabolic disease, stroke, and type-2 diabetes)[55,56,59,60]. Since its advent, this hypothesis has found application in explaining fetal health outcomes under various adverse intrauterine conditions including malnutrition, overnutrition, infection, chemical exposure, hypoxia, and hormonal perturbations[57,61].

Research has tried to elucidate the underlying mechanism behind these programmatic changes. While there is no single mechanism that will explain the multitude of observed phenomena, three primary mechanisms have been identified, although there are likely more that exist.

First is through permanent structural changes to fetal tissues[61]. Suboptimal delivery, both elevations and reductions, of nutrients, hormones, or substrates needed for appropriate development may permanently alter the structure of the fetal tissues[61–65]. This effect is particularly prominent in the brain, where a reduction in neurotrophin delivery can reduce cell proliferation and alter neurogenic processes, thus altering the structural connectivity of the brain as has been shown in multiple animal models[47,61,64,66].

Second is through the epigenetic regulation of multiple genes[61]. Epigenetics refers to DNA modifications that do not alter the DNA sequence itself but instead alter its expression[67]. These modifications include DNA methylation/acetylation, histone acetylation and non-coding RNA[67]. They contribute to cellular memory, eliciting change that persists even following multiple rounds of cell division[67]. When transcription factors are the target of this regulation, epigenetic marks on a single gene can influence an entire network of genes. For example, nutritional pregnancy challenges have been found to alter methylation patterns in various genes involved in carbohydrate and lipid metabolism, eliciting changes that may only become apparent when the individual is exposed to post-natal dietary stress (ex over or under nutrition) [67–69].

Finally, adverse events can alter the expression of proteins related to cellular senescence and can induce oxidative stress, both of which accelerate cellular ageing[61,70]. Consequently, this increases the risk of developing conditions that are associated with ageing, which includes many metabolic and neurodegenerative diseases[61]. Pre-natal adverse events can induce oxidative stress either by increasing fetal hypoxia, reducing anti-oxidant defence mechanisms, or altering mitochondrial function[57,71]. The latter two mechanisms are common features of maternal obesity and WD consumption, both of which increase ROS production[57,72]. Elevated levels of ROS damage lipid membranes, proteins, and DNA, thus accelerating the rate of tissue ageing and elevating the risk of developing age-related pathologies[57,61].

Collectively these mechanisms help to explain some of the long-term health outcomes noted under various forms of maternal stress. While the DOHaD hypothesis was initially used to explain the prevalence of metabolic health conditions, research has found evidence of its importance in programming fetal brain development as well[73–75].

1.2.2 Placental Development

Placental development begins following the implantation of the blastocyst, the outer layer of which is made up of trophoblast cells that will eventually derive the placental structures[76]. Trophoblasts differentiate into an outer layer of syncytiotrophoblasts and an inner layer of cytotrophoblasts[76]. The syncytiotrophoblasts are highly invasive cells that erode maternal tissues and blood vessels, filling the lacunae—empty spaces within the syncytiotrophoblast layer—with maternal blood[76]. Cytotrophoblasts are proliferative cells that continuously grow to form projections that extend into the surrounding syncytiotrophoblast cells[76]. With further development, extra-embryonic mesoderm and embryonic blood vessels form within these projections forming what is termed the tertiary chorionic villi[76]. As pregnancy advances, these villi undergo expansive branching, forming tree-like structures within the intervillous space that contain a network of fetal blood vessels and are optimized for gas and nutrient exchange[76,77]

In addition to these “floating” villi, anchoring villi extend to the basal plate (maternal side of the placenta) and provide support to the placental structure[77]. From the distal ends of these villi, cytotrophoblasts differentiate into extravillous trophoblasts (EVT), which migrate into

the maternal decidua and invade the myometrium where they remodel uteroplacental arteries[77]. This process ensures adequate blood flow and nutrients are accessible to the developing fetus[77].

By the end of placental development, a network of developed branching villi protrudes into the intervillous space where they are bathed in nutrient-rich maternal blood[76]. Syncytiotrophoblasts, which cover the surface of these placental villi are in direct contact with maternal blood and are critical for mediating many of the placenta's functions, including hormone production and nutrient/gas exchange[78].

Across various animal species, there are differences in the structure of the placenta, one of which being in the cellular layers making up the placental barrier. The placentae of common rodent models like the rat or mouse possess a haemotrichorial barrier with three layers of trophoblast cells separating the maternal blood space from the fetal capillaries[79]. This structure contrast that of the human placenta, which is haemomonochorial with only a single layer of syncytiotrophoblast cells at the maternal-fetal interface[79]. The guinea pig model is often used as a pre-clinical animal model of pregnancy because, unlike other rodents, it too possesses a haemomonochorial placenta[79,80]. In addition to their similarities at the exchange interface, both human and guinea pig trophoblast cells invade deeply into the decidua, contrasting the shallower invasion noted in other rodent models[80]. Despite its similarities to the human placenta, some key differences distinguish the two placentae[80]. For example, the guinea pig placenta includes a subplacenta, which is a region of the placenta that connects the placenta to the junctional zone, analogous to the anchoring villi of the human placenta[80]. Rather than branching placental villi, the guinea pig placenta possesses a labyrinth region made up of a mass of syncytiotrophoblast cells that surround maternal lacunae and fetal capillaries[80]. This structure supports a countercurrent exchange system that differs from the concurrent exchange system of the human placenta[80]. Regardless of the species, it is these various placental structures and cell types that mediate exchange and communication between the maternal and fetal environment, making their formation a critical determinant of healthy fetal development.

1.2.3 Brain Development

Development of the fetal nervous system begins on the 4th week of gestation following neurulation and continues into early adulthood[81]. The neurodevelopmental timeline begins with

the rapid proliferation of neuroprogenitor cells in the ventricular and subventricular regions of the fetal brain through symmetrical cell division[81]. Neuroprogenitor cells gradually transition to asymmetrical division, producing an identical neuroprogenitor cell to replenish the progenitor pool, as well as an additional cell with a distinct cell fate[81]. These neuroprogenitor cells, also referred to as radial glia, give rise to three major cell types: neurons, oligodendrocytes, and astrocytes. Neuroprogenitors destined to become neurons will give rise to immature neurons, which migrate out to the developing neocortex where they differentiate into specialized neurons, creating multiple layers and neural structures that contain functionally distinct cell types[81]. The axonal and dendritic processes of positioned neurons begin to develop, forming synapses and allowing them to integrate into a growing network of cells[81]. With the development of other neural cell types, namely oligodendrocytes, the axons become myelinated in specific patterns that ensure proper conduction across neural circuits[81,82].

Oligodendrocytes are a type of macroglial cell, much like astrocytes. The generation of these cells, referred to as gliogenesis, begins late in the embryonic stage following the start of neurogenesis and continues, although to a much smaller extent, into adulthood[83]. Glial cells make up most of the brain cells and are essential for maintaining the homeostasis of the mature brain, as well as supporting neurodevelopmental processes like cell migration, myelination, and synaptogenesis[83].

In addition to the macroglia cells, another type of glial cell exists called microglia. Contrary to neurons and macroglia, these cells do not arise from ectoderm-derived neuroprogenitor cells; rather, these cells are derived from primitive macrophages of the yolk sac that begin to populate the developing nervous system early in development alongside the neurons[84]. While microglia are primarily known for their immune function, they also mediate regressive developmental events like neuronal apoptosis and synaptic pruning[81,85]. Following expansive cell proliferation and synaptogenesis, the microglia help to eliminate excessive or dysfunctional cells and synaptic connections, which refines neural circuitry[81]. Synaptic pruning is an example of a developmental process that continues well into adulthood, contributing to what is termed synaptic plasticity, a process that is essential for learning and memory[81,83].

A second component contributing to synaptic plasticity is hippocampal neurogenesis. While most neuroproliferation occurs *in utero*, in select regions of the brain, specifically the

hippocampus, the process similarly continues into adulthood[81,86,87]. The hippocampus is one component of the limbic-cognitive system, a system involved in learning and memory, attentional processes, motivational states, and emotions[88,89]. Hippocampal neurogenesis specifically, is largely involved in memory formation, learning, and behaviour, and deficits in this process are thought to underlie the development of cognitive deficits and mood disorders like depression and schizophrenia[87–89].

Progression through this developmental timeline (**Fig 1.2.3-1**) *in utero* and beyond is guided largely by neurotrophic factors and neurotransmitters, alterations of which can alter long-term brain connectivity and function[81]. Similarly, aberrations in non-pathological regressive processes can pose significant detriments to the developing brain. As previously mentioned, microglia are immune cells in the central nervous system that protect the neural tissues from pathogens and maintain homeostasis[81,90]. During early development, these cells play an additional role in refining neuronal circuits by mediating neurogenesis and synaptic pruning[81,90]. While a certain level of neuroinflammation is essential for neurodevelopment, aberrant chronic early-life inflammation may alter the programming of the microglial population such that the threshold required for reactivation is reduced, predisposing the individual to inflammatory damage later in life[90]. Healthy neurodevelopment thus depends on a delicate balance of trophic signals promoting neurogenesis, and the activation of microglial-mediated inflammatory and anti-inflammatory pathways that "clean-up" neural circuitry[90].

Many of these developmental processes are conserved across species; however, the timing of these events can differ (**Fig 1.2.3-2**)[91]. Animals like the rat or mouse are postnatal brain developers, meaning that they are born with relatively immature brains that undergo significant development postnatally[91]. Unlike most other rodents, guinea pigs give birth to precocial young and are considered prenatal brain developers[91]. Their young are born with highly developed brains comparable to that of a mouse approximately 30 days after birth[91]. Human neurodevelopment is somewhere in the middle; while significant brain development does occur *in utero*, it begins to slow towards the end of pregnancy, then continuing postnatally[91]. Understanding the differences between developmental timelines is essential in interpreting and translating scientific findings on neurodevelopment across species.

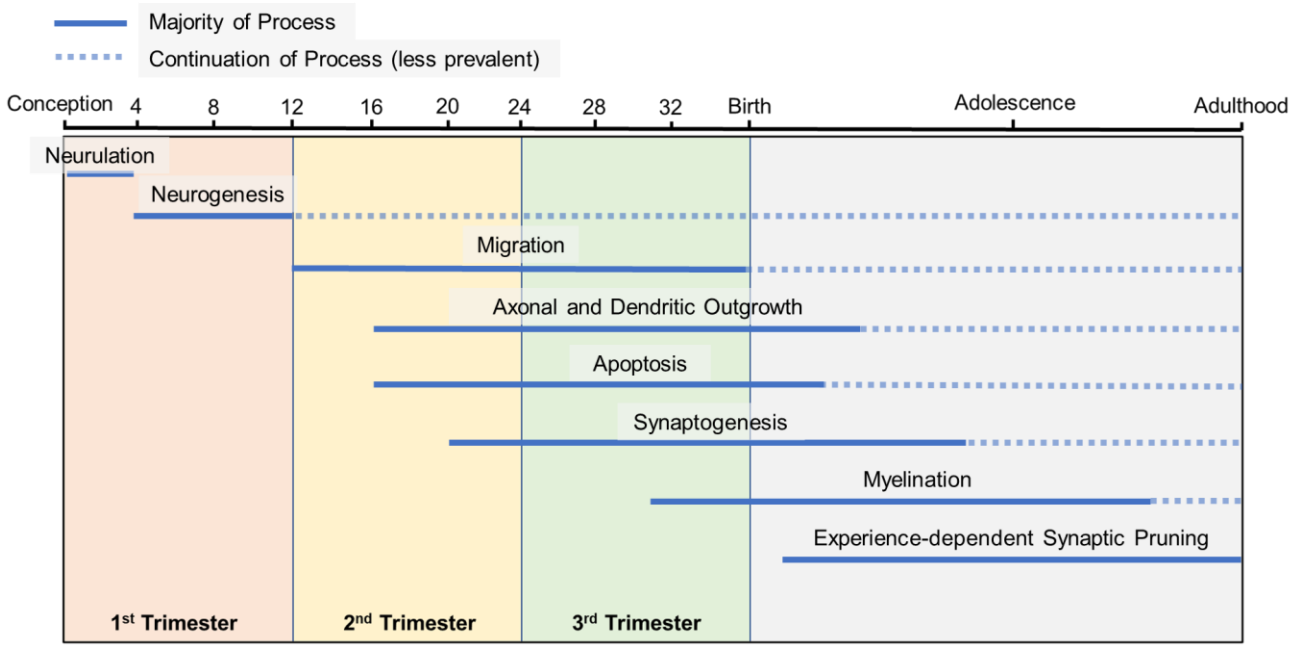

Figure 1.2.3-1. Timeline of human brain development.

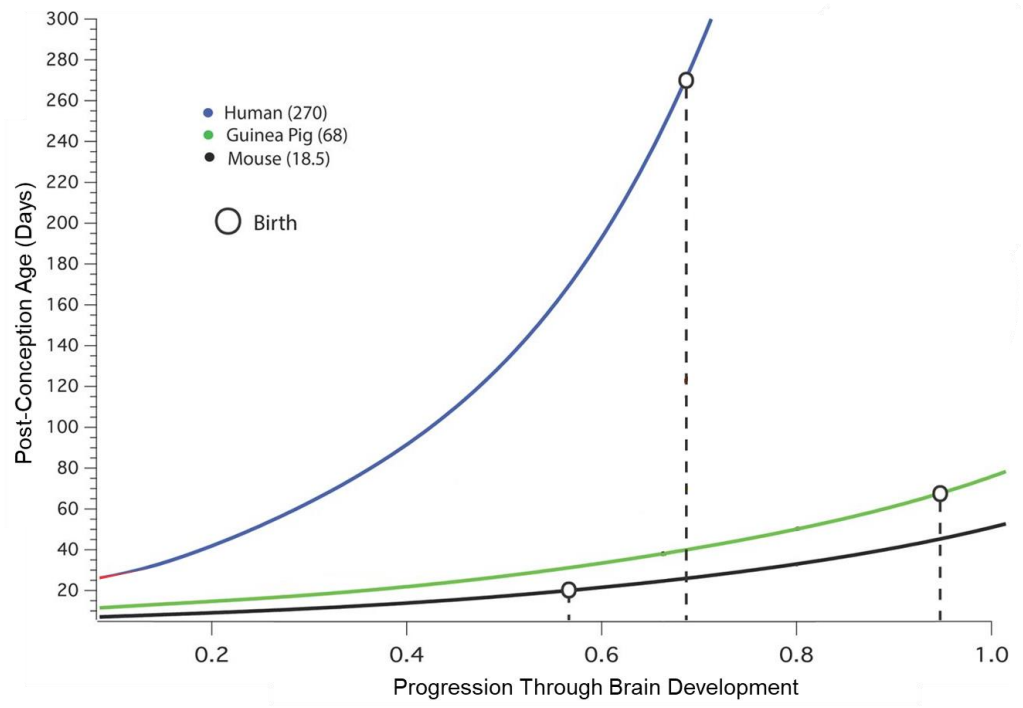

Figure 1.2.3-2. Comparison of brain development timeline between humans, guinea pigs, and mice. Figure adapted from Workman *et al*[91].

1.2.4 The Placenta's Role in Fetal Neurodevelopment

It is impossible to discuss the DOHaD hypothesis without recognizing the importance of the placenta. The placenta is a transient, but vital organ acting as an interface between mother and fetus. Aside from the exchange of nutrients and waste, the placenta produces critical hormones, growth factors, and proteins, acts as an immunological barrier that protects the fetus from xenobiotic chemicals, and supplies the fetus with molecular substrates needed for processes like neurodevelopment[92,93]. These processes depend on the proper structural development of the placenta.

As a dynamic sensor of the maternal-fetal environment, the placenta can adapt to changes in the maternal environment to prioritize gas and nutrient transport to the fetus[92,93]. While these adaptations ultimately preserve the life of the fetus, they may still disrupt normal placental and fetal development[92,93]. Abnormal placental morphology that alters blood flow and nutrient exchange, as well as alterations in nutrient transporter expression that occur in response to maternal perturbations, can reduce placental efficiency and have detrimental effects on fetal development[93,94]. Brain development is resource-intensive, relying on nutrients supplied from the placenta to support the rapid expansion of the fetal nervous system[95]. This makes neurodevelopment particularly susceptible to aberrations in placental architecture that disrupt nutrient exchange[95].

Just as brain development demands adequate nutrient supply, it also relies on the delivery of neurotrophic factors and neurotransmitters that direct progression through the neurodevelopment timeline, some of which may be impacted by imbalances in nutrient availability[93,94]. For example, placental-derived BDNF directs cell proliferation, among other developmental processes in the fetal brain, and its expression is tightly regulated by maternal energy status[96–98]. Consequently, disrupting the early supply of this factor may then alter early brain development, affecting its receptors' expression and local production in the adult brain, eliciting future cognitive impairment[96,97].

It is postulated that many of these changes in placental function are manifestations of altered gene expression occurring because of epigenetic changes[93,99,100]. The exact mechanism underlying these differences is not fully known. It is suspected that altered energy balance, inflammation, or ROS, resulting from adverse maternal conditions (obesity, WD, malnutrition,

stress, infection, hypoxia) may alter epigenetic machinery and consequently the epigenetic markings of various placental genes[99–101]. Subsequent alterations in placental function may then directly or indirectly program neurodevelopmental change[92,101].

It is also postulated that many maternal stressors, both infectious and non-infectious, induce some form of inflammation within the placenta[93]. Although the underlying mechanisms remain unknown, it has been shown that maternal inflammation is detrimental to fetal neurodevelopment as it has been associated with greater risk of neurodevelopmental disorders like Schizophrenia and Autism spectrum disorder, as well as cognitive impairement[102–106]. It is postulated that placental inflammation may modify fetal neurodevelopment by altering placental function as described above, or by releasing pro-inflammatory cytokines into fetal circulation[93,107]. In the fetal brain, inflammatory cytokines are involved in signal transduction pathways, which may be disrupted by their aberrant expression[107]. Moreover, these cytokines program microglial activation, enhancing their future inflammatory response[90].

The placenta has a formative role in fetal neurodevelopment. Consequently, alterations in its morphological development or function lead to significant changes in brain function in later life. While questions remain around the exact mechanisms behind placental-mediated neural programming, inflammation and epigenetic changes are likely implicated.

1.2.5 Maternal Overnutrition and Brain Development

It has been well established that maternal undernutrition is associated with alterations in fetal neurodevelopment that communicate serious risks for long-term cognitive deficit[108]. On the other end of the spectrum, studying maternal overnutrition poses greater challenges as obesity and diet are often inseparable. Many obese models are achieved through an obesogenic diet regimen and studies investigating the dietary patterns (WD, high fat, or high sugar) themselves are typically confounded by weight gain or obesity, making it difficult to identify the risks of the diet independent of maternal obesity[109].

Much like the aforementioned trends in obesity and WD consumption, the prevalence of neurodevelopmental disorders has increased dramatically[110]. This led researchers to question the association between maternal metabolic disease and prenatal diet on offspring behaviour. Indeed, epidemiological studies have found correlations between maternal obesity, metabolic

dysfunction, and diet and the development of multiple cognitive disorders, namely attention deficit hyperactivity disorder, autism spectrum disorder, schizophrenia, anxiety, and depressive disorders[111].

In animal models, including both rodents and non-human primates, offspring exposed to maternal overnutrition have shown additional deficits in learning and memory, as well as abnormalities in behaviour[97,112,113]. Animal models are advantageous in that they offer additional insight into the neurological changes underlying these outcomes. At a molecular level, maternal overnutrition has been associated with reduced hippocampal neurogenesis and synaptogenesis in rodent models, processes that greatly contribute to learning and memory[97,114]. Moreover, one study found evidence of increased lipid peroxidation in the hippocampus of mice as a result of maternal obesity (induced by a high-fat diet), which also led to impaired branching of hippocampal neurons[112,114]. This is aligned with previous studies which suggest that obesity and insulin resistance are associated with increased levels of oxidative stress[1,109].

Similarly, both metabolic dysfunction and WD consumption lead to 'metainflammation', a form of chronic low-grade inflammation arising from metabolically active tissues like the adipose, muscle, and liver experiencing metabolic challenges[93,115,116]. In animal and human studies, maternal overnutrition was associated with increased expression of placental genes related to inflammation and oxidative stress and similarly, increased production of inflammatory cytokines like tumour necrosis factor, interleukin-6, and toll-like receptor-4[117,118]. Similar findings have been noted in the brain. Third-trimester fetuses of high-fat-fed macaque mothers have shown enhanced microglial activation and elevated levels of proinflammatory cytokines[119]. Increases in microglial activation have also been noted in the hippocampus of adult rodent offspring born from high-fat-fed dams and these changes were accompanied by increased anxiety-like behaviours and deficits in spatial learning[113].

Research has just begun to uncover the effects of maternal overnutrition on fetal brain development. Given the strong correlation between overnutrition and cognitive dysfunction in the adult brain, as well as the preliminary evidence of its perinatal influence described above, it is likely that overnutrition experienced during the critical period of intrauterine development would confer similar detriment.

1.3 Brain-Derived Neurotrophic Factor (BDNF)

1.3.1 Overview

BDNF is a member of the neurotrophin family, a group of small secreted proteins that play an important role in directing neurodevelopment *in utero* and into adulthood[48]. Aside from its critical role in the brain, BDNF has multiple functions in peripheral tissues. Both BDNF and its receptors are expressed in tissues outside of the central nervous system including, but not limited to, the skeletal muscle, heart, liver, adipose tissue, and the placenta [120–122]

Within most tissues BDNF is expressed constitutively; however, in the brain, production and release of BDNF are activity-dependent; a calcium influx stimulated by membrane depolarization triggers downstream signalling pathways resulting in the binding of transcription factors that stimulate BDNF expression[123,124]. Synthesis begins with a 32kDa precursor called pro-BDNF, which is then processed intra-or extra-cellularly into the mature 14kDa protein[48]. The extracellular cleavage of pro-BDNF is mediated by plasmin and metalloproteinases, while intracellular cleavage is mediated by furin and prohormone convertase 1 within the trans golgi network [48]. Secreted BDNF and its precursor mediate their effects through receptor binding and signal transduction.

The two primary receptors with which BDNF binds are the tropomyosin receptor kinase B (TrkB) and the p75 neurotrophin receptor (p75NTR)[46]. The TrkB receptor is a high-affinity receptor from the tyrosine kinase receptor family that is highly selective for mature BDNF[46]. Ligand-binding stimulates receptor dimerization and kinase activation, initiating complex intracellular signalling cascades[48]. These signalling pathways have previously been discussed in detail by Sandhya *et al*[125]. Among these pathways, MAPK, PLCγ, and PI3K are most often highlighted as they mediate the primary cell survival and regenerative functions of BDNF-TrkB signalling[48,123]. P75NTR is a low-affinity receptor belonging to the tumour necrosis factor receptor superfamily[46]. Unlike the TrkB receptor, it shows minimal binding selectivity in that it binds multiple analogous neurotrophins; however, it does show a higher affinity for proBDNF[46,126].

Understanding the exact molecular functions of BDNF is made complicated by the opposing actions of mature BDNF and its precursor, which are often stored and secreted together

with cellular activity[126]. Contrary to the function of TrkB, p75NTR generally has pro-apoptotic functions; but when both receptors are co-expressed, p75NTR promotes ligand discrimination and increases the binding affinity of TrkB[46,123]. Evidence also suggests that truncated isoforms of the TrkB receptor exert dominant negative inhibition of p75NRT, thus preventing cell death[127]. Thus, while its primary functions are trophic in nature, it is likely the unique expression pattern of BDNF, its precursor, and its receptors that define signalling in any given tissue[126].

In sum, BDNF is an activity-dependent growth factor, expressed within and beyond the central nervous system. Through complex signalling pathways, it balances cell growth and survival with programmed cell death making it an essential aspect of fetoplacental development.

1.3.2 The Role of BDNF in Placental Development

BDNF and its corresponding TrkB receptor are both expressed by decidua and trophoblast cells of the placenta at various stages of pregnancy [122,128,129].

As early as embryonic development, BDNF-TrkB interactions promote embryo growth and suppress apoptosis through paracrine signalling mechanisms[122]. Moreover, they promote trophoblast proliferation and outgrowth of the blastocyst prior to implantation[122].

As pregnancy advances post-implantation, BDNF continues to support the growth and survival of placental cells[122,128]. BDNF is expressed by both syncytiotrophoblasts and EVT, while the TrkB receptors are expressed in cytotrophoblasts and EVTs[128]. TrkB signalling promotes the differentiation, proliferation, and survival of cytotrophoblasts and EVTs, two integral cell types involved in establishing placental structure[128,130]. Consequently, inhibition of this signalling pathway has been previously associated with decreased human cytotrophoblast proliferation and differentiation, and suppressed outgrowth of human EVTs[128]. Moreover, this inhibition led to reduced cytotrophoblast cell viability, marked by reduced glucose metabolism and increased apoptosis, underscoring the importance of BDNF in maintaining placental integrity[128].

A major determinant of fetal growth and development is the placenta's ability to supply nutrients, an ability that is dependent on placental size, morphology, blood flow, and transporter availability[131]. By promoting the growth and development of the placenta, placental BDNF effectively supports the growth and development of the fetus. This is evidenced by *in vivo*

inhibition of BDNF-TrkB signalling, which was associated with reduced placental and fetal weights in a murine model[122]. In addition to placental size, BDNF expression is positively correlated with gestational age. As pregnancy advances, both BDNF and TrkB expression are increased in animal and human placentae[129]. It follows that BDNF expression in the placentae and fetal cord blood is reduced in preterm human pregnancies[132,133].

Aside from its role in cell growth and survival, BDNF has been classified as an angioneurin—a molecule that affects both neural and vascular functions[134]. Through binding to TrkB receptors expressed on endothelial cells, BDNF stimulates angiogenesis in the heart, skeletal muscle, and skin[135]. In addition, the absence of p75NTR receptor signalling can lead to defects in vascular systems, including blood vessel dilation and rupture, and blood cell leakage[136]. It is postulated that BDNF may also exhibit the same angiogenic effects in the placenta, promoting angiogenesis and maintaining the health of vasculature in the fetoplacental unit[133,136,137]. Preeclampsia is a pregnancy complication with unknown origin; however, its etiology is thought to involve altered uteroplacental perfusion resulting from abnormal placental development[138]. Reductions in placental BDNF expression have been noted in the placentae of pre-eclamptic mothers, further suggesting the neurotrophin's role in supporting vascular development in the placenta[137,138].

Stressors in the maternal environment have a notable impact on placental BDNF expression. Maternal energy status of either extreme decreases placental BDNF expression[130,139]. Maternal obesity specifically was associated with reduced expression of both BDNF and its receptor and disrupted signalling that occurred in a sexually dimorphic manner in the human placenta[139,140]. Moreover, both acute and chronic maternal psychosocial stress have been associated with reduced BDNF expression in rodent models[141].

In addition to supporting overall placental development, placental BDNF also plays a role in fetal development. In the absence of BDNF, mouse fetuses either failed to reach term or were non-viable at birth[98]. The primary sources of BDNF are endogenous fetal synthesis, placental production, and maternal passage[142]. Contributions from these sources differ throughout pregnancy. Early in gestation, the placenta is a major source of BDNF[98]. As pregnancy advances the fetus begins to produce its own supply of BDNF and its reliance on placental BDNF diminishes[98]. This period aligns with the cerebral development and maturation of the fetus

occurring in the latter half of gestation[132]. Despite the fetus' ability to synthesize the neurotrophin, maternally-derived BDNF has been identified in the fetal brains of mice at term and concentrations of BDNF in these fetal brains show dose-dependent increases in response to exogenous maternal BDNF administration[98]. The importance of the maternal BDNF supply is further supported by the sharp decline in BDNF in fetal blood noted immediately after birth, reflecting the abrupt removal of its exogenous sources[143]. Thus, placental BDNF is a major contributor of fetal BDNF and alterations in its expression or production may prove detrimental to fetal development and programming.

In summary, BDNF and its primary receptor are expressed across maternal and fetal compartments of the placenta. Through signalling mechanisms, BDNF promotes the growth and development of placental tissue and likely its vascular system. Stressors in the external and maternal environment can have detrimental impacts on placental BDNF expression, contributing to poor pregnancy outcomes. As a primary source of BDNF for the developing fetus, the impact of these alterations likely extends to the fetus itself, programming altered developmental outcomes.

1.3.3 The Role of BDNF in Early Brain Development

BDNF is most well known for its role in the brain, both during *in utero* development and into adulthood. Before the fetal brain can produce its own endogenous supply of BDNF, the neurotrophin is supplied by the mother and placenta[98]. As pregnancy advances, the fetus increasingly relies on endogenous BDNF produced by the cells of its expanding central nervous system, even though maternally-derived BDNF continues to be supplied across the placental barrier[98]. Both BDNF and its precursor are stored in the axons and dendrites of neurons and released in an activity-dependent manner[126]. Within the brain, expression is highest in the hippocampus and hypothalamus with moderate expression in the cerebral cortex, thalamus, amygdala, and cerebellum[46,48].

Throughout fetal development, BDNF is involved in nearly all neuronal processes including neurogenesis, myelination, synaptogenesis, and synaptic refinement[46,144]. During the rapid expansion of the nervous system during the first two trimesters, BDNF mediates cortical neuro- and glial-genesis by promoting cell proliferation, differentiation, and migration[46,126]. The

balance between BDNF and its precursor pro-BDNF helps to determine cell fate and regulate apoptosis following expansive cell proliferation[124,145]. This form of regulated apoptosis is essential for controlling the size of neuron and glial cell populations[145]. While cortical neurogenesis is typically completed by gestational week 25 in humans, neurogenesis within the hippocampus continues throughout gestation and after birth into adulthood through a process that is similarly supported by BDNF[146–148]. Both hippocampal infusion and peripheral injection of BDNF have been associated with dramatic stimulation of hippocampal neurogenesis in various rodent models[149–151]. This form of continued neurogenesis is one way in which BDNF contributes to learning, memory, and neuronal plasticity, chief functions of the hippocampus.

Beginning in the latter half of gestation, BDNF promotes myelination and synaptogenesis, generating a functional neural network[124,144]. The majority of myelination occurs by the first two years of life, although it has been reported that myelination processes may persist into early adulthood[82,144]. In addition to promoting the proliferation and survival of oligodendrocytes, the myelin-producing cells of the central nervous system, BDNF promotes the synthesis of myelin itself[144]. Consequently, BDNF-heterozygous mouse models have been associated with reduced myelin basic protein and altered myelination patterns in the hippocampus that disrupt cognitive function[144].

Much like hippocampal neurogenesis, both synaptogenesis and synaptic refinement begin *in utero*, but continue throughout adult-life, and are important mediators of synaptic plasticity[152,153]. Specifically, BDNF signalling through the TrkB receptor promotes the growth and branching of dendritic processes, increasing their size, number, and complexity[126,153]. Much like its regulation of neuronal apoptosis, the balance between BDNF and pro-BDNF signalling is thought to control long-term potentiation and long-term depression by strengthening or eliminating connections in an activity-dependent manor[124,153]. Malfunctioning or ineffective connections are thus removed, while new connections are created and strengthened[124]. These functions overlap with the functions attributed to regulated microglial activation. Microglia, like neurons, regulate their own BDNF secretion, through which it is suspected they modulate synapse refinement and tissue re-modelling[154].

Evidently, BDNF is heavily involved in neurodevelopment *in utero* and beyond. As such, deficits in BDNF communicate serious cognitive detriments. Complete BDNF knock-out models

rarely reach adulthood and typically have severe sensory impairment, reflecting the importance of the neurotophin[32,104]. The Val66Met single nucleotide polymorphism is a human mutation in the BDNF gene that causes decreased BDNF trafficking into activity-dependent secretory pathways resulting in its reduced expression[152]. Both Val66Met models and experimental BDNF inhibition or downregulation have been associated with impairments in learning and memory, as well as increased susceptibility to psychiatric and neurodegenerative disorders, including schizophrenia, bipolar disorder, major depressive disorder, and Alzheimer's disease[32,33,104,126,152].

Most of these cognitive outcomes are attributed to deficits in the hippocampus, a region that is chiefly responsible for learning, memory, and behaviour. It is also a brain structure with one of the highest levels of BDNF expression, making it a natural target of BDNF inhibition[46]. The hypothalamus, which regulates multiple metabolic processes, likewise shows high levels of expression[46]. Hyperphagic obesity is a common phenotype attributed to the Val66Met mutation and is common to animal models with suppressed BDNF or TrkB receptor expression[30,152]. Within the hypothalamus, BDNF acts as an anorexigenic agent suppressing food intake[30,155]. The role of the hippocampus in eating behaviour has also been described. Specifically, impairing hippocampal-dependent memory is suspected to affect appetite control by interfering with the cognitive response to satiety signals[41]. Apart from appetite suppression, other findings suggest BDNF may have more direct effects on peripheral energy metabolism through unknown central nervous system functions and through tissues outside of the central nervous system, namely the pancreas, liver, heart, and skeletal muscle[156].

The actions of BDNF *in utero* are diverse and expansive. As a key mediator of fetal neurodevelopment, it programs neural structure and connection, setting the stage for future brain development. In addition to its roles in learning, memory, and behaviour, BDNF is also intertwined with metabolic health and energy status.

1.3.4 BDNF, Neuroinflammation, and Neuroprotection

In addition to neuronal expression, BDNF is secreted from and regulated by microglia[157–159]. Apart from facilitating synaptic refinement, the primary function of microglia is monitoring and responding to damage-associated proteins and inflammatory cytokines in the neuronal environment[49,158].

Traditional classification defines microglia as either “resting” or “activated”[160]. Resting microglia have long ratified processes and small cell bodies, which are ideal for their surveillance function[160]. When a pathogen or cytokine is detected, these microglia will become activated; the cell body swells and processes retract as the cell begins releasing inflammatory cytokines[160]. This traditional classification fails to account for the broad range of functions attributed to activated microglia. More recent literature proposes that activated microglia be divided into two classes, pro-inflammatory (M1) and anti-inflammatory (M2) activated microglia[49,158]. As the name suggests, pro-inflammatory microglia release pro-inflammatory cytokines that recruit and activate microglia at the site of damage, while anti-inflammatory microglia mediate anti-inflammatory functions through cytokines and various neurotrophic factors, including BDNF[49,158]. The M1 class produces ROS, and these cells express a greater number of antigen-presenting receptors[49]. The M2 class includes a broader range of activation states that are differentiated by their receptor profiles, cytokine production, chemokine secretion, and overall function[49]. Collectively this group regulates anti-inflammatory functions such as cellular clean-up, tissue remodelling, and angiogenesis[49].

For healthy tissue repair, acute pro-inflammatory activation is followed by anti-inflammatory activation[49]. BDNF produced by microglia acts as a mediator in this secondary response. Following tissue damage induced by local ischemia, BDNF is suspected to promote post-lesion plasticity, and attenuating BDNF levels reduces recovery of function following acute brain injury[158,161–163]. Even during the peri-natal period, BDNF is capable of counteracting acute hypoxic-ischemic brain injuries[164,165].

A continuous influx of systemic inflammatory cytokines or failure to transition into M2 activation may lead to a sustained pro-inflammatory state with the continued presence of ROS and pro-inflammatory cytokines[49]. Pathological neuroinflammation of this nature causes severe detriment to cognitive function as it leads to neurodegeneration. Unlike acute inflammation where BDNF may become upregulated, chronic inflammation appears to suppress BDNF and its TrkB receptor[104,166,167]. This in turn may also be an attributing factor to the damage induced by chronic neuroinflammation[104].

Taken together, the regulated release of BDNF from activated microglia is a mechanism underlying tissue repair following acute damage and inflammation; however, prolonged neuroinflammation suppresses BDNF expression and signalling.

1.3.5 Impact of Environmental Stressors on BDNF

Similar to BDNF expression in the placenta, neural expression is sensitive to various forms of environmental stimuli, including stress, physical activity, brain injury, and diet[104]. While BDNF likely has protective effects in combatting the detrimental effects of acute stressors, chronic stressors have been found to impair BDNF expression and signalling. These effects are largely concentrated at the hippocampus, which is both a site of abundant BDNF expression and a brain structure often targeted by extended exposure to environmental stressors[46,168].

While much of the literature points to downregulation in its expression, the exact impacts of chronic stress on BDNF-related pathways are likely dependent on the animal model, the type of stress, and the duration exposure[169]. Chronic inflammation, intermittent hypoxia, and both physiological and psychological forms of stress have been found to downregulate BDNF or its signalling pathway in the hippocampus across various murine and non-human primate models[34,50,154,170–173]. Moreover, these downregulations were associated with impaired synaptic plasticity, decreased hippocampal neurogenesis, increased hippocampal atrophy, altered behaviour, and cognitive impairment[34,50,170].

Just as environmental stressors can impact the expression of BDNF in the adult brain, so too can they influence its expression in the fetal brain when encountered during the prenatal period. The hippocampus remains a major target of the damage induced by prenatal stress and it has been postulated that the vulnerability of the hippocampus may be attributed to alterations in BDNF signalling[174]. Both physiological and psychosocial forms of maternal stress during pregnancy, including maternal nutrient restriction, placental insufficiency, chronic hypoxia, social isolation, and variable unpredictable stress, have been associated with reduced expression of BDNF in the fetal hippocampus in multiple rodent models, including the guinea pig[174–178]. In some of these studies, the effect persisted into offspring adulthood, communicating long-term deficits in brain structure and function[174,176,177]. For example, rats exposed to chronic unpredictable prenatal stress showed decreased BDNF expression, reduced hippocampal cell

proliferation, and increased apoptotic signalling, thus reducing hippocampal volume[174,176]. Moreover, this reduction was paralleled by increased memory impairment in adult offspring[176].

More insidious forms of systemic stress, such as those related to energy status and metabolic health, have also been found to regulate BDNF expression and signalling. Both diabetes and obesity have been associated with reduced levels of BDNF in humans and rodent models, although it is suspected that impaired BDNF expression is both a contributing factor to, and a by-product of, metabolic dysfunction[30,48,179,180]. Independent of metabolic dysfunction, lifestyle factors such as diet and exercise that impact energy status also influence BDNF signalling. Multiple studies have suggested that diets high in saturated fats and/or simple sugars reduce hippocampal BDNF levels, impede its signalling, and alter cognitive function[47,181–184]. In experimental models, WD feeding for as little as two months reduced hippocampal expression of BDNF and its downstream effectors of neurogenesis and synaptogenesis, which coincided with impaired spatial learning performance in rodents and non-human primates[47,181,184]. Moreover, the WD was found to worsen cognitive outcomes following brain injury or sleep deprivation with reduced learning performance that matched the reduction in BDNF signalling in rat models[182,183]

Much like other maternal stressors, the impacts of maternal metabolic dysfunction and WD consumption are not limited to the mother. The extent to which these forms of maternal stress extend to BDNF expression in the fetal brain is less well described; however, a similar reduction in BDNF expression has similarly been reported in the fetal rat hippocampus following a high-fat maternal diet, which was also linked to worsened learning performance postnatally[97,140].

Detriments associated with this dietary pattern are likely due to the specifics of its macronutrient composition. While the saturated fats and refined sugars characteristic of the WD suppress BDNF expression, omega-3 fatty acids appear to mitigate this effect in rats[181]. The beneficial impact of omega-3 fatty acids on hippocampal BDNF expression has also been noted in the fetal mouse brain following maternal docosahexaenoic acid (omega-3) supplementation[185]. Positive effects on BDNF expression have also been attributed to exercise, which has an impact so striking that it has been proposed as a non-invasive way to mimic the effects of direct BDNF administrations[126,186]. This offers greater support to suggest that BDNF is highly related—positively and negatively—to metabolic health and energy status.

Hippocampal BDNF expression and signalling, and consequently cognitive function, are impacted by a broad range of environmental conditions, that include diet and metabolic status. In the context of pregnancy, maternal metabolic health and dietary patterns may then communicate neurological deficits through alterations in BDNF expression.

1.4 Rationale, Hypothesis, And Objectives

1.4.1 Rationale

The increasing availability of cheap processed foods has made the WD widely accessible, resulting in its habitual consumption. The rise in WD popularity is paralleled by increasing rates of obesity and metabolic dysfunction[42]. This is unsurprising given the association of habitual WD consumption with the risk of various cardiovascular and metabolic diseases, including NAFLD[42]. While prevalent across society, this dietary pattern is of particular concern for the pregnant population given that many of these metabolic outcomes convey an increased risk of pregnancy-related complications[187–189].

Research investigating the impact of maternal overnutrition during pregnancy is often focused on obesity, irrespective of overall metabolic health[109]. This is problematic because there is strong evidence to suggest that under the broad term of obesity are two classes of individuals: the metabolically unhealthy and the metabolically healthy, defined based on the presence/absence of other metabolic morbidities (impaired glucose tolerance, dyslipidemia, hypertension, and systemic inflammation)[15,16,18,190]. Analogous classifications exist amongst a lean population, with some possessing equal or greater metabolic and cardiovascular risks than those who are metabolically healthy and obese[15,16,18,190]. Energy-rich diets are associated with the development of metabolic dysfunction in both obese and non-obese individuals, but typically only the obese are captured by most study designs[109]. Consequently, it is difficult to identify the risks associated with such a dietary pattern, independent of obesity. This represents a major gap in the research as ~20% of the population is estimated to fall into this lean metabolically unhealthy classification and in some conditions, such as NAFLD, this lean phenotype sustains a greater risk of further metabolic dysfunction than its obese counterpart[15,18,24]. Collectively this highlights the importance of understanding the risks associated with maintaining a lifestyle (ex,

habitual WD consumption) that perpetuates both lean and obese metabolically unhealthy phenotypes—a topic on which research is scarce, particularly in the context of pregnancy.

The DOHaD hypothesis posits that adverse events experienced *in utero* are capable of programming fetal development such that it alters the long-term health outcomes of the individual[109,191]. While initial research was focused on the implications of maternal stressors on fetal metabolic health outcomes, more recent research has found similar detriments in fetal neurodevelopment[73,95,102,192]. Brain development is resource-intensive, demanding nutrients and trophic factors from the mother and placenta to support and direct its processes[95]. Consequently, aberrations in placental development and function likely link maternal stressors to the cognitive impairment noted in their offspring. The hippocampus is particularly vulnerable to maternal stressors during pregnancy[168]. As a mediator of learning, memory, and behaviour, alterations in its development may explain cognitive abnormalities noted in offspring exposed to adversity *in utero*[35,168].

Metabolic dysfunction, such as that associated with habitual WD consumption, is an example of intrauterine adversity that may convey detriment to the fetus. In addition to fostering chronic low-grade inflammation and ROS production, the WD alters energy balance, all of which have been shown to interfere with fetal growth and neurodevelopment[93,102,192]. While the exact mechanism behind this relationship remains unknown, BDNF has emerged as a possible candidate linking maternal energy status with placental and fetal brain development as its expression in both tissues is impacted by both maternal under- and over-nutrition[97,112,193]. Once again, studies focused on maternal over-nutrition often fail to capture the subset of metabolically unhealthy individuals who are lean, instead focusing on the consequences of diet-induced maternal obesity[97,112]. Consequently, it is not clear whether BDNF expression in the placenta or the brain is subject to regulation by the WD, independent of obesity.

The impact of such changes could be severe. BDNF plays a formative role in the development of both tissues, supporting cell growth and proliferation[128,148]. Postnatally, BDNF continues to support learning and memory through its effects on hippocampal neurogenesis and synaptogenesis, and to facilitate tissue remodelling following neuroinflammation through its microglia-mediated release[148,152,153]. It follows that a reduction in BDNF expression in either the placenta or the brain *in utero* may impact their structural and functional development, and in the

brain specifically, may convey long-term changes to the offspring's cognition. Understanding the relationship between placental BDNF expression and that of the fetal hippocampus is valuable because placental expression at term may serve as a non-invasive indicator of its relative expression in a newborn's brain, potentially indicating risk of future cognitive dysfunction.

Taken together, the present research is focused on understanding the impact of adverse intrauterine conditions, specifically WD-induced metabolic dysfunction, on placental development and fetal neurodevelopment. Studies of this nature far and few between; of those that have previously been conducted, clinical applicability is often hindered by limitations of the animal model and their exclusive focus on the obese phenotype. The guinea pig is considered a superior model of human pregnancy and neurodevelopment due to similarities in placental structure and its relatively long gestational period (~68 days)[80,194]. Unlike other rodent models, guinea pigs give birth to precocial young, meaning that extensive neurodevelopment occurs *in utero* and is thus more susceptible to pregnancy conditioning like maternal dietary patterns[80]. Additionally, studies have shown the guinea pig's propensity to adopt a lean NAFLD phenotype in response to chronic WD consumption, reflecting the elusive lean metabolically unhealthy phenotype[195,196]. These features set the guinea pig apart as one of the top models for the developmental origins of neurological dysfunction, particularly in the context of WD-associated lean metabolic dysfunction.

1.4.2 Hypothesis

It was postulated that chronic maternal WD exposure, resulting in a lean metabolically unhealthy phenotype, would alter fetoplacental development in a guinea pig model. Moreover, it was hypothesized to impact the expression of BDNF in the placenta and fetal brain, potentially communicating alterations in neurodevelopmental processes such as hippocampal neuro- and glia-genesis, and microglial activation.

1.4.3 Objectives

The objectives of this work are as follows:

1. To characterize maternal and fetal guinea pig growth characteristics for the body and select organs in near-term pregnancies featuring chronic maternal WD exposure using magnetic resonance imaging (MRI) segmentations and weights at collection.

2. To determine the presence and extent of changes in placental pathology in placentae of WD-fed mothers using a semi-quantitative method to score placental necrosis and fibrin thrombi on H&E stained placentae.

3. To determine the presence and extent of change in the relative expression of BDNF in the placenta and hippocampus of fetal guinea pigs born to WD-fed sows using Western Blot and immunohistochemistry.

4. To determine the presence and extent of changes in cell proliferation and microglial activation states in the hippocampal region of fetal guinea pigs born to WD-fed sows, using immunohistochemistry to: a) quantify the number of Ki67 (marker of cell proliferation) positive cells, b) quantify the number of Iba1 (marker of microglia) positive cells and characterize cell morphology as an indicator of microglial activation.

CHAPTER 2: MATERIALS AND METHODS

2.1 Animal Care

2.1.1 Ethics Statement

All animal care protocols and procedures were conducted in accordance with guidelines and standards of the Canadian Council on Animal Care. The Western University Animal Care Committee approved Animal Use Protocol (AUP #2019-116; Appendix A) and conducted post-approval monitoring. All investigators understood and followed the ethical principles outlined by Grundy[197] and the study design was informed by ARRIVE guidelines[198]. A single cohort of guinea pigs was used for all experiments in this thesis.

2.1.2 Animal Feeding, Breeding, and Pregnancy

The female offspring of commercially bred guinea pigs (Dunkin-Hartley, Charles River Laboratories, Senneville, QC) were weaned at 15 days of age and housed in individual cages in a small animal care facility with a 12-hour light-dark cycle and controlled temperatures (20 ± 2°C) and humidity (30–40%). At weaning, female pups were randomized to either the Control Diet (CD; TD.110240; Harlan Laboratories, Madison, WI) (n=3) or the Western Diet (WD; TD.110239; Harlan Laboratories, Madison, WI) (n=13) (**Fig 2.1.2-1**) (See **Table 2.1.2-1** for detailed nutrient breakdown). WD was designed to represent human WD consumption[199]. Kilocalorie density differed between diets (3.4 vs 4.2 kcal/g); however, protein levels were matched between the diets based on kilocalorie density. Proteins in both diets were supplied by isolated soy proteins and made up 21.6% and 21.4% of the total kilocalories for CD and WD respectively. In the CD, carbohydrates accounted for 60% of total kilocalories, containing sucrose (100 g/kg), cornstarch (354 g/kg), and cellulose (130 g/kg). In the WD, carbohydrates comprised 33% of total kilocalories, containing greater proportions of sucrose (190 g/kg) as well as the addition of straight fructose (65g/kg). In the CD, fats made up 18.4% of the total kilocalorie density and were derived from soybean oil (60 g/kg), with the following fatty acid distribution (% total fats): 16.7% saturated fatty acids (SFA), 22.2% monounsaturated fatty acids (MUFA), and 61.1% polyunsaturated fatty acids (PUFA). Fats in the WD were sourced from a combination of coconut oil (95 g/kg), cocoa butter (50 g/kg), and lard (55 g/kg), making up 33% of total kilocalorie density derived largely from SFAs (69.6%) and MUFAs (26.1%), rather than

PUFAs (4.3%). In addition, the WD contained cholesterol (2.5g/kg). From the point of weaning, animals were weighed twice weekly and total feed consumption was monitored daily.

The estrous cycles of sows were tracked. A nulliparous female found in estrous was placed in a breeding cage with a male for ~48-72 hours until the vaginal membrane was closed. Pregnancies were then confirmed 18-25 days later by ultrasound. The day prior to vaginal membrane closure was considered the day of conception. Animals that were unsuccessful at conceiving were rebred at their subsequent estrous cycle(s). Age of sows at conception averaged (mean ± SEM) at 139 ± 10.21 days for CD animals and 163 ± 9.6 days for WD (difference in age was not significant between groups). A second ultrasound was performed on gestational day 57-60 to confirm litter size prior to MRI and subsequent tissue collection. MRIs were conducted at day 60 of pregnancy. Animals were sacrificed by CO_2 asphyxiation on gestational day 65 (term ~68 days)[200].

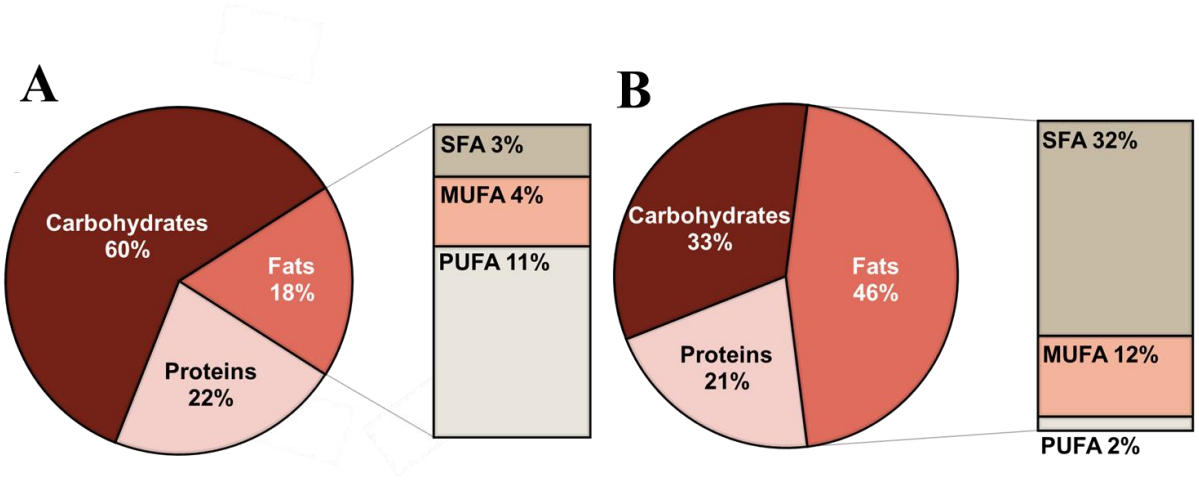

Figure 2.1.2-1. Macronutrient breakdown of (**A**) Control diet and (**B**) Western diet.

Table 2.1.2-1 Composition of the experimental Control diet (CD) and Western diet (WD**)**

Item	CD∗ (TD.110240)	WD* (TD.110239)
Main ingredients (g kg−1)		
Isolated soy protein	210	255
L-Methionine	2.47	3
Sucrose	100	190
Fructose	—	65
Corn starch	354	—
Maltodextrin	93	93
Cellulose	130	130
Soybean oil	60	—
Cocoa butter	—	50
Lard	—	55
Coconut oil	—	95
Cholesterol	—	2.5
Vitamin Mix Teklad (40060)	10	12.3
Vitamin C, L-ascorbyl-2-polyphosphate (35%)	0.61	0.75
Folic acid	0.008	0.01
Calcium phosphate dibasic	17.66	21.5
Potassium citrate, monohydrate	9.85	12
Magnesium oxide	2.63	3.2
Potassium chloride	3.29	4
Sodium chloride	1.64	2
Calcium carbonate	4.1	5
Ferric citrate	0.33	0.4
Manganese sulfate, monohydrate	0.164	0.2
Zinc carbonate	0.05	0.06
Cupric sulfate	0.0164	0.02
Potassium iodate	0.0008	0.001
Chromium potassium sulfate, dodecahydrate	0.008	0.01
Sodium selenite, pentahydrate	0.0008	0.001
Ammonium paramolybdate, tetrahydrate	0.0002	0.0003
Chemical composition		
Protein (% kcal)	21.6	21.4
Fat (% kcal)	18.4	45.3
Carbohydrates (% kcal)	60	33.3
Energy (kcal g^{-1})	3.4	4.2
Fatty acid composition (% of total fatty acids)		
Lauric acid (C12:0)	—	23.12
Myristic acid (C14:0)	—	8.83
Palmitic acid (C16:0)	11	17.32
Stearic acid (C18:0)	4	13.24
Oleic acid (C18:1*cis*9)	23.5	24.4
Linoleic acid (C18:2*n*−6)	53.4	4.2
α-Linoleic acid (C18:3*n*−3)	8	0.03

∗Diets were formulated in the Harlan Laboratories (Madison, WI, USA).

2.1.3 Tissue Collection and Sample Preparation

At collection maternal bodies and livers were weighed immediately upon sacrifice (CD: n=3, WD: n=13). Placentae were weighed and then sectioned in half longitudinally; half was immediately flash-frozen in liquid nitrogen for use in western blotting experiments, while the second half was prepared for fixation for use in immunohistochemistry. Fetuses were weighed and sexed before undergoing full necropsy (CD: n=9, WD: n=24). Fetuses that showed evidence of *in utero* demise (degrading brain and/or evidence of reabsorption) were excluded from the collection. Fetal brains were weighed and coronally sectioned at the optic chiasm through the mamillary body. Fetal brains and placentae were immersion fixed using 4% paraformaldehyde for 24 hours, rinsed 3X with phosphate-buffered saline (PBS) for 2-hour intervals, and then washed with 70% ethanol for 14 days. The blocks were processed in paraffin wax, embedded, and sectioned using a rotary microtome at a thickness of 5 μm. Tissue sections were then mounted on 1.5mm Superfrost Plus Slides (VWR Scientific, Westchester, PA).

To ensure fetal hippocampal structures were preserved in all sections, a single section from each caudal brain block was stained with hematoxylin and eosin (H&E). Using the *Comparative Mammalian Brain Collection – Guinea Pig* (University of Wisconsin) as a reference, each brain was systematically re-sectioned and stained with H&E until the correct brain region (#840; **Fig 2.1.3-1**) was achieved.

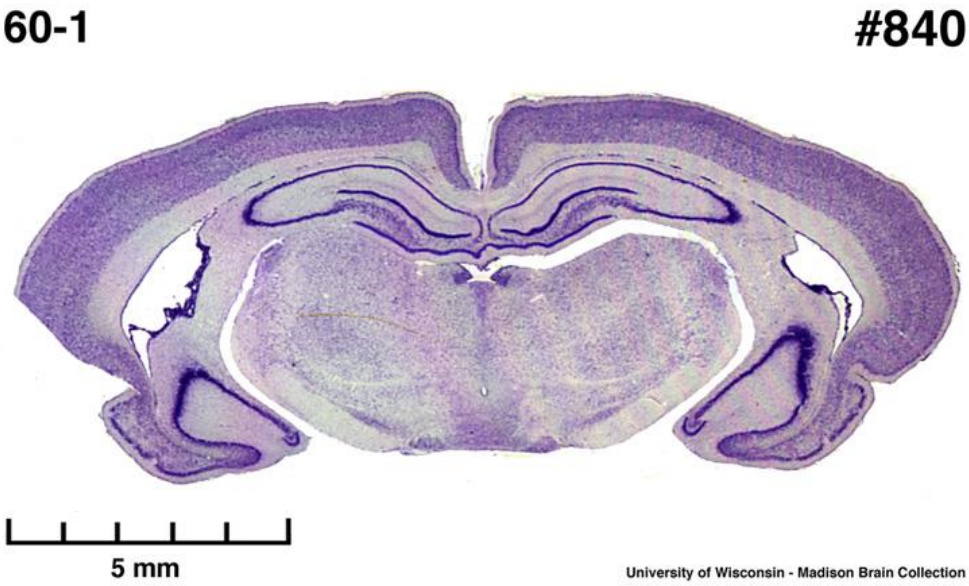

Figure 2.1.3-1. Representative image of coronally-sectioned guinea pig brain stained with Nissl. Obtained from *Comparative Mammalian Brain Collection* created by the University of Wisconsin.

2.2 Magnetic Resonance Imaging

2.2.1 Maternal Magnetic Resonance Imaging

All magnetic resonance imaging (MRI) was performed by members of the McKenzie Laboratory as described by Sinclair, *et al* on ~day 60 gestation[196]. Images were used to quantify body and tissue volumes, as well as hepatic fat fractions for both maternal and fetal animals.

Briefly, animals were fasted for 2 hours prior to MRI. Glycopyrrolate (0.01mg/kg body weight) was administered to animals 30 minutes prior to MRI via subcutaneous injection to reduce saliva production and minimize the risk of asphyxiation while anesthetized. Following glycopyrrolate administration, sows were anaesthetized using a constant flow of 4.5% isoflurane and then maintained with 1.5-2.5% isoflurane. Sows were supplied with 100% O_2 (2L/min) for the duration of the experiment. Vital signs (heart rate, electrocardiogram waveform, respiratory rate, respiratory waveform, oxygen saturation, and temperature) were monitored throughout the

experiment using an animal monitoring kit (Small Animal Instruments, Stony Brook, NY). The core body temperature of the sows was maintained with a water-bath system circulating heated water beneath them. Foam earplugs were inserted, and protective lubricant was applied to both eyes.

A series of anatomical T1 and IDEAL water-fat MRI images were collected with a 3 Tesla MRI (MR750, General Electric, Waukesha, WI, USA) as described by Sinclair *et al*[196]. For T1-weighted gradient-echo images (repetition time/echo time [TR/TE] = 5.1ms/2.4ms, flip angle = 15°, number of averages = 4, total scan time ~ 7min) were acquired with 0.875 × 0.875 mm^2 in-plane spatial resolution and 0.9 mm slice thickness. IDEAL water-fat images (TR/ΔTE = 9.4ms/0.974ms, 6 echoes, flip angle = 4°, number of averages = 4, total scan time ~ 13min) were also collected for each guinea pig with 0.933 × 0.933 mm^2 in-plane spatial resolution and 0.9 mm slice thickness. IDEAL images were accelerated with parallel MRI by a factor of 1.75 in both the phase and slice directions. For all images, the field of view was centred on the sow's abdomen, ensuring all fetoplacental units were in view. Following MRI, the sows were monitored while recovering from anesthesia, after which they were returned to their cages.

2.2.2 MRI Image Analysis

Manual, full-volume segmentation was performed on T1 anatomical images and water-fat images using 3D Slicer software (version 4.9.0)[201] (**Figure 2.2.2-1**). Whole-body and organ volume measurements for maternal (CD: n=3, WD: n=13) and fetal populations (CD: n=11, WD: n=26) were obtained from T1 anatomical images, while maternal visceral adipose tissue (VAT) and fetal total adipose tissue (TAT) volumes were derived from water-fat images. Hepatic proton-density fat fractions (HPFF), representing a quantitative measure of the fraction of hepatic tissue composed of triglycerides, were likewise acquired[202]. To obtain the HPFF, the liver was segmented using anatomical images and then triglycerides were quantified using water-fat images.

Fetuses that showed evidence of intrauterine demise (3 fetuses from the WD group) were excluded from analyses. Sex was not a variable considered in the fetal analysis because it was unidentifiable at the time of MRI.

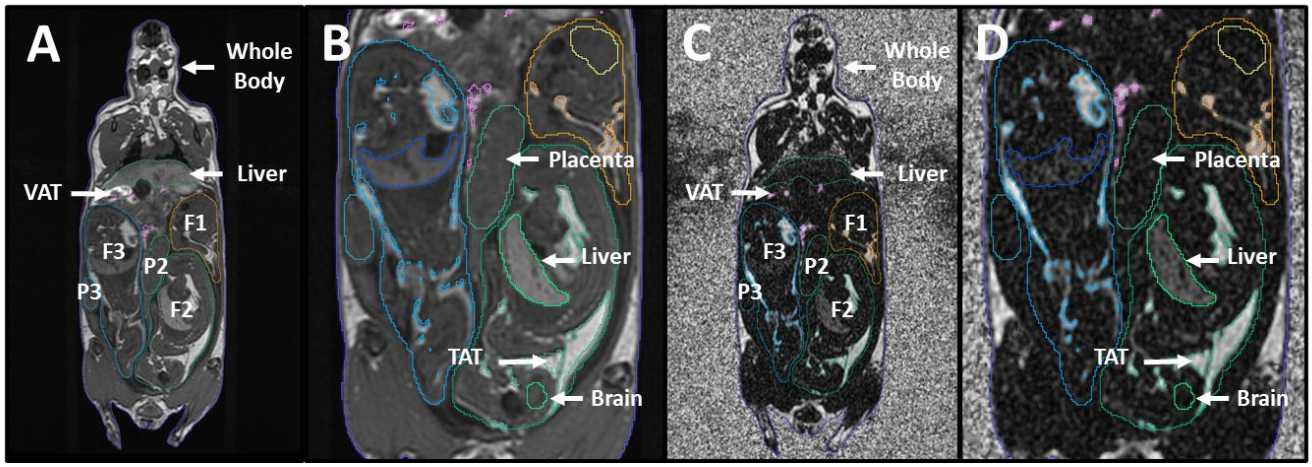

Figure 2.2.2-1. Whole-body segmentation conducted on (**A&B**) T1 anatomical images and (**C&D**) IDEAL water-fat images. Images represent single frames of MRI sequence. Outlined regions represent whole-body, organ, or tissue volumes. VAT: visceral adipose tissue; F: fetus; P: placenta; TAT: total adipose tissue.

2.3 Placental Pathology Scoring

2.3.1 Hematoxylin and Eosin Staining

Following deparaffinization, placental sections were rinsed for 5 minutes in pure water and then immersed for 10 seconds in Harris modified hematoxylin stain (Fisher Scientific, Hampton, NH). The stain was differentiated in 1% acid ethanol (2 mL HCl + 198 mL 70% ethanol) for 1 second and then immediately rinsed under running water for 1 minute. Tissue sections were then stained with eosin (1% eosinY in 95% ethanol and 0.5% glacial acetic acid; Fisher Scientific, Hampton, NH) for approximately 1 second. The tissue sections were then dehydrated and DPX mountant was applied before mounting the slides with a coverslip.

2.3.2 Pathology Scoring

To characterize the health of placental tissue, H&E stained sections of placentae were semi-quantitatively analyzed at multiple magnifications by an experienced pathologist. The

scoring system used was established for the present study, although similar schemes have previously been used to characterize placental pathology[203,204]. To eliminate any experimental bias, the scorer was blinded to the diet group of each animal.

Sections were scored from 0 to 3 for the distribution and the degree of necrosis or fibrin thrombi (**Table 2.3.2-1**). Individual scores of distribution and degree were summated to yield a necrosis score and a fibrin score, each ranging from 0 to 6. The total pathology score represents the sum of necrosis and fibrin scores, in which a score of 0 represents a healthy placenta and a score of 12 represents a placenta with wide-spread, severe necrosis, and fibrin thrombi.

Table 2.3.2-1. Pathology Scoring System

	Score	0	1	2	3
Necrosis	**Degree**	Minimal	<25%	25-50%	>50%
	Distribution	None	Focal	Multifocal	Multifocal to coalescing or locally extensive
Fibrin thrombi	**Frequency**	None	1-2	<5	>10
	Degree	None/minimal	Small, discrete	Large	Extensive area

2.4 Western Blotting

2.4.1 BDNF Western Blot Analysis

Flash-frozen placentae were ground and homogenized in RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Na-deoxycholate, pH 7.4) with protease and phosphatase inhibitors. Samples were sonicated and then centrifuged at 10,000g for 10 min at 4°C. The supernatants were collected, and their protein levels were quantified using the Pierce BCA Protein Assay Reagent Kit (ThermoFisher, Waltham, CA), following the manufacturer's instructions.

A sample from each supernatant was mixed with 5X laemmli sample buffer and beta-mercaptoethanol (BME), and then heated for 10 min at 75°C. From each sample, 30 µg of total protein were loaded into individual wells of polyacrylamide gels for SDS-PAGE (4% stacking gel, 10% separating gel). The prepared placental samples were distributed across three gels such that each gel had samples from both sexes and from both diet groups. One lane of each gel was loaded with the same pooled sample for subsequent normalization, and another lane from each gel was left empty to serve as a negative control. Gels were run at 120V until the dye fronts reached the bottom of gels (~60-90 mins) in 1X running buffer. Once separated, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Hybond P 0.2 µm PVDF) using the wet transfer method at 100V for 90 minutes in 1X transfer buffer.

Following protein transfer, membranes were incubated in Ponceau S stain for 5 minutes, and then rinsed briefly with methanol. The Ponceau S stain binds to protein transferred to PVDF membranes, which confirms the extent and success of the protein transfer, and visible protein lanes can then be quantified and used for total protein normalization[205]. Bands representing total protein were visualized on ChemiDoc (Bio-Rad Laboratories, Hercules, CA) and show efficient transfer and allow for total protein normalization.

Membranes were blocked with 5% milk diluted in tris-buffered saline/tween 20 (0.01%) (TBS-T) for 1 hour at room temperature on a shaker. Blots were then incubated with anti-rabbit polyclonal BDNF (1:1000; PA5-85730, Invitrogen, Waltham, CA) diluted with 5% milk in TBS-T overnight at 4°C on an orbital shaker. The following day, blots were probed with horseradish peroxidase-conjugated anti-rabbit IgG (1:10000; #7074, Cell Signalling Technologies, Danvers, MA) secondary antibody diluted with 5% milk in TBS-T for 1 hour at room temperature.

Immunoreactive bands were visualized on ChemiDoc (Bio-Rad Laboratories, Hercules, CA) using electrochemiluminescent substrate Clarity Max (Bio-Rad Laboratories, Hercules, CA) and ensuring exposure times were kept constant across all blots. A single band was detected at ~14 kDa representing mature BDNF expression. Bands representing total BDNF protein abundance were quantified using the ImageLab software (Bio-Rad Laboratories, Hercules, CA) and then normalized by the total protein (Ponceau S stain) as previously described by Sander *et al*[206].

2.5 Immunostaining and Image Analysis

2.5.1 BDNF Immunohistochemistry, Image Acquisition, and Analysis

All staining was performed on the same day with the same batch of reagents to limit staining variability. The tissue sections were deparaffinized in three sequential 5-minute xylene washes and then rehydrated in a graded series of ethanol washes (2X at 100%, 2X at 90%, and 1X at 70%) for 2 minutes each, followed by running tap water for 5 minutes. Heat-induced epitope retrieval (HIER) was performed using 10mM Sodium Citrate buffer pH 6.0 in the 2100 Antigen Retriever Device (Aptum Biologics Ltd, Southampton, UK). Following HIER, the samples were washed in PBS (2X 5mins) and then 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. After two rinses in phosphate-buffered saline (PBS), background SNIPER (Biocare Medical, Pacheco, CA) was applied to the samples for 7 minutes to reduce non-specific background staining. Samples were then incubated with anti-rabbit polyclonal BDNF primary antibody (1:500; PA5-85730, Invitrogen, Waltham, CA) overnight at 4℃ in a covered humidity chamber. Antibodies were diluted using DAKO diluent (Agilent Technologies, Santa Clara, CA) and for one slide diluent was substituted for primary antibody to serve as a negative control.

The following day, samples were rinsed in PBS and then incubated with ImmPRESS anti-rabbit horseradish-peroxidase polymer solution (MP-6401-15, Vector Laboratories, Burlington, ON) for 45 minutes at room temperature in a covered humidity chamber. Slides were rinsed with PBS and bound antibody was visualized by incubating slides with brown diaminobenzidine (DAB) chromogen (Sigma Aldrich, St. Louis, MO) for 6 minutes. After a rinse in tap water, samples were counterstained with brief (1 minute) exposure to modified Mayers Hematoxylin stain, after which they were rinsed again with tap water. Samples were then dehydrated in an increasing series of ethanol washes (2X at 70%, 2X at 90%, 2X at 100%) for 30 seconds each, followed by three 5-minute xylene washes. Slides were immediately cover slipped with DPX Mountant.

Analyses were conducted on coronal sections at the level of the optic chiasm and mamillary bodies. Brain regions being analyzed include the CA3 and dentate gyrus of the hippocampus, along with the thalamus—sites known to express BDNF in abundance[46]. At each

brain region, 4-6 different high-power field images were captured with the Zeiss AxioImager Z1 microscope (Carl Zeiss Canada Ltd, North York, ON) using a 40X oil objective. Identical illumination settings were used for all captured images to ensure an accurate comparison.

Image analysis was performed using Image-Pro Premier 9.2 software (Media Cybernetics Inc., Rockville, MD) ensuring that the analyst was blind to the diet group of each animal. Images were analyzed based on the percent area stained and integrated optical density, which considers both the area stained as well as the average intensity of the pixels. A threshold established through the smart selection feature was used to detect areas of positive staining based on intensity and colour. Thresholds were tested on a variety of images including negative control to ensure specificity and then applied uniformly to all images to obtain data. Software was calibrated to specific magnification, prior to analysis.

2.5.2 Hematoxylin Staining, Image Acquisition, and Analysis

All staining was performed on the same day with the same batch of reagents to limit staining variability. The tissue sections were deparaffinized as described above. Samples were stained for 90 seconds with modified Mayers Hematoxylin stain, after which slides were washed in tap water. Samples were then dehydrated and mounted as previously described for BDNF immunohistochemistry.

Analyses were conducted on coronal sections at the level of the optic chiasm and mamillary bodies. Brain regions being analyzed include the CA1, CA3, dentate gyrus of the hippocampus, along with the thalamus. At each brain region, 4-6 different high-power field images were captured with the Nikon Eclipse Ti2E Inverted Deconvolution Microscope (Nikon Canada, Mississauga, ON) using a 60X oil objective. Identical illumination settings were used for all captured images to ensure an accurate comparison.

Image analysis was performed using Image-Pro Premier 9.2 software (Media Cybernetics Inc., Rockville, MD) ensuring that the analyst was blind to the diet group of each animal. Cells were counted semi-automatically with a threshold established through the smart selection feature that was trained to detect areas of hematoxylin staining based on intensity and colour. Software was calibrated to specific magnification, prior to analysis.

2.5.3 Ki67 Immunohistochemistry, Image Acquisition, and Analysis

All staining was performed on the same day with the same batch of reagents to limit staining variability. The Ki67 antigen is a marker of cell proliferation expressed by cell nuclei in the active phase of the cell cycle that has been previously used to quantify neurogenesis[207].

Ki67 immunostaining was performed as described for BDNF immunohistochemistry with the following modifications: HIER was performed using 10mM Tris-EDTA buffer pH 8.0, and monoclonal mouse anti-human Ki67 primary antibody (1:4000; IR626, Agilent Technologies, Santa Clara, CA) and ImmPRESS anti-mouse horseradish-peroxidase polymer solution (MP-7422-15, Vector Laboratories, Burlington, ON) were used as primary and secondary antibodies, respectively.

Analyses were conducted on coronal sections at the level of the optic chiasm and mamillary bodies. The brain regions being analyzed include hippocampal regions CA1, CA3, and dentate gyrus, as the hippocampus is a primary site of neurogenesis[88]. At each brain region, 4-6 different high-power field images were captured with the Zeiss AxioImager Z1 microscope (Carl Zeiss Canada Ltd, North York, ON) using a 40X oil objective. Identical illumination settings were used for all captured images to ensure an accurate comparison.

Image analysis was performed using Image-Pro Premier 9.2 software (Media Cybernetics Inc., Rockville, MD) ensuring that the analyst was blind to the diet group of each animal. Images were analyzed based on the number of Ki67-positive cells per mm^2. A threshold established through the smart selection feature was used to detect positive staining based on intensity, colour, and morphology. Thresholds were tested on a variety of images, including the negative control, to ensure specificity, and then applied uniformly to all images to obtain data. Software was calibrated to specific magnification, prior to analysis. Using calibration, raw data expressing values per μm^2 was scaled to mm^2.

2.5.4 Iba1 Immunohistochemistry, Image Acquisition, and Analysis

All staining was performed on the same day with the same batch of reagents to limit staining variability. Ionized calcium-binding adapter molecule 1 (Iba1) is a protein expressed in

both the cell processes and soma of microglia cells. As such, the Iba1 marker is commonly used to study microglial morphology, which is heavily tied to microglial activation state[160,208].

The tissue sections were deparaffinized as described for BDNF immunohistochemistry. Heat-induced epitope retrieval (HIER) was performed using 10mM Sodium Citrate buffer pH 6.0 in the 2100 Antigen Retriever device (Aptum Biologics Ltd, Southampton, UK). Following HIER, the samples were washed in PBS and then 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. After a phosphate-buffered saline (PBS) rinse, background SNIPER (Biocare Medical, Pacheco, CA) was applied to the samples for 7 minutes to reduce non-specific background staining. Samples were then incubated with primary antibody, anti-rabbit polyclonal Iba1 primary antibody (1:3000; 019-19741, FUJIFILM, College Station, TX) overnight at 4℃ in a covered humidity chamber. Antibodies were prepared in a solution of 50% background SNIPER and 50% PBS with horse serum (Vector Laboratories, Burlington, ON). One slide was incubated with this diluent preparation rather than the primary antibody to serve as a negative control.

The following day, samples were rinsed in PBS and then incubated with ImmPRESS anti-rabbit horseradish-peroxidase polymer solution (MP-6401-15, Vector Laboratories, Burlington, ON) for 45 minutes at room temperature in a covered humidity chamber. Slides were washed with PBS and then bound antibody was visualized by incubating slides with black diaminobenzidine (DAB) chromogen (Sigma Aldrich, St. Louis, MO) for 6 minutes. After subsequent washes in tap water and PBS, slides were incubated with DAPI counterstain (1:300: Life Technologies, Burlington, ON, Canada) prepared in PBS for 2 minutes at room temperature with minimal light exposure. Following PBS and tap water washes, samples were dehydrated in an increasing series of ethanol washes (1X at 70%, 1X at 90%, 1X at 100%) for 15 seconds each, followed by a 15-second dip in toluene. Slides were immediately cover slipped with DePex mountant.

Analyses were conducted on coronal sections at the level of the optic chiasm and mamillary bodies. Brain regions being analyzed include hippocampal regions CA1, CA3, and dentate gyrus, as well as the corpus callosum and thalamus. A fluorescent channel was used to visualize DAPI stain and to navigate to brain regions of interest, while a brightfield channel was

used to capture all images. At each brain region, Z-stack images were taken at 4-6 different high-power fields per brain region with a Zeiss AxioImager Z1 microscope (Carl Zeiss Canada Ltd, North York, ON) using a 40X oil objective. Illumination settings used for all brain regions were identical. Each stack of images was converted to a two-dimensional image for subsequent image analysis.

Image analysis was performed using Image-Pro Premier 9.2 software (Media Cybernetics Inc., Rockville, MD) ensuring that the analyst was blind to the diet group of each animal. Two thresholds were established through the smart selection feature. The first threshold identified positive-staining cell bodies along with attached large processes. The second threshold identified small processes. Both identified objects based on morphology and intensity of greyscale images. Thresholds were tested on a variety of images, including the negative controls, to ensure specificity, and then they were applied uniformly to all images as follows (**Fig 2.5.4-1**). The first threshold was used to capture cell bodies and their attached processes. Large processes were manually segmented, and cell bodies and large processes data were then captured independently. The second threshold was used to capture small processes that were not attached to a visible cell body.

Images were analyzed based on the number of Iba1-positive cells per mm^2, and by the morphological feature of positive staining cells. Specifically, cell body size, roundness, and cell body to process ratio were investigated as measures of microglial activation. Software was calibrated to specific magnification, prior to analysis. Using calibration, raw data expressing values per μm^2 was scaled to mm^2.

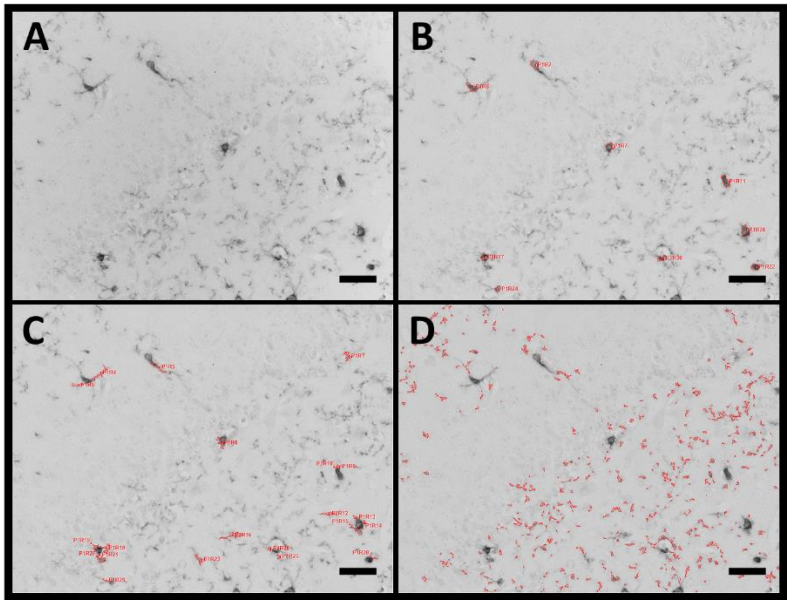

Figure 2.5.4-1. Representative images of ionized calcium-binding adapter molecule 1 (Iba1) image analysis process on high-power field (40X) Z-stack imaged of the dentate gyrus region of Control diet brain: (**A**) original image, (**B**) collect: cell body, (**C**) collect: large processes, (**D**) collect: small processes. Red outlines reflect Iba1-positive staining area being identified by software intensity and size threshold. Scale bar = 20µm

2.6 Statistical Analyses

2.6.1 Data Acquisition and Statistical Analyses

Maternal weight at conception was taken prior to breeding, while the maternal weight at sacrifice was measured prior to fetal collection minus the weights of all respective fetoplacental units. Litter sizes were based on the number of viable fetuses at collection. Gestational weight gain represents the difference between maternal weight at sacrifice, weight at conception, and the weight of all fetoplacental units. Likewise, maternal volumes attained by MRI were normalized by subtracting the volumes of all fetoplacental units. Three WD fetuses were excluded from all analyzes because they showed evidence of *in utero* demise both in MRI images and at collection. These fetuses could be identified from MRI analyses based on the integrity of their central nervous system relative to neighbouring fetuses as well as the quality of their placenta[209]. Two WD placentae were excluded from BDNF western blot analysis due to improper tissue freezing.

Three WD brains were excluded from immunohistochemistry analyses due to hippocampal damage that occurred during tissue sectioning.

All data are presented as group means ± SEM. The normality of data was confirmed using a Shapiro Wilks test (Graphpad Software, San Diego, CA). To compare between maternal CD and WD populations a Welch's two-tailed t-test (Graphpad Software, San Diego, CA) was used. For all fetal data, a linear mixed model (LMM) emmeans pairwise analysis (RStudio Software, Boston, MA) was performed averaged over sex to compare between diet groups with maternal ID as the fixed effect (See appendix for code)[210]. A LMM was used because it accounts for a fixed variable that differs within the sample populations—in the present analysis, maternal ID—that might otherwise confound results. While the sex of fetuses was recorded, the sample population was not large enough to achieve high statistical power based on power calculations. For all correlation analyses a Pearson correlation coefficient analysis was performed (Graphpad Software, San Diego, CA). Statistical significance was assumed when $p<0.05$ for all analyses.

CHAPTER 3: RESULTS

3.1 Maternal and fetal population characteristics

3.1.1 Maternal and fetal MRI volume measurements

MRIs were conducted on Control diet (CD) and Western diet (WD) sows around gestational day 60 to determine the maternal and fetal body and organ volumes. Although not significant, WD sows had smaller whole-body volumes compared to those in the CD group (CD: 818.40cm^2 ± 54.55cm^2 vs WD: 720.30cm^2 ± 15.33cm^2) (**Fig 3.1.1-1A**). Maternal VAT and liver volumes were both compared as a percentage of maternal body volume. Maternal VAT volumes were similar between diet groups (CD: 2.49% ± 0.82% vs WD: 2.83% ± 0.16%) (**Fig 3.1.1-1B**). As for the maternal liver, volumes did not differ significantly (CD: 4.56% ± 0.18% vs WD: 5.17% ± 0.32%) **(Fig 3.1.1-1C)**; however, the hepatic fat fraction of the WD sows was significantly greater than that of the CD sows ($p<0.01$; CD: 4.33% ± 0.33% vs WD: 7.38% ± 0.88%) (**Fig 3.1.1-1D**).

Analogous volumetric measurements were also obtained from CD and WD fetuses. Fetal whole-body volumes did not differ significantly between groups (CD: 56.16cm^2 ± 2.57cm^2 vs WD: 65.53cm^2 ± 2.06cm^2) (**Fig 3.1.1-2A**). WD placentae were ~34% larger by volume than the CD placentae ($p<0.001$; CD: 6.84cm^2 ± 1.59cm^2 vs WD: 9.19cm^2 ± 1.44cm^2) (**Fig 3.1.1-2B**). Fetal TAT, brain, and liver volumes were all compared as a percentage of fetal body volumes. Neither TAT volumes (CD: 7.30% ± 0.50% vs WD: 9.56% ± 0.38%), nor brain volumes (CD: 3.11% ± 0.08% vs WD: 3.68% ± 0.22%) were significantly different between the fetal groups (**Fig 3.1.1-2C**). Conversely, liver volumes were greater in the WD fetuses compared to the CD fetuses ($p<0.05$; CD: 5.17% ± 0.62% vs WD: 7.53% ± 0.26%) (**Fig 3.1.1-2C**). Additionally, the fetal WD group had an average hepatic fat fraction that was nearly double that of the CD fetuses ($p<0.01$; CD: 6.91 ± 1.00% vs WD: 14.00% ± 0.71%) (**Fig 3.1.1-2D**).

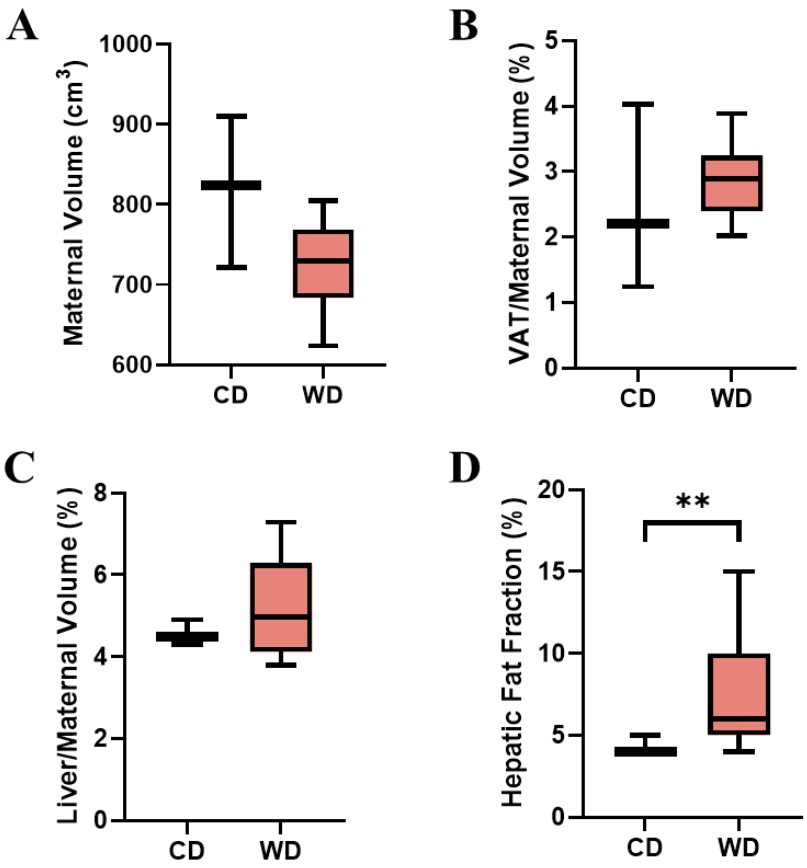

Figure 3.1.1-1. Maternal MRI measurements of Control diet (CD) (n=3) compared to Western diet (WD) (n=13) sows; data present distribution of (**A**) maternal volume (cm^3) normalized by volume of fetoplacental unit, (**B**) visceral adipose tissue (VAT) volume relative to maternal volume (%), (**C**) liver volume relative to maternal volume (%), (**D**) hepatic fat fraction (%).**; $p<0.01$. Statistical significance of the difference between means determined using Welch's T Test. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

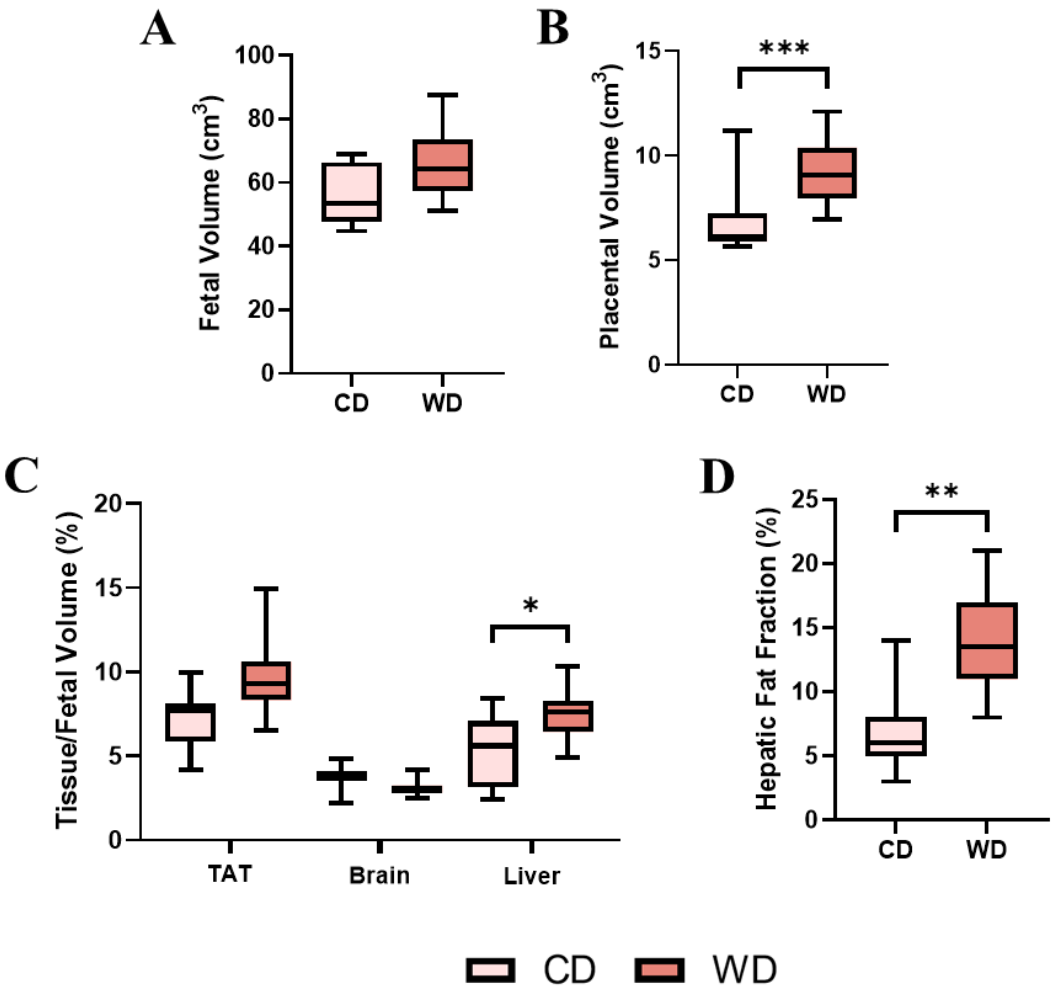

Figure 3.1.1-2. Fetal (~60 days) MRI measurements of Control diet (CD) (n=11) compared to Western diet (WD) (n=26) fetuses; data present distribution of (**A**) fetal volume (cm^3), (**B**) placental volume (cm^3), (**C**) tissue-fetal volume ratios expressed as percentage of fetal volume (%), and (**D**) hepatic fat fraction (%); * $p<0.05$, **; $p<0.01$, ***; $p<0.001$. Statistical significance of the difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect. TAT: Total Adipose Tissue. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

3.1.2 Maternal and fetal growth measurements

Maternal CD (N=3) and WD (N=13) fed sows underwent full necropsy at gestational day 65. While there was no significant difference between the body weights of CD and WD sows at conception (CD: 707.0g ± 52.08g vs WD: 665.5g ± 16.01g), nor at sacrifice when normalized by the weight of their respective fetoplacental units (CD: 803.1g ± 40.11g vs WD: 746.7g ± 14.68g) (**Fig 3.1.2-1A**). Similarly normalized weight gain across pregnancy did not differ between groups (CD: +96.15g ± 19.86g vs WD: +81.20g ± 10.51g) (**Fig 3.1.2-1B**). Moreover, while not significant, WD sows had only 1-3 viable pups per litters, whereas CD sows consistently had 3-5 pups per litter (**Fig. 3.1.2-1C**). The livers of WD sows were ~25% larger by weight compared to CD sows ($p<0.05$; CD: 32.62g ± 2.36g vs WD: 40.88g ± 2.10g) (**Fig 3.1.2-1D**).

While there was no significant difference in body weight between fetuses (CD: 84.92g ± 5.80g vs WD: 88.16g ± 2.89g) (**Fig 3.1.2-2A**), the placentae of the WD group were ~56% larger by weight than CD ($p<0.01$; CD: 4.89g ± 0.42g vs WD: 7.62g ± 0.25g) (**Fig 3.1.2-2B**). In addition, the fetal to placental weight ratio, a measure of placental efficiency, was reduced in the WD group relative to the CD ($p<0.001$; CD: 17.71g ± 0.77g per g vs WD: 11.78g ± 0.47g per g) (**Fig 3.1.2-2C**). All fetal organ weights were analyzed as a percentage of fetal body weight. While fetal brains did not differ significantly in weight between groups (CD: 2.89% ± 0.15% vs WD: 2.67% ± 0.10%) (**Fig 3.1.2-2D**), fetal livers were larger in WD fetuses compared to CD fetuses ($p<0.05$; CD: 4.68% ± 0.34% vs WD: 6.69% ± 0.25%) (**Fig 3.1.2-2D**).

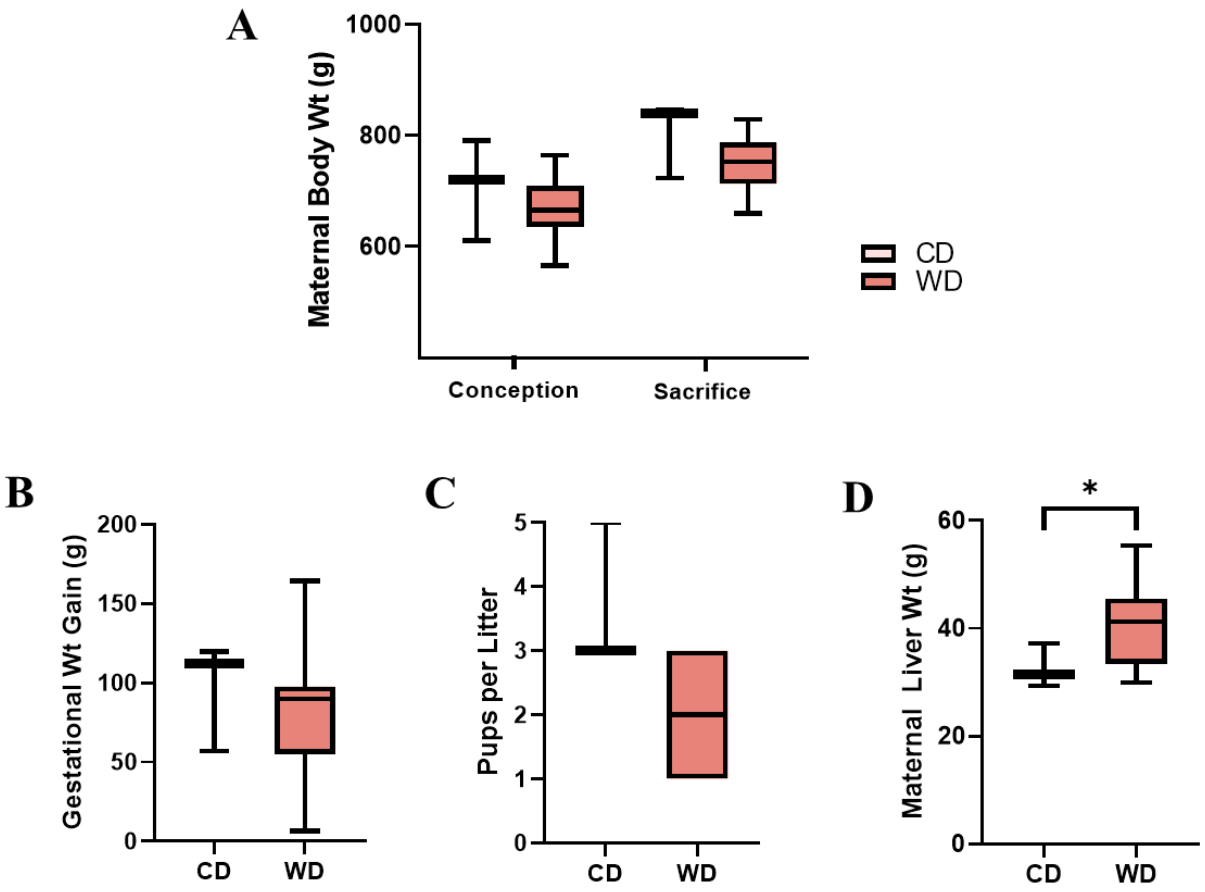

Figure 3.1.2-1. Maternal growth measurements of Control diet (CD) (n=3) compared to Western diet (WD) (n=13) sows; data present distribution of (**A**) body weight at conception and at sacrifice (g), (**B**) gestational weight gain (g) normalized by weight of fetoplacental unit, (**C**) pups per litter, and (**D**) liver weight (g). * $p<0.05$, ***; $p<0.001$. Statistical significance of the difference between means determined using Welch's T Test. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

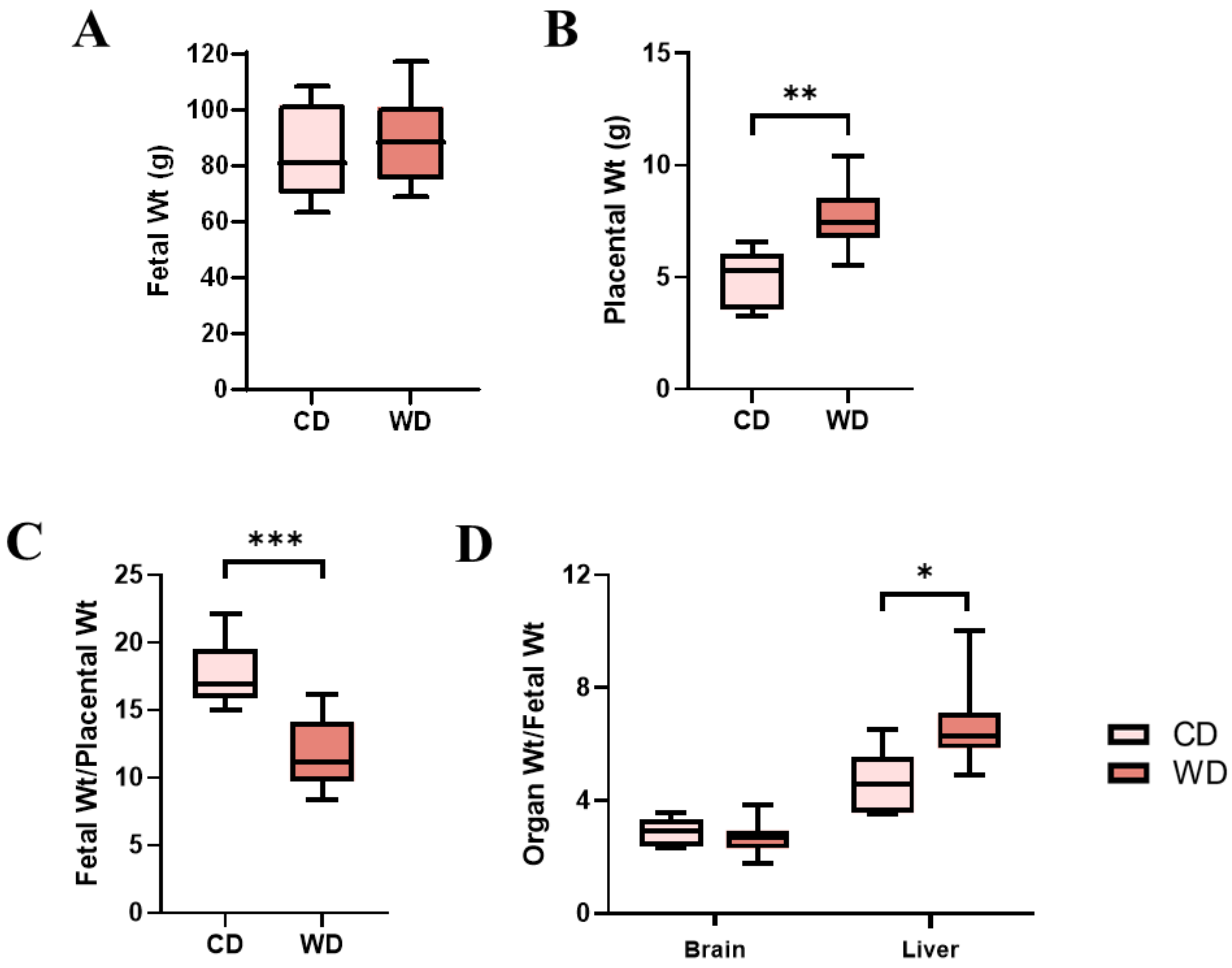

Figure 3.1.2-2. Fetal (~65 days) growth measurements of Control diet (CD) (n=9) compared to Western diet (WD) (n=24) fetuses; data present distribution of (**A**) fetal body weight (g), (**B**) placental weight (g), (**C**) fetal-placental weight ratio expressed as percentage of placental weight (%), (**D**) organ-fetal weight ratios expressed as percentage of fetal body weight (%); * $p<0.05$, **; $p<0.01$, ***; $p<0.001$. Statistical significance of the difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

3.2 Placental Pathology

3.2.1 Placental Pathology Scores

To characterize placental tissue, H&E stained sections of placentae (**Fig 3.2.1-1**) were semi-quantitatively analyzed by a certified veterinary pathologist (Dr. Patti Kiser). Necrosis and fibrin were scored individually on a scale of 0-6 that reflected the severity and distribution of either feature. Individual necrosis and fibrin scores were summated to establish a total pathology score for each placenta with 0 representing a healthy placenta and 12 representing a placenta with wide-spread, severe necrosis, and fibrin thrombi. Although scale was established for this study specifically, similar scoring schemes have previously been used[203,204].

WD placental pathology scores were 5-times higher than that of the CD placentae ($p<0.05$; CD: 1.00 ± 0.41 vs WD: 5.08 ± 0.66) (**Fig 3.2.1-2**). Individually, both fibrin and necrosis scores were higher in the WD placentae (Fibrin: 2.58 ± 0.37; Necrosis: 0.67 ± 0.29) compared to CD placentae (Fibrin: 0.67 ± 0.29; Necrosis: 0.33 ± 0.24); however, only the difference in necrosis scores achieved significance ($p<0.05$) (**Fig 3.2.1-2**).

A correlation analysis was performed to understand the relationship between placental weight and corresponding pathology scores. These data had a significant positive correlation ($p<0.001$) with an R value of 0.55 (**Fig 3.2.1-3A)**. The relationship between fibrin and necrosis scores was also investigated. The two pathology scores had a significant positive correlation ($p<0.0001$) with an R value of 0.64 (**Fig 3.2.1-3B**).

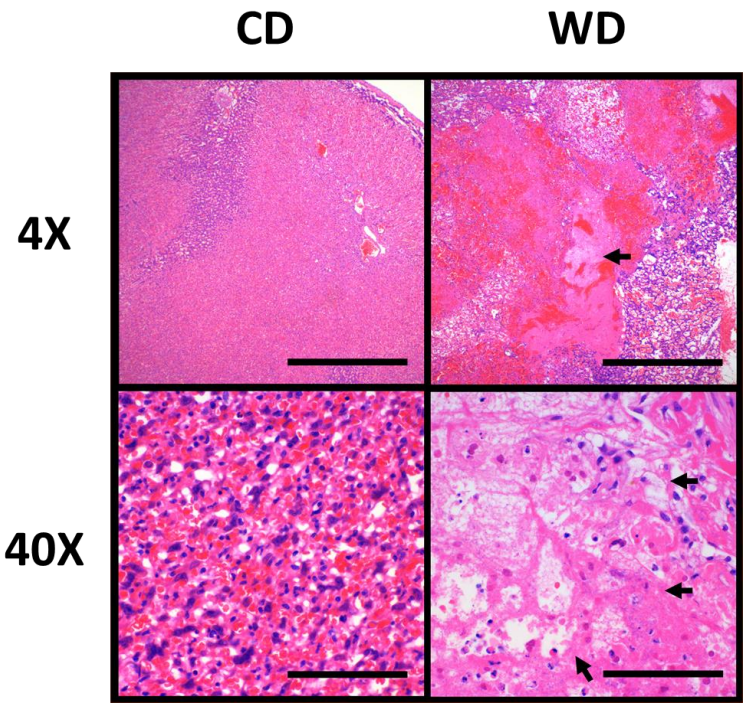

Figure 3.2.1-1. Representative high-power field of H&E stained placentae in the Control diet (CD) and Western diet (WD) groups (**A&B**) at 4X and (**C&D**) at 40X. Arrow represents region of necrosis. Scale bars = 1000µm (A&B) and 100µm (C&D).

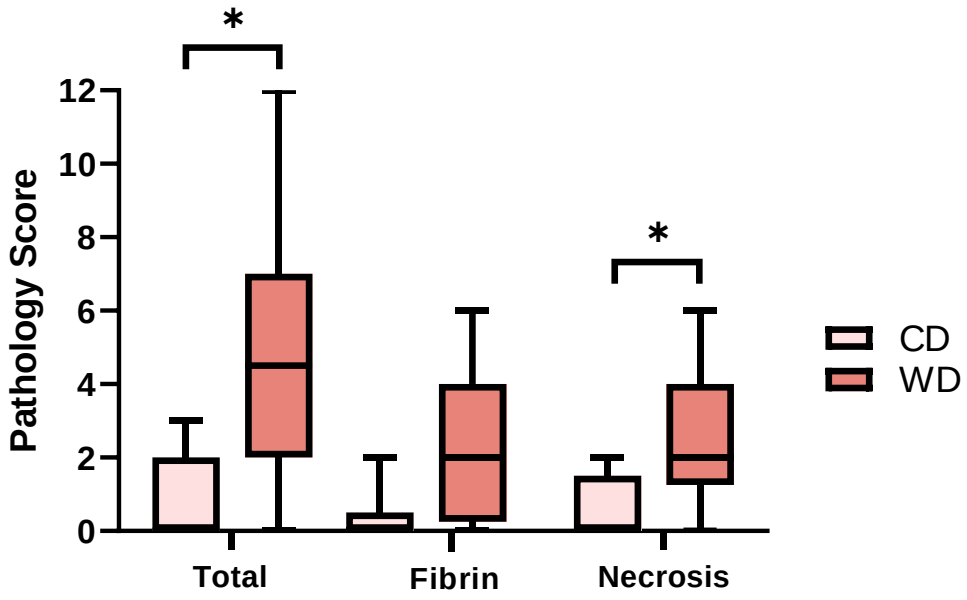

Figure 3.2.1-2. Data represent distribution of total pathology scores, fibrin scores, and necrosis scores of Control diet (CD) (n=9) compared to Western diet (WD) (n=24) placentae. Necrosis and fibrin were each scored on numerical scale from 0 (no necrosis or thrombi) to 6 (extensive, wide-spread necrosis or fibrin thrombi) and summated for a total pathology score; * $p<0.05$. Statistical significance of the difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

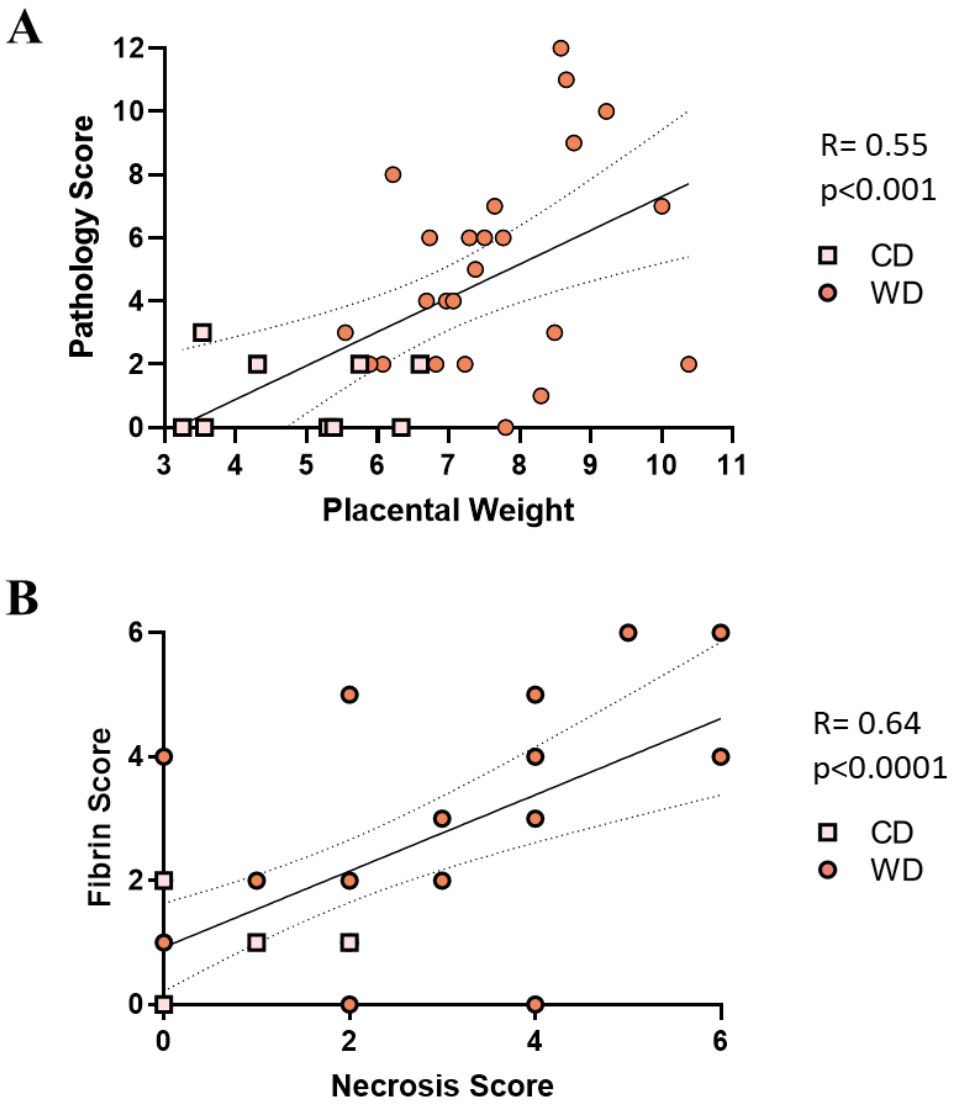

Figure 3.2.1-3. Data represent correlation of (**A**) placental pathology score vs placental weight (g) and (**B**) placental fibrin score vs placental necrosis score in total fetal population (n=33). Statistical significance of the difference between means determined using a Pearson r correlation analysis. Linear fit lines for all data are shown with 95% confidence intervals. CD: Control diet; WD: Western diet.

3.3 BDNF protein expression

3.3.1 Western blot analysis of placental BDNF expression

Western blots were performed to compare the relative level of BDNF protein expression in the placentae of each diet group. BDNF expression was normalized to total protein detected using PonceauS stain as described by Sander *et al*[206] (**Fig 3.3.1-1A**). A single band representing mature BDNF was detected at 14kDa (**Fig 3.3.1-1B**). Relative BDNF expression was reduced by ~62% in the placentae of WD fetuses compared to CD placentae ($p<0.001$; CD: 0.62 ± 0.11 vs WD: 0.23 ± 0.03) (**Fig 3.3.1-1C**).

To understand the relationship between placental pathology scores and relative BDNF expression a correlation analysis was performed. Placental variables showed a significant ($p<0.05$) negative correlation with an R value of -0.40 (**Fig 3.3.1-1D**).

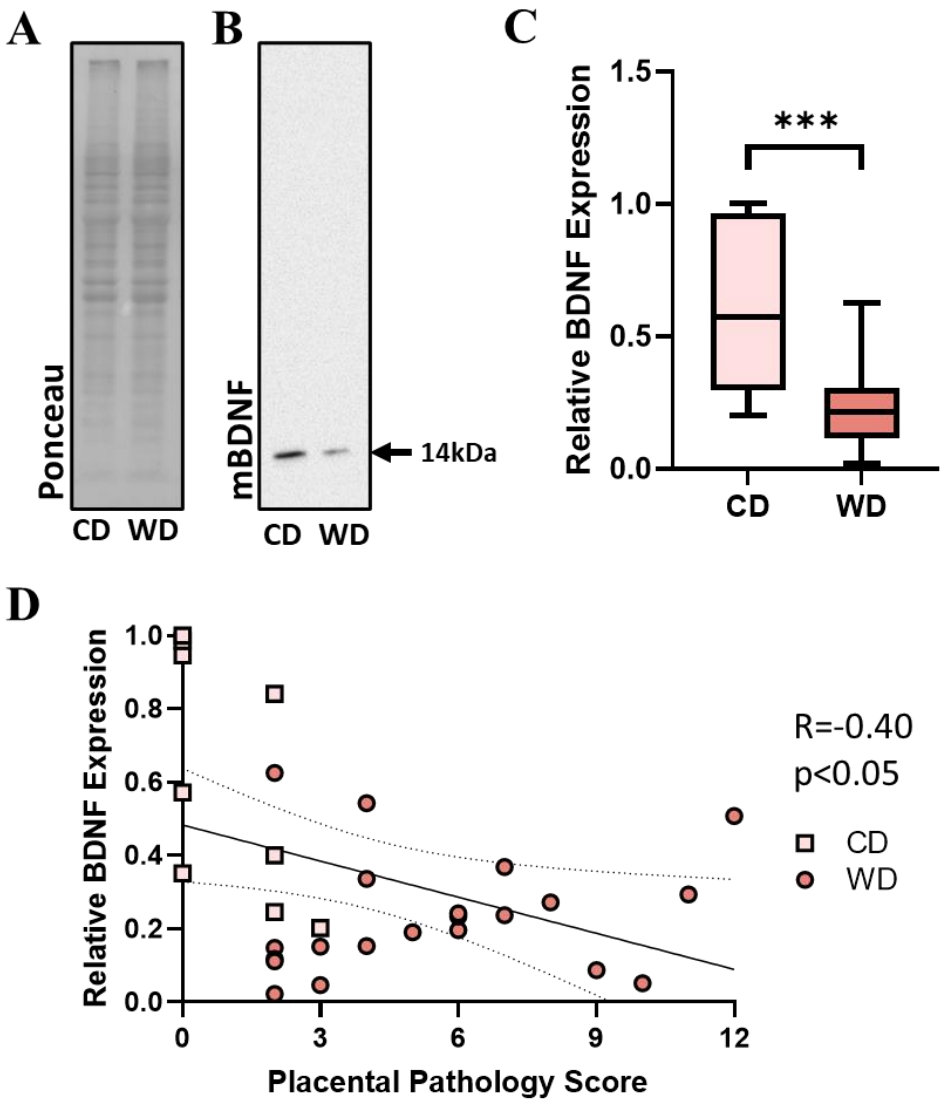

Figure 3.3.1-1. Data represent (**A**) distribution of relative placental BDNF expression in Control diet (CD) (n=9) compared to Western diet (WD) (n=21) placentae and (**B**) correlation of relative placental BDNF expression and placental pathology score (n=31); ***; p<0.001. Statistical significance of the difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect, while significance of the correlation between pathology scores and BDNF expression and a Pearson r correlation analysis, respectively. Linear fit lines for all data are shown with 95% confidence intervals. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

3.3.2 Immunohistochemical analysis of BDNF in fetal brain

Coronal sections of the fetal brain were immunostained to identify changes in BDNF protein expression between diet groups. In each region (DG, CA3, thalamus), 4-6 high-power fields were acquired. BDNF expression was quantified by the percent area stained, and the integrated optical density, which integrates both the total area stained as well as the pixel intensity. BDNF immunoreactivity was primarily localized extracellularly near neuronal cells with the most prominent expression being found in the DG and CA3 regions and moderate expression found in the thalamus (**Fig 3.3.2-1**).

BDNF immunoreactivity showed reduced percent area stained in the WD fetuses across the DG ($p<0.001$; CD: 10.13% ± 1.31% vs WD: 3.08% ± 0.76%), CA3 ($p<0.001$; CD: 13.15% ± 1.75% vs WD: 4.60% ± 1.04%), and thalamus ($p<0.001$; CD: 3.14% ± 0.84% vs WD: 0.77% ± 0.65%) (**Fig 3.3.2-2A**). Similarly, the integrated optical density was significantly lower in the WD fetuses across the DG ($p<0.001$; CD: $1.16x10^{10}$ ± $1.58x10^{9}$ vs WD: $3.66x10^{9}$ ± $1.05x10^{9}$), CA3 ($p<0.001$; CD: $1.51x10^{10}$ ± $2.49x10^{9}$ vs WD: $5.41x10^{9}$ ± $1.44x10^{9}$), and thalamus ($p<0.01$; CD: $3.23x10^{9}$ ± $7.82x10^{8}$ vs WD: $9.84x10^{8}$ ± $8.58x10^{8}$) (**Fig 3.3.2-2B**)

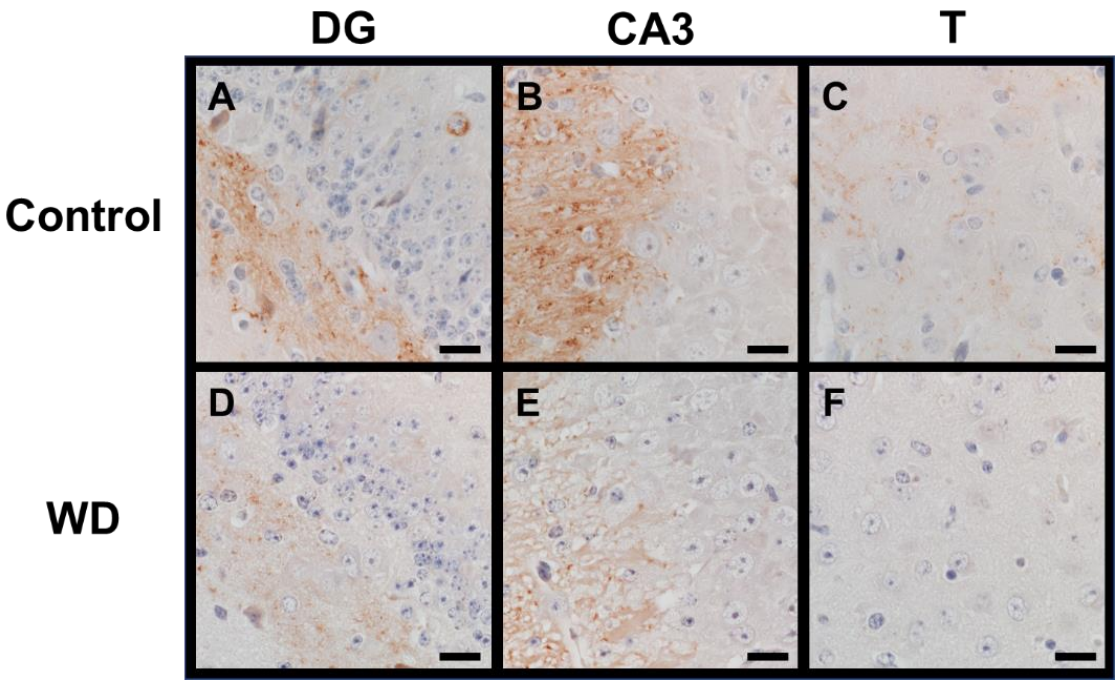

Figure 3.3.2-1. Representative high-power field (40X) images of brain regions exposed to anti-brain derived neurotrophic factor (BDNF) immunohistochemistry with hematoxylin counterstain. Brown staining in the Control diet (CD) (**A**) dentate gyrus (DG), (**B**) CA3, and (**C**) thalamus (T) and the Western diet (WD) (**D**) DG, (**E**) CA3, and (**F**) T. Brown staining represents BDNF protein expression. Scale bar = 20µm.

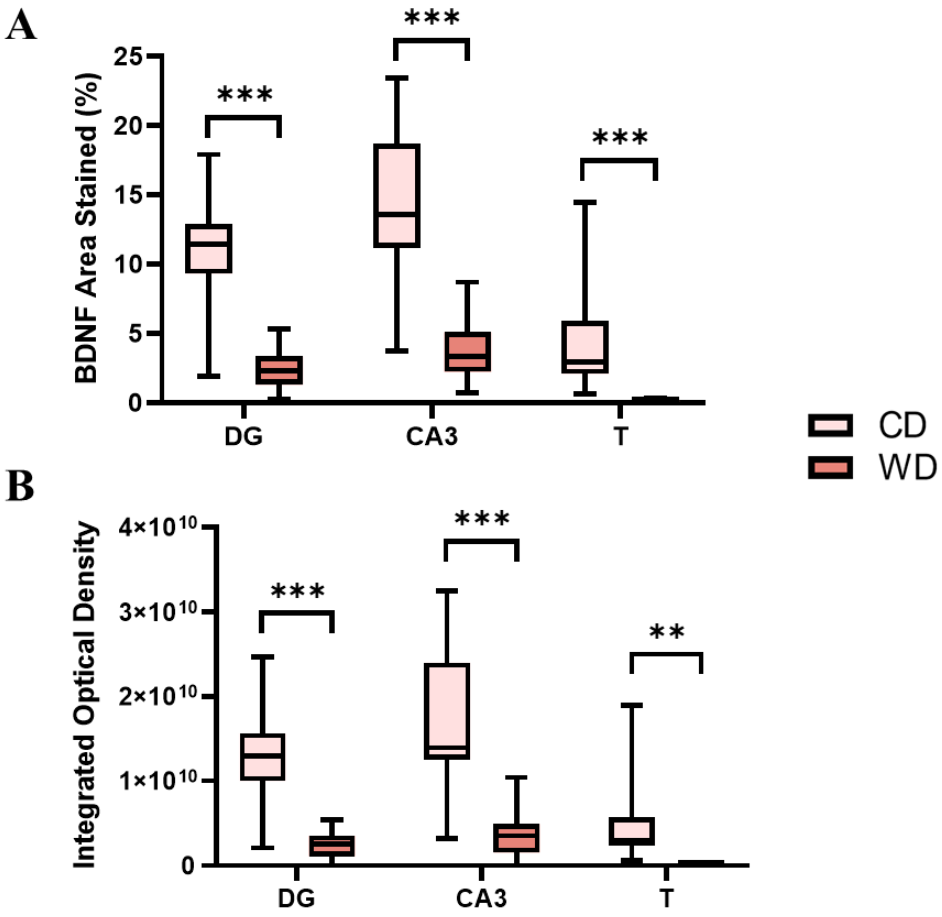

Figure 3.3.2-2. Data represent the distribution of (**A**) brain derived neurotrophic factor (BDNF) area stained (%) (**B**) integrated optical density in the dentate gyrus (DG), CA3, and thalamus (T) of Control diet (CD) (n=9) and Western diet (WD) (n=21) fetuses. ***; $p<0.001$. Statistical significance of the difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

3.4 Cellular changes in fetal brain

3.4.1 Immunohistochemical analysis of Ki67 expression in the fetal hippocampus

Ki67 is a protein expressed by cells that are in the active phase of the cell cycle and it is a marker that has been previously used to quantify neurogenesis[207]. To determine the effects of lifelong maternal WD exposure on fetal hippocampal neurogenesis, Ki67 expression was quantified in the hippocampus using immunohistochemistry. In each hippocampal region (dentate gyrus (DG), CA1, and CA3) 4-6 high-power fields were acquired and Ki67 was quantified as a positive cell count per mm^2 (**Fig. 3.4.1-1**).

While the mean Ki67-positive cell count was 10-30% lower in the various hippocampal regions of fetal WD brains compared to those of the CD group, none of the differences were significant (Table 3.4.1-1).

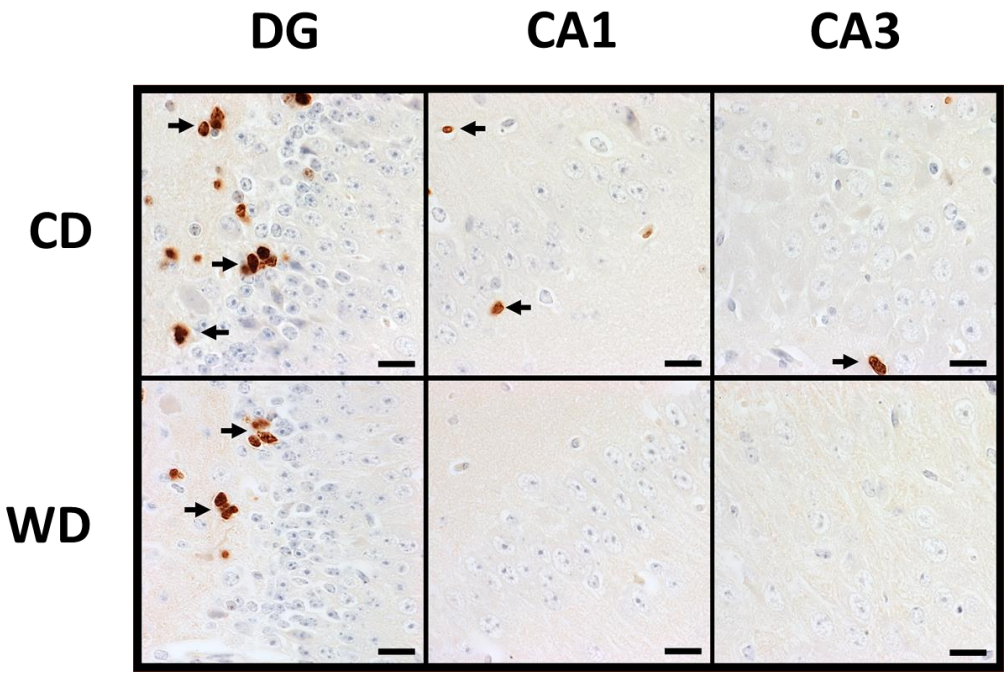

Figure 3.4.2-1. Representative high-power field (40X) images of hippocampal brain regions exposed to anti-Ki67 immunohistochemistry with hematoxylin counterstain. Brown staining in the Control diet (CD) (**A**) dentate gyrus (DG), (**B**) CA1, and (**C**) CA3; and the Western diet (WD) (**D**) DG, (**E**) CA1, and (**F**) CA3 represents Ki67 protein expression, a marker of cell proliferation. Arrows indicate Ki67-positive cells. Scale bar = 20µm.

Table 3.4.1-1. Immunohistochemical analysis of Ki67 in fetal brain

	Control diet	**Western diet**
Ki67-positive cell count per mm^2		
DG	1976.75 ± 222.39	1760.11 ± 104.71
CA1	376.93 ± 100.06	263.10 ± 34.35
CA3	414.41 ± 100.46	274.25 ± 36.19

Ki67-positive cell count of fetal brains (Control diet: n=9; Western diet: n=21) are presented as means ± SEM for the dentate gyrus (DG), CA1, and CA3. Statistical significance determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect.

3.4.2 Cell counting

Hematoxylin staining was used to label the cell nuclei in coronal brain sections to identify changes in cell density between diet groups. In each region (DG, CA1, CA3, thalamus), 4-6 high-power fields were acquired, and cell density was quantified as a cell count per mm^2 (**Figure 3.4.2-1**).

Cell density was decreased across all regions of WD fetal brains compared to those of the CD group, which includes the DG ($p<0.01$; CD: $8790.99mm^2 \pm 669.87mm^2$ vs WD: $6320.50mm^2 \pm 226.06mm^2$), CA1 ($p<0.05$; CD: $4323.93mm^2 \pm 474.16mm^2$ vs WD: $2763.04mm^2 \pm 93.40mm^2$), CA3 ($p<0.05$; CD: $3271.33mm^2 \pm 269.29mm^2$ vs WD: $2469.49mm^2 \pm 59.34mm^2$), and thalamus ($p<0.05$; CD: $3543.47mm^2 \pm 482.81mm^2$ vs WD: $2084.73mm^2 \pm 91.62mm^2$) (**Figure 3.4.2-2**).

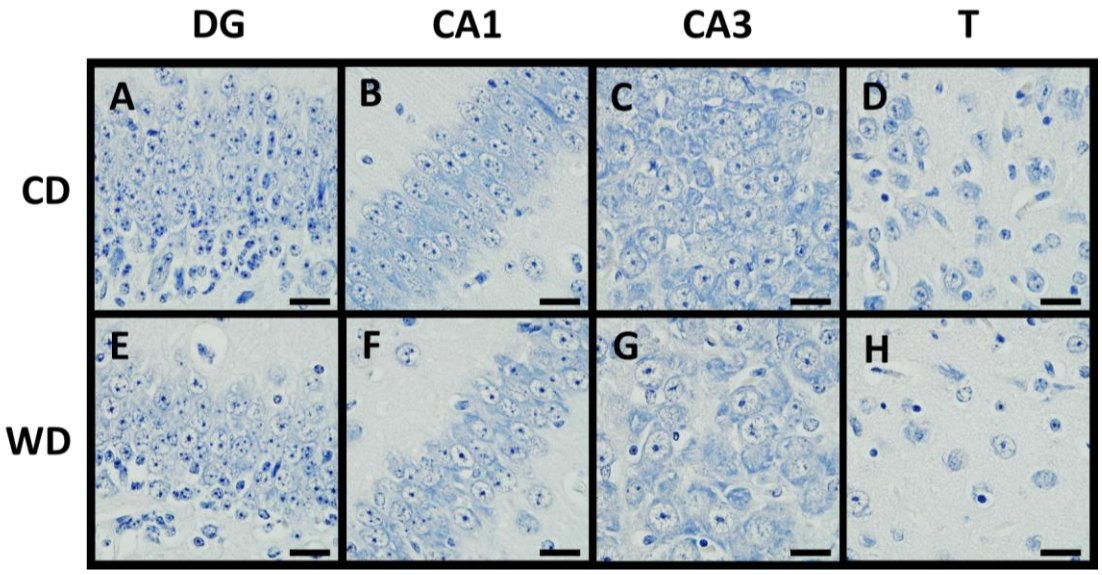

Figure 3.4.2-1. Representative high-power field (60X) images of brain regions stained with hematoxylin in the Control diet (CD) (**A**) dentate gyrus (DG), (**B**) CA1, (**C**) CA3, (**D**) thalamus (T) and the Western diet (WD) (**E**) DG, (**F**) CA1, (**G**) CA3, and (**H**) T. Scale bar = 20µm.

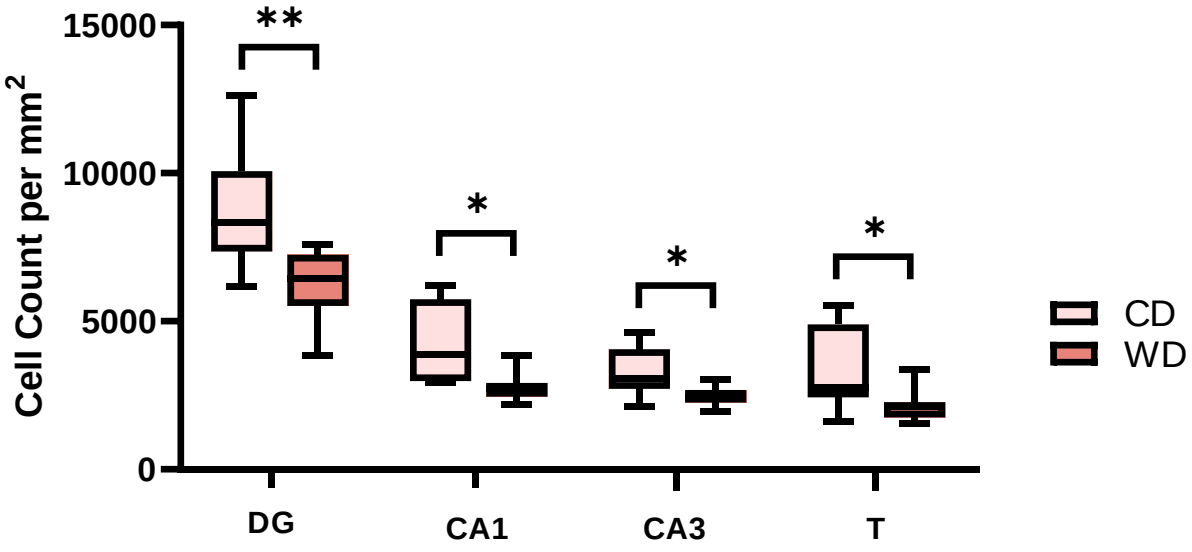

Figure 3.4.2-2. Data represent the distribution of cell density (per mm^2) in the dentate gyrus (DG), CA1, CA3, and thalamus (T) of Control diet (CD) (n=9) and Western diet (WD) (n=21) fetuses.*; $p<0.05$, **; $p<0.01$ Statistical significance of difference between means determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect. Box plot legend: median (midline), box (25th and 75th percentiles), and whiskers (extrema).

3.4.3 Immunohistochemical analysis of Iba1 to measure microglial activation

The ionized calcium-binding adapter molecule 1 (Iba1) is a protein expressed by glial cells that is useful for investigating microglial morphology, which changes from highly ramified to amoeboid shaped as the cells transition from resting to activated states. Coronal sections of the fetal brain were immunostained for Iba1. In each region (DG, CA1, CA3, T, and corpus callosum (CC)) 4-6 high-power field Z-stack images were acquired (**Fig 3.4.3-1**). Iba1-positive cells were counted and morphological characteristics indicative of microglial activation, including cell body to process ratio, circularity, and cell body size, were investigated.

There were no significant differences in the number of Iba1-positive cells between CD and WD fetal brains in the DG, CA1, CA3, T, or CC. In addition, microglia in the CD and WD fetal brains showed no significant differences in cell to process ratio, circularity, or cell body size across any of the brain regions studied (Table 3.4.3-1).

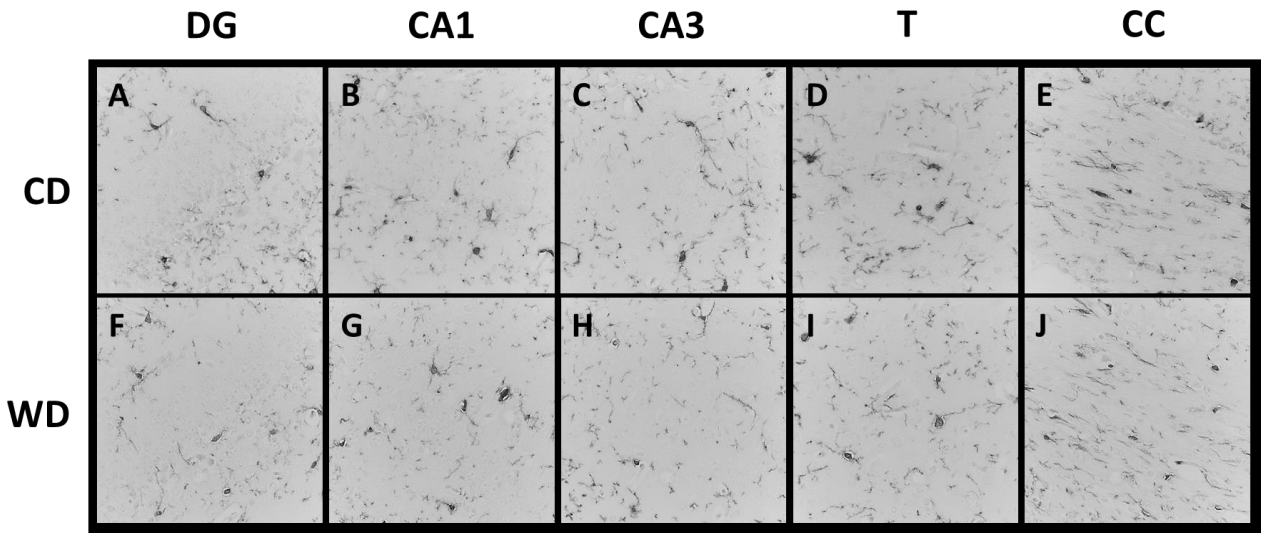

Figure 3.4.3-1. Representative high-power field (40X) merged Z-stack images of brain regions exposed to anti-ionized calcium-binding adapter molecule 1 (Iba1) immunohistochemistry. Black staining in the Control diet (CD) (**A**) dentate gyrus (DG), (**B**) CA1, and (**C**) CA3, (**D**) thalamus (T), (**E**) corpus callosum (CC); and the Western diet (WD) (**F**) DG, (**G**) CA1, and (**H**) CA3, (**I**) T, and (**J**) CC represents Iba1 protein expression, a marker of microglia. Scale bar = 20µm.

Table 3.4.3-1. Immunohistochemical analysis of Iba1 in fetal brain

	Control Diet	**Western Diet**
Iba1-positive cell count per mm^2		
DG	94.20 ± 10.79	79.54 ± 5.31
CA1	103.90 ± 8.36	93.66 ± 6.27
CA3	95.19 ± 6.97	91.31 ± 6.85
T	80.69 ± 5.80	81.89 ± 4.63
CC	146.80 ± 18.87	148.30 ± 9.95
Cell body to process ratio		
DG	0.171 ± 0.017	0.175 ± 0.0097
CA1	0.177 ± 0.015	0.170 ± 0.0093
CA3	0.171 ± 0.011	0.163 ± 0.0078
T	0.180 ± 0.015	0.201 ± 0.014
CC	0.158 ± 0.016	0.188 ± 0.011
Circularity		
DG	0.519 ± 0.013	0.496 ± 0.010
CA1	0.515 ± 0.014	0.479 ± 0.0070
CA3	0.529 ± 0.016	0.491 ± 0.0075
T	0.519 ± 0.018	0.493 ± 0.0097
CC	0.397 ± 0.023	0.394 ± 0.016
Cell body size (pixels)		
DG	547.5 ± 43.01	583.4 ± 13.07
CA1	593.8 ± 41.10	598.7 ± 15.61
CA3	649.7 ± 46.99	595.4 ± 15.58
T	631.3 ± 47.02	658.3 ± 17.57
CC	593.0 ± 36.72	589.9 ± 18.16

Microglia (Iba1-positive) cell count and morphological features within fetal brains (Control diet: n=9; Western diet: n=21) are presented as means ± SEM for the dentate gyrus (DG), CA1, CA3, thalamus (T), and corpus callosum (CC). Statistical significance determined using a linear mixed effects model emmeans pairwise analysis with maternal ID as the fixed effect.

CHAPTER 4 DISCUSSION

4.1 Impact of Maternal WD on population characteristics

4.1.1 Maternal population characteristics

Habitual WD consumption is associated with metabolic dysfunction in both obese and non-obese phenotypes[42]. To investigate the impact of this dietary pattern on a pregnant guinea pig model, sows were subjected to a diet high in saturated fats and simple sugars from the point of weening and throughout pregnancy. Their metabolic phenotype was subsequently explored through MRI and growth measurements collected near term to elucidate differences resulting from the dietary pattern.

At conception, body weights were comparable between groups. Similarly, when the weights/volumes of all fetoplacental units were accounted for, body weight and volume did not differ between the groups. While these measurements suggest both groups maintained a lean phenotype, these measurements alone offer little indication of internal body composition. Further investigation found that the diet groups showed no significant differences in VAT. Conversely, WD-fed sows had larger livers by weight and volume. These measurements were accompanied by elevated hepatic proton density fat fractions, suggesting an increased proportion of the hepatic tissue was comprised of lipid. While the triglyceride content of these livers was not analyzed in this study, previous reports from our lab have reported elevations in the hepatic triglyceride content of WD-fed guinea pigs[195]. Taken together these findings implicate hepatic triglyceride accumulation as a probable cause of the current increases in liver weight and volume noted in the WD sows. This type of hepatic lipid accumulation is reflective of NAFLD, a prominent indicator of metabolic dysfunction that communicates the further risk of metabolic disease, even in a lean phenotype[15]. Based on these features, the maternal WD-fed population phenotype can be classified as lean and metabolically unhealthy, specifically reflecting lean NAFLD[15].

While diets high in saturated fats and simple sugars are often associated with an obese phenotype, similar reports implicating the WD with hepatic lipid accumulation in the absence of obesity have also been described[195,211,212]. It is speculated that these findings may be attributable, at least in part, to the high proportion of fructose found in the WD. Dietary fructose, sucrose, and high-fructose corn syrup have all demonstrated a tendency of inducing hepatic lipid

accumulation in various experimental animal models and humans[213–218]. These findings have also been observed in the absence of weight gain and even when total calories were restricted[213,214].

In the small intestine, fructan (polymer of fructose) and sucrose (disaccharide of glucose and fructose) molecules are cleaved to release free fructose monosaccharides[219]. Following its absorption through transporters on the intestinal epithelium, fructose enters the portal system and is directly transported to the liver where it is metabolized[220]. Unlike glucose, only a small fraction of fructose enters the systemic circulation as it is almost completely extracted from the portal blood upon its first pass[220]. The primary step of fructose metabolism is its phosphorylation by ketohexokinase (KHK), which has no negative feedback system[219]. Unlike glucose metabolism, fructolysis bypasses enzyme-mediated (phosphofructokinase) regulation, allowing it to occur at a far faster rate[219]. The product of fructolysis, a trios phosphate intermediate, may then enter gluconeogenesis, lipogenesis, or oxidation pathways[219]. It follows that fructose greatly increases the availability of intrahepatic carbohydrate stores and promotes a shift towards carbohydrate oxidation, which results in reduced fatty acid oxidation[220]. Diets high in fructose result in significant amounts of carbohydrates entering glycolytic pathways, and thus an excess of glycolytic intermediates and by-products, which may then be used as substrates for de novo lipogenesis at the liver[219]. In addition to acting as a substrate for hepatic de novo lipogenesis, chronic fructose consumption also induces hepatic lipogenic programs, further promoting the accumulation of hepatic triglycerides[219,220]. Moreover, the metabolism of fructose by KHK greatly decreases the ATP:AMP ratio, resulting in the rapid production of uric acid and consequently, hepatotoxicity that drives progression of NAFLD to NASH[219].

Despite its propensity for promoting the development and progression of NAFLD, fructose may have opposing actions on peripheral adipose tissue. In rats specifically, an overload of dietary fructose has been found to increase the release of free fatty acids from peripheral adipose tissue into the bloodstream, allowing them to be taken up by hepatocytes and stored within the liver as triglycerides[221]. Moreover, the combination of saturated fats and refined sugars, like sucrose and added fructose, have been associated with hyperinsulinemia and insulin resistance [222]. While insulin typically has an anti-lipolytic effect, insulin resistance—particularly in adipose tissue—can lead to dysregulated lipolysis in peripheral tissues, thus increasing the supply of free fatty acids arriving at the liver[220]. Adipocyte insulin resistance is similarly related

to the development of VAT[220]. Although the present findings were not significant, greater levels of VAT were noted in WD sows. This is of note because VAT releases free fatty acids directly into the portal circulation, offering a direct path to the liver[220].

These findings highlight the often-insidious nature of the metabolic dysfunction resulting from habitual WD consumption, supporting the concept that a lean phenotype does not equate to superior metabolic health. Moreover, these findings reflect adverse alterations in the maternal environment which, based on the robust literature surrounding the DOHaD hypothesis, are expected to confer metabolic or cognitive deficits to the future offspring. Maternal NAFLD specifically is a strong risk factor for both adverse maternal and perinatal outcomes[188].

4.1.2 Fetal population characteristics

In addition to investigating maternal phenotypes, fetal (65 days) MRI and growth measurements were similarly analyzed to characterize the impact of maternal WD consumption on the gross development of the fetus. Interestingly, the fetal WD population mirrored the lean NAFLD phenotype of their mothers. There was no difference in fetal size (by weight or volumes), nor in TAT; however, the livers of WD fetuses were larger and showed greater levels of intrahepatic triglyceride accumulation as measured by the proton density fat fraction.

These findings may be the result of primitive adaptive mechanisms regulating nutrient flow between mother and fetus. The delivery of nutrients to the fetus is influenced by multiple factors including umbilical blood flow, available surface area for exchange, placental metabolism, concentration gradients, and nutrient transporter expression[223]. In addition to increasing the concentration gradient of nutrients between mother and fetus, maternal overnutrition (obesity, hyperglycemia, energy-dense diets) has also been shown to alter the expression of placental nutrient transporters altering nutrient availability to the fetus[223,224].

Mismatches in materno-placental nutrient supply and fetal nutrient demand affect the distribution of umbilical venous blood flow, such that it preferentially delivers nutrients to either the fetal brain or liver, eliciting changes to fetal body composition[225]. In environments with fewer resources than those of the current developed world, these mechanisms served to optimize fetal development; however, under conditions of abundant nutrient supply (obesity or energy-rich diets), these same mechanisms are speculated to promote prenatal fat deposition to preserve

nutrient supply and buffer brain development from possible nutrient restriction postnatally[225]. Much like humans, guinea pigs do not start laying down adipose tissue until later in gestation[80]. Given the chronic nature of maternal overnutrition in the present study, it is possible that during early pregnancy excess nutrients are being deposited findings preferentially in the liver and other organs[196,225]. These present findings are supported by the previous works of our collaborators and others who have similarly reported that maternal diets high in fat and/or refined sugar during pregnancy were related to increased lipid accumulation in the liver either at birth or postnatally[63,72,196,226,227].

Collectively, these studies along with our present findings underscore the severe metabolic risks associated with maternal WD consumption independent of obesity and highlight the importance of maternal diet in programming fetal metabolic health, seeing as fetal hepatic lipid accumulation increases the individual's risks of various cardiometabolic diseases[17].

4.2 Placental development

4.2.1 Placental growth

The placenta acts as an interface between mother and fetus that regulates communication and is critical in supporting the development of the fetus. As such, placental MRI and growth measurements were assessed, alongside the maternal and fetal population characteristics described above. Maternal WD consumption was associated with increased placental volumes and weights at collections. Presumably, this increase in placental size was a result of increased nutrient availability during critical periods of placental development.

The impact of maternal diet on placental size has been investigated in other animal models; however, their results are often inconsistent due to variability in model animal/strain, duration of diet exposure, and the specific compositions of both control and experimental diets[228]. Conversely, our findings are supported by multiple prospective large-scale cohort studies, which have consistently found that maternal obesity and gestational weight gain, in the absence of other complications, are associated with increased placental size [229–233]. While not a identical representation of the present model, these human studies support the notion that maternal overnutrition may be a driving factor of abnormal placental growth.

Clinically placental size has been largely correlated with the size of the fetus, reflecting increased nutrient transport capacity; yet, the present findings showed only minor increases in the body weight and volumes of WD fetuses[230,231]. This led to questioning the placental efficiency of the described WD model. Fetal to placental weight ratios were compared between diet groups as a proxy of placental efficiency, which was found to be reduced in the WD placentae. Thus, it is evident that the additional placental weight and volume associated with WD exposure did not increase the functional capacity of placentae in the current model. Similar reductions in placental efficiency have been noted in human pregnancies complicated by obesity and maternal high-fat diets[229,232–234]. The mechanisms underlying these changes are unclear, although it was suggested that maternal overnutrition may lead to structural abnormalities and/or functional disruptions in the placenta that limit fetal nutrient supply[231,232]. Alternatively, it has been postulated that increases in placental weight in response to overnutrition may be attributed to tissue remodelling in an attempt to adapt to tissue damage and preserve placental function[231].

4.2.2 Placental Pathology

In view of the foundational role of the placenta in fetal development, understanding the etiology of the noted reductions in placental efficiency was essential to elucidating the impact of maternal WD consumption. To investigate the quality of the placental tissues, the placentae were scored for necrosis and fibrin thrombi. WD placentae had greater total pathology scores, suggesting greater levels of widespread and severe placental pathology. Necrosis specifically was frequently observed in these placentae and was correlated with the occurrence of organizing fibrin and/or fibrin thrombi, although there were no dramatic changes in fibrin alone. Furthermore, these pathology scores were positively correlated with placental size, suggesting that the larger placentae showed greater distribution and severity of necrosis and fibrin deposition. This lends support to the theory that increased placental weights may be the consequence of tissue remodelling or edema following placental infarcts induced by maternal overnutrition.

These features may also be an indication of disrupted integrity of the placental labyrinth, which in the guinea pig placenta houses fetal capillaries and is the structure chiefly responsible for nutrient and gas exchange[235]. Defects in the integrity of the labyrinth can lead to edema and thrombosis, leading to infarcts which may disrupt blood flow[235]. Inadequate perfusion of the

labyrinth can lead to tissue fibrosis and necrosis, which ultimately limits surface area for nutrient and gas exchange[235]. Typically these findings—particularly fibrin deposition and thrombosis—are associated with impaired fetal growth, which was not an outcome observed in the current study population[235–237]. Previous unpublished work in our lab conducted on 40-day placentae of the same WD model found reduced fetal capillary size, which might further implicate altered labyrinth structure in the noted reduction in placental efficiency. It is yet to be discovered if these outcomes are consistent in the current 65-day model used in the present study.

While the exact findings differed between studies, maternal overnutrition has been previously associated with various pathological findings and altered villous integrity in the placentae of humans, non-human primates, and guinea pigs; but, the etiology of these changes is poorly understood[117,209,230]. Oxidative stress and/or inflammation are possible suspects of mediating the structural and functional changes observed in the placentae, given that they have both been found to increase in the placentae of over-nourished mothers[117,238,239]. The present findings cannot confirm nor deny whether the current observed placental changes are attributable to placental inflammation or oxidative stress; however, this is a question that warrants further research to further characterize this maternal WD model.

Nonetheless, the present findings reflect severe changes in placental structure occurring in association with lifelong maternal WD consumption independent of obesity. These changes likely contribute to functional disruptions in the placenta and may be involved in altering the trajectory of fetal development.

4.3 BDNF expression

4.3.1 BDNF expression in the placenta

One of the placenta's major functions is the production of hormones and growth factors, one of which being BDNF, a growth factor with trophic effects in both the placenta and fetal brain[46,128]. As such, relative placental BDNF protein expression was investigated by western blot analysis, which highlighted lower protein levels in the placentae of fetuses exposed to maternal WD consumption. Furthermore, correlation analysis revealed a negative correlation between placental BDNF expression and pathology scores, pointing to a relation between BDNF expression and the health of the placental tissue.

It is difficult to understand the nature of this relationship since it is unclear which was the precipitating factor. For one, the cytotrophoblasts and syncytiotrophoblasts, are thought to constitutively express BDNF[128,240]. Thus, apoptosis or cellular damage such as necrosis would likely reduce the placenta's functional capacity to produce hormones and neurotrophic factors such as BDNF. On the other hand, placental cells like the cytotrophoblasts and EVTs, which express the TrkB receptor, are responsive to its local expression. BDNF supports the growth and development of the placenta by promoting cytotrophoblast proliferation, differentiation, and survival, supporting the formation of the placental villi and their exchange surface area[122]. Furthermore, the role of BDNF in angiogenesis has been established in multiple tissues; it has been proposed that it may likewise contribute to the development and maintenance of the placental vasculature, which is further supported by the finding that the TrkB receptor is expressed on cells of the fetal endothelium within the placental villi [120,136,138,241–243]. This theory is strengthened by the negative correlation previously reported between placental BDNF and blood pressure (systolic and diastolic), as well as its noted reduction in preeclamptic human placentae[138]. These proposed mechanisms reflect pathways through which placental pathology may both contribute to, and be induced by, reduced BDNF expression.

The argument that the reductions in BDNF preceded placental infarct in the current model is strengthened by BDNF's reported sensitivity to maternal nutritional challenges, showing altered expression patterns following both maternal over- and under-nutrition in both humans and animal models[139,241]. With regards to maternal overnutrition specifically, previous clinical studies have reported reduced placental expression of BNDF, its receptors, and downstream effectors in the context of maternal obesity[139]. These changes were attributed to alterations in BDNF gene methylation, which is consistent with outcomes reported following exposure to perinatal maternal stress[48,133,139]. The present findings are novel in that they are the first to extend the association of maternal overnutrition and reduced placental BDNF expression to include maternal WD consumption in the absence of obesity.

Given BDNF's apparent sensitivity to nutritional imbalances, it is speculated that maternal WD consumption alters BDNF expression, diminishing its trophic signalling in the placenta. This in turn, may lead to abnormal placental development and subsequent tissue

damage, which further impedes BDNF production in a positive feedback loop that perpetuates placental damage.

4.3.2 BDNF expression in the fetal brain

In the fetal brain, BDNF derived from both the placenta and endogenous fetal production is involved in nearly all developmental functions; thus, alterations in its expression, particularly in regions where it is abundantly expressed, may lead to severe cognitive deficits[46,48]. To investigate whether the observed changes in placental BDNF expression in response to maternal overnutrition extended to the fetal brain, immunostaining was used to measure BDNF protein levels in three regions where its expression is established[46,48]. The localization of BDNF protein was confirmed in the extracellular environment of hippocampal regions DG and CA3, as well as the thalamus. Across all regions, both the area and integrated optical density of BDNF immunoreactivity were reduced in the WD fetuses, representing lower BDNF protein expression.

The validity of these findings is supported by others who have reported similar reductions in BDNF mRNA and protein levels postnatally in the hippocampus of murine offspring exposed to maternal high-fat or high fructose diets, even when switched to a control diet postnatally[97,112,244]. Interestingly these outcomes, along with the present fetal findings, mirror those which were previously observed in adult rodent models who adhered to the western dietary pattern[183]. As previously noted, BDNF has been proposed as a nutrient sensor, although this relationship has largely been discussed with regards to its expression in the hypothalamus[155]. Collectively these findings suggest that BDNF expression in the hippocampus and thalamus are similarly regulated by nutritional status, although the underlying mechanisms and implications are still to be defined. Epigenetic modifications of the BDNF gene are a promising theory given that altered methylation patterns of the BDNF promoter have been reported in the brains of offspring exposed to maternal high-fructose diets, as well as the brains of mature animals exposed to chronic high-fat diets, either through increased methylation or decreased demethylation[181,244]. Moreover, there is evidence that suggests epigenetic modifications of BDNF are triggered by alterations in energy status, specifically the NAD^+/NADH ratio and mTOR pathway[181,245]. As outlined by these studies, increased fetal nutrient availability resulting from maternal WD consumption may promote epigenetic modifications—likely alterations to DNA methylation patterns—that suppress BDNF expression in the fetal brain.

On the other hand, these reductions may be the product of the specific macronutrient composition unique to the WD. In the present study, the experimental WD contained approximately 32% SFA, 12% MUFAs and 2% PUFAs, contrasting the composition of the CD which contained 3% SFA, 4% MUFAs, and 11% PUFAs. One study in mice reported reductions in fetal hippocampal BDNF and its TrkB receptor in response to maternal diets low in omega-3 PUFAs independent of the diet's total fat content[246]. A similar study conducted in mice reported upregulation of BDNF and its downstream effectors in response to high maternal omega-3 PUFA consumption[185]. Moreover, maternal diets deficient in omega-3 PUFAs confer increased DNA methylation of the BDNF gene in the brains of mouse offspring postnatally, thus reducing its long-term expression[247]. Taken together it is speculated that the fatty acid ratio of the WD is in part responsible for the detrimental effects presently observed on BDNF expression in the fetal brain.

It would be remiss not to mention the potential influence of oxidative stress and inflammation. Previously it was shown in an adult murine model that the WD reduced the expression of BDNF and its downstream effectors; however, these effects were mitigated with aerobic exercise[47,248]. In addition to reversing the diet's effect on BDNF expression, levels of oxidative stress were also reduced. While the relationship between maternal oxidative stress and its prevalence within the fetal brain is not clear, there is evidence that maternal diet may be increasing ROS production in the fetal brain. For example, one study noted that lipid peroxidation was accompanied by decreased BDNF expression in the hippocampus of mouse offspring born to high-fat-fed dams[112]. Just as overnutrition and metabolic dysfunction have been found to increase oxidative stress, so too are they associated with chronic low-grade inflammation, termed "metainflammation'[93]. This may be relevant to the present findings given that experimentally induced inflammation is capable of suppressing BDNF expression and signalling[166,167]. Perhaps more convincing is that neuroinflammation has been found in association with reductions in BDNF expression following chronic exposure of mice to a high-fat diet[249]. It has been postulated that maternal inflammation in the context of metabolic dysfunction or dietary patterns may confer similar inflammation in the fetus, possibly through placental production of pro-inflammatory cytokines[93,117]. The presence of oxidative stress and inflammation remains a possible suspect of mediating changes in fetal BDNF expression following maternal WD consumption and metabolic dysfunction; however, these claims are

made with the caveat that currently the oxidative and inflammatory status of the present model remains to be discovered.

Finally, reductions in BDNF expression in the fetal brain may be directly attributed, at least in part, to its reduced expression in the placenta. Maternal-derived BDNF has been found to cross the uteroplacental barrier and has been noted in the fetal brains of mice at multiple timepoints during pregnancy, including at term[98]. Moreover, the same study found levels of BDNF in the placenta to be positively correlated with that of the fetal cord blood, supporting the notion that placental-derived BDNF is directly supporting fetal development[98]. A reduction in placental production or delivery of BDNF to the fetus may then be reflected in reduced protein levels in the brain, as was currently reported. This is unlikely to be the sole cause of BDNF reduction given that the fetuses' reliance on maternally-derived BDNF diminishes as pregnancy progresses and the current model reflects a fetus near term[98]. It is more likely that reduced supply early in pregnancy communicates deficits to early fetal brain development that alter its intrinsic expression of BDNF later in adulthood. For example, these differences may reflect decreased cell density. As a regulator of cell proliferation and survival, higher levels of placental-derived BDNF during early periods of neurogenesis would promote larger cell populations[46]. Neurons and microglia are both responsible for the production and secretion of BDNF, thus a reduction in cell density may be both cause and effect of reduced BDNF expression.

At present, there is not enough information to conclusively determine the cause of noted BDNF reduction, although it is likely the culmination of one or more of the factors discussed above. Nonetheless, this remains an important point of discussion given its vital role in neurodevelopment both *in utero* and beyond.

4.4 Impact of Maternal WD on fetal neurodevelopment

4.4.1 Cell proliferation

BDNF is involved in nearly all developmental processes throughout gestation, some of which continue into late gestation and later life. One such process is hippocampal neurogenesis, which along with synaptic pruning and synaptogenesis, is responsible for brain plasticity[152]. To understand the implications of lifelong maternal WD consumption on fetal hippocampal neurogenesis, brain sections were stained for Ki67, a marker of cell proliferation. Between fetal

diet groups, no changes were noted in the number of Ki67 immunoreactive cells in any region of the hippocampus, suggesting there were no changes in cell proliferation. These results were surprising. Given that BDNF has been shown to promote cell proliferation and neurogenesis in the brain, it was expected that cell proliferation would decrease in parallel with BDNF expression in the hippocampus of WD Fetuses[148,250]. These suspicions are further grounded in evidence from previous studies that found diets rich in saturated fats and refined sugars consumed postnatally or experienced through maternal consumption during gestation, reduced hippocampal neurogenesis[184,244,251]

BDNF exerts its effects on proliferation through its TrkB receptor[46]. As such, hippocampal cell proliferation has shown positive correlations to the receptor's expression[252]. In one previous study conducted in guinea pigs, fetal hippocampal expression of BDNF and its receptor showed an inverse relationship—albeit under different adverse intrauterine conditions than that which was explored in the present study[253]. They proposed the observed upregulation of TrkB receptor expression was a compensatory mechanism responding to reduced BDNF expression, which has been similarly documented under other post-natal conditions[254]. While reductions in fetal hippocampal BDNF expression were noted in the current study following maternal WD consumption, TrkB expression was not reported and remains unknown. Differential expression or localization of the TrkB receptor may modify the impact of reduced BDNF expression, although these changes would not be without their own consequences.

The present results may otherwise be a product of the time point studied. Previous studies that have reported a relationship between reduced BDNF and hippocampal neurogenesis have been primarily conducted on post-natal animals[184,244,251]. This makes it easy to speculate that detriments to hippocampal neurogenesis may not manifest until later in postnatal life. However, an important caveat to interpreting these studies is recognizing their use of a rat or mouse model, which both exhibit delayed neuronal maturation relative to guinea pigs and humans[91,255]. Unlike most rodents, guinea pigs undergo expansive neurodevelopment *in utero;* thus, when comparing developmental timelines, the aforementioned rodent studies may be comparable to the time point presently studied[91,255,256]. In agreement with this statement, approximately 80% of the granule cells in the hippocampus are produced prenatally in the guinea pig much like humans[255]. As such, one might expect hippocampal neurogenesis to steadily decline throughout gestation and

postnatal development; however, this is not the case. Hippocampal neurogenesis in the guinea pig shows a postnatal peak on day 3 after birth, showing a 1.5-fold increase between post-natal days 1 and 3[255]. It has been proposed that cell genesis in the guinea pig brain is temporarily inhibited near term and then resumed postnatally, as is the case for the rat[255]. The present study observed the fetal brain at gestational day 65, which is 3 days prior to expected delivery and 1 week prior to when hippocampal neurogenesis is expected to peak. Greater differences in cell proliferation may have been noted at either an earlier period of prenatal development or during postnatal life.

In utero, BDNF is sourced from the mother, placenta, or endogenous fetal production[142]. Without understanding which of these factors is primarily driving the observed reduction in BDNF noted in the fetal brain, it is difficult to draw conclusions. Even if maternal and/or placental sources were somewhat reduced, their supply of BDNF may help to mitigate the effects of reduced endogenous production in the fetal brain (assuming endogenous production is reduced). If this were the case, the effects of reduced BDNF expression on hippocampal neurogenesis may not manifest until the alternative sources are eliminated, as is the case after birth[142].

From the findings reported here, hippocampal neurogenesis appears unaffected by lifelong maternal WD consumption in near-term fetuses; however, this does not absolve the fetal brain from developmental deficits resulting from the WD-induced declines in BDNF expression.

4.4.2 Cell density

The role of BDNF in promoting neurogenesis begins early in gestation with the rapid expansion of the fetal brain. Given that hippocampal cell proliferation appeared unchanged near term, cells were counted to determine whether differences existed in cell density between diet groups. Hematoxylin-stained cells were counted in the hippocampus and thalamus, which was included in this analysis as it showed similar reductions to the hippocampus in BDNF expression.

WD fetuses showed reduced cell density across all brain regions, which was expected given that maternal overnutrition has been shown to negatively impact the size and cell density of the brain, particularly the hippocampus. For example, it was observed that children (7-11

years) born to obese mothers had reduced hippocampal volumes as determined by MRI[257]. Similarly, rodent models have demonstrated that maternal diets high in saturated fat are associated with reduced neurogenesis, which may drive reduced cell density[114,184,250]. On the other side of the macronutrient spectrum, the work of Erbas *et al* demonstrated that a diet high in fructose was associated with increased hepatic lipid accumulation in a maternal rat population, just as it was noted in the guinea pig in the present study following WD consumption[258]. Moreover, following *in utero* exposure to this maternal metabolic environment, offspring showed reduced hippocampal cell density in tandem with increased expression of pro-inflammatory cytokines[258]. Collectively these findings support the notion that maternal WD consumption is the catalyst of the observed reduction in cell density, although the mechanisms behind this change are less clear.

Healthy neurodevelopment involves both cell proliferation and apoptosis, a delicate dichotomy that is maintained by the balance of pro-survival and pro-apoptotic signals[81]. As previously discussed, WD consumption and related metabolic dysfunction, including the NAFLD phenotype observed in the present maternal population, are associated with increased levels of inflammation and oxidative stress in the maternal and fetal environments[93,258,259]. In addition to their detrimental impact on BDNF signalling, inflammation and oxidative stress appear to impede neurogenesis and promote cellular damage, potentially altering the balance between cell survival and death[260]. As it relates to BDNF, the neurotrophic factor's role in neurogenesis has been well established; however, some have suggested its trophic effects in the brain are attributable to increased cell survival of the newly generated neurons, rather than increased cell proliferation[147,250]. BDNF may then reduce rates of apoptosis in the brain by acting as a pro-survival signal. Given that cell proliferation appeared unchanged in the hippocampus of the currently studied WD fetuses, the current WD model may be perpetuating cellular damage through the induction of inflammation and/or oxidative stress, while simultaneously reducing pro-survival signalling through the suppression of BDNF expression. Together these mechanisms would enhance the susceptibility of the brain cells to cell death leading to reduced cell density.

In light of the fact that the presence of inflammation and oxidative stress in the present model can only be speculated, a simpler explanation may be that the noted decrease in cell

density is a product of earlier disruption in neurodevelopment resulting from altered placental function. As previously discussed, the placenta is an important source of BDNF early in gestation prior to the expansion of the fetal brain[98]. Considering that the present model features lifelong (including pregnancy) maternal WD consumption, the noted reductions in placental BDNF expression may be consistent throughout gestation. It would then be plausible that the initial formation of brain structures like the hippocampus and thalamus could be disrupted, either by reduced cell proliferation or decreased cell survival, resulting from decreased BDNF delivery from the placenta. Given that changes in cell density were noted in the thalamus, a region where continued neurogenesis is not expected in a term fetus, it is likely that the reported changes in cell density arose at an earlier period of neurodevelopment during global cell proliferation. To this end, evaluating cellular proliferation at an earlier time point in pregnancy may better elucidate whether the current reductions in cell density are a product of early changes in proliferation or rather increased levels of cell death at a later timepoint in pregnancy.

These findings reflect that maternal WD consumption independent of obesity can confer significant changes to cell density in vital brain structures such as the hippocampus and thalamus. As the center regulating learning, memory, and mood, these changes may confer severe detriment to the cognitive function of offspring and potentially predispose them to the development of future neurological disorders.

4.4.3 Microglia cell density and activation state

As the resident immune cells of the brain, microglia are often discussed in relation to neuroinflammation and disease pathology. While aberrant microglial activation does perpetuate neuroinflammation and consequently neurological pathology, microglia are also beneficial in supporting cell proliferation, survival, and differentiation[49]. The exact effects of microglia are dependent on the extent of their activation as well as their specific phenotype or activation state, which is heavily related to their morphology and cell-surface receptor expression[49]. Consequently, morphological characteristics of microglial activation are often used as an indicator of neuroinflammation, specifically by quantifying their transition from a highly ramified state to an ameoboid-like morphology that is characteristic of activated microglia[160,208]. In the present study, both microglia cell density and morphology were evaluated by immunostaining for Iba1, a microglia cell marker. Microglial morphology was evaluated based

on cell body size and roundness, and the cell body size to process length ratio to reflect changes in activation state. Across all the brain regions studied, there was no difference noted in microglial cell density, nor any of the morphological parameters, suggesting that microglial populations in the fetal brain regions studied were unaffected by lifelong maternal WD consumption.

An important caveat to the present findings is that there are no standardized classifications for the various microglial phenotypes. Beyond the traditional "activated" vs "resting" state classification that is often used to describe microglia populations, alternative morphological phenotypes are not fully understood[49,208]. The activated state, for example, encompasses multiple phenotypes with varying functions[49]. This is an important distinction because certain populations of activated microglia have been shown to mediate anti-inflammatory responses that are vital to brain repair following acute inflammation or ischemia, including the secretion of BDNF[49,158,163]. Nonetheless, it still follows that neuroinflammation would likely result in an increase to both the total and the activated microglia cell populations, with the specific activation states of the population being dependent on the duration and nature of the precipitating insult[158]. Given that no changes were observed in microglia cell density or morphology, this suggests that neuroinflammation may not be present in fetal brains challenged with maternal gestational WD consumption.

These findings were surprising given the associations of maternal WD consumption and subsequent metabolic inflammation with increased offspring expression of inflammatory cytokines. In the offspring of high-fat-fed macaque mothers, there were noted increases in both the population and activation of microglia, as well as in the expression of interleukin 1 beta (IL-1β) inflammatory cytokines and the IL-1 receptor[119]. In a similar rat model of maternal high-fat consumption, these findings were corroborated with offspring showing enhanced microglial activation in tandem with increased expression of IL-1β and toll-like receptor 4 (TLR4), signalling through the latter of which enhances inflammatory cytokine production[113]. Conversely, in rat offspring born from mothers with fructose-induced hepatic lipid accumulation, the expression of tumour necrosis factor-alpha (TNF-α) was increased[258]. These studies implicate maternal consumption of diets like the WD in enhancing the inflammatory state of the fetal brain. Variability in animal models, diet composition, and age of subject offspring between

studies may likely account for inconsistencies in the current results and those previously reported. Additionally, it should be noted that in the absence of additional markers of neuroinflammation, such as the inflammatory cytokines and receptors discussed above, conclusions and comparisons regarding the inflammatory state of the fetal brain are made difficult.

The lack of change in microglial cell number and activation state helps to contextualize some of the present findings. As mentioned, BDNF can be secreted by activated microglia, typically following acute inflammation, to facilitate tissue repair[158]. The reported findings eliminate microglia from being considered as a major source of the BDNF observed in the brains of CD fetuses. Secondly, these findings strengthen the argument that the observed reduction in cell density is attributable to a decrease in neuronal cell populations, although other glial cell types (astrocytes and oligodendrocytes) cannot be completely discounted.

Taken together, while no changes in the microglial population were observed, this analysis provided valuable insights that helped to contextualize other findings contained within the present study. The analysis also highlighted the importance of utilizing additional markers to better understand microglia activation and to characterize the inflammatory state of the brain.

4.5 Limitations

While care was taken to ensure the quality and utility of the present research, this study is not without limitations. First, the present study was focused on the outcomes of near-term fetuses. As such these findings represent only a snapshot of development that may not reflect additional changes occurring earlier in gestation or immediately post-birth during other critical windows of brain development. In the present study, *in utero* fetal demise occurring near the end of pregnancy was noted amongst the WD group and due to the integrity (or complete reabsorption) of tissues, these fetuses were excluded from all analyses. As a result, valuable fetal specimens were lost that may have provided greater insight into more severe consequences related to maternal diet. Moreover, it is difficult to form conclusions about the long-term cognitive outcomes of these fetuses, given that brain development is a fluid process that continues postnatally and is subject to further modifications by environmental factors.

Moreover, interpretation of this study's results is limited by the diet itself. The experimental WD was designed to mimic a human WD as described by Cordain et al, which is characterized by a particular proportion of fatty acids and simple sugars[199]. Consequently, the current study cannot distinguish between the effects of added simple sugars versus added saturated fats. Tangentially, food was provided to the sows in solid pellet form, which does not account for the prevalent intake of fructose/sucrose sweetened beverages involved in a typical WD. This is a limitation because there is epidemiological evidence to suggest that simple sugars may have enhanced metabolic detriment when consumed in liquid form[261,262]. Thus, by nature of the current experimental diet design, detriments of true WD consumption may be minimized.

In addition, this study was limited by sample size, which prevented fetal sex from being included in the analysis. There is a plethora of evidence to suggest that the outcomes from intrauterine adversity differ between sexes[257,263,264]. As it pertains to the present study, sex differences have been specifically noted in BDNF expression[139]. Thus, while there were notable differences in features of fetal and placental development, the current study cannot statistically speak to sex differences in vulnerability to maternal overnutrition. It should also be noted that maternal populations were also particularly small, which drastically reduced statistical power of the maternal analysis; however, analyses were included as they were consistent with phenotypes previously reported by our lab in larger sample populations[195,196,265].

Finally, the interpretation of this study is limited by the prevalent use of immunohistochemistry for fetal brain characterizations. Although useful for determining the localization of protein expression and for visualizing changes in brain morphology, this technique identifies changes in only a single cross-section of the tissue. Consequently, there may be changes in adjacent brain regions that are not captured. Analyses of cell proliferation and cell density were further limited because they were conducted without the use of additional neuronal markers. Neither Ki67 nor hematoxylin is specific to neurons, thus any changes in their expression cannot be wholly attributed to changes in the neuronal cell population. This lack of specificity muddies the findings making it difficult to form conclusions around the impact of maternal WD on fetal neurogenesis and it highlights the importance of using additional markers that identify neurons like NeuN.

4.6 Future works

The present study revealed several fascinating avenues of future research, some of which expand on the current findings and others that help to fill in the remaining gaps in knowledge. First, this study highlighted reduced BDNF expression in the placenta and fetal brain. While it can be assumed that this would convey detriments to brain development, BDNF is only the catalyst of what is a complex signalling pathway. To fully understand the implication of this reduction, further investigation is required. Of particular interest would be the expression of the TrkB receptor in the placenta and fetal brain, expression of which has previously been shown to change following perinatal adversity[130,139,246,266]. Changes in receptor expression may ultimately be mitigating or exacerbating the changes noted in BDNF expression, thus such an analysis would complement the current findings and would offer insight into the extent to which the noted reduction in BDNF might affect either tissue.

Secondly, as has been alluded to numerous times, a major gap in the presented research is the missing characterization of inflammation in the placental and fetal brain of the current model. The WD has been described as pro-inflammatory and maternal overnutrition has likewise been associated with chronic low-grade inflammation[1,93]. Moreover, in such circumstances, the placenta itself may produce its own inflammatory cytokines[117]. Microglial activation was explored in the fetal brain as a proxy for neuroinflammation, but in the absence of additional inflammatory markers, it is not clear whether maternal WD consumption is affecting levels of inflammation in the fetal brain. Given the antagonistic effects of chronic inflammation on BDNF expression, identifying the inflammatory state of both tissues would be pertinent to clarifying the underlying etiology of the reported placental and neurological changes. To this end, future studies should aim to characterize the cytokine profile of both the placenta and fetal brain in the current WD model.

While characterizing the above features would be of use in the current population, expanding the present research to include a larger sample size would allow sex to be adequately considered in future analyses, improving the relevance and applicability of the present research.

Finally, this study highlighted changes in the fetal brain at term. The noted reduction of BDNF expression in the hippocampus is of particular interest because as a region involved in

memory, learning, and mood regulation, such changes are expected to bring about behavioural and cognitive deficits. Neurobehavioural studies in a future cohort of neonates born to WD-fed sows would be of value to better understand how these changes in BDNF expression affect cognition and behaviour. Beyond cognitive function, evaluating the food intake of these neonates would also be relevant. Hyperphagic obesity is a well-established phenotype of the Val66Met mutation and inhibition of BDNF-TrkB signalling has led to similar outcomes[48,53]. The observed decreases in BDNF expression may not only confer cognitive deficits but also dysregulate satiety signalling further perpetuating metabolic dysfunction.

4.7 Conclusion

The present study was guided by the governing hypothesis that lifelong maternal WD consumption, even in the absence of obesity, would alter fetoplacental development in a guinea pig model, specifically as it pertained to placental and fetal neurodevelopment. The findings reported here stand to support this hypothesis.

The first objective was to characterize the phenotypes of maternal and fetal animals to elucidate potential changes that may be associated with chronic exposure to a western dietary pattern. Within the mothers themselves, lifelong WD consumption was associated with hepatic lipid accumulation, independent of additional extrahepatic adiposity. This phenotype was conserved in both mother and fetus, although it is speculated these outcomes may be driven by different mechanisms. Maternal NAFLD is postulated to be largely a result of the additional fructose found in the WD, as an added sugar that has been known to reduce hepatic oxidation and increase de novo lipogenesis in the liver, while promoting the oxidation of peripheral adipose tissues[219–221]. Regarding the fetal population, these findings are more likely the response of primitive adaptive mechanisms that promote lipid storage *in utero* in response to a mismatch between maternal supply and fetal demand[225]. Given that the guinea pig, much like humans, do not lay down adipose tissue until late gestation, mismatches present earlier in gestation, as is the case with lifelong maternal WD consumption, may instead promote triglyceride accumulations in fetal organs such as the liver, setting the stage for young adulthood NAFLD[80,196,225]

Attention was then shifted to the second component of the fetoplacental unit, to the vital organ regulating fetal development. The second objective was to identify changes in placental

development arising from maternal adherence to WD consumption. The diet was associated with larger, less-efficient placentae and the detriments in placental efficiency were likely attributable to some form of placental infarct, as there was a higher prevalence of necrosis and fibrin thrombi in these functionally deficient placentae. The exact cause of these findings remains a fascinating avenue for future study.

Among the many functions of the placenta is its production of hormones such as BDNF, a trophic factor that supports the development of both the placenta and the fetal brain and is largely regulated by changes in nutritional status[46,128,137]. The third objective was to characterize its expression in both the placenta and the fetal brain to determine whether expression in either tissue was being influenced by maternal diet. To this end, it was demonstrated that BDNF expression was notably reduced in the placentae and in multiple regions of the fetal brain. Given BDNF's established regulation by energy status, it is speculated that maternal overnutrition may have reduced its expression in the early developing placenta[155,267,268]. Such a reduction would attenuate trophic support of placental development, which may cause placental infarcts that further limit the functional capacity of placental cells to produce BDNF later in pregnancy. Within the brain, the cause of BDNF reduction is less clear and likely results from the combination of reduced placental delivery and suppressed fetal endogenous production. Regardless these findings are grounds for concern, particularly if maintained after birth given the multiple roles of BDNF in the developing brain[46].

The noted reductions in BDNF expression highlighted the potential impact of maternal WD consumption on fetal brain development. This led to the fourth and final objective, to broadly investigate potential disruptions in the balance of neurogenesis and neuroinflammation during neurodevelopment. No changes in cell proliferation were observed in the hippocampus, suggesting that in a near-term fetus, hippocampal neurogenesis remains unchanged despite the concurrent reduction in BDNF. Conversely, cell density in the hippocampus and thalamus were lower under the WD condition. Together these findings may indicate disruption of early neurogenesis or increased rates of apoptosis following the suppression of BDNF expression. Originally it was speculated that the changes in the fetal brain may be explained by the presence of inflammation, as is commonly found in association with maternal metainflammation[93]. The present study does not support this claim given that microglia populations remained unchanged

both in cell density and activation levels; however, further investigations of pro-inflammatory cytokine expression in the fetal brain would be useful to confirm these results.

Collectively, here it was shown that lifelong maternal WD consumption in the context of a metabolically unhealthy but lean maternal phenotype, may confer metabolic and cognitive detriments to the fetus that are in part related to alterations in the placental function. The present study has only scratched the surface of the neurodevelopmental changes that may be born out of a reduction in fetoplacental BDNF expression. While the impact of these changes postnatally remains to be determined, they are likely to confer long-term detriments to memory, learning, and behaviour. These findings underscore the importance of viewing maternal metabolic health holistically, which means considering lifestyle factors such as dietary patterns, independent of body composition, as key predictors of metabolic health. Habitual WD consumption before and during pregnancy, even with a lean phenotype, is a risk factor of future metabolic and cognitive disruption in offspring through *in utero* fetal reprogramming.

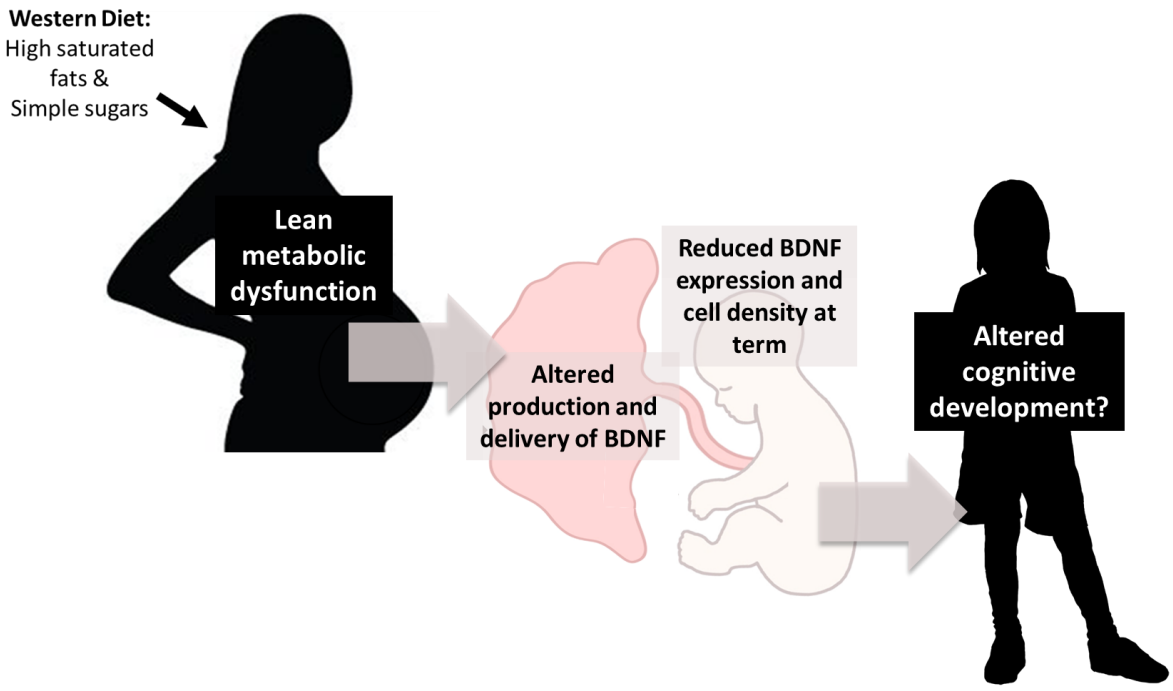

Figure 4.7.1-1. Lifelong maternal western diet consumption, even in the absence of obesity, can have detrimental effects on both placental and fetal brain development. These changes are likely attributed in part to alterations in BDNF protein expression as a consequence of maternal overnutrition.

REFERENCES

1. Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. *Diabetes, Metab Syndr Obes Targets Ther*. 2019;12:2221-2236.

2. Azzam A. Is the world converging to a "Western diet"? *Public Health Nutr*. 2021;24(2):309-317.

3. Fritsche KL. The Science of Fatty Acids and Inflammation. *Adv Nutr*. 2015;6(3):293S-301S.

4. FAO. WHO | Fats and fatty acids in human nutrition. *WHO*. 2010.

5. Gammone MA, Riccioni G, Parrinello G, D'orazio N. Omega-3 polyunsaturated fatty acids: Benefits and endpoints in sport. *Nutrients*. 2019;11(1).

6. Rakhra V, Galappaththy SL, Bulchandani S, Cabandugama PK. Obesity and the Western Diet: How We Got Here. *Mo Med*. 2020;117(6):536-538.

7. Johnson RK, Yon BA. Weighing in on added sugars and health. *J Am Diet Assoc*. 2010;110(9):1296-1299.

8. Ma J, Fox CS, Jacques PF, et al. Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. *J Hepatol*. 2015;63(2):462-469.

9. Gangwisch JE, Hale L, Garcia L, et al. High glycemic index diet as a risk factor for depression: Analyses from the Women's Health Initiative. *Am J Clin Nutr*. 2015;102(2):454-463.

10. Crane PK, Walker R, Hubbard RA, et al. Glucose levels and risk of dementia. *Forsch Komplementarmed*. 2013;20(5):386-387.

11. Barber TM, Kabisch S, Pfeiffer AFH, Weickert MO. The health benefits of dietary fibre. *Nutrients*. 2020;12(10):1-17.

12. Noble EE, Hsu TM, Kanoski SE. Gut to brain dysbiosis: Mechanisms linking western diet

consumption, the microbiome, and cognitive impairment. *Front Behav Neurosci*. 2017;11.

13. Sarma S, Sockalingam S, Dash S. Obesity as a multisystem disease: Trends in obesity rates and obesity-related complications. *Diabetes, Obes Metab*. 2021;23(S1):3-16.

14. Ataide Lima RP, Neto Hayashi D, de Farias Lima KQ, et al. The Role of Epigenetics in the Etiology of Obesity: A Review. *J Clin Epigenetics*. 2017;03(04):41.

15. Stefan N, Schick F, Häring HU. Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans. *Cell Metab*. 2017;26(2):292-300.

16. Stefan N, Kantartzis K, Machann J, et al. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med*. 2008;168(15):1609-1616.

17. Osadnik K, Osadnik T, Lonnie M, et al. Metabolically healthy obese and metabolic syndrome of the lean: The importance of diet quality. Analysis of MAGNETIC cohort. *Nutr J*. 2020;19(1):1-13.

18. Wildman RP, Muntner P, Reynolds K, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: Prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med*. 2008;168(15):1617-1624.

19. Riazi K, Raman M, Taylor L, Swain MG, Shaheen AA. Dietary patterns and components in nonalcoholic fatty liver disease (NAFLD): What key messages can health care providers offer? *Nutrients*. 2019;11(12).

20. Bertot LC, Adams LA. The natural course of non-alcoholic fatty liver disease. *Int J Mol Sci*. 2016;17(5).

21. Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2020;5(8):739-752.

22. Hagström H, Nasr P, Ekstedt M, et al. Risk for development of severe liver disease in lean

patients with nonalcoholic fatty liver disease: A long-term follow-up study. *Hepatol Commun*. 2018;2(1):48-57.

23. VanWagner LB, Armstrong MJ. Lean NAFLD: A not so benign condition? *Hepatol Commun*. 2018;2(1):5-8.

24. Sinn DH, Kang D, Cho SJ, et al. Lean non-alcoholic fatty liver disease and development of diabetes: A cohort study. *Eur J Endocrinol*. 2019;181(2):185-192.

25. Wang AY, Dhaliwal J, Mouzaki M. Lean non-alcoholic fatty liver disease. *Clin Nutr*. 2019;38(3):975-981.

26. Stephenson K, Kennedy L, Hargrove L, et al. Updates on Dietary Models of Nonalcoholic Fatty Liver Disease: Current Studies and Insights. *Gene Expr*. 2017;18(1):5-17.

27. Ishimoto T, Lanaspa MA, Rivard CJ, et al. High-fat and high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase. *Hepatology*. 2013;58(5):1632-1643.

28. Leung JCF, Loong TCW, Wei JL, et al. Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology*. 2017;65(1):54-64.

29. Albhaisi S, Chowdhury A, Sanyal AJ. Non-alcoholic fatty liver disease in lean individuals. *JHEP Reports*. 2019;1(4):329-341.

30. Xu B, Goulding EH, Zang K, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci*. 2003;6(7):736-742.

31. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: Pathways to obesity. *DMM Dis Model Mech*. 2017;10(6):679-689.

32. Taliaz D, Stall N, Dar DE, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Mol Psychiatry*. 2010;15(1):80-92.

33. Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB

and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder. *Neurobiol Dis*. 2015;77:220-227.

34. Kim DM, Leem YH. Chronic stress-induced memory deficits are reversed by regular exercise via AMPK-mediated BDNF induction. *Neuroscience*. 2016;324:271-285.

35. Walsh TJ, Emerich DF. The hippocampus as a common target of neurotoxic agents. *Toxicology*. 1988;49(1):137-140.

36. Jurdak N, Lichtenstein AH, Kanarek RB. Diet-induced obesity and spatial cognition in young male rats. *Nutr Neurosci*. 2008;11(2):48-54.

37. Eskelinen MH, Ngandu T, Helkala EL, et al. Fat intake at midlife and cognitive impairment later in life: A population-based CAIDE study. *Int J Geriatr Psychiatry*. 2008;23(7):741-747.

38. Berrino F. Western diet and Alzheimer's disease. *Epidemiol Prev*. 2002;26(3):107-115.

39. Pasinetti GM, Eberstein JA. Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. *J Neurochem*. 2008;106(4):1503-1514.

40. Kanoski SE, Zhang Y, Zheng W, Davidson TL. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. *J Alzheimer's Dis*. 2010;21(1):207-219.

41. Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. *Physiol Behav*. 2011;103(1):59-68.

42. Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. *Diabetes, Metab Syndr Obes Targets Ther*. 2019;12:2221-2236.

43. Park CR, Seeley RJ, Craft S, Woods SC. Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol Behav*. 2000;68(4):509-514.

44. Reger MA, Watson GS, Green PS, et al. Intranasal insulin administration dose-

dependently modulates verbal memory and plasma amyloid-β in memory-impaired older adults. *J Alzheimer's Dis*. 2008;13(3):323-331.

45. Gold SM, Dziobek I, Sweat V, et al. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia*. 2007;50(4):711-719.

46. Tapia-Arancibia L, Rage F, Givalois L, Arancibia S. Physiology of BDNF: Focus on hypothalamic function. *Front Neuroendocrinol*. 2004;25(2):77-107.

47. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience*. 2002;112(4):803-814.

48. Rosas-Vargas H, Martínez-Ezquerro JD, Bienvenu T. Brain-Derived Neurotrophic Factor, Food Intake Regulation, and Obesity. *Arch Med Res*. 2011;42(6):482-494.

49. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: The good, the bad, and the inflamed. *J Neuroinflammation*. 2014;11(1):1-15.

50. Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R. Brain-derived neurotrophic factor: A bridge between inflammation and neuroplasticity. *Front Cell Neurosci*. 2014;8(DEC).

51. Wang H, Golob EJ, Su MY. Vascular volume and blood-brain barrier permeability measured by dynamic contrast enhanced MRI in hippocampus and cerebellum of patients with MCI and normal controls. *J Magn Reson Imaging*. 2006;24(3):695-700.

52. Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. Blood-brain barrier impairment in Alzheimer disease: Stability and functional significance. *Neurology*. 2007;68(21):1809-1814.

53. Yeo GSH, Hung CCC, Rochford J, et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat Neurosci*. 2004;7(11):1187-1189.

54. Davidson TL, Kanoski SE, Chan K, Clegg DJ, Benoit SC, Jarrard LE. Hippocampal Lesions Impair Retention of Discriminative Responding Based on Energy State Cues. *Behav Neurosci*. 2010;124(1):97-105.

55. Lucas A. Role of nutritional programming in determining adult morbidity. *Arch Dis Child*. 1994;71(4):288-290.

56. Barker DJP. *In Utero Programming of Chronic Disease*. Vol 115.; 1998.

57. Alfaradhi MZ, Ozanne SE. Developmental programming in response to maternal overnutrition. *Front Genet*. 2011;2(JUNE):27.

58. Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *Br J Prev Soc Med*. 1977;31(2):91-95.

59. Barker DJP, Osmond C, Golding J, Kuh D, Wadsworth MEJ. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *Br Med J*. 1989;298(6673):564-567.

60. Hales CN, Barker DJP, Clark PMS, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *Br Med J*. 1991;303(6809):1019-1022.

61. Martin-Gronert MS, Ozanne SE. Mechanisms underlying the developmental origins of disease. *Rev Endocr Metab Disord*. 2012;13(2):85-92.

62. Oben JA, Mouralidarane A, Samuelsson AM, et al. Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *J Hepatol*. 2010;52(6):913-920.

63. McCurdy CE, Bishop JM, Williams SM, et al. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. *J Clin Invest*. 2009;119(2):323-335.

64. Samuelsson AM, Matthews PA, Argenton M, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: A novel murine model of developmental programming. *Hypertension*. 2008;51(2):383-392.

65. Lucas A. Long-term programming effects of early nutrition — implications for the preterm infant. *J Perinatol*. 2005;25:S2-S6.

66. Plagemann A, Harder T, Rake A, et al. Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome X-like alterations in adulthood of neonatally overfed rats. *Brain Res*. 1999;836(1-2):146-155.

67. Geraghty AA, Lindsay KL, Alberdi G, McAuliffe FM, Gibney ER. Nutrition during Pregnancy Impacts Offspring's Epigenetic Status—Evidence from Human and Animal Studies. *Nutr Metab Insights*. 2015;8s1(Suppl 1):NMI.S29527.

68. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr*. 2005;135(6):1382-1386.

69. Li M, Sloboda DM, Vickers MH. Maternal obesity and developmental programming of metabolic disorders in offspring: Evidence from animal models. *Exp Diabetes Res*. 2011;2011.

70. Chen JH, Hales CN, Ozanne SE. DNA damage, cellular senescence and organismal ageing: Causal or correlative? *Nucleic Acids Res*. 2007;35(22):7417-7428.

71. Simmons RA. Developmental origins of diabetes: The role of oxidative stress. *Free Radic Biol Med*. 2006;40(6):917-922.

72. Bruce KD, Cagampang FR, Argenton M, et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology*. 2009;50(6):1796-1808.

73. Bale TL. Epigenetic and transgenerational reprogramming of brain development. *Nat Rev Neurosci*. 2015;16(6):332-344.

74. Rosenfeld CS. The placenta-brain-axis. *J Neurosci Res*. 2021;99(1):271-283.

75. Kratimenos P, Penn AA. Placental programming of neuropsychiatric disease. *Pediatr Res*.

2019;86(2):157-164.

76. Turco MY, Moffett A. Development of the human placenta. *Dev*. 2019;146(22).

77. Pollheimer J, Vondra S, Baltayeva J, Beristain AG, Knöfler M. Regulation of placental extravillous trophoblasts by the maternal uterine environment. *Front Immunol*. 2018;9(NOV):2597.

78. Wang Y, Zhao S. *Vascular Biology of the Placenta*. Morgan & Claypool Life Sciences; 2010.

79. Mikkelsen E, Lauridsen H, Nielsen PM, et al. The chinchilla as a novel animal model of pregnancy. *R Soc Open Sci*. 2017;4(4).

80. Morrison JL, Botting KJ, Darby JRT, et al. The Journal of Physiology Guinea pig models for translation of the developmental origins of health and disease hypothesis into the clinic. *J Physiol*. 2018;596:5535-5569.

81. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychol Rev*. 2010;20(4):327-348.

82. Williamson JM, Lyons DA. Myelin Dynamics Throughout Life: An Ever-Changing Landscape? *Front Cell Neurosci*. 2018;12:424.

83. Jiang X, Nardelli J. Cellular and molecular introduction to brain development. *Neurobiol Dis*. 2016;92(Part A):3-17.

84. Menassa DA, Gomez-Nicola D. Microglial Dynamics During Human Brain Development. *Front Immunol*. 2018;0(MAY):1014.

85. Casano AM, Albert M, Peri F. Developmental Apoptosis Mediates Entry and Positioning of Microglia in the Zebrafish Brain. *Cell Rep*. 2016;16(4):897-906.

86. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. *Mol Psychiatry*. 2019;24(1):67-87.

87. Jun H, Mohammed Qasim Hussaini S, Rigby MJ, Jang MH. Functional role of adult hippocampal neurogenesis as a therapeutic strategy for mental disorders. *Neural Plast*. 2012;2012.

88. Koehl M, Abrous DN. A new chapter in the field of memory: adult hippocampal neurogenesis. *Eur J Neurosci*. 2011;33(6):1101-1114.

89. Toda T, Parylak SL, Linker SB, Gage FH. The role of adult hippocampal neurogenesis in brain health and disease. *Mol Psychiatry*. 2019;24(1):67-87.

90. Cowan M, Petri WA. Microglia: Immune Regulators of Neurodevelopment. *Front Immunol*. 2018;9(NOV):2576.

91. Workman AD, Charvet CJ, Clancy B, Darlington RB, Finlay BL. Modeling transformations of neurodevelopmental sequences across mammalian species. *J Neurosci*. 2013;33(17):7368-7383.

92. Zeltser LM, Leibel RL. Roles of the placenta in fetal brain development. *Proc Natl Acad Sci U S A*. 2011;108(38):15667-15668.

93. Burton GJ, Fowden AL, Thornburg KL. Placental Origins of Chronic Disease. *Physiol Rev*. 2016;96:1509-1565.

94. Fowden AL, Forhead AJ. Hormones as epigenetic signals in developmental programming. *Exp Physiol*. 2009;94(6):607-625.

95. Shallie PD, Naicker T. The placenta as a window to the brain: A review on the role of placental markers in prenatal programming of neurodevelopment. *Int J Dev Neurosci*. 2019;73(1):41-49.

96. Hsu MH, Sheen JM, Lin IC, et al. Effects of maternal resveratrol on maternal high-fat diet/obesity with or without postnatal high-fat diet. *Int J Mol Sci*. 2020;21(10).

97. Page KC, Jones EK, Anday EK. Maternal and postweaning high-fat diets disturb hippocampal gene expression,learning, and memory function. *Am J Physiol Regul Integr*

Comp Physiol. 2014;306:527-537.

98. Kodomari I, Wada E, Nakamura S, Wada K. Maternal supply of BDNF to mouse fetal brain through the placenta. *Neurochem Int*. 2009;54(2):95-98.

99. Nugent BM, Bale TL. The omniscient placenta: Metabolic and epigenetic regulation of fetal programming. *Front Neuroendocrinol*. 2015;39:28-37.

100. Mourier E, Tarrade A, Duan J, et al. Non-invasive evaluation of placental blood flow: Lessons from animal models. *Reproduction*. 2017;153(3):R85-R96.

101. Bronson SL, Bale TL. The Placenta as a Mediator of Stress Effects on Neurodevelopmental Reprogramming. *Neuropsychopharmacology*. 2016;41(1):207-218.

102. Goeden N, Velasquez J, Arnold KA, et al. Maternal inflammation disrupts fetal neurodevelopment via increased placental output of serotonin to the fetal brain. *J Neurosci*. 2016;36(22):6041-6049.

103. Hsiao EY, Patterson PH. Placental regulation of maternal-fetal interactions and brain development. *Dev Neurobiol*. 2012;72(10):1317-1326.

104. Lima Giacobbo B, Doorduin J, Klein HC, Dierckx RAJO, Bromberg E, de Vries EFJ. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. *Mol Neurobiol*. 2019;56(5):3295-3312.

105. Bilbo SD. Early-life infection is a vulnerability factor for aging-related glial alterations and cognitive decline. *Neurobiol Learn Mem*. 2010;94(1):57-64.

106. Bronson SL, Bale TL. The Placenta as a Mediator of Stress Effects on Neurodevelopmental Reprogramming. *Neuropsychopharmacology*. 2016;41(1):207-218.

107. Meyer U, Feldon J, Yee BK. A review of the fetal brain cytokine imbalance hypothesis of schizophrenia. *Schizophr Bull*. 2009;35(5):959-972.

108. Yan X, Zhao X, Li J, He L, Xu M. Effects of early-life malnutrition on neurodevelopment and neuropsychiatric disorders and the potential mechanisms. *Prog Neuro-*

Psychopharmacology Biol Psychiatry. 2018;83:64-75.

109. Alfaradhi MZ, Ozanne SE, Schwartz J, Stocker CJ, Mcmullen S. Developmental programming in response to maternal overnutrition. 2011.

110. Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res*. 2009;65(6):591-598.

111. Sullivan EL, Riper KM, Lockard R, Valleau JC. Maternal high-fat diet programming of the neuroendocrine system and behavior. *Horm Behav*. 2015;76:153-161.

112. Tozuka Y, Kumon M, Wada E, Onodera M, Mochizuki H, Wada K. Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. *Neurochem Int*. 2010;57(3):235-247.

113. Bilbo SD, Tsang V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *FASEB J*. 2010;24(6):2104-2115.

114. Tozuka Y, Wada E, Wada K. Diet-induced obesity in female mice leads to peroxidized lipid accumulations and impairment of hippocampal neurogenesis during the early life of their offspring. *FASEB J*. 2009;23(6):1920-1934.

115. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol*. 2011;29:415-445.

116. Christ A, Lauterbach M, Latz E. Western Diet and the Immune System: An Inflammatory Connection. *Immunity*. 2019;51(5):794-811.

117. Frias AE, Morgan TK, Evans AE, et al. Maternal High-Fat Diet Disturbs Uteroplacental Hemodynamics and Increases the Frequency of Stillbirth in a Nonhuman Primate Model of Excess Nutrition. *Endocrinology*. 2011;152(6):2456-2464.

118. Radaelli T, Varastehpour A, Catalano P, Hauguel-De Mouzon S. Gestational Diabetes Induces Placental Genes for Chronic Stress and Inflammatory Pathways. *Diabetes*. 2003;52(12):2951-2958.

119. Grayson BE, Levasseur PR, Williams SM, Smith MS, Marks DL, Grove KL. Changes in melanocortin expression and inflammatory pathways in fetal offspring of nonhuman primates fed a high-fat diet. *Endocrinology*. 2010;151(4):1622-1632.

120. Kermani P, Hempstead B. BDNF actions in the cardiovascular system: Roles in development, adulthood and response to injury. *Front Physiol*. 2019;10(APR).

121. Genzer Y, Chapnik N, Froy O. Effect of brain-derived neurotrophic factor (BDNF) on hepatocyte metabolism. *Int J Biochem Cell Biol*. 2017;88:69-74.

122. Kawamura K, Kawamura N, Sato W, Fukuda J, Kumagai J, Tanaka T. Brain-Derived Neurotrophic Factor Promotes Implantation and Subsequent Placental Development by Stimulating Trophoblast Cell Growth and Survival. *Endocrinology*. 2009;150(8):3774-3782.

123. Zhao H, Alam A, San CY, et al. Molecular mechanisms of brain-derived neurotrophic factor in neuro-protection: Recent developments. *Brain Res*. 2017;1665:1-21.

124. Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. *Cell Mol Neurobiol*. 2018;38(3):579-593.

125. Sandhya VK, Raju R, Verma R, et al. A network map of BDNF/TRKB and BDNF/p75NTR signaling system. *J Cell Commun Signal*. 2013;7(4):301-307.

126. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Front Cell Neurosci*. 2019;13:363.

127. Michaelsen K, Zagrebelsky M, Berndt-Huch J, et al. Neurotrophin receptors TrkB.T1 and p75NTR cooperate in modulating both functional and structural plasticity in mature hippocampal neurons. *Eur J Neurosci*. 2010;32(11):1854-1865.

128. Kawamura K, Kawamura N, Kumazawa Y, Kumagai J, Fujimoto T, Tanaka T. Brain-derived neurotrophic factor/tyrosine kinase B signaling regulates human trophoblast

growth in an in vivo animal model of ectopic pregnancy. *Endocrinology*. 2011;152(3):1090-1100.

129. Garcés MF, Sanchez E, Torres-Sierra AL, et al. Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. *Clin Endocrinol (Oxf)*. 2014;81(1):141-151.

130. Mayeur S, Silhol M, Moitrot E, et al. Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human. *Placenta*. 2010;31(9):785-791.

131. Fowden AL, Ward JW, Wooding FPB, Forhead AJ, Constancia M. Programming placental nutrient transport capacity. In: *Journal of Physiology*. Vol 572. John Wiley & Sons, Ltd; 2006:5-15.

132. Flöck A, Weber SK, Ferrari N, et al. Determinants of brain-derived neurotrophic factor (BDNF) in umbilical cord and maternal serum. *Psychoneuroendocrinology*. 2016;63:191-197.

133. Dhobale M V., Pisal HR, Mehendale SS, Joshi SR. Differential expression of human placental neurotrophic factors in preterm and term deliveries. *Int J Dev Neurosci*. 2013;31(8):719-723.

134. Zacchigna S, Lambrechts D, Carmeliet P. Neurovascular signalling defects in neurodegeneration. *Nat Rev Neurosci*. 2008;9(3):169-181.

135. Kermani P, Rafii D, Jin DK, et al. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. *J Clin Invest*. 2005;115(3):653-663.

136. He T, Katusic ZS. Brain-derived neurotrophic factor increases expression of MnSOD in human circulating angiogenic cells. *Microvasc Res*. 2012;83(3):366-371.

137. Sahay AS, Sundrani DP, Joshi SR. Neurotrophins: Role in Placental Growth and Development. In: *Vitamins and Hormones*. Vol 104. Academic Press Inc.; 2017:243-261.

138. D'Souza V, Patil V, Pisal H, et al. Levels of brain derived neurotrophic factors across gestation in women with preeclampsia. *Int J Dev Neurosci*. 2014;37(1):36-40.

139. Prince CS, Maloyan A, Myatt L. Maternal obesity alters brain derived neurotrophic factor (BDNF) signaling in the placenta in a sexually dimorphic manner. *Placenta*. 2017;49:55-63.

140. Hsu MH, Sheen JM, Lin IC, et al. Effects of maternal resveratrol on maternal high-fat diet/obesity with or without postnatal high-fat diet. *Int J Mol Sci*. 2020;21(10).

141. Kertes DA, Bhatt SS, Kamin HS, Hughes DA, Rodney NC, Mulligan CJ. BNDF methylation in mothers and newborns is associated with maternal exposure to war trauma. *Clin Epigenetics*. 2017;9(1):1-12.

142. Rao R, Mashburn CB, Mao J, Wadhwa N, Smith GM, Desai NS. Brain-derived neurotrophic factor in infants <32 weeks gestational age: Correlation with antenatal factors and postnatal outcomes. *Pediatr Res*. 2009;65(5):548-552.

143. Nikolaou KE, Malamitsi-Puchner A, Boutsikou T, et al. The Varying Patterns of Neurotrophin Changes in the Perinatal Period.

144. Fletcher JL, Murray SS, Xiao J. Brain-derived neurotrophic factor in central nervous system myelination: A new mechanism to promote myelin plasticity and repair. *Int J Mol Sci*. 2018;19(12).

145. Kristiansen M, Ham J. Programmed cell death during neuronal development: The sympathetic neuron model. *Cell Death Differ*. 2014;21(7):1025-1035.

146. Budday S, Steinmann P, Kuhl E. Physical biology of human brain development. *Front Cell Neurosci*. 2015;9(JULY):257.

147. Linnarsson S, Willson CA, Ernfors P. Cell death in regenerating populations of neurons in BDNF mutant mice. *Mol Brain Res*. 2000;75(1):61-69.

148. Katoh-Semba R, Asano T, Ueda H, et al. Riluzole enhances expression of brain-derived

neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus. *FASEB J*. 2002.

149. Shirayama Y, C-H Chen A, Nakagawa S, Russell DS, Duman RS. *Brain-Derived Neurotrophic Factor Produces Antidepressant Effects in Behavioral Models of Depression*.; 2002.

150. Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, Croll S. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Exp Neurol*. 2005;192(2):348-356.

151. Schmidt HD, Duman RS. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology*. 2010;35(12):2378-2391.

152. Casey BJ, Glatt CE, Tottenham N, et al. Brain-derived neurotrophic factor as a model system for examining gene by environment interactions across development. *Neuroscience*. 2009;164(1):108-120.

153. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Prog Neurobiol*. 2005;76(2):99-125.

154. Jin Y, Sun LH, Yang W, Cui RJ, Xu SB. The role of BDNF in the neuroimmune axis regulation of mood disorders. *Front Neurol*. 2019;10:515.

155. Rios M. BDNF and the central control of feeding: Accidental bystander or essential player? *Trends Neurosci*. 2013;36(2):83-90.

156. Briana DD, Malamitsi-Puchner A. Developmental origins of adult health and disease: The metabolic role of BDNF from early life to adulthood. *Metabolism*. 2018;81:45-51.

157. Jin Y, Sun LH, Yang W, Cui RJ, Xu SB. The role of BDNF in the neuroimmune axis regulation of mood disorders. *Front Neurol*. 2019;10(JUN).

158. Lai AY, Todd KG. Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury. *Glia*. 2008;56(3):259-270.

159. Nieto R, Kukuljan M, Silva H. BDNF and schizophrenia: From neurodevelopment to neuronal plasticity, learning, and memory. *Front Psychiatry*. 2013;4:45.

160. Hovens I, Nyakas C, Schoemaker R. A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: cell body to cell size ratio. *Neuroimmunol Neuroinflammation*. 2014;1(2):82.

161. Madinier A, Bertrand N, Mossiat C, et al. Microglial involvement in neuroplastic changes following focal brain ischemia in rats. *PLoS One*. 2009;4(12).

162. Ploughman M, Windle V, MacLellan CL, White N, Doré JJ, Corbett D. Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. *Stroke*. 2009;40(4):1490-1495.

163. Nagamoto-Combs K, Mcneal DW, Morecraft RJ, Combs CK. Prolonged Microgliosis in the Rhesus Monkey Central Nervous System after Traumatic Brain Injury.

164. Cheng Y, Gidday JM, Yan Q, Shah AR, Holtzman DM. Marked age-dependent neuroprotection by brain-derived neurotrophic factor against neonatal hypoxic-ischemic brain injury. *Ann Neurol*. 1997;41(4):521-529.

165. Beck T, Lindholm D, Castren E, Wree A. *Journal of Cerebral Blood Flow and Metabolism Brain-Derived Neurotrophic Factor Protects Against Ischemic Cell Damage in Rat Hippocampus*.; 1994.

166. Frühauf-Perez PK, Temp FR, Pillat MM, et al. Spermine protects from LPS-induced memory deficit via BDNF and TrkB activation. *Neurobiol Learn Mem*. 2018;149:135-143.

167. Gibney SM, McGuinness B, Prendergast C, Harkin A, Connor TJ. Poly I: C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression. *Brain Behav Immun*. 2013;28:170-181.

168. Kim EJ, Pellman B, Kim JJ. Stress effects on the hippocampus: A critical review. *Learn*

Mem. 2015;22(9):411-416.

169. Bath KG, Schilit A, Lee FS. Stress effects on BDNF expression: Effects of age, sex, and form of stress. *Neuroscience*. 2013;239:149-156.

170. Xie H, Yung WH. Chronic intermittent hypoxia-induced deficits in synaptic plasticity and neurocognitive functions: A role for brain-derived neurotrophic factor. *Acta Pharmacol Sin*. 2012;33(1):5-10.

171. Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci*. 2006;9(4):519-525.

172. Niknazar S, Nahavandi A, Peyvandi AA, Peyvandi H, Akhtari AS, Karimi M. Comparison of the Adulthood Chronic Stress Effect on Hippocampal BDNF Signaling in Male and Female Rats. *Mol Neurobiol*. 2016;53(6):4026-4033.

173. Lakshminarasimhan H, Chattarji S. Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PLoS One*. 2012;7(1).

174. Van Den Hove DLA, Steinbusch HWM, Scheepens A, et al. Prenatal stress and neonatal rat brain development. *Neuroscience*. 2006;137(1):145-155.

175. Dieni S, Rees S. BDNF and TrkB protein expression is altered in the fetal hippocampus but not cerebellum after chronic prenatal compromise. *Exp Neurol*. 2005;192(2):265-273.

176. Huang Y, Shi X, Xu H, et al. Chronic unpredictable stress before pregnancy reduce the expression of brain-derived neurotrophic factor and N-methyl-D-aspartate receptor in hippocampus of offspring rats associated with impairment of memory. *Neurochem Res*. 2010;35(7):1038-1049.

177. Boersma GJ, Lee RS, Cordner ZA, et al. Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. *Epigenetics*. 2013;9(3):437-447.

178. Coupé B, Dutriez-Casteloot I, Breton C, et al. Perinatal Undernutrition Modifies Cell Proliferation and Brain-Derived Neurotrophic Factor Levels During Critical Time-Windows for Hypothalamic and Hippocampal Development in the Male Rat. *J Neuroendocrinol*. 2009;21(1):40-48.

179. Krabbe KS, Nielsen AR, Krogh-Madsen R, et al. Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia*. 2007;50(2):431-438.

180. Tang L, Kang YT, Yin B, Sun LJ, Fan XS. Effects of weight-bearing ladder and aerobic treadmill exercise on learning and memory ability of diabetic rats and its mechanism. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2017;33(5):436-440.

181. Tyagi E, Zhuang Y, Agrawal R, Ying Z, Gomez-Pinilla F. Interactive actions of Bdnf methylation and cell metabolism for building neural resilience under the influence of diet. *Neurobiol Dis*. 2015;73:307-318.

182. Alzoubi KH, Khabour OF, Salah HA, Abu Rashid BE. The combined effect of sleep deprivation and western diet on spatial learning and memory: Role of BDNF and oxidative stress. *J Mol Neurosci*. 2013;50(1):124-133.

183. Wu A, Molteni R, Ying Z, Gomez-Pinilla F. A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. *Neuroscience*. 2003;119(2):365-375.

184. Stranahan AM, Norman ED, Lee K, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus*. 2008;18(11):1085-1088.

185. Akerele OA, Cheema SK. Maternal diet high in Omega-3 fatty acids upregulate genes involved in neurotrophin signalling in fetal brain during pregnancy in C57BL/6 mice. *Neurochem Int*. 2020;138:104778.

186. Szuhany KL, Bugatti M, Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J Psychiatr Res*. 2015;60:56-64.

187. Bedrick BS, Eskew AM, Chavarro JE, Jungheim ES. Dietary Patterns, Physical Activity, and Socioeconomic Associations in a Midwestern Cohort of Healthy Reproductive-Age Women. *Matern Child Health J*. 2020;24:1299-1307.

188. Sarkar M, Grab J, Dodge JL, et al. Non-alcoholic fatty liver disease in pregnancy is associated with adverse maternal and perinatal outcomes. *J Hepatol*. 2020;73(3):516-522.

189. Grieger JA, Bianco-Miotto T, Grzeskowiak LE, et al. Metabolic syndrome in pregnancy and risk for adverse pregnancy outcomes: A prospective cohort of nulliparous women. *PLoS Med*. 2018;15(12).

190. Mathew H, Farr OM, Mantzoros CS. Metabolic health and weight: Understanding metabolically unhealthy normal weight or metabolically healthy obese patients. *Metabolism*. 2016;65(1):73-80.

191. Godfrey KM, Jp Barker D. Fetal programming and adult health. 2021.

192. Bangma JT, Hartwell H, Santos HP, O'Shea TM, Fry RC. Placental programming, perinatal inflammation, and neurodevelopment impairment among those born extremely preterm. *Pediatr Res*. November 2020:1-10.

193. Coupé B, Dutriez-Casteloot I, Breton C, et al. Perinatal undernutrition modifies cell proliferation and brain-derived neurotrophic factor levels during critical time-windows for hypothalamic and hippocampal development in the male rat. *J Neuroendocrinol*. 2009;21(1):40-48.

194. Gomez-Pinilla F, Vaynman S. A "deficient environment" in prenatal life may compromise systems important for cognitive function by affecting BDNF in the hippocampus. *Exp Neurol*. 2005;192(2):235-243.

195. Smith LM, Pitts CB, Friesen-Waldner LJ, et al. In vivo magnetic resonance spectroscopy of hyperpolarized [1-13 C] pyruvate in a male guinea pig model of life-long Western diet consumption and non-alcoholic fatty liver disease development. *bioRxiv*. February 2021:2021.02.05.429612.

196. Sinclair KJ, Friesen–Waldner LJ, McCurdy CM, et al. Quantification of fetal organ volume and fat deposition following in utero exposure to maternal Western Diet using MRI. Thompson L, ed. *PLoS One*. 2018;13(2):e0192900.

197. Grundy D. Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology. *J Physiol*. 2015;593(12):2547-2549.

198. du Sert NP, Hurst V, Ahluwalia A, et al. The arrive guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):e3000410.

199. Cordain L, Eaton SB, Sebastian A, et al. Origins and evolution of the Western diet: Health implications for the 21st century. *Am J Clin Nutr*. 2005;81(2):341-354.

200. Harrell MI, Burnside K, Whidbey C, Vornhagen J, Adams Waldorf KM, Rajagopal L. Exploring the Pregnant Guinea Pig as a Model for Group B Streptococcus Intrauterine Infection. *J Infect Dis Med*. 2017;02(02).

201. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging*. 2012;30(9):1323-1341.

202. Reeder SB, Robson PM, Yu H, et al. Quantification of Hepatic Steatosis With MRI: The Effects of Accurate Fat Spectral Modeling. *J Magn Reson Imaging*. 2009;29:1332-1339.

203. Faralla C, Rizzuto GA, Lowe DE, et al. InlP, a new virulence factor with strong placental tropism. *Infect Immun*. 2016;84(12):3584-3596.

204. Chatterjee A, Harrison CJ, Britt WJ, Bewtra C. *Modification of Maternal and Congenital Cytomegalovirus Infection by Anti-Glycoprotein B Antibody Transfer in Guinea Pigs*. Vol 183.; 2001.

205. Goldman A, Harper S, Speicher DW. Detection of proteins on blot membranes. *Curr Protoc Protein Sci*. 2016;2016:10.8.1-10.8.11.

206. Sander H, Wallace S, Plouse R, Tiwari S, Gomes A V. Ponceau S waste: Ponceau S

staining for total protein normalization. *Anal Biochem*. 2019;575:44-53.

207. Kee N, Sivalingam S, Boonstra R, Wojtowicz JM. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. *J Neurosci Methods*. 2002;115(1):97-105.

208. Davis BM, Salinas-Navarro M, Cordeiro MF, Moons L, De Groef L. Characterizing microglia activation: a spatial statistics approach to maximize information extraction.

209. Empey M-E. Quantifying Mid-pregnancy Placental Metabolism in Guinea Pigs Fed a Lifelong Western Diet. *Electron Thesis Diss Repos*. August 2020.

210. Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: An alternative to least squares means. *Am Stat*. 1980;34(4):216-221.

211. Kim D, Kim WR. PERSPECTIVES IN CLINICAL GASTROENTEROLOGY AND HEPATOLOGY Nonobese Fatty Liver Disease. 2017.

212. MacQueen HA, Sadler DA, Moore SA, et al. Deleterious effects of a cafeteria diet on the livers of nonobese rats. *Nutr Res*. 2007;27(1):38-47.

213. Roncal-Jimenez CA, Lanaspa MA, Rivard CJ, et al. Sucrose induces fatty liver and pancreatic inflammation in male breeder rats independent of excess energy intake. *Metabolism*. 2011;60(9):1259-1270.

214. Schultz A, Neil D, Aguila MB, Mandarim-de-Lacerda CA. Hepatic adverse effects of fructose consumption independent of overweight/obesity. *Int J Mol Sci*. 2013;14(11):21873-21886.

215. Cydylo MA, Davis AT, Kavanagh K. Fatty liver promotes fibrosis in monkeys consuming high fructose. *Obesity*. 2017;25(2):290-293.

216. Sánchez-Lozada LG, Mu W, Roncal C, et al. Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver. *Eur J Nutr*. 2010;49(1):1-9.

217. Maersk M, Belza A, Stødkilde-Jørgensen H, et al. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: A 6-mo randomized intervention

study. *Am J Clin Nutr*. 2012;95(2):283-289.

218. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol*. 2008;48(6):993-999.

219. Jegatheesan P, De Bandt JP. Fructose and NAFLD: The multifaceted aspects of fructose metabolism. *Nutrients*. 2017;9(3).

220. Ter Horst KW, Serlie MJ. Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients*. 2017;9(9):1-20.

221. Bar-On H, Stein Y. Effect of glucose and fructose administration on lipid metabolism in the rat. *J Nutr*. 1968;94(1):95-105.

222. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. *Am J Clin Nutr*. 2007;85(3):910-918.

223. Gaccioli F, Lager S, Powell TL, Jansson T. Placental transport in response to altered maternal nutrition. *J Dev Orig Health Dis*. 2013;4(2):101-115.

224. Parrettini S, Caroli A, Torlone E. Nutrition and Metabolic Adaptations in Physiological and Complicated Pregnancy: Focus on Obesity and Gestational Diabetes. *Front Endocrinol (Lausanne)*. 2020;11:937.

225. Godfrey KM, Haugen G, Kiserud T, et al. Fetal liver blood flow distribution: Role in human developmental strategy to prioritize fat deposition versus brain development. *PLoS One*. 2012;7(8):e41759.

226. Pruis MGM, Lendvai Á, Bloks VW, et al. Maternal western diet primes non-alcoholic fatty liver disease in adult mouse offspring. *Acta Physiol*. 2014;210(1):215-227.

227. Wesolowski SR, El Kasmi KC, Jonscher KR, Friedman JE. Developmental origins of NAFLD: a womb with a clue. 2017.

228. Christians JK, Lennie KI, Wild LK, Garcha R. Effects of high-fat diets on fetal growth in

rodents: A systematic review. *Reprod Biol Endocrinol*. 2019;17(1):1-12.

229. Bianchi C, Taricco E, Cardellicchio M, et al. The role of obesity and gestational diabetes on placental size and fetal oxygenation. *Placenta*. 2021;103:59-63.

230. Kovo M, Zion-Saukhanov E, Schreiber L, et al. The Effect of Maternal Obesity on Pregnancy Outcome in Correlation with Placental Pathology. *Reprod Sci*. 2015;22(12):1643-1648.

231. Ouyang F, Parker M, Cerda S, et al. Placental weight mediates the effects of prenatal factors on fetal growth: The extent differs by preterm status. *Obesity*. 2013;21(3):609-620.

232. Wallace JM, Horgan GW, Bhattacharya S. Placental weight and efficiency in relation to maternal body mass index and the risk of pregnancy complications in women delivering singleton babies. *Placenta*. 2012;33(8):611-618.

233. Berglund SK, García-Valdés L, Torres-Espinola FJ, et al. Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: An observational cohort study (PREOBE). *BMC Public Health*. 2016;16(1):1-12.

234. Lager S, Samulesson A-M, Taylor PD, Poston L, Powell TL, Jansson T. Diet-induced obesity in mice reduces placental efficiency and inhibits placental mTOR signaling. *Physiol Rep*. 2014;2(2):e00242.

235. Woods L, Perez-Garcia V, Hemberger M. Regulation of Placental Development and Its Impact on Fetal Growth—New Insights From Mouse Models. *Front Endocrinol (Lausanne)*. 2018;9.

236. Biswas S, Ghosh SK. Gross morphological changes of placentas associated with intrauterine growth restriction of fetuses: A case control study. *Early Hum Dev*. 2008;84(6):357-362.

237. Spinillo A, Gardella B, Muscettola G, Cesari S, Fiandrino G, Tzialla C. The impact of placental massive perivillous fibrin deposition on neonatal outcome in pregnancies complicated by fetal growth restriction. *Placenta*. 2019;87:46-52.

238. Malti N, Merzouk H, Merzouk SA, et al. Oxidative stress and maternal obesity: Feto-placental unit interaction. *Placenta*. 2014;35(6):411-416.

239. Salati JA, Roberts VHJ, Schabel MC, et al. Maternal high-fat diet reversal improves placental hemodynamics in a nonhuman primate model of diet-induced obesity. *Int J Obes*. 2019;43(4):906-916.

240. Chow R, Wessels JM, Foster WG. Brain-derived neurotrophic factor (BDNF) expression and function in the mammalian reproductive Tract • Introduction • Methods • Results Circulating and reproductive fluid concentrations of BDNF Regulation of BDNF in the reproductive system BDNF in the female reproductive tract BDNF in the male reproductive tract Reproductive pathologies and BDNF • Conclusions and future directions. *Hum Reprod Update*. 2020;26(4):545-564.

241. Mayeur S, Silhol M, Moitrot E, et al. Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human. *Placenta*. 2010;31(9):785-791.

242. Jiang H, Liu Y, Zhang Y, Chen ZY. Association of plasma brain-derived neurotrophic factor and cardiovascular risk factors and prognosis in angina pectoris. *Biochem Biophys Res Commun*. 2011;415(1):99-103.

243. Fujita K, Tatsumi K, Kondoh E, et al. Differential expression and the anti-apoptotic effect of human placental neurotrophins and their receptors. *Placenta*. 2011;32(10):737-744.

244. Yamazaki M, Yamada H, Munetsuna E, et al. Excess maternal fructose consumption impairs hippocampal function in offspring via epigenetic modification of BDNF promoter. *FASEB J*. 2018;32(5):2549-2562.

245. Han Y, Luo Y, Sun J, et al. AMPK Signaling in the Dorsal Hippocampus Negatively Regulates Contextual Fear Memory Formation. *Neuropsychopharmacol 2016 417*. 2015;41(7):1849-1864.

246. Balogun KA, Cheema SK. The expression of neurotrophins is differentially regulated by omega-3 polyunsaturated fatty acids at weaning and postweaning in C57BL/6 mice cerebral cortex. *Neurochem Int*. 2014;66(1):33-42.

247. Fan C, Fu H, Dong H, Lu Y, Lu Y, Qi K. Maternal n-3 polyunsaturated fatty acid deprivation during pregnancy and lactation affects neurogenesis and apoptosis in adult offspring: associated with DNA methylation of brain-derived neurotrophic factor transcripts. *Nutr Res*. 2016;36(9):1013-1021.

248. Molteni R, Wu A, Vaynman S, Ying Z, Barnard RJ, Gómez-Pinilla F. Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience*. 2004;123(2):429-440.

249. Cavaliere G, Trinchese G, Penna E, et al. High-Fat Diet Induces Neuroinflammation and Mitochondrial Impairment in Mice Cerebral Cortex and Synaptic Fraction. *Front Cell Neurosci*. 2019;13.

250. Lee J, Duan W, Mattson MP. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem*. 2002;82(6):1367-1375.

251. Lindqvist A, Mohapel P, Bouter B, et al. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur J Neurol*. 2006;13(12):1385-1388.

252. Allen KM, Purves-Tyson TD, Fung SJ, Shannon Weickert C. The effect of adolescent testosterone on hippocampal BDNF and TrkB mRNA expression: Relationship with cell proliferation. *BMC Neurosci*. 2015;16(1):1-15.

253. Dieni S, Rees S. BDNF and TrkB protein expression is altered in the fetal hippocampus but not cerebellum after chronic prenatal compromise. *Exp Neurol*. 2005;192(2):265-273.

254. Baek JK, Heaton MB, Walker DW. Up-regulation of high-affinity neurotrophin receptor, trk B-like protein on Western blots of rat cortex after chronic ethanol treatment. *Mol Brain Res*. 1996;40(1):161-164.

255. Guidi S, Ciani E, Severi S, Contestabile A, Bartesaghi R. Postnatal neurogenesis in the dentate gyrus of the guinea pig. *Hippocampus*. 2005;15(3):285-301.

256. Yang P, Zhang J, Shi H, et al. Developmental profile of neurogenesis in prenatal human hippocampus: An immunohistochemical study. *Int J Dev Neurosci*. 2014;38:1-9.

257. Alves JM, Luo S, Chow T, Herting M, Xiang AH, Page KA. Sex differences in the association between prenatal exposure to maternal obesity and hippocampal volume in children. *Brain Behav*. 2020;10(2).

258. Erbas O, Erdogan MA, Khalilnezhad A, et al. Neurobehavioral effects of long-term maternal fructose intake in rat offspring. *Int J Dev Neurosci*. 2018;69:68-79.

259. Bordeleau M, Lacabanne C, Fernández De Cossío L, et al. Microglial and peripheral immune priming is partially sexually dimorphic in adolescent mouse offspring exposed to maternal high-fat diet. *J Neuroinflammation*. 2020;17(1):1-28.

260. Hester MS, Tulina N, Brown A, Barila G, Elovitz MA. Intrauterine inflammation reduces postnatal neurogenesis in the hippocampal subgranular zone and leads to accumulation of hilar ectopic granule cells. *Brain Res*. 2018;1685:51-59.

261. Sundborn G, Thornley S, Merriman TR, et al. Are Liquid Sugars Different from Solid Sugar in Their Ability to Cause Metabolic Syndrome? *Obesity*. 2019;27(6):879-887.

262. Mastrocola R, Ferrocino I, Liberto E, et al. Fructose liquid and solid formulations differently affect gut integrity, microbiota composition and related liver toxicity: a comparative in vivo study. *J Nutr Biochem*. 2018;55:185-199.

263. Bale TL. The placenta and neurodevelopment: Sex differences in prenatal vulnerability. *Dialogues Clin Neurosci*. 2016;18(4):459-464.

264. Tarrade A, Panchenko P, Junien C, Gabory A. Placental contribution to nutritional programming of health and diseases: Epigenetics and sexual dimorphism. *J Exp Biol*. 2015;218(1):50-58.

265. Sarr O, Mathers KE, Zhao L, et al. Western diet consumption through early life induces microvesicular hepatic steatosis in association with an altered metabolome in low birth weight Guinea pigs. *J Nutr Biochem*. 2019;67:219-233.

266. Garcés MF, Sanchez E, Torres-Sierra AL, et al. Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. *Clin Endocrinol (Oxf)*. 2014;81(1):141-151.

267. Briana DD, Malamitsi-Puchner A. Developmental origins of adult health and disease: The metabolic role of BDNF from early life to adulthood. *Metabolism*. 2018;81:45-51.

268. Nonomuraa T, Tsuchidaa A, Ono-Kishinoa M, Nakagawaa T, Taiji A' M, Noguchi H. *Brain-Derived Neurotrophic Factor Regulates Energy Expenditure Through the Central Nervous System in Obese Diabetic Mice*. Vol 2.; 2001.

APPENDICES

Appendix A:

Animal Use Protocol:

AUP Number: 2019-116

AUP Title: Hyperpolarized 13C MRI of Placental Metabolic Abnormalities Resulting from the Western Diet

Yearly Renewal Date: 02/01/2022

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2019-116 has been approved by the Animal Care Committee (ACC), and will be approved through to the above review date.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:

1) Animals used in this research project will be cared for in alignment with:

   a) Western's Senate MAPPs 7.12, 7.10, and 7.15 http://www.uwo.ca/univsec/policies_procedures/research.html

   b) University Council on Animal Care Policies and related Animal Care Committee procedures http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.html

2) As per UCAC's Animal Use Protocols Policy,

   a) this AUP accurately represents intended animal use;

   b) external approvals associated with this AUP, including permits and scientific/departmental peer approvals, are complete and accurate;

c) any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and

d) AUP form submissions - Annual Protocol Renewals and Full AUP Renewals - will be submitted and attended to within timeframes outlined by the ACC. http://uwo.ca/research/services/animalethics/animal_use_protocols.html

3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will

a) be made familiar with and have direct access to this AUP;

b) complete all required CCAC mandatory training (training@uwo.ca); and

c) be overseen by me to ensure appropriate care and use of animals.

4) As per MAPP 7.15,

a) Practice will align with approved AUP elements;

b) Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;

c) UCAC policies and related ACC procedures will be followed, including but not limited to:

i. Research Animal Procurement

ii. Animal Care and Use Records

iii. Sick Animal Response

iv. Continuing Care Visits

5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have completed in advance the appropriate

institutional OH&S training, facility-level training, and reviewed related (M)SDS Sheets, http://www.uwo.ca/hr/learning/required/index.html

Submitted by: Copeman, Laura

on behalf of the Animal Care Committee

University Council on Animal Care

Appendix B:

R Code for Fetal Statistical Analyses

```
install.packages("lme4")
install.packages("learnr")
install.packages("emmeans")
library(lme4)
library(lmerTest)
library(emmeans)

MRIdata <- read.csv("C:/Users/cciho/OneDrive/Desktop/MRIdata.csv")
MRIdata
fetal.v_model <- lmer(fetal.v~diet + (1|maternal_id), MRIdata)
placenta.v_model <- lmer(placenta.v~diet + (1|maternal_id), MRIdata)
TAT.bodyv_model <- lmer(TAT.bodyv~diet + (1|maternal_id), MRIdata)
brain.bodyv_model <- lmer(brain.bodyv~diet + (1|maternal_id), MRIdata)
liver.bodyv_model <- lmer(liver.bodyv~diet + (1|maternal_id), MRIdata)
pdff_model <- lmer(pdff~diet + (1|maternal_id), MRIdata)
emmeans(object=fetal.v_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=placenta.v_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=TAT.bodyv_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=brain.bodyv_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=liver.bodyv_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=pdff_model, pairwise ~ diet, adjust= "t-test")

animaldata <- read.csv("C:/Users/cciho/OneDrive/Desktop/animaldata.csv")
animaldata
fetal.w_model <- lmer(fetal.w~diet + (1|maternal_id), animaldata)
placenta.w_model <- lmer(placenta.w~diet + (1|maternal_id), animaldata)
fetal.placenta_model <- lmer(fetal.placenta~diet + (1|maternal_id), animaldata)
brain.bodyw_model <- lmer(brain.bodyw~diet + (1|maternal_id), animaldata)
liver.bodyw_model <- lmer(liver.bodyw~diet + (1|maternal_id), animaldata)
emmeans(object=fetal.w_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=placenta.w_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=fetal.placenta_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=brain.bodyw_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=liver.bodyw_model, pairwise ~ diet, adjust= "t-test")

placentaldata <- read.csv("C:/Users/cciho/OneDrive/Desktop/placentaldata.csv")
placentaldata
pathology_model <- lmer(pathology~diet + (1|maternal_id), placentaldata)
necrosis_model <- lmer(necrosis~diet + (1|maternal_id), animaldata)
fibrin_model <- lmer(fibrin~diet + (1|maternal_id), animaldata)
bdnf_model <- lmer(bdnf~diet + (1|maternal_id), animaldata)
emmeans(object=pathology_model, pairwise ~ diet, adjust= "t-test")
```

```
emmeans(object=necrosis_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=fibrin_model, pairwise ~ diet, adjust= "t-test")
emmeans(object=bdnf_model, pairwise ~ diet, adjust= "t-test")

bdnfdata <- read.csv("C:/Users/cciho/OneDrive/Desktop/bdnfdata.csv")
bdnfdata
dgarea_model <- lmer(dgarea~diet + (1|maternal_id), placentaldata)
ca1area_model <- lmer(ca1area~diet + (1|maternal_id), placentaldata)
ca3area_model <- lmer(ca3area~diet + (1|maternal_id), placentaldata)
tarea_model <- lmer(tarea~diet + (1|maternal_id), placentaldata)
emmeans(object=dgarea_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1area_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3area_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=tarea_model, pairwise ~ diet, adjust= "t.test")
dgod_model <- lmer(dgod~diet + (1|maternal_id), placentaldata)
ca1od_model <- lmer(ca1od~diet + (1|maternal_id), placentaldata)
ca3od_model <- lmer(ca3od~diet + (1|maternal_id), placentaldata)
tod_model <- lmer(tod~diet + (1|maternal_id), placentaldata)
emmeans(object=dgod_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1od_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3od_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=tod_model, pairwise ~ diet, adjust= "t.test")

cellcountdata <- read.csv("C:/Users/cciho/OneDrive/Desktop/cellcountdata.csv")
cellcountdata
dg_model <- lmer(dg~diet + (1|maternal_id), cellcountdata)
ca1_model <- lmer(ca1~diet + (1|maternal_id), cellcountdata)
ca3_model <- lmer(ca3~diet + (1|maternal_id), cellcountdata)
t_model <- lmer(t~diet + (1|maternal_id), cellcountdata)
emmeans(object=dg_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=t_model, pairwise ~ diet, adjust= "t.test")

Ki67data <- read.csv("C:/Users/cciho/OneDrive/Desktop/Ki67data.csv")
Ki67data
dg_model <- lmer(dg~diet + (1|maternal_id), Ki67data)
ca1_model <- lmer(ca1~diet + (1|maternal_id), Ki67data)
ca3_model <- lmer(ca3~diet + (1|maternal_id), Ki67data)
emmeans(object=dg_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3_model, pairwise ~ diet, adjust= "t.test")

Iba1data <- read.csv("C:/Users/cciho/OneDrive/Desktop/Iba1data.csv")
Iba1data
dg.cell.count_model <- lmer(dg.cell.count~diet + (1|maternal.id), Iba1data)
```

```
ca1.cell.count_model <- lmer(ca1.cell.count~diet + (1|maternal.id), Iba1data)
ca3.cell.count_model <- lmer(ca3.cell.count~diet + (1|maternal.id), Iba1data)
t.cell.count_model <- lmer(t.cell.count~diet + (1|maternal.id), Iba1data)
cc.cell.count_model <- lmer(cc.cell.count~diet + (1|maternal.id), Iba1data)
emmeans(object=dg.cell.count_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1.cell.count_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3.cell.count_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=t.cell.count_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=cc.cell.count_model, pairwise ~ diet, adjust= "t.test")
dg.body.size_model <- lmer(dg.body.size~diet + (1|maternal.id), Iba1data)
ca1.body.size_model <- lmer(ca1.body.size~diet + (1|maternal.id), Iba1data)
ca3.body.size_model <- lmer(ca3.body.size~diet + (1|maternal.id), Iba1data)
t.body.size_model <- lmer(t.body.size~diet + (1|maternal.id), Iba1data)
cc.body.size_model <- lmer(cc.body.size~diet + (1|maternal.id), Iba1data)
emmeans(object=dg.body.size_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1.body.size_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3.body.size_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=t.body.size_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=cc.body.size_model, pairwise ~ diet, adjust= "t.test")
dg.body.circularity_model <- lmer(dg.body.circularity~diet + (1|maternal.id), Iba1data)
ca1.body.circularity_model <- lmer(ca1.body.circularity~diet + (1|maternal.id), Iba1data)
ca3.body.circularity_model <- lmer(ca3.body.circularity~diet + (1|maternal.id), Iba1data)
t.body.circularity_model <- lmer(t.body.circularity~diet + (1|maternal.id), Iba1data)
cc.body.circularity_model <- lmer(cc.body.circularity~diet + (1|maternal.id), Iba1data)
emmeans(object=dg.body.circularity_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1.body.circularity_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3.body.circularity_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=t.body.circularity_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=cc.body.circularity_model, pairwise ~ diet, adjust= "t.test")
dg.body.process_model <- lmer(dg.body.process~diet + (1|maternal.id), Iba1data)
ca1.body.process_model <- lmer(ca1.body.process~diet + (1|maternal.id), Iba1data)
ca3.body.process_model <- lmer(ca3.body.process~diet + (1|maternal.id), Iba1data)
t.body.process_model <- lmer(t.body.process~diet + (1|maternal.id), Iba1data)
cc.body.process_model <- lmer(cc.body.process~diet + (1|maternal.id), Iba1data)
emmeans(object=dg.body.process_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca1.body.process_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=ca3.body.process_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=t.body.process_model, pairwise ~ diet, adjust= "t.test")
emmeans(object=cc.body.process_model, pairwise ~ diet, adjust= "t.test")
```

CURRICULUM VITAE

Name: Carlene Cihosky

Post-secondary Education and Degrees:
Western University
London, Ontario, Canada
2015-2019 B.MSc.

Western University
London, Ontario, Canada
2019-2021 M.Sc.

Honours and Awards:
Honour Roll
Western University
2016, 2017, 2018, 2019

Hugh B. Anderson Award
Western University, Department of Physiology and Pharmacology
2019

Obstetrics and Gynaecology Graduate Scholarship (OGGS)
Western University, Department of Obstetrics and Gynaecology
2019

19th Annual Paul Harding Research Day Oral Presentation Award
Western University, Department of Obstetrics and Gynaecology
2021

5th Annual Developmental Origins of Health and Disease (DOHaD) Canada Meeting Flash Talk Award
DOHaD Canada
2021

Related Work Experience:
Teaching Assistant
The University of Western Ontario
2019-2021

Publications:

Cihosky C, Regnault T, McKenzie C, Lee T, de Vrijer B, Richardson B. Imaging Technologies in Pregnancy and Advances in Pre-clinical Animal Models– a Review for the Future. *To be published (2021)*

Research Presentations:

Cihosky C, Nygard K, Kiser P, Richardson B, Regnault T. Impact of Life-Long Maternal Western Diet Consumption and Altered Fetoplacental Development. 5th Annual Developmental Origins of Health and Disease (DOHaD) Canada Webinar 2021, Virtual, May 31st – June 2nd, 2021.

Cihosky C, Nygard K, Kiser P, Richardson B, Regnault T. Impact of Maternal Western Diet on Placental Development, Fetal Hippocampal Neuroproliferation, and Neuroinflammation. 2021 London Health Research Day (LHRD), Virtual, May 11th, 2021.

Cihosky C, Nygard K, Kiser P, Richardson B, Regnault T. Understanding the Impact of Maternal Western Diet on Placental Development and Fetal Neurodevelopment. 19th Annual Paul Harding Research Day, Virtual, April 28th, 2021.

Cihosky C, Nygard K, Kiser P, Richardson B, Regnault T. Understanding the Link Between Maternal Western Diet and Ki67 Expression in the Fetal Hippocampus. 8th Annual Canadian National Perinatal Research Meeting (CNPRM), Virtual, February 8th-12th 2021.

Cihosky C, Nygard K, Richardson B, Regnault T. Understanding the Link Between Maternal Gestational Energy Status and Ki67 Expression in the Fetal Hippocampus.14th Annual Physiology and Pharmacology Research Day, Virtual, November 3rd, 2020.

Hashimoto T, Cihosky C, Nygard K, Richardson B, Regnault T. Western Diet Exposure and Neurodegenerative Changes of Alzheimer's Disease. 1st Annual Neuroscience Research Day, May 9th-10th 2019.

Cihosky C, Hashimoto T, Nygard K, Richardson B, Regnault T. Western Diet Exposure and Neurodegenerative Changes of Alzheimer's Disease. 4th Year Physiology and Pharmacology Thesis Day, April 2019.