Spring 6-2022

The Impact of Maternal Nutrition on the Development and Severity of Generalized Anxiety Disorders in Rodent and Non-Human Primate Studies

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The Impact of Maternal Nutrition on the Development and Severity of Generalized Anxiety Disorders in Rodent and Non-Human Primate Studies

by

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An undergraduate honors thesis submitted in partial fulfillment of the

requirements for the degree of

Bachelor of Science

in

University Honors

and

Public Health Studies: Pre-Clinical

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2022

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
CACFP	Child and Adult Care Food Program
CB	Clean bedding
CD11b	Cluster of differentiation molecule 11b
CHD	Control chow diet
CORT	Cortisol
Cort	Corticosterone
CRH/ Crh	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
DOHaD	Developmental Origins of Health and Disease
GABA	Gamma-Aminobutyric acid
GAD	Generalized anxiety disorder
GAD1	Glutamic acid decarboxylase 1
GR	Glucocorticoid receptor
HFD	High-fat diet
HPA	Hypothalamic-pituitary-adrenal axis
IL-1Ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IkBa	I-kappa-B-alpha
MB	Soiled bedding
MKP-1	Mitogen-activated protein kinase phosphatase-1
MnR	Median raphe nucleus
MR	Mineralocorticoid receptor
mRNA	Messenger RNA
NFkB	Nuclear factor kappa beta
Nr3c1	Glucocorticoid receptor

Nr3c2	Mineralocorticoid receptor
PFC	Prefrontal cortex
PND	Postnatal day
PVN	Paraventricular nucleus of the hypothalamus
rDR/ DR	Dorsal raphe nucleus
RNA	Ribonucleic acid
SERT	Serotonin transporter
SNAP	Supplemental Nutrition Assistance Program
TPH2	Tryptophan hydroxylase 2
TRP	Tryptophan
USV	Ultrasonic vocalizations
VMAT2	Vesicular monoamine transporter isoform 2
WIC	Special Supplemental Nutrition Program for Women, Infants, and Children
Ywhaz	14-3-3 protein zelta/delta
5-HT	Serotonin/ 5-hydroxytryptophan
5-HT1AR	Serotonin 1A receptor subtype

Abstract

Generalized anxiety has become more prevalent among children and adolescents in the United States which impacts their health and livelihood. Maternal nutrition during pregnancy is purported to play a role in cognitive development and mental health during childhood, adolescence, and into adulthood. The Developmental Origins of Health and Disease theory suggests maternal nutrition before and during pregnancy influences transgenerational susceptibility, onset, and severity of disease through epigenetic mechanisms that then impact the child's overall health, including cognitive development and mental health later in life. A literature review of rodents and non-human primate studies was conducted to explore the association between fetal exposure to a maternal high-fat diet and the development and severity of anxiety among offspring. Perturbations in the serotonergic neurotransmitter system were observed among offspring exposed to a maternal high-fat diet that altered the expression of Tryptophan hydroxylase 2, serotonin transporter, and serotonin 1A receptor subtype. In contrast to offspring of mothers fed a standard chow diet, those born to a mother fed a high-fat diet experienced early activation of the hypothalamic-pituitary-adrenal axis, impaired negative feedback mechanisms, increased expression of glucocorticoid receptors in the limbic system, and dysregulation of pro- and anti-inflammatory gene expression. These studies suggest that fetal exposure to a maternal high-fat diet increases the risk for the development of generalized anxiety disorders among children and adolescents. To break this transgenerational cycle of cognitive impairment, public health policies and interventions must be implemented to enhance maternal dietary patterns and improve the health of future generations.

Keywords: generalized anxiety disorder, maternal nutrition, maternal high-fat diet, rodent, non-human primate, developmental origins of health and disease, epigenetics, transgenerational health

Introduction

Epidemiological studies have identified anxiety disorders as one of the most prevalent mental health disorders in children and adolescents with generalized anxiety disorders (GAD) being the most common (Merikangas et al., 2009). A GAD is characterized by uncontrollable and diffuse worry that is excessive or unrealistic and persists for 6 months or longer (Racine et al., 2021). The rates of GAD among children and adolescents have increased over time from 5.5% in 2007 to 6.4% in 2011–2012 (Bistko et al., 2018). According to Racine et al. (2021), the COVID-19 pandemic has doubled the global prevalence of GAD from 11.6% in 2020 to 20.5% in 2021. This increased prevalence of GAD is especially prominent among children and adolescents who experienced significant life-changing disruptions. Social isolation, inadequate peer interaction, financial loss in the family, lack of social support such as teachers and coaches, school closures, and missed memorable events or opportunities are some of the life-changing disruptions that impacted their daily lives (Racine et al., 2021).

Other vulnerable groups also experience higher rates of GAD that may not become apparent until adulthood. For instance, children and adolescents of ethnic minorities have lower anxiety rates than non-Hispanic whites (American Psychological Association, 2017). However, Kessler et al. (2001) reported that the prevalence of GAD is higher among adult ethnic minorities than their non-minority peers due to systematic racism that perpetuates a cycle of inequity and disparity. Often, the impact of these inequities and disparities is not truly realized until much later in life, after an anxiety disorder has developed. This may explain, in part, the different rates of GAD among children, adolescents, and adult ethnic minorities. Passal et al. (2021) state that another

population vulnerable to mental health disorders and with higher rates of GAD are sexual minorities. GAD can develop in adolescence and continue into adulthood among transgender and gender diverse individuals because those with same-sex or both-sex attractions are often negatively impacted by structural and social stigma and discriminatory policies that reject and isolate this vulnerable group (Passal et al., 2021).

In the end, living with a GAD is a significant determinant of health and livelihood. Living with a GAD the decreases quality of life, contributes to the burden of disability, makes it challenging to learn and manage emotions, and can lead to depression and suicide (Wittchen, 2002; Merikangas et al., 2009). Hill et al. (2011) reported that a GAD is a risk marker for suicidal behaviors. Knox et al. (2004) recognized that suicide is “one of the leading causes of death in the world” and proposed that living with a GAD is a significant public health problem due to inadequate suicide prevention programs and a shortage of mental healthcare resources.

Numerous factors likely contribute to the increasing rates of anxiety disorders among adolescents and children. One proposed etiology is poor or inadequate maternal nutrition during fetal development. A relatively new field of research examines how maternal nutrition during gestation impacts the development of chronic disease and mental health disorders such as anxiety in offspring. This newer field of study is known as the Developmental Origins of Health and Disease (DOHaD). To lower the risk of conditions associated with the DOHaD, health-promoting interventions must be initiated before conception, maintained during pregnancy, and included in early postnatal life to reduce offspring susceptibility to and delay the onset and severity of disease among individuals later in life (Barker, 2008). In other words, the DOHaD theory suggests that

maternal nutrition before and during pregnancy affects not only the offspring's health during childhood but also the offspring's health during adulthood (Barker, 2008). Barker (2008) explains that certain diseases, including mental health disorders that develop during childhood or adulthood, may result from an excess or lack of "nutrition in the womb." During gestation, the fetus is highly "plastic" and adjusts to intrauterine environmental conditions impacted by the environment in which the mother resides. This tailoring process is where the "origins of lifelong health" lie (Barker, 2008).

The "first 1000 days" movement which refers to the time between conception and the second year of life, supports the concept of DOHaD because it recognizes that early fetal and infant development is a crucial period that influences neurodevelopment, mental health, and risk for chronic diseases (Schwarzenberg et al., 2018). Bekdash (2021) adds that epigenetics, the post-translational modification of genetic material, e.g. methylation of DNA, influences the interaction between nutrition and genes and subsequently brain health and neurologic diseases. For instance, the maternal diet must provide necessary and adequate amounts of micronutrients such as folate and choline to support the developing fetus. Folate and choline are methyl donors which play an essential role in DNA and histone (the proteins involved in DNA packaging) methylation; histone methylation regulates gene expression, and subsequently, offspring phenotype. Numerous studies have and continue to examine how maternal nutrition affects gene expression during early fetal development and later health and disease (Dauncey, 2012). Maternal nutrition and what is now referred to as "transgenerational nutrition" is crucial to explore and understand because it acknowledges how maternal nutrition and

the intrauterine environment can influence fetal and early infant development and the health of future generations.

In the United States, improving maternal nutrition to promote health and lower rates of disease is a significant public health priority. The 2020-2025 Dietary Guidelines for Americans recommend that pregnant and lactating women consume 2 ½ - 3 ½ cups of vegetables per day, 1 ½ - 2 ½ cups of fruit per day, 6-10 ounces of grains per day, 3 cups of dairy per day, 5-7 ounces of protein per day, and 24-36 grams of oil/fat per day depending on their caloric needs. However, in general, the current intake of vegetables, fruits, and dairy is low among pregnant or lactating mothers while fat intake, especially saturated fat intake, exceeds the recommended intake by about 75-77% (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020). This pattern of low intake of recommended food groups and nutrients is consistent among pregnant and lactating women and the general United States population. The pattern of undernutrition can be attributed at least in part to the mass production of highly- or ultra-processed food that are high in fat, sugar, and salt with lower amounts of essential nutrients (Nestle & Trueman, 2020). Therefore, in this review, a high-fat diet is a diet high in saturated fats and lower in nutrients derived from fruits and vegetables that represent a “typical Western diet.”

Another explanation for this pattern of lower nutrient intake is through the concept of “food apartheids” which is a new concept that is replacing “food deserts” (Lu, 2014). Lu (2014) defines food deserts as geographical areas with lower numbers of retail food outlets resulting in limited access to wholesome food. Food apartheids refer to how discrimination, racism, and other forms of oppression influence the availability of

food within a community. For example, Fleming-Milici et al. (2018) explained how food industries target vulnerable populations such as ethnic minorities, children, and adolescents through advertising, marketing, and zoning. Food corporations have the power to do this due to inadequate marketing regulations which lead to health disparities among vulnerable populations including pregnant women and their children (Fleming-Milici et al., 2018). Overall, the food system plays a significant role in food apartheids and influences food corporations. This is because the food system is a complex and interconnected process that involves the production, processing, distribution, and consumption of food (Sobal et al., 1998). Therefore, to improve maternal nutrition and health outcomes of offspring, understanding how consumption of a westernized eating pattern before and during pregnancy affects childhood and adult mental and physical health is important to study. With this information, evidence-based, effective interventions can be implemented and policies enacted to improve the health of future generations.

Objective

This literature review aims to summarize how maternal prenatal high-fat dietary patterns, similar to commonly consumed westernized dietary patterns, impact the development and severity of generalized anxiety disorders in early infant, childhood, and adolescent animal models. Because few studies have been performed or published exploring this relationship in humans, empirical research conducted with rodents and non-human primates will be described. Non-human primate studies are especially relevant to this thesis because of the similarities that exist with humans. This thesis will conclude with a discussion of the public health implications and suggest interventions

and policies that could mitigate the rising rates and severity of generalized anxiety disorders and its mental health consequences.

Methods

The databases used to select the research on animal models included *PubMed, PsycINFO, Health Source: Nursing/Academic edition, ScienceDirect, PLoS Journals, Clinical Psychology Review, American Journal of Public Health, JAMA Pediatrics, ProQuest, Frontiers, American Academy of Pediatrics, the Journal of Neuroscience,* and *Clinical Psychology Review*. These databases were identified using *Google scholar* and the Portland State University (PSU) library. In the PSU library, the subject guides of public health and psychology were used to locate relevant databases.

The process used to find the empirical research was based on specific keywords including generalized anxiety disorders, prenatal diet, child development, nutritional status, epigenetics, high-fat diet, rodents, primates, and maternal diet. The publications selected mentioned a high-fat maternal diet; animal studies; were published in English; discussed the interaction of nutrition and genes; empirical research; discussed the development of generalized anxiety; and focused on children and adolescents. In this literature review, generalized anxiety disorders was defined as uncontrollable and diffuse worry that is excessive or unrealistic and persists for 6 months or longer (Kessler et al., 2001). In addition, when analyzing generalized anxiety disorders, this literature review focuses on symptoms and behaviors characteristic of generalized anxiety disorders instead of diagnoses. Research that was excluded targeted a low-fat or high-protein diet; social or separation anxiety; human studies; articles written in foreign languages; non-empirical research, conducted experiments using adults, and did not

discuss the complex interaction between nutrition and genes. As part of this process, an evidence table, included in the Appendix, was created that describes all the studies identified and used in this review and the evidence collected.

Literature Review

Numerous studies have examined the impacts of early nutrition on the development of generalized anxiety disorders. However, this literature review focuses on four studies with two highlighting rodent studies, and two targeting non-human primate studies. The first is a non-human primate study by Sullivan et al. (2010) while the second is a non-human primate study by Thompson et al. (2017). The review of these two non-human primate studies is then followed by a review of the rodent studies which consists of research done by Abuaish et al. (2018) and Sasaki et al. (2014). Before analyzing the empirical research, a foundation of biology must be established to comprehensively understand metabolic processes and systems; therefore, this literature review will begin with a biology lesson on the serotonin system the hypothalamic-pituitary-adrenal (HPA) axis.

Biology

Serotonin System

Serotonin is a neurotransmitter that allows serotonergic neurons to communicate and modulate physiologic functions within the body as illustrated in **Figure 1**. According to Pourhamzeh et al. (2021), serotonin is synthesized when tryptophan (TRP), an essential amino acid, is converted to 5-hydroxytryptophan (5-HT) via tryptophan hydroxylase 2 (TPH2). TPH2 is the rate-limiting enzyme of this metabolic pathway

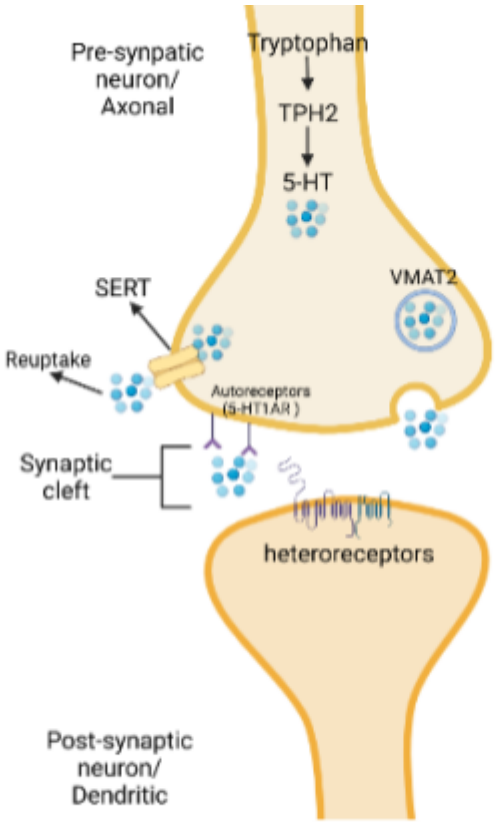

Figure 1. The Synthesis and Metabolism of Serotonin. The conversion of tryptophan by tryptophan hydroxylase 2 (TPH2) into serotonin (5-HT), a neurotransmitter. Serotonin is transported by vesicular monoamine transporters, isoform 2 (VMAT2) and secreted into the synaptic cleft to induce a positive and/or negative feedback loop. The binding of serotonin to the heteroreceptors on the Post-synaptic/Dendritic Neuron causes a positive feedback loop while binding to the autoreceptors or serotonin 1A receptor subtype (5-HT1AR) of the Pre-synaptic/Axonal Neuron leads to a negative feedback loop. A negative feedback loop causes the serotonin transporter (SERT) to reuptake serotonin into the Pre-synaptic Neuron for later use.
Source: Created using a BioRender.com; template adapted from Pourhamzeh, M., Moravej, F.G., Arabi, M. *et al.* The Roles of Serotonin in Neuropsychiatric Disorders. *Cell Mol Neurobiol* (2021). https://doi.org/10.1007/s10571-021-01064-9

which takes place in the axon and cell body of serotonergic neurons found in the midbrain, and the axon of serotonergic neurons found in the prefrontal cortex. Serotonin is then transported into intracellular vesicles by vesicular monoamine transporters, isoform 2 (VMAT2). Serotonin-containing vesicles are then transported to the axon terminal end of the neuron. When a serotonergic neuron is stimulated via an action potential, the intracellular vesicles translocate to and combine with the neuronal membrane and release serotonin into the synaptic cleft, the extracellular space between the axonal and dendritic regions of adjacent neurons. Serotonin released into the synaptic cleft binds to receptors on the post-synaptic membrane of the dendritic terminal end of the receiving neuron. Receptors on the post-synaptic neuron are called heteroreceptors while receptors on the pre-synaptic serotonergic neuron are called autoreceptors. Serotonin bind to receptors like two pieces of a puzzle to initiate intercellular communication through a second messenger system. In addition, serotonin that has been released into the synaptic cleft can bind to autoreceptors on the axonal terminal of the pre-synaptic neuron, such as the serotonin 1A receptor subtype (5-HT1AR), to initiate a negative feedback loop that activates serotonin transporter (SERT). The binding of serotonin to the SERT causes a structural conformational change that allows the SERT receptor protein to reuptake serotonin back into the serotonergic neuron; thereby lowering the concentration of serotonin in the synaptic cleft. Serotonin that enters the pre-synaptic neuron through this reuptake process is then encapsulated into intracellular vesicles to be reused in response to a subsequent action potential (Pourhamzeh et al., 2021).

Hornung et al. (2012) explain how the midbrain consists of raphe nuclei (**Figure 2)** where a majority of serotonin is synthesized; more specifically, the raphe nuclei are located along the brainstem from the midbrain to the spinal cord. The raphe nuclei is the primary location for the production of serotonin within the brain and the Central Nervous System because they contain numerous serotonergic neurons. The raphe nuclei are divided into rostral and caudal groups (**Figure 3)**, and the rostral raphe contains about 85% of all serotonin neurons in the brain. The rostral raphe is further divided into the caudal linear nucleus, dorsal raphe nucleus (rDR), and the median raphe nucleus which is clustered within the midbrain. While the caudal raphe consists of the raphe magnus nucleus found in the pons, and the raphe obscurus nucleus and raphe pallidus nucleus are located in the medulla.

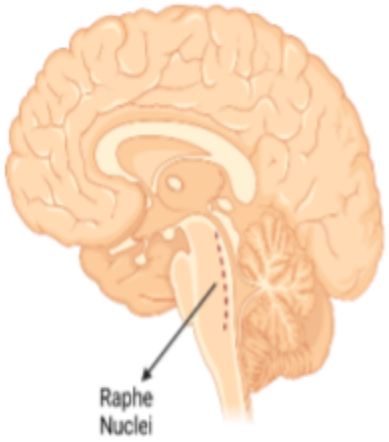

Figure 2. The Location of the Raphe Nuclei. The raphe nuclei are indicated by the dashed line.
Source: Created with BioRender.com; template adapted from Hornung, JP. Raphe Nuclei. In: Mai JK and Paxinos G, eds. The Human Nervous System. 3rd ed. New York: Elsevier; 2012.

The serotonergic neurons of the rostral and caudal raphe innervates and distributes serotonin throughout the Centeral Nervous System (**Figure 4**) (Hornung et al., 2012). For instance, Area 10 of the prefrontal cortex (PFC), known as Brodmann Area 10, interacts with the raphe nuclei due to serotonergic innervation (**Figure 4)** (Peng et al., 2018; Puig & Gulledge, 2011). This area of the prefrontal cortex has a variety of functions including risk and decision making, reward and conflict, pain, and working memory (Peng et al., 2018). In general, the prefrontal cortex also regulates

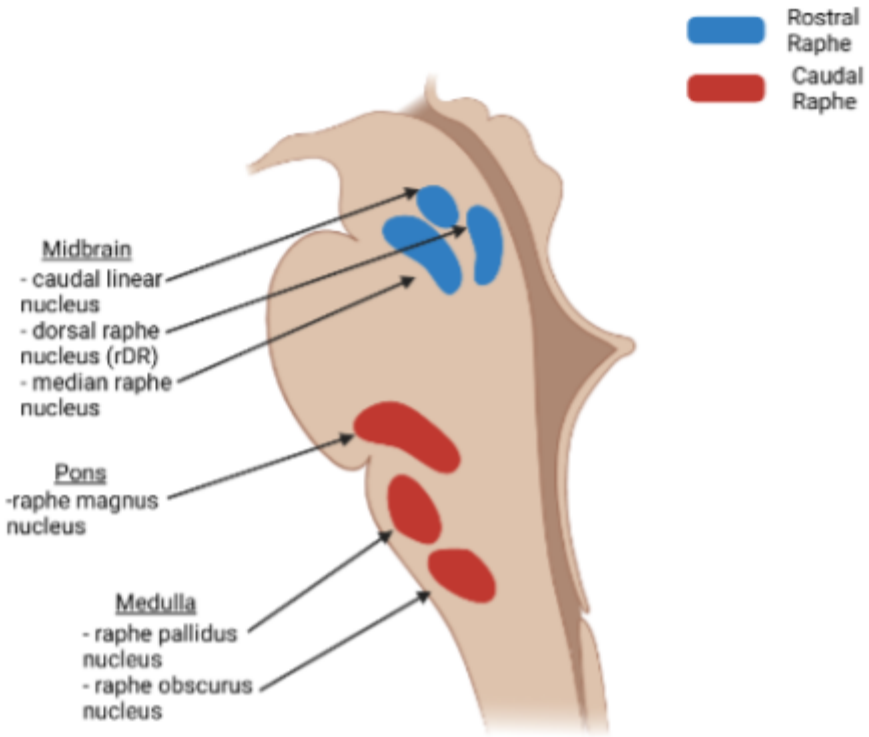

Figure 3. The Raphe Nuclei divided into Rostral and Caudal Raphe. The rostral and caudal raphe are further divided into their nucleus within the midbrain, pons, and medulla.
Source: Created with BioRender.com; template adapted from Carpenter, M.B. and Sutin, J. (1983). Human Neuroanatomy, 8th Ed. Baltimore. Williams & Wilkins. p. 330

behavioral inhibition which is associated with anxiety disorders (Puig & Gulledge, 2011). Overall, serotonin plays a crucial role in the brain by modulating mood, cognition, anxiety, learning, memory, reward processing, and sleep (Pourhamzeh et al., 2021). It also regulates energy homeostasis and is impacted by energy status or diet, explaining why maternal diet can impact the offspring's outcomes (Tecott, 2007; Hassanain & Levin, 2002).

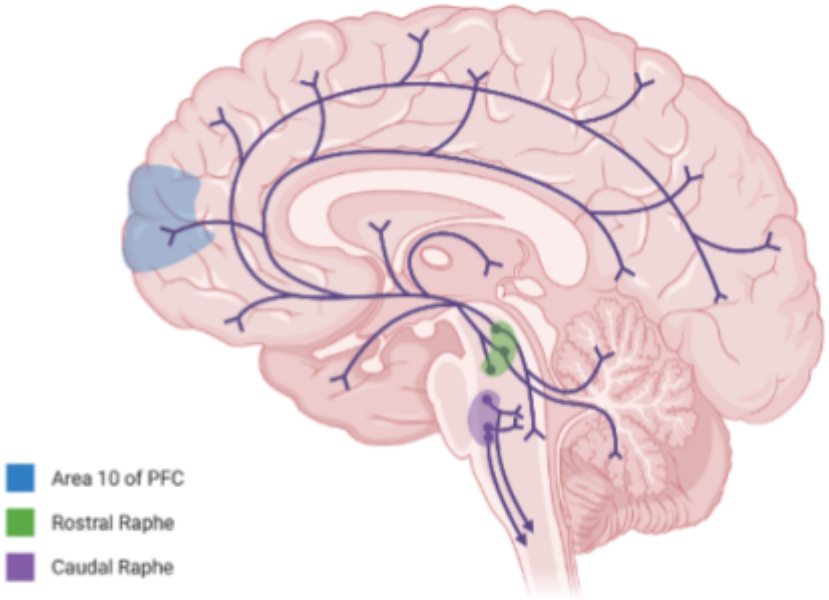

Figure 4. The Serotonin Pathways in the Brain. The innervation and distribution of serotonin from the rostral raphe and caudal raphe throughout the Central Nervous System. This includes Area 10 of the prefrontal cortex (PFC) due to serotonergic innervation of the raphe nuclei.
Source: Created by BioRender.com; template adapted from Pourhamzeh, M., Moravej, F.G., Arabi, M. *et al.* The Roles of Serotonin in Neuropsychiatric Disorders. *Cell Mol Neurobiol* (2021). https://doi.org/10.1007/s10571-021-01064-9

Hypothalamic-Pituitary-Adrenal (HPA) Axis

Cortisol, a steroid hormone, promotes and maintains the stress response system by increasing blood pressure, cardiac output, and glucose levels in the bloodstream (Chrousos, 2009). Chrousos (2009) described how cortisol is regulated and secreted as part of the hypothalamic-pituitary-adrenal (HPA) axis which is critical for behavioral regulation. The HPA axis encompasses the interactions between the hypothalamus, pituitary gland, and adrenal glands (**Figure 5**). The HPA axis is triggered when a stressful event activates the sympathetic nervous system which then secretes the hormones/ neurotransmitters epinephrine and norepinephrine. Higher concentrations of circulating norepinephrine signal the hypothalamus to secrete corticotropin-releasing hormone (CRH) which then communicates with the pituitary gland to release adrenocorticotropic hormone (ACTH) into the bloodstream. From the bloodstream, ACTH travels to and binds to receptors on the outer layer of the adrenal gland, the adrenal cortex. The binding of ACTH to adrenal cortex receptors triggers the adrenal cortex to secrete cortisol (CORT) which enhances and maintains the stress response by impacting metabolic responses such as heart rate; cortisol also is used to establish a negative feedback loop (Chrousos, 2009). This process is important to understand since the activation of the HPA axis and increased circulating cortisol concentrations have been associated with anxiety disorders in human and non-human primates.

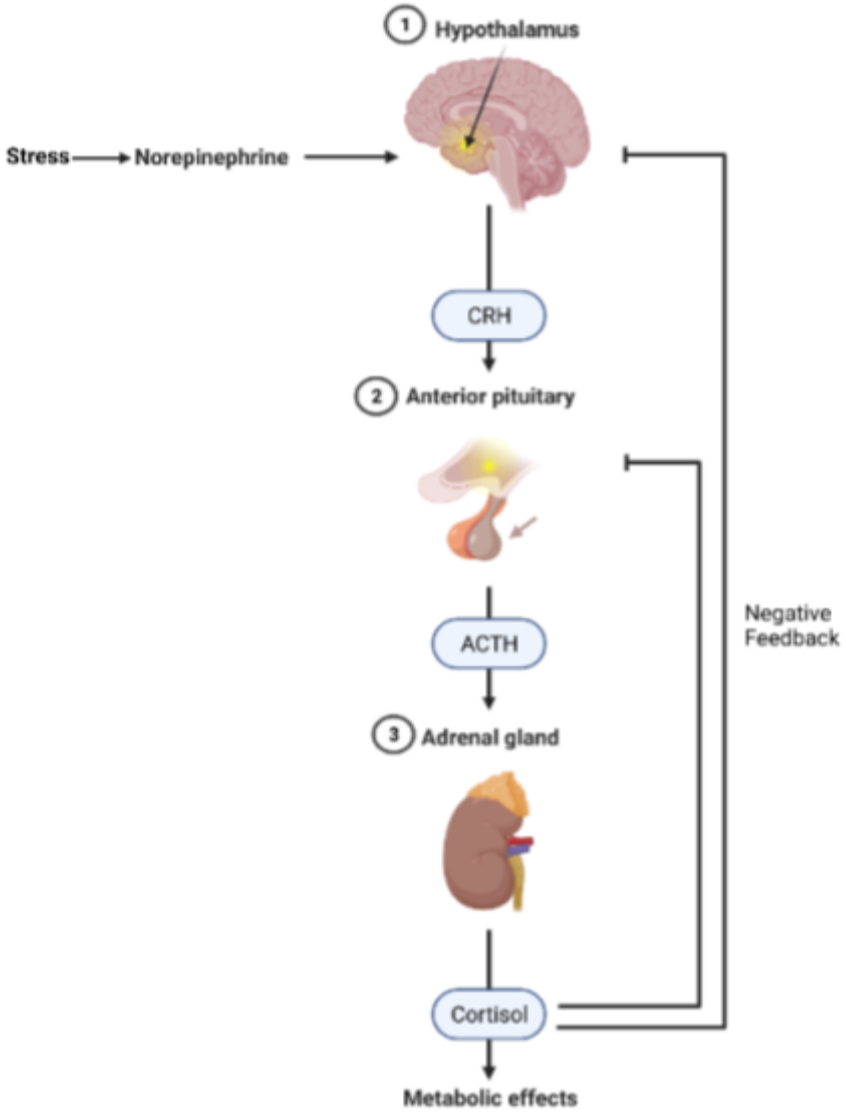

Figure 5. The Hypothalamic-Pituitary-Adrenal (HPA) Axis. The hypothalamic-pituitary-adrenal (HPA) axis is activated by norepinephrine, a hormone/ neurotransmitter that is secreted due to a stress response. This hormone then triggers the release of corticotropin-releasing hormone (CRH) from the hypothalamus, then adrenocorticotropic hormone (ACTH) from the pituitary gland, and finally, the adrenal gland produces cortisol. Cortisol then interacts with the pituitary gland and hypothalamus to activate a negative feedback loop if cortisol concentrations are high. Overall, the hypothalamic-pituitary-adrenal axis enhances and maintains the stress response through metabolic changes.
Source: Created with BioRender.com; template adapted from Camilla Maria Fontana PhD Student, University of Padova and Chrousos G. P. (2009). Stress and disorders of the stress system. Nature reviews. Endocrinology, 5(7), 374–381. https://doi.org/10.1038/nrendo.2009.106

Non-Human Primate Studies

Sullivan et al. (2010) suggest that fetal exposure to a maternal high-fat diet (HFD) alters the central serotonergic system which increases the risk of developing an anxiety disorder. The authors demonstrated that exposure to a maternal high-fat diet caused infant primates to exhibit increased anxiety behaviors when introduced to threatening novel objects. To explore this relationship, adult female Japanese macaques were fed a control (CTR) or high-fat diet for up to 4 years. The offspring of the macaques underwent behavioral testing on postnatal day 130 which included the human intruder and novel objects tests. These tests measured anxiety-like behaviors based on vocalizations, exploration, movement, and response to the human intruder while latency, movement, exploration, vocalizations, and other responses were scored in relation to exposure to novel objects.

On gestational day 130, five to six fetuses in each diet group were surgically removed and euthanized so that *in situ* hybridization experiments could be performed using fetal hypothalamus and midbrain tissue to identify the presence of Tryptophan hydroxylase 2 (TPH2), serotonin transporter (SERT), and serotonin 1A receptor subtype (5-HT1AR). Fluorescent immunohistochemistry was used to identify serotonin (5-HT)-ir projections in sections containing the arcuate nucleus of the hypothalamus and TPH2-ir in hypothalamic raphe-containing sections. The number of immunoreactive cells in the raphe-containing sections was determined. Through these *in vitro* experiments, the authors examined several components of the serotonin system including TPH2, SERT, and 5-HT1AR.

Compared to fetuses whose mothers were fed the control diet, fetuses exposed to a maternal high-fat diet had upregulated expression of 5-HT1AR in the rostral dorsal raphe (rDR) but not the caudal dorsal raphe (cDR). The expression of TPH2 mRNA in the rostral dorsal raphe was higher while 5-HT concentrations in cerebrospinal fluid (CSF) were lower in fetuses whose mothers were fed a high-fat diet compared to fetuses whose mothers were fed the control diet. There was no difference in SERT mRNA expression among fetuses of mothers fed the high-fat or control diets in either the rostral dorsal raphe or caudal dorsal raphe.

The rostral dorsal raphe contains the largest number of serotonergic neurons (Hornung et al., 2012) which helps explain why Sullivan et al. (2010) examined the midbrain and discovered a higher density of TPH2 and 5-HT1AR in the rostral dorsal raphe of offspring exposed to a maternal high-fat diet. According to Sullivan et al. (2010), when the synthesis of serotonin is decreased, it can lead to pathological disorders or behavioral disorders such as anxiety. This decrease in serotonin synthesis is due, at least in part, to increased expression of 5-HT1AR in the rostral raphe which results in higher rates of serotonin reuptake and prevents neuronal communication. Sullivan et al. (2010) also identified lower concentrations of 5-HT in CSF. In response to lower concentrations of serotonin in CSF, concentrations of THP2 increase to rectify the deficiency of serotonin, but 5-HT1AR prevents serotonin concentration from rising too high within the synaptic cleft. In this way, Sullivan et al. (2010) propose that a maternal diet high in fat causes epigenetic changes leading to changes in the central serotonergic system of offspring that lead to behavioral disorders such as anxiety disorders.

The research published by Thompson et al. (2017) is a continuation of the Sullivan et al. (2010) research to study the long-term impacts of fetal exposure to a maternal high-fat diet on behavior and brain development in juvenile non-human primates offspring. The non-human primate mothers were fed either a control diet or a high-fat diet for 1.2-8.5 years. The offspring of each maternal diet group were maintained on their mother's diet until weaning at about 8 months of age then they either continued to consume the same chow as their mother or were transitioned to the alternate diet; this created four groups of offspring: CTR/CTR, CTR/ HFD, HFD/HFD, and HFD/CTR. At 11 months of age, offspring in each group underwent behavioral testing which included the novel object and intruders tests. Behaviors were scored based on locomotive state, exploration, vocalizations, anxious or abnormal behaviors, responses to strangers, responses to objects, and other behaviors that are not typical of anxiety behaviors but were clearly defined. After each behavior test, blood samples were collected to measure plasma cortisol concentrations. Physical activity was measured before, during, and after the behavioral tests by attaching accelerometers to the collars worn by each juvenile offspring. Then, at 13 months of age and after weaning, hair samples were collected to measure cortisol concentrations as a marker of chronic stress. The offspring were then euthanized and *in situ* hybridization studies were performed on the midbrain samples. Each midbrain was divided into rostral, medial, and caudal sections, and Tryptophan hydroxylase 2 (TPH2), serotonin transporter (SERT), and serotonin 1A receptor subtype (5-HT1AR) were quantified. In addition, coronal sections from the prefrontal cortex (PFC) were collected to identify the presence of serotonin.

The authors reported that early infant nutritional interventions such as the consumption of the control diet after being exposed to a maternal high-fat were not sufficient to ameliorate anxiety-like behaviors in juvenile offspring. Maternal high-fat diet exposure increased active anxiety and caused abnormal exploratory behaviors among offspring as shown by reduced cage interactions during the behavioral tests which are indicative of anxiety. In contrast, a post-weaning high-fat diet independent of maternal diet exposure exacerbated anxiety-like behaviors such as stereotypy which is an extreme reaction to stress that is not indicative of standard anxiety behaviors. The high-fat weaning diet was also associated with decreased interactions during the novel object and cage tests, which has been linked to behavioral inhibition; behavioral inhibition has been associated with anxiety not only in animal studies but in human studies as well. Behavioral inhibition is also impacted by a maternal high-fat diet exposure as was noted in the abnormal cage interactions during the behavioral tests. Therefore, the connection between a post-weaning high-fat diet and fetal exposure to a maternal high-fat diet is strongly associated with different types of anxiety disorders such as social anxiety or generalized anxiety disorders. Overall, fetal exposure to a high-fat maternal diet accounted for most anxiety exhibited during the behavioral testing while a post-weaning high-fat diet was primarily associated with the development of stereotypy. Anxiety-like behaviors are further supported by higher cortisol concentrations found in the blood and hair samples and aberrations of the serotonin system identified in the midbrain and area 10 of the prefrontal cortex. Post-weaning high-fat diet consumption was associated with higher plasma cortisol concentrations, but prenatal exposure to a maternal high-fat diet was associated with dramatically

higher plasma cortisol concentrations. However, hair cortisol concentrations, a marker of chronic stress, were even higher when exposed to both a maternal high-fat diet and a post-weaning high-fat diet. Overall, increased cortisol concentrations in primates fed a post-weaning high-fat diet exacerbates anxiety behaviors. This increase in cortisol concentrations also explains how fetal exposure to a maternal high-fat diet and post-weaning high-fat diet can contribute to different types of anxiety disorders. For example, fetal exposure to a maternal high-fat diet is more likely to result in a generalized anxiety disorder while consumption of a post-weaning high-fat diet is more likely to result in social anxiety.

The serotonin system also played a role in the Thompson et al. (2017) study where TPH2 mRNA expression in the dorsal raphe decreased with exposure to a maternal high-fat diet regardless of a healthy post-weaning diet while a post-weaning high fat diet caused an increase in TPH2 mRNA expression in the median raphe. The authors measured this rate-limiting enzyme in the cell bodies of the serotonergic neurons found in the dorsal raphe and median raphe instead of the axonal terminal as was done in the Sullivan et al. (2010) study. Therefore, a reduction of TPH2 mRNA expression suppresses the ability to synthesize serotonin (**Figure 1**); these results were consistent with rodent studies that showed increased anxiety-like behaviors. Thompson et al. (2017) explain how the dorsal raphe and median raphe also impact metabolism and behavior since their serotonergic neurons innervate and interact with areas of the brain that impact anxiety disorders such as the amygdala, the ventral prefrontal cortex, and the medial prefrontal cortex.

The authors also observed that a post-weaning high-fat diet caused a decreased serotonin immunoreactivity in area 10 of the prefrontal cortex. This lack of immunoreactivity in area 10 of the prefrontal cortex explains why Thompson et al. (2017) observed behavioral inhibition in offspring exposed to a post-weaning high-fat diet during the behavioral tests. This is because the prefrontal cortex regulates behavioral inhibition; as a result, consumption of a high-fat diet by non-human primates during childhood can lead to anxiety disorders such as social anxiety. Decreased serotonergic immunoreactivity may also lead to increased stereotypy since other non-human primate studies have shown impairment in the prefrontal cortex leading to increased stereotypy and the development of anxiety-like behaviors. Fetal exposure to a maternal high-fat diet exposure can also impact this area of the prefrontal cortex but through a more indirect mechanism. For instance, area 10 of the prefrontal cortex is connected to the dopamine system because it contains tyrosine hydroxylase and dopamine receptor 1 and 2 proteins. Tyrosine hydroxylase is another rate-limiting enzyme like tryptophan hydroxylase 2 that converts tyrosine, an essential amino acid, into dopamine (Daubner et al., 2011). However, fetal exposure to a maternal high-fat diet reduces the immunoreactivity of these enzymes and receptors within this area, leading to anxiety-like behaviors. The serotonin system also modulates the dopamine system with both playing significant roles in regulating anxiety.

The Thompson et al. (2017) study also proposes that these behavioral and serotonergic impairments induced by a high-fat diet are because of pro-inflammatory factors such as inflammatory cytokines. Inflammation can have detrimental effects on neurodevelopment such as degeneration of serotonergic neurons that disrupt the

serotonin system. Overall, these results reveal that in comparison to the Sullivan et al. (2010) study, offspring exposed to a maternal high-fat diet have a higher risk of anxiety that continues throughout the juvenile period with a post-weaning high-fat diet exacerbating these anxiety-like behaviors and responses.

Rodent Studies

Abuaish et al. (2018) examined the impacts of perinatal high-fat diet (HFD) exposure on the development of the hypothalamic-pituitary-adrenal (HPA) axis in neonatal rodents during and after the hyporesponsiveness period. The perinatal high-fat diet exposure spanned from maternal pregestation, through gestation, into the lactation period, and throughout the stress hyporesponsive period of the offspring. This hyporesponsive period is unique to neonatal rodents and begins at postnatal day (PND) 2 and ends on PND14. It is a period in which animals are not responsive to stimuli or stressors, yet the HPA axis has been shown to be activated even during this period due to maternal deprivation, ether exposure, and endotoxin injections. The authors theorize that a perinatal high-fat diet can cause early activation of the HPA axis during the hyporesponsive period which can intensify the response to stress in neonates. Overall, this study further solidifies and expands on the research done by Thompson et al. (2017) by examining the HPA axis in more detail and its impact on the development of anxiety disorders.

The Abuaish et al. (2018) study began with adult female rats maintained on either a high-fat diet or a control chow diet (CHD). Food intake and weight were measured before the adult female rats were mated; once they conceived, the dams (pregnant or parent females) and their offspring were weighed and received nose-to-anal length measurements. There were three cohorts with each undergoing a specific type of test. Cohort 1 consisted of 11 litters from mothers fed a standard chow diet and 9 litters from mothers fed a high-fat diet who were then tested on PND7 and PND13. This test contained two operant conditioning chambers that were set up with

either clean bedding (CB) or soiled bedding (MB) from a male sexual interaction. On PND7 and PND13, Cohort 1 completed an isolating test procedure in either the chamber with clean bedding or the chamber with soiled bedding. The offspring in the chamber with soiled bedding were then euthanized to collect blood and tissue from the brain. This process was similar for PND7 and PND13 except for PND7 ultrasonic vocalizations (USV) and behaviors were also recorded. Cohort 2 had 7 litters from a mother who consumed a control chow diet and 8 litters from a mother who consumed a high-fat diet that were then used to obtain tissues for basal and baseline measures on PND7 of the isolating test. Cohort 3 had 6 pregnant adult female Long Evans rats (7 week) on a control chow diet and 8 adut females on a high-fat diet to assess HPA axis reactivity during gestation. To assess reactivity, cohort three dams underwent a 20-minute restrain challenge on gestation day 20 with blood samples collected before and after the stress reactivity test. Maternal behavior was also recorded which included total licking, total nursing, dam away from the nest, nesting, total care of offspring, and self-directed behaviors. Blood samples collected during the restraint challenge and blood samples collected from the soiled bedding exposure in offspring were used to measure corticosterone (Cort) and adrenocorticotropic hormone (ACTH) concentrations. Corticosterone is a glucocorticoid that is secreted by the adrenal gland similar to cortisol except rodents are unable to produce cortisol (Sheng et al., 2021). Lastly, the brains of offspring collected during euthanization on PND7 and PND 13 were sectioned into the paraventricular nucleus of the hypothalamus (PVN) and the ventral hippocampus. Five genes within these areas were analyzed including 14-3-3 protein zelta/delta (Ywhaz),

corticotropin-releasing hormone (Crh), glucocorticoid receptor (Nr3c1), mineralocorticoid receptor (Nr3c2), and glutamic acid decarboxylase 1 (Gad1).

During the analysis, the authors observed that neonates born to a dam who consumed a high-fat diet during gestation experienced early activation of the HPA axis during the hyporesponsive period (PND7) due to alterations in corticosterone concentrations, ultrasonic vocalizations, Crh transcript levels in the the paraventricular nucleus of the hypothalamus, and glucocorticoid receptor expression. On PND7, the offspring whose dams were exposed to a high-fat diet had higher corticosterone levels after the soiled bedding exposure compared to offspring exposed to a control chow diet. This increase in corticosterone also occurred in high-fat diet dams during the maternal tests. In contrast, ultrasonic vocalizations decreased among high-fat diet-exposed offspring during this hyporesponsive period while Crh transcript levels increased. The outcome of Crh and ultrasonic vocalizations is consistent since Crh regulates ultrasonic vocalization production in an inverted U-shaped relationship. Therefore, if Crh transcript levels are significantly increasing, ultrasonic vocalizations decrease. There was also a decrease in glucocorticoid receptor expression among high-fat diet offspring which has been known to negatively influence Crh expression. Overall, this increase in Crh commonly occurs in response to early postnatal stress which in this case is a high-fat diet that leads to early activation of the HPA axis during the hyporesponsive period.

In PND13, this early activation brought on by a maternal high-fat diet exposure was amplified due to increased ACTH levels, increased ultrasonic vocalizations, decreased exploration, increased immobility, reduced glucocorticoid receptor transcript abundance in the ventral hippocampus, and increased Gad1 transcript abundance in

the ventral hippocampus. After the hyporesponsive period (PND13), offspring exposed to a maternal high-fat diet showed only increased ACTH levels and no change in corticosterone levels in response to the soiled bedding exposure which continued even after the test ended. The isolation test also showed increased ultrasonic vocalizations, decreased exploration, and increased immobility among offspring exposed to a maternal high-fat diet regardless if there was a clean bedding or soiled bedding exposure; these outcomes have been associated in other rodent studies as an index of anxiety-like behaviors.

The perturbations in ACTH levels and anxiety-like behaviors indicate an alteration in the negative feedback of the HPA axis. The presence of glucocorticoid receptors can explain this disturbance in the negative feedback of the HPA axis. Glucocorticoid receptors regulate the negative feedback of the HPA axis within the paraventricular nucleus of the hypothalamus and ventral hippocampus (Herman et al., 2016). However, on PND13, offspring exposed to a maternal high-fat diet showed a lower glucocorticoid receptor transcript abundance in the ventral hippocampus. The hippocampus indirectly inhibits the HPA axis due to excitatory glutamatergic neurons that extend from the hippocampus to inhibitory regions surrounding the paraventricular nucleus of the hypothalamus (Gunn et al., 2015). In contrast, offspring exposed to a maternal high-fat diet during the emergence from the hyporesponsive period had an increase in Gad1 transcript abundance in the ventral hippocampus. According to Dent et al. (2007), Gad1 is a rate-limiting factor needed to synthesize gamma-Aminobutyric acid (GABA) and determines the presence of GABAergic neurons. These neurons innervate the hippocampus and regulate the excitatory output from the hippocampus.

However, an increase in GAD1 inhibits the hippocampus which then inhibits the excitatory output of the GABAergic neurons that control the negative feedback of the HPA axis (Dent et al., 2007). This alteration in the negative feedback of the HPA axis plays a significant role in the onset of anxiety (Faravelli et al., 2012). More specifically, this inhibition leads to persistent activation of the HPA axis. Abuaish et al. (2018) observed that a high-fat diet acts as a stressor during gestation for the mother and the offspringl. As a result, the maternal HPA axis can be impaired due to the consumption of a high-fat diet which then programs the stress response of the offspring. Overall, Abuaish et al. (2018) suggest that perinatal high-fat diet exposure can increase stress-related behaviors due to early and persistent activation of the HPA axis which has been associated with generalized anxiety disorders (Faravelli et al., 2012).

In the Sasaki et al. (2104) study, the authors used a rodent model to examine the impact of maternal high-fat diet exposure during gestation on several measures of anxiety behavior and gene expression in offspring during adolescence. The study consisted of adult male and female Long Evans rats (7 weeks old) with the female rats provided either a high-fat diet (HFD) or a control chow diet (CHD). The dams were fed their assigned diets 4 weeks before mating and during gestation and lactation. All offspring, regardless of maternal diet exposure, were placed on a control chow diet upon weaning on PND21, and body weight measurements were taken between PND35 and PD45.

Two groups of offspring took part in different behavioral tests such as the Light-Dark transition task, the Elevated Plus Maze task, and the Open Field task. The first group (Group 1) consisted of HFD females (n = 19); HFD males (n = 13); CHD

females (n = 19); and CHD males (n = 19). This first group was used to conduct the Light-Dark transition task and the Elevated Plus Maze task. The Light-Dark transition task measured the duration and frequency in which the rats entered an opaque white Plexiglas box (light zone) connected to an opaque black box (dark zone). During the Elevated Plus Maze task, rodents were placed in the center of the maze, and their movements into predefined zones were measured. The predefined zones include the center zone, two open arm zones attached to the center zone, and two closed arm zones attached to the center zone with the entire maze elevated above the floor. The open and closed arms were further divided into proximal and distal zones, and the amount of time and frequency of entries into each zone were calculated and compared.

The second group of offspring (Group 2) had HFD females (n = 13); HFD males (n = 7); CHD females (n = 13); and CHD males (n = 13) that underwent the Open Field task in a white opaque square box. The box also had predefined zones including the center zone, the edge zone along the wall of the box, and the corner zone. Each offspring's movements were tracked to calculate the number of entries and time spent in each predefined zone.

After the behavioral tests, another group of offspring (Group 3) consisted of 6 offspring per sex and per diet group that were decapitated to collect the brains for gene expression analysis. The whole hippocampus and amygdala were dissected, and RNA was extracted from both the adolescent high-fat diet-exposed and control chow diet-exposed offspring. Gene expression analysis of eight transcripts using quantitative reverse transcriptase-polymerase chain reaction (PCR) was carried out using hippocampal and amygdala samples. The relative abundance of transcripts for

glucocorticoid receptor (GR), mineralocorticoid receptor (MR), nuclear factor kappa beta (NFkB), interleukin-6 (IL-6), cluster of differentiation molecule 11b (CD11b), I-kappa-B-alpha (IkBa), mitogen-activated protein kinase phosphatase-1 (MKP-1), and interleukin-1 receptor antagonist (IL-1Ra) was determined.

The authors reported that during the Elevated Plus Maze task high-fat diet-exposed adolescent rodents explored the open arms more frequently than control chow diet-exposed offspring, suggesting decreased anxiety-like behaviors. In the Open Field task, the frequency of entries into the center zone occurred more often among high-fat diet-exposed offspring which indicates decreased anxiety-like behaviors. The Light-Dark transition task was the only behavioral test that suggested high-fat diet-exposed offspring exhibited increased anxiety-like behaviors due to increased time spent in the lighted portion of the box.

These results from the behavioral tests contrast with other previous studies that have identified that offspring exposed to a maternal high-fat diet are more likely to develop anxiety into adulthood (Sasaki et al., 2013; Jacobson-Pick & Richter-Levin, 2010; Jacobson-Pick et al., 2011; McCormick and Green, 2013). These studies have also recognized that behaviors characteristic of anxiety in adulthood are consistent with impulsive/risk-taking exploratory behaviors in adolescent rodents. For example, one study identified that adolescent rodents spent more time in the center of the Open Field and the lighted portion of the Light–Dark transition box when put through an anxiety-producing experience that is known to increase anxiety behavior in adulthood (Colorado et al., 2006). It has also been suggested that higher exploration in the open arms of the Elevated Plus Maze is indicative of impulsive behavior (Almeida et al.,

1996). Animal and human studies have also recognized a difference in stress response and reactivity between adolescents and adults that may explain the difference in symptoms of anxiety-like behaviors (Pine et al., 1998; Stein et al., 2001; Bostic et al., 2005). Impulsive behavior in adolescents now becomes synonymous with anxiety behaviors in adults. Another possible reason for the difference in anxiety-like behaviors could be due to a maternal high-fat diet exposure advancing the onset of puberty, especially in females. According to Sasaki et al. (2014), the higher amounts of adipose tissue from a high-fat diet exposure can signal estrogen production. Estrogen plays a role in anxiety and the response to stress which may explain how the early onset of puberty and the variation of estrogen influence impulsive anxiety behaviors in offspring exposed to a maternal high-fat diet. In the Thompson et al. (2017) study, the authors also observed a deviation of behaviors typical of anxiety among juvenile non-human primates. This led to the creation of more inclusive anxiety behaviors that are more indicative of anxiety-like behaviors.

During the gene expression analysis, a perinatal high-fat diet exposure was associated with increased glucocorticoid receptor expression within the hippocampus, especially among female offspring. While the expression of NFkB and IL-6 pro-inflammatory genes and IkBa and MKP-1 anti-inflammatory genes were upregulated in the hippocampus of offspring exposed to a maternal high-fat diet. There was also a decreased expression of NFkB pro-inflammatory gene and IL-1Ra anti-inflammatory gene expression in the amygdala among high-fat diet-exposed offspring.

Glucocorticoid receptors are corticosteroid receptors found in the limbic regions of the hippocampus and amygdala and facilitates the response of the HPA axis by

regulating circulating corticosterone levels during stress response (Timmermans et al., 2019). This means that increased concentrations of glucocorticoid receptors in the hippocampus inhibit the HPA axis while an increase in glucocorticoid receptor concentrations in the amygdala enhances the HPA axis (Groeneweg et al., 2011). Therefore, the observed decrease in anxiety-like behavior among high-fat diet-exposed adolescent rodents in this study is consistent with the increased glucocorticoid receptor concentrations in the hippocampus that inhibit the HPA axis. These results further indicate a developmental shift between adolescence into adulthood due to the impact that a high-fat diet has on limbic regions of the hippocampus and amygdala which are linked to HPA axis regulation and determine anxiety behaviors.

Corticosteroid receptors influence inflammatory processes in the hippocampus and amygdala (Groeneweg et al., 2011). Studies have identified that chronic stress increases glucocorticoid receptor expression to enhance the inflammatory response, elevating the expression of NFkB a pro-inflammatory gene (Sorrells et al., 2009). The increased expression of glucocorticoid receptor was consistent with the increased expression of NFkB and IL-6 pro-inflammatory genes in the hippocampus of offspring exposed to a maternal high-fat diet. However, the anti-inflammatory genes of IkBa and MKP-1 were also upregulated in the hippocampus while in the amygdala there was a decreased expression of NFkB pro-inflammatory gene and IL-1Ra anti-inflammatory gene expression. This suggests that anti-inflammatory genes respond to pro-inflammatory genes in a direct relationship. These results also indicate a dysregulation of pro- and anti-inflammatory genes due to a high-fat diet exposure. Overall, the interaction between glucocorticoid receptors and inflammatory genes

reveals possible brain region-specific effects of perinatal high-fat diet exposure that suggest adolescent anxiety behavior is determined by changes in hippocampal glucocorticoid receptors and inflammatory genes. Sasaki et al. (2104) also observed that body weight was similar between the high-fat diet-exposed and control chow diet-exposed offspring, suggesting the impulsive behaviors and changes in the pro- and ant-inflammatory genes are due to perinatal high-fat diet exposure and not to differences in body weight.

Overall, the Sasaki et al. (2014) study emphasized the connection between the HPA axis and the limbic system by acknowledging how corticosteroid receptors in the limbic regions of the hippocampus and amygdala regulate circulating corticosterone concentrations during a stress response. It also expands the knowledge of how corticosteroid receptors in the limbic regions influence inflammatory processes which support the hypothesis proposed by Thompson et al. (2017) that the effects of a maternal high-fat diet exposure are due to pro-inflammatory factors. Pro-inflammatory factors are detrimental to neurodevelopment because they cause degeneration of serotonergic neurons that disrupt the serotonin system. Although this study showed a decrease in anxiety behaviors among adolescent rats exposed to a high-fat diet in utero, it also recognized that the developmental shift from adolescence to adulthood is an additional period for developmental reprogramming that may determine mental health trajectories.

Public Health Implications

Overall, this research is relevant and applicable to many populations because it recognizes how maternal nutrition impacts the health of offspring. However, we must first acknowledge that social, economic, and environmental factors influence maternal nutrition which then affects maternal health and offspring health and their risk for diseases inlcuding generalized anxiety disorders. Transgenerational nutrition can alter gene expression and subsequently the health of the child and later their health as an adult. This is why it is important to address environmental and social factors that impact the health of the mother and their children as well.

Maternal, child, and adult health outcomes are a priority among public health officials and primary care providers who strive to prevent or eliminate chronic diseases including mental health disorders to promote good health and wellness. The relationship between maternal nutrition and child and adult health and disease is also relevant to health educators, obstetricians, family practitioners, and nurse midwives who inform future parents about the importance of reproductive health. Parents and future parents will also find this research valuable because they want to optimize the health of their children and grandchildren. Therefore, the health of future generations depends on how society and the social, economic, and environment supports expecting mothers and promotes reproductive health for current and future generations.

To public health officials, this research can be beneficial in implementing future policies or interventions that strive to improve the health of mothers and children. Programs and organizations can use this literature review to create policies and receive more financial assistance such as the Special Supplemental Nutrition Program for

Women, Infants, and Children (WIC); Supplemental Nutrition Assistance Program (SNAP); Child and Adult Care Food Program (CACFP); Maternal, Infant, and Early Childhood Home Visiting Program; and Baby-Friendly Hospital Initiative (Schwarzenberg et al., 2018). Pediatricians and other health care providers can also use this knowledge to inform parents better and provide resources (Schwarzenberg et al., 2018). This research is especially important to dissuade the government who has continued to “undermine women's health, roll back women's rights, and defund programs and institutions that provide support for women” (Waxman, 2012). Therefore, this research will encourage the government and the community to play a more prominent role in implementing policies and supporting organizations and programs that improve the health of mothers and their children.

Policies or interventions that could reduce the severity and development of generalized anxiety disorders due to exposure to a high-fat maternal diet include policies or interventions that target the individual, interpersonal, organizational, community, and policy levels. At the individual level, breastfeeding promotion programs can be established to educate and inform mothers about breastfeeding throughout pregnancy and postnatal care (CDC, 2013). Breastfeeding is beneficial because the mother provides all the necessary nutrients, fat, and other minerals that the infant needs for healthy growth and development (Giugliani et al., 2015). It is also an accessible source of food that can prevent health disparities among low-income populations and ethnic minorities (Louis-Jacques et al., 2017).

A possible intervention at the interpersonal level is the promotion and access to support groups for nursing mothers to encourage and support mothers while they are

breastfeeding (CDC, 2013). Another interpersonal intervention is the implementation of Centering Pregnancy programs which incorporate group maternity care that provides health assessment, education, and a support group of mothers (Tanner-Smith et el., 2013).

At the organizational level, numerous interventions can be implemented such as workplace support for breastfeeding, school nutrition standards, universal school meal programs, and school-based nutrition education programs. These interventions will ensure that schools meet the nutritional needs of a growing child and promote healthy eating behaviors while a supportive work environment will allow mothers to continue breastfeeding.

The community level plays an integral role in the mother's health and her offspring because this is where people are born, grow, work, play, and live. According to Gittelsohn et al. (2017), implementating competitive pricing for healthy foods in schools, worksites, grocery stores, and other food retailers and businesses is an effective community intervention. It increases the cost of non-nutritious food and decreases the cost of nutritious foods so that individuals in the community have access to nutrient-dense food. Other community interventions include establishing Early Head Start programs and group-based parenting programs; these programs provide family support and parent education (Furlong et al., 2012).

Lastly, at the policy level, some possible policies that should be implemented include mandatory paid paternity and maternity leave and living wages that are based on the family's needs (Isaacs et al., 2017; Luce, 2017). These policies will provide mothers with a secure income, so they can support their and their children's nutritional

needs. Another recommended policy is to modify the guidelines of the Farm Bill to support more production of fruits and vegetables and broaden distribution (Hodgson, 2012). This will ensure communities, especially mothers and their children, have access to a variety of food that can meet their nutritional needs. Overall, each intervention overlaps and ensures that mothers and future mothers recieve the necessary nutrition from their environment and society to lower the risk of developing generalized anxiety disorders in their children, grandchildren, and great-grandchildren.

In return, these interventions can save money for society, healthcare, and the individual. Wittchen (2002) recognizes that the annual cost of anxiety disorder was $42-$47 billion in 1990; $23 billion contributed to non-psychiatric medical treatment while $13 billion was used in psychiatric treatment, and over $4 billion in indirect workplace costs (Wittchen, 2002). These costs will continue to increase as more populations become diagnosed with a generalized anxiety disorder. Therefore, we must invest in the health of our children because they are the future.

Conclusion

In conclusion, this literature review highlights the association between fetal exposure to a maternal high-fat diet while in utero and the development and severity of generalized anxiety disorders as explored in early infancy, childhood, and adolescent rodent and non-human primate models. This thesis also describes how diet, a marker of environmental exposure, interacts with genes to influence neurodevelopment and the likelihood of developing generalized anxiety. These epigenetic changes can create a downstream effect that alters the function of metabolic processes and systems that regulate behavior. As summarized in both the Sullivan et al. (2010) and Thompson et al. (2017) studies, differences in expression of Tryptophan hydroxylase 2 (TPH2), serotonin transporter (SERT), and serotonin 1A receptor subtype (5-HT1AR) altered serotonin concentrations among offspring exposed to a high-fat diet in utero. The Thompson et al. (2017) study also acknowledged that the serotonin system impacted the function of the hypothalamic-pituitary-adrenal (HPA) axis due to higher cortisol concentrations among juveniles fed high-fat diets. The Abuaish et al. (2018) study described dysregulation of the hypothalamic-pituitary-adrenal axis due to early activation and impaired negative feedback of the hypothalamic-pituitary-adrenal axis among rodent neonates exposed to a maternal high-fat diet in utero. Sasaki et al. (2014) suggest that the limbic system also interacted with the hypothalamic-pituitary-adrenal axis through corticosteroid receptors. Sasaki et al. (2014) showed that in adolescent rodents fed a high-fat diet there was increased expression of the glucocorticoid receptors in the hippocampus and dysregulation of pro- and anti-inflammatory genes in the hippocampus and amygdala. Overall, these studies demonstrate associations between fetal exposure to a maternal

high-fat diet and the development of anxiety-like behaviors in offspring (except for the Sasaki et al. (2014) study). Sasaki et al. (2014) reported that anxiety-like behaviors in adolescents might be expressed differently than anxiety symptoms among adults due to a developmental shift between adolescence and adulthood in rodents.

The strengths of this thesis include the incorporation of non-human primate studies since they are more closely representative of human behaviors. Another strength is the inclusion of recently reported empirical research. However, a limitation of this review is that the dietary composition of the high-fat diets and the control diets were not consistent with the dietary guideline for humans; a difference that could make the results less transferable or generalizable to humans. In addition, this thesis reviewed studies performed in animal models, not humans. Some results were similar to human studies such as females exhibiting more anxiety-like behaviors than males in rodent and non-human primate studies.

Still, human studies are needed to fill in the discourse gap. Human studies linking maternal diet to the development of generalized anxiety disorders in offspring are scarce; controlled feeding studies in humans are expensive, complicated, and difficult to perform, especially studies of women during pregnancy and postpartum. Regardless, additional research is needed to better understand how maternal diet, epigenetics, and the Developmental Origins of Health and Disease theory apply to the development of generalized anxiety disorders in humans. For instance, ongoing research by Bert Boyer and Scarlett Hopkins at Oregon Health & Science University aims to better understand how nutritional and behavioral factors in Alaska Native mothers impact their children's health. A native community that has a history of eating a high-fat diet due to the types of

foods available within their environment. This current research and other natural history studies focusing on populations that have historically eaten healthier high-fat diets should be used to explore the development of generalized anxiety disorders. The research findings presented here and future research will then inform implementation trials to provide evidence for public health policies to improve the health of future generations.

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Appendix

Non-Human Primate Study	
Citation	Sullivan et al.
Year	2010
Topic	Chronic Consumption of a High-Fat Diet during Pregnancy Causes Perturbations in the Serotonergic System and Increased Anxiety-like Behavior in Nonhuman Primate (NHP) Offspring
Population	-Adult female and male NHPs were housed together in groups of 10 to 12 with the ratio of male to female was 2/ 9-10 - After 2-4 years on the control (CTR) or high-fat (HFD) diet, the dams underwent a caesarian section to collect fetal brains on gestational day 130. Five fetuses were collected in the CTR group while 6 were gathered in the HFD group - On postnatal day 130 (PND130), behavioral exams were conducted on 4 male and 4 female offspring in the CTR group, and 12 male and 11 female offspring in the HFD group.
Methods	-in situ hybridization of fetal hypothalamus and midbrain was performed. Midbrain images were further divided into rostral, medial, and caudal to identify the presence of Tryptophan hydroxylase 2 (TPH2), serotonin transporter (SERT), and serotonin 1A receptor subtype (5-HT1AR) - Fluorescent immunohistochemistry was used to determine serotonin (5-HT)-ir projections in sections containing the arcuate nucleus of the hypothalamus and TPH2-ir in raphe-containing sections. The immunofluorescent images were captured using confocal laser microscopy. - the number of immunoreactive cells in the raphe-containing sections was counted using the Immunohistochemical images -Behavioral testing included the human intruder and novel objects test to measure anxiety-like behaviors based on vocalizations, exploration, movement, and response to the human intruder while latency, movement, exploration, vocalizations, and other responses were scored in relation to the novel objects test.
Measurement Tools	-normality and homogeneity of variance for all analyses -T-test -Mann– Whitney U test -Univariate ANOVA -used the SPSS software package, version 16.0 for all analyses
Results/ Evidence	- 5-HT1AR was upregulated in the rostral dorsal raphe (rDR), but not in the caudal dorsal raphe (cDR). Cerebrospinal fluid (CSF) 5-HT in the juvenile (PND130) offspring exposed to a maternal HFD was lower, suggesting that maternal HFD exposure suppresses the serotonergic system. -Increased TPH2 mRNA expression in the rDR region of fetal HFD offspring occurs in response to the decreased expression of the 5-HT1AR in the DR, a deficiency of 5-HT neurotransmission, and the stress induced by the maternal HFD -55% of female HFD offspring experienced increased anxiety while the male offspring, in contrast, demonstrated aggressive behavior; this occurs commonly, especially with a decreased serotonergic tone

	-It was also demonstrated that obesity did not account for the risk of developing anxiety disorders but rather the exposure to a maternal HFD -Overall, both female and male NHP offspring who were exposed to a maternal HFD experienced perturbations in the central serotonergic system that increased the risk of developing behavioral disorders such as anxiety.

Non-Human Primate Study	
Citation	Thompson et al.
Year	2017
Topic	Exposure to a High-Fat Diet during Early Development Programs Behavior and Impairs the Central Serotonergic
Population	- In outdoor or indoor pens, adult female Japanese macaques were grouped into 4-12 individuals with the male-to-female of 1-2/ 3-10. - There were 135 offspring born from 65 mothers that were included in the study with no more than 6 offspring per mother.
Methods	- Mothers/ Dams were maintained on either a control diet (CTR) which contained less fat in the overall diet and less energy consumption, or a high-fat diet (HFD) that resembles a Western-style diet. They stayed on this diet for 1.2-8.5 years before and during parturition. - The offspring born to mothers of either an HFD or CTR primarily consumed their mother's diet by 6 months of age. Once they were weaned from their mothers at about 7.99 months of age, half of the offspring maintained their mother's diet while the other half switched diets. This created four diet groups: CTR/CTR, CTR/HFD, HFD/CTR, and HFD/HFD. -At 11 months of age, juveniles underwent behavioral testing and their reactions were videotaped in an adjacent room. The tests included the human intruder test, the novel object test, and a blood sample collection of cortisol after these tests. The video scoring of these behaviors consisted of two blind observers who scored the behaviors based on locomotive state, exploration, vocalizations, anxious or abnormal behaviors, responses to strangers, response to objects, and other behaviors not clearly defined were subject to review by both observers in order to be classified. The tests also scored responses that were not typical of anxiety behaviors but were unique to that individual by conducting a composite variable determination. - Physical activity was measured by accelerometers attached to collars worn continuously on the offspring. Data was specifically collected before, during, and after the behavioral testing. - An intravenous glucose tolerance test was collected at 13 months of age on the offspring. First, a baseline was collected from the vein, and then, a glucose bolus was injected into the vein to measure glucose levels at 1, 3, 5, 10, 20, 40, and 60 min after infusion. All blood collected was also used to measure insulin levels, and in the end, the area under the curve of glucose and insulin (GUAC and IUAC) was calculated. -Juiveline blood samples were collected at preweaning and post-weaning with both gathered after the behavioral tests. However, the preweaning sample also included a sample separated from the mother and prior to weaning. The blood was then centrifuged to obtain the plasma for the assay.

	-Hair samples were collected prior to weaning and at 13 months of age until assay time. - Hair assays measured the chronic stress in the hair samples. -At 13 months of age, offspring were necropsied to collect brain tissue from the hypothalamus and midbrain along with a terminal blood sample from the aorta or caudal vena cava. - Using the plasma collected before, they conducted assay tests to identify cortisol, insulin, glucagon, and leptin. - in situ hybridization of fetal midbrain that was then exposed to film. Midbrain images were further divided into rostral, medial, and caudal to identify the presence of Tryptophan hydroxylase 2 (TPH2), serotonin transporter (SERT), and serotonin 1A receptor subtype (5-HT1AR). - In the 1:24 series, coronal sections from the prefrontal cortex (PFC) were collected. Immunohistochemistry and immunofluorescent was used to provide images for the presence of serotonin in six fields of view per section throughout area 10 of the right PFC and two fields of view each of the dorsal, medial, and ventral regions.
Measurement Tools	- all statistical tests were run using the using SPSS Version 22 - Kolmogorov–Smirnov tests of normality - all graphs were created using GraphPad Prism Version 6 software - Pearson correlations - parametric analysis -non-parametric analysis - three-factor univariate ANCOVAs - a three-factor univariate ANOVA - a two-factor univariate ANOVA - Kendall's correlations - Mann–Whitney U tests - Kruskal–Wallis tests - the Jonckheere–Terpstra test
Results/ Evidence	- The effects of maternal HFD exposure caused a reduced expression of TPH2 mRNA in the dorsal raphe. This increased anxiety-like behaviors due to developmental reprogramming in juvenile non-human primates. -These outcomes occurred regardless of post-weaning diet, showing that early nutritional intervention is insufficient to mitigate the outcomes caused by a maternal HFD exposure - A post-weaning HFD independent of maternal exposure continued to exacerbate behavioral abnormalities for juvenile primates while also reducing serotonin immunoreactivity in area 10 of the PFC and increasing the occurrence of stereotypy. - This study recognized that female offspring were more prone to anxiety than male offspring which correlates with human studies. - During early development, any HFD exposure can lead to increased active anxiety and stereotypy in adolescents. Active anxiety was a primary determinant that arose due to maternal HFD exposure during the behavioral exams. Stereotypy is an extreme reaction to stress that is not indicative of standard anxiety behaviors but is independently associated with a post-weaning high-fat diet. - A maternal HFD also caused abnormal levels of cage interaction that are indicative of anxiety. - The connection between maternal exposure and post-weaning exposure is that they impact the development of certain kinds of anxiety disorders such as social anxiety.

	- Postweaning HFD caused behavioral inhibition based on decreased interaction with the novel object and cage test; this is associated with anxiety in animal models and children. - The reprogramming of the stress response due to a maternal HFD exposure is more prominent among female offspring while male offspring are more impacted by a postweaning HFD consumption. - Postweaning HFD caused plasma cortisol to increase, but these levels increased dramatically with a maternal HFD exposure. - Hair cortisol, which is indicative of chronic stress, was only present in the males, but it was also higher at weaning if exposed to a maternal HFD early on. Overall, these cortisol levels are impacted by the HPA axis where increased cortisol is associated with anxiety and abnormal behaviors. - The decrease in serotonin immunoreactivity in area 10 of the PFC is impacted by a postweaning HFD exposure that can lead to behavioral inhibition since serotonergic innervation of this area regulates behavioral inhibition. It also may explain the increase of stereotypy based on non-human primate studies showing impairment in the PFC can lead to increased stereotypy. Overall, these outcomes cause anxiety-like behaviors. - TPH2 mRNA expression in the DR decreased with exposure to a maternal HFD regardless of a healthy post-weaning diet. In contrast, a post-weaning HFD caused an increased expression of TPH2 mRNA in the MnR instead of the DR. These results were consistent with rodent studies that showed an increase in anxiety-like behaviors. The TPH2 mRNA expression was measured in the cell bodies of the DR instead of the axonal terminal; therefore, the reduction of TPH2 mRNA expression suppresses serotonin synthesis. The DR and MnR also play an important role in metabolism and behavior since they innervate and interact with areas of the brain that impact anxiety disorders such as the amygdala, the ventral PFC, and the medial PFC. - Post-weaning HFD decreased the serotonergic innervation from the MnR to the area 10 of the medial PFC which could lead to anxiety disorders such as social anxiety -The results indicate that pro-inflammatory factors are the reason that HFD-induced behavioral and serotonergic impairments were observed. Inflammation can have detrimental effects on neurodevelopment such as degeneration of serotonergic neurons that disrupt the serotonin system.

Rodent Study	
Citation	Abuaish et al.
Year	2018
Topic	Perinatal high fat diet induces early activation of endocrine stress responsivity and anxiety-like behavior in neonates
Population	-Three different cohorts of rodent neonates were used in this study. Cohort one consisted of 11 litters maternally exposed to a control chow diet (CHD) and 9 litters maternally exposed to a high-fat diet (HFD). -Cohort 2 had 7 litters from a maternal CHD and 8 litters from a maternal HFD. -Cohort three had 6 pregnant adult female Long Evans rats (7 weeks) on a CHD and 8 on a high-fat diet.

Methods	- Adult female rats were maintained on either a CHD or HFD. The CHD contains a higher percentage of protein and carbohydrates than fat while the HFD has more fat than protein or carbohydrates. - The food intake and weight gain of adult female rats were monitored by measuring the food and their weight once a week before mating. - Dams and their offspring were then weighed, and nose-to-anal lengths were recorded on PND0, PND7, and PND13. - Cohort three underwent a 20-minute restrain challenge on gestation day 20 with blood samples collected before and after the stress reactivity test. - Maternal behavior was also recorded from PND1-6 for 1 hour and 6 times during the day. The behaviors identified included total licking, total nursing, dam away from the nest, nesting, total care of offspring, and self-directed behaviors. -Two operant conditioning chambers were set up with either clean bedding (CB) or soiled bedding (MB) from a male sexual interaction. - On PND7 and 13, the offspring took an isolating test procedure with either CB or MB. The offspring in the isolating chamber with soiled bedding were then euthanized to collect blood and tissue from the brain. Baseline measures were also recorded along with ultrasonic vocalizations (USV) and behaviors but only on PND7. - Ultrasonic vocalizations of offspring were also measured and analyzed by using Avisoft SASLab Pro while behavior during the isolation test was analyzed using the Observer XT 10 software (Noldus). - Corticosterone (Cort) levels and adrenocorticotropic hormone (ACTH) in both dams and offspring were measured from the blood samples collected during the dams' restraint challenge and MB exposure in offspring; This was done using a Corticosterone Double Antibody RIA Kit. -The brains collected during the euthanization were sectioned into the paraventricular nucleus of the hypothalamus (PVN) and the ventral hippocampus along with RNA extraction. - Reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to identify five genes measured in the PVN and hippocampus of the offspring. These genes included 14-3-3 protein zelta/delta (Ywhaz), Corticotropin-releasing hormone (Crh), Glucocorticoid receptor (Nr3c1), Mineralocorticoid receptor (Nr3c2), and glutamic acid decarboxylase 1 (Gad1).
Measurement Tools	- Statistical analysis was done using SPSS (IBM). - A Shapiro-Wilk test identified normality for data sets and was examined by using box plots. - Factorial linear mixed models -Factorial general linear model - Two-tailed t-tests were used to determine the effect of diet on PND7 Cort, ACTH levels, and relative transcript abundance. - Fisher's least significant difference (LSD) post hoc testing helped with pairwise comparison among the diet groups at the same time while Bonferroni post hoc testing accounted for different time points across diet groups. - The Pearson correlation analysis evaluated the relationship between dam caloric intake, weight change, and the amount of time spent self-feeding during the maternal recording.
Results/Evidence	- A HFD caused early activation of the HPA axis during the hyporesponsive period (PND7) which is supposed to be unaffected by stimuli or stressors. The activation of the HPA axis caused alterations in USVs and Crh transcript levels in the PVN.

	-In PND13, this early activation brought on by a HFD exposure was further enhanced due to an increased ACTH response. -The lower Nr3c1 transcript levels in the ventral hippocampus also caused this early activation in PND13 since a low Nr3c1 can impair HPA negative feedback. - Offspring exposed to a HFD during maternal nutrition showed increased USVs and immobility on PND13 which is a measure that indicates increased anxiety-like behaviors. - Overall, these results suggest that a perinatal HFD exposure can impact the development of the HPA axis which can increase stress-related behaviors such as anxiety disorders. - A HFD can act as a stressor during gestation for not only the mother but also the offspring. - A perinatal HFD can lead to early activation of the HPA axis during the hyporesponsive period. On PND7, the offspring had higher (Cort) levels after the exposure to MB; this increase in Cort also appeared during the maternal tests. - During the hyporesponsive period (PND7), there was an increase of CrH in the PVN which regulates USVs. This regulation is an inverted U-shaped relationship where a dramatic increase in Crh can decrease USVs. During their analysis, the authors observed an increase CrH and a decreased USVs in HFD-exposed offspring in contrast to the CHD-exposed offspring. There was also a decrease in Nr3c1 expression among HFD offspring which has known to negatively influence Crh expression. - After the hyporesponsive period (PND13), HFD offspring showed only increased ACTH levels and no change in Cort levels, unlike the CHD offspring who showed both a Cort and ACTH response to the MB exposure. This response among HFD offspring continued even an hour after the test ended. - During the isolation tests in PND13, HFD offspring had increased USVs, decreased exploration, and increased immobility regardless if there was a CB or MB exposure. An increased USVs has been associated in other rodent studies as an index of anxiety-like behaviors, and the same goes for increased immobility and decreased exploration. - The perturbations in ACTH levels and anxiety-like behaviors observed in the HFD offspring during the emergence from the hyporesponsive period indicate an alteration in the negative feedback of the HPA axis. Nr3c1 regulates the negative feedback of the HPA axis within the PVN and ventral hippocampus. However, HFD offspring showed a lower Nr3c1 transcript abundance in the ventral hippocampus. - HFD offspring during the emergence from the hyporesponsive period also had an increase in Gad1 transcript abundance in the ventral hippocampus. This increase can lead to the inhibition of the PVN which is part of and controls the HPA axis negative feedback loop. Again, a HFD becomes a stressor in neonates that can upregulate Gad1. -Overall, neonates exposed to a maternal HFD caused early activation of the HPA axis during the hyporesponsive period along with the increased Cort levels, decreased USVs, and increased Crh transcript abundance in the PVN. In contrast, after the hyporesponsive period, these same neonates showed abnormal HPA axis function, increased ACTH levels, impaired negative feedback, increased anxiety-like behaviors, and reduced Nr3c1 transcript abundance in the ventral hippocampus. -This perinatal HFD can impact the development of a stress-related phenotype due to early activation of the HPA axis which has been associated with anxiety disorders.

Rodent Study	
Citation	Sasaki et al.
Year	2014
Topic	Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring
Population	-Adult male and female Long Evans rats (7 weeks old) -The first group of offspring consisted of HFD females n = 19; HFD males n = 13; CHD females n = 19; CHD males n = 19 -The second group of offspring contained HFD females n = 13; HFD males n = 7; CHD females n = 13; CHD males n = 13 - Another group of offspring included n = 6 offspring per sex and per diet group.
Methods	- Adult female rats were given access to either a control chow diet (CHD) or a high-fat diet (HFD). The CHD contains a higher percentage of protein and carbohydrates than fat while the HFD has more fat than protein or carbohydrates. - The dams (pregnant or parent female rodents) stayed on their diets for 4 weeks before mating and continued to stay on this diet throughout gestation and lactation. The offspring were automatically placed on a CHD diet once they were born to test the impacts of a HFD maternal exposure. - Body weights of the offspring were measured between postnatal day (PD) 35 and PD45 before the behavioral tests - The first group of offspring was used to conduct the Light-Dark transition task which consisted of an opaque white Plexiglas box (light zone) connected through a small opening to an opaque black box (dark zone). Duration and frequency of entries within the light zone were measured since offspring started in the dark zone. - The first group of offspring also underwent the Elevated Plus Maze task which started with the rodents placed in the center of the maze. The movements were then measured based on predefined zones; this included the center zone, two open arms attached to the center zone, and two closed arms attached to the center with the whole maze elevated above the floor. The open and closed arms were further divided into proximal and distal zones with the time and frequency of entries into each zone calculated and compared; open zones relative to the closed zones. -Group two was used for the Open Field task which was a white opaque square box. The box also had predefined zones such as the center zone, the edge zone along the wall of the box, and the corner zone. ANY-maze software tracked the offspring's movement and calculated the number of entries and time spent in the predefined zones to measure the duration and frequency of entries for each zone and the center zone relative to the edge. - The last group of offspring (n=9) was decapitated to collect the brains for gene expressions analysis after the behavioral testing. The whole hippocampus and amygdala were dissected along with RNA extraction from the juvenile HFD- and CHD-exposed offspring. - Quantitative reverse transcriptase-polymerase chain reaction was then performed to analyze the expression of eight transcripts within the hippocampus and amygdala; this included the relative abundance of transcripts for glucocorticoid receptor (GR),

	mineralocorticoid receptor (MR), nuclear factor kappa beta (NFkB), interleukin-6 (IL-6), cluster of differentiation molecule 11b (CD11b), I-kappa-B-alpha (IkBa), mitogen-activated protein kinase phosphatase-1 (MKP-1), and interleukin-1 receptor antagonist (IL-1Ra).
Measurement Tools	-Statview statistically analyzed the results. -Student's t-test compared body weight between HFD- and CHD-exposed offspring. - Behavioral measures were examined through 2x2 diet-by-sex mix-model along with repeated measures analysis of ANOVA and Bonferroni post hoc testing. - All behavioral measures also used the Shapiro–Wilk method in SPSS to test for normality. - A 2x2 factorial ANOVA and Bonferroni post hoc tests were used for gene expression analysis to examine the influence of diet and sex.
Results/Evidence	-The study revealed that a maternal HFD diet exposure decreased anxiety-like behaviors on several measures of anxiety in adolescence. -One measure that showed a decrease in anxiety-like behaviors was the increased exploration of the open arms of the Elevated Plus Maze and entries in the center portion of the Open Field among HFD exposed offspring. These behaviors are uncharacteristic of anxiety-like behavior, but previous studies have identified that offspring exposed to a maternal high-fat diet are still more likely to develop anxiety into adulthood. Therefore, these studies have recognized that behaviors characteristic of anxiety in adulthood are consistent with impulsive/risk-taking exploratory behaviors in adolescent animals. -These differences in anxiety behaviors among adolescents and adults can be due to differences in the stress response or reactivity. In both animal models and human studies, the differences in anxiety disorder symptoms between adolescents and adults are prominent. Impulsive behavior in adolescents is synonymous with anxiety behaviors in adults. - Another possible explanation for these differences in anxiety behaviors could be due to a maternal HFD exposure advancing the onset of puberty, especially the hormone estrogen. - A perinatal HFD exposure was associated with an increase in GR expression within the hippocampus, especially among female offspring. - In the hippocampus, the expression of NFkB and IL-6 pro-inflammatory genes and IkBa and MKP-1 anti-inflammatory genes were upregulated in HFD-exposed offspring, while in the amygdala, there was a decreased expression of NFkB pro-inflammatory gene and IL-1Ra anti-inflammatory gene expression among HFD-exposed offspring. -Weight was similar between the HFD-exposed and CHD-exposed offspring.