

The long-term impact of obesity in pregnancy on offspring hypothalamic feeding pathways

Pit Shan (Rosanne) Chong¹ and Laura Dearden¹

¹ *University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Level 4, Box 289, Addenbrooke's Hospital, Cambridge, CB20QQ, United Kingdom*

Abstract

An accumulating body of evidence shows that offspring exposure to maternal obesity in the peri-natal period causes an increased risk to develop obesity later in life. Animal models have demonstrated that increased weight gain in offspring exposed to maternal obesity is preceded by increased food intake, implicating altered brain control of food intake as a likely cause. The hypothalamus is crucial for regulating feeding behaviour and energy homeostasis. This article reviews findings from human and animal studies to provide an updated perspective on how maternal obesity alters fetal hypothalamic development, predisposing offspring to long-term metabolic dysfunction. We discuss how maternal obesity impacts on hypothalamic development and the key molecular mechanisms, including epigenetic modifications, hormonal disruption, neuroinflammation, and gut-brain axis interactions, which may mediate these changes. We highlight the critical gaps that remain in understanding the specific molecular pathways driving neurodevelopmental alterations in offspring, as well as emerging areas of research, including the role of extracellular vesicles in maternal-fetal communication. An in depth understanding of the molecular mechanisms that mediate the link between maternal metabolic state and offspring hypothalamic control of feeding is crucial in informing public health policies and clinical interventions aimed at reducing the intergenerational transmission of obesity.

1. Introduction

1.1. Multi-faceted causes of the global rise in obesity rates

In recent decades worldwide obesity levels have increased exponentially, leaving us in the midst of an obesity epidemic. Obesity is a major risk factor for a number of chronic diseases, including cardiovascular disease, diabetes and cancer and can also have a negative impact on mental health and well-being. Due to these associated health concerns, obesity represents a significant financial burden (1). The financial and societal cost of obesity is particularly concerning for low and middle income countries that are starting to see rising obesity rates, in addition to countries like the UK and USA that have been experiencing rising obesity for decades.

There are many environmental factors driving increased obesity in the population. A changing food environment is a major driver of the increasing prevalence of obesity, as our modern industrialized food system allows increased availability of inexpensive and convenient ultra-processed foods (2). Furthermore, socioeconomic status is strongly associated with obesity rates. Food insecurity (or food poverty) is associated with obesity among women in high-income countries (3). Experiments on animals suggest that unpredictable food causes animals to store more energy in adipose tissue, and lower energy expenditure sufficient to fuel weight gain in the absence of increased food intake (4). An individual's body weight and metabolic health is a sum of their energy input (diet) and energy output (energy expenditure including physical activity). Whilst some studies have suggested that a decline in physical activity levels in recent decades is to blame for rising body weight in the population (5), others have argued that there has not been a decline in energy expenditure (6), and it is instead a decline in basal expenditure in cells that has contributed to a reduction in daily energy expenditure (7).

52
53 However, not all individuals gain weight in the face of the “obesogenic” environment that many
54 of us live in. Obesity is therefore increasingly being recognised as a disease determined by
55 risk factors linked to an individual’s biological make-up that interact with societal and
56 environmental factors. Large genome-wide association studies have identified more than 200
57 common genetic variants linked to increased weight gain, primarily in genes related to brain
58 function (8), which support the theory that obesity is caused primarily by changes in feeding
59 behaviour that lead to excess food consumption and adipose tissue deposition. Although an
60 individual’s genetics undoubtedly play a strong part in determining body weight, to date, the
61 identified genetic changes can only account for small increases in body weight, and even
62 when multiple genetic variants are combined into a genetic risk score, they explain a very
63 small amount of the variance in BMI (9). There must therefore be additional mechanisms that
64 can alter an individual’s biology- independent of their genetics- and increase the susceptibility
65 to develop obesity and associated metabolic diseases. Indeed, it has recently been shown
66 that the genetic heritability of child Body Mass Index (BMI) is altered by an obesogenic home
67 environment (10), demonstrating the intricacy and importance of gene and environment
68 interactions in determining obesity rates.
69

70 Increasing evidence suggests that some of the variation in biology that underlies altered
71 obesity risk comes from the environment experienced by an individual during their early life,
72 roughly from conception to age two. Changes to normal development during this time can
73 influence long-term obesity risk (among other diseases), a concept embodied within the
74 Developmental Origins of Health and Disease (DOHaD). An association between the fetal
75 environment and later incidence of metabolic disease was first reported by Hales and Barker,
76 who proposed the “Thrifty Phenotype Hypothesis” based on their observations of a link
77 between reduced birth weight and increased cardio-metabolic disease in adulthood in a UK-
78 based cohort of babies born in the 1950s (11, 12). Further studies examining individuals who
79 were *in utero* during the Dutch Hunger Winter confirmed an association between *in utero*
80 nutrition state and the development of metabolic disease, and suggested it was a causative
81 relationship dependent on specific windows of exposure (13).
82

83 **1.2. Rising obesity rates during pregnancy and associated health outcomes**

84 Early studies in the DOHaD field investigated the long-term consequences of under-nutrition
85 or intra-uterine growth restriction on long-term metabolic health. Over the past 20 years,
86 numerous studies in human and animal models have explored the relationship between
87 maternal obesity and/or gestational diabetes mellitus (GDM) in pregnancy and long-term
88 health in offspring (14). The global rise in obesity means there has inevitably been an increase
89 in the number of pregnancies in women living with obesity. In the year 2024, obesity rates in
90 women entering pregnancy were as high as 31% in areas of the UK, with a high percentage
91 of the population living in deprived quintiles (15). Women with type 2 diabetes now make up
92 56% of diabetic pregnancies (compared to 47% in 2014); they face additional healthcare
93 inequalities, with 63% living in the 2 most deprived quintiles (compared to 7% in the least
94 deprived quintile) and 53% belonging to ethnic minorities (16). Thus negative health outcomes
95 associated with obesity and GDM in pregnancy are likely to impact most heavily on the most
96 deprived people in society, and compound other socio-economic risk factors associated with
97 obesity.
98

99 Obesity during pregnancy is associated with negative health outcomes for the mother and the
100 baby both during pregnancy and birth. For the mother, obesity is associated with greater odds
101 of developing GDM, which affects 10-20% of pregnancies in the UK and is itself associated
102 with health risks for mother and baby (17). Obesity in pregnancy also increases maternal
103 hypertension and the risk of developing the life-threatening condition pre-eclampsia. For the
104 fetus, maternal obesity is associated with an increased risk of stillbirth, being born both small
105 or large for gestational age, and an increased incidence of shoulder dystocia and emergency

caesarean birth (17). As well as these immediate effects on the health of the mother and the baby, exposure to maternal obesity during pregnancy is associated with long-term health problems in offspring. There is now strong evidence that exposure to maternal obesity causes an increased risk to develop obesity, diabetes, and cardiovascular disease later in life.

1.3. Increased obesity risk in children born from pregnancies complicated by obesity and gestational diabetes

1 in 3 children leaving primary school in the UK are overweight or have obesity by the age of 11 (18). This is concerning for the future health of the nation, as childhood obesity is a strong predictor of adult obesity and associated co-morbidities. Exposure to maternal obesity and/or GDM in pregnancy may be a contributing factor to rising childhood obesity rates. A recent meta-analysis of 67 birth cohorts from around the world calculated a 264% increase in odds of childhood obesity when mothers have obesity before conception (19). Whilst birth cohorts of this type also capture the effects of inherited BMI determining genetics and the shared household environment, studies in siblings discordant for exposure to maternal obesity or GDM have shown that there is an additional impact from the *in utero* environment (20, 21).

1.4. Feeding behaviour and neuronal responses to food cues in offspring born from pregnancies complicated by obesity or GDM

Increased BMI in children born from pregnancies complicated by obesity or GDM could be driven by increased food intake. A recent study has shown increased food cue activation in the pre-frontal cortex of children of parents with obesity- which could underlie over-consumption of food. Offspring pre-frontal cortex activation shows a stronger association with maternal rather than paternal weight (22). This suggests that there are additional factors resulting from maternal metabolic state that can influence brain responses to food in the offspring in addition to inherited genetics. A unique study of children born from pregnancies complicated by GDM demonstrated that GDM-exposed children have increased hypothalamic blood flow (a marker of hypothalamic activation) in response to glucose when compared with unexposed children, and that a greater hypothalamic response to glucose predicted greater increases in later BMI. These results remain significant after adjustments for child age, sex, current BMI, and maternal pre-pregnancy BMI (23). Fetal exposure to GDM is also associated with greater daily energy intake, and children exposed to GDM that was diagnosed early in pregnancy displayed greater food cue reactivity in their orbital-frontal cortex. Children exposed to GDM also had a larger waist-to-hip ratio, which was explained by their higher energy intake (22, 24).

Unfortunately, there are few studies in humans examining whether altered feeding behaviour is a driver of obesity in individuals exposed to maternal obesity, due to the difficulty in studying the direct consequences of obesity in pregnancy independently of genetics and household environment. Animal models in which genetic and environmental factors can be more tightly controlled have shown conclusively that maternal obesity in pregnancy, with or without GDM, results in offspring hyperphagia and that this is a driver of increased adiposity. This altered feeding behaviour is evident from a young age; rodent offspring of obese dams consume more milk during experimental tests of independent feeding, even after correcting for their body weight (25). In rodents, independent feeding by pups (i.e., calorie intake other from dam milk) typically emerges during the third postnatal week. The offspring of obese dams transition earlier to independent feeding and although they consume the same amounts of milk from the mother, they supplement milk intake with additional solid food consumption, suggesting impaired satiety signals (26). Animal models have shown that not only does maternal obesity during pregnancy program hyperphagia, but also increases preference for high-fat foods in non-human primates (NHP) (27), rats (28) and mice (29).

1.5. Importance of the hypothalamus and correct hypothalamic development in maintaining energy homeostasis

Animal models that show increased weight gain in offspring exposed to maternal obesity are preceded by increased food intake, implicating altered neuronal control of food intake as an underlying cause. The hypothalamus is the primary site in the brain for maintaining energy homeostasis, which it does by appropriately adjusting parameters- including food intake- based on signals of nutrition state received from the periphery. The primary neuronal populations in the hypothalamus involved in food intake control are orexigenic neurons expressing Neuropeptide-Y (NPY) and/ or Agouti-Related Peptide (AgRP), and anorexigenic neurons expressing Pro-opiomelanocortin (POMC). Both of these populations of neurons are resident in the Arcuate nucleus of the hypothalamus (ARC) adjacent to the median eminence (MEE) where the blood-brain barrier (BBB) is leaky, allowing cells to sense peripheral circulating nutrients (e.g. glucose, fatty acids) and hormones (e.g. leptin, insulin, ghrelin). Interestingly, these neuronal populations that are well known to control opposing actions for food intake after birth, initially form from a common lineage (30) and therefore developmental disruption around the time point of switch from a POMC- defined neuron to an NPY-defined neuron (E13 in mice) is a window for potential disruption. In rodents, melanocortin neurons are born between days E11-12 (30, 31) and the circuitry of projections from these neurons is completed in the post-natal period (32). In humans and NHP, which give birth to offspring with more mature brains, the majority of the circuit formation is completed by birth. However there is still evidence of refinement of the melanocortin circuitry in NHP in the early post-natal period (33).

AgRP/NPY and POMC neurons project intra-hypothalamically to regions, including the Para-Ventricular nucleus of the hypothalamus (PVH) where they synapse onto melanocortin 4 receptor (MC4R) expressing neurons. It is well established in rodents and humans that disruption of signalling within these key pathways in the hypothalamus results in obesity (34). More recent studies have shown that genetic disruption of the cell signalling pathways required for correct hypothalamic development- specifically axon guidance pathways- result in severe early onset obesity in humans (35) and impaired glucose tolerance in rodents (36). Therefore, changes to hypothalamic development caused by an altered *in utero* milieu of a pregnancy complicated by obesity could be a contributing cause to rising obesity rates via life-long changes in hypothalamic function.

2. Impact of maternal obesity on development of the hypothalamus

Changes to offspring hypothalamic development in the perinatal period as a result of altered nutrition and/ or hormonal signalling in response to maternal obesity is a likely cause of long-term metabolic phenotypes in offspring. Examining molecular and detailed anatomical changes in the hypothalamus requires access to brain tissue and animal models must be used for this purpose. Murine hypothalamic development is well defined, allowing researchers to distinguish changes from the normal process of neurodevelopment. Therefore, the following section refers primarily to studies examining the impact of maternal obesity on hypothalamic development, primarily in rodents.

2.1. Neurogenesis and cell fate decisions

The neuroendocrine portion of the hypothalamus- which is the primary site of feeding regulatory pathways- comprises the anterior and tuberal hypothalamus. In the mouse tuberal hypothalamus, all neurons are produced during a period of only a few days (37). Experiments using BrdU labelling of newborn cells have shown that the majority of neurons in the PVH and dorsomedial nucleus (DMH) are generated between E12-E14, whereas the ARC and

ventromedial nucleus (VMH) have longer periods of neuronal generation from E12-E16 (30, 31).

2.1.1 Decreased proliferation of neural progenitor cells in the hypothalamus

Maternal obesity causes a reduction in the proliferative potential of hypothalamic neural progenitor cells generated from the fetal hypothalamus and decreased neurogenic markers in newborn mice in the mid-gestation period (38, 39). Although this period is when the majority of hypothalamic cells are born, hypothalamic cell turnover and neurogenesis continue in the post-natal period and into adulthood in mice (40). A recent study found that in male offspring of HFD-fed mothers, newly born cells (as labelled by BrdU) were significantly decreased in the ARC, but not other hypothalamic regions, such as the VMH or DMH, on postnatal day (PND) 9. However, by PND21 decreased proliferation was observed in all hypothalamic regions of both male and female offspring exposed to a maternal HFD (41). These results suggest there are different time windows of vulnerability within the hypothalamus, with the ARC being sensitive at an earlier time point than other regions of the hypothalamus. Furthermore, it also suggests time differences in windows of sensitivity between male and female offspring. This study also reported upregulation of pathways related to neuronal death in offspring, suggesting that maternal nutrition may interfere with cell survival as well as proliferation (41). This is consistent with previous reports that HFD induces apoptosis of hypothalamic neurons in rodents (42).

2.1.2 Changes in pathways that regulate differentiation of neural progenitor cells

The Notch signalling pathway is a key regulator of neurogenesis in the CNS. As Notch signalling inhibits pro-neural genes, models lacking Notch signalling exhibit an increase in neurons throughout the embryo (43). Mice which lack Notch signalling in the hypothalamus demonstrate that Notch signalling is essential for the differentiation of late ARC neurons in the mouse from E13 (44). Exposure to maternal obesity results in an up-regulation of Notch signalling in the neonatal period (38), and a concomitant decrease in expression of the pro-neural transcription factor Mash1. As the Notch/ Mash1 pathway contributes to neuronal differentiation into POMC and NPY lineages (45) (44), these signalling disruptions could therefore be an underlying cause of altered anorexigenic/ orexigenic cell populations in the offspring of obese mothers. Rodent models of obese pregnancies have consistently shown that offspring of obese or HFD-fed mothers have a higher ratio of AgRP/NPY to POMC-expressing neurons (38, 46), which may be caused by a reduction in the differentiation of newborn neurons into POMC neurons (41).

Changes in proliferation of neural stem cell populations and downstream differentiation pathways may also result in long-term changes to cell types in the hypothalamus. Astrocytes, once viewed merely as support cells, are now recognised as active modulators of neuronal function and synaptic plasticity. Several studies in rodents have shown a decrease in cells adopting a neuronal fate, and concomitant increase in the proportion of newly born astrocytes in the developing fetal and neonatal mouse hypothalamus caused by maternal obesity in pregnancy (47). This abnormal increase in astrocyte numbers may disrupt the proper assembly and connectivity of hypothalamic circuits, which are critical for regulating feeding behaviour and energy homeostasis. Interestingly, recent single-cell sequencing analysis in offspring exposed to maternal obesity only in the post-natal period showed no difference in relative cell populations (48), likely due to the fact that neurogenesis and differentiation are mostly completed by the post-natal period. This again highlights specific windows of vulnerability for different components of the hypothalamic machinery that controls energy homeostasis.

2.2. Hypothalamic circuitry and anatomical development

The hypothalamus is a highly connected region of the brain. Animal models of maternal obesity have shown numerous changes to the wiring of the hypothalamus, primarily focused on intra-hypothalamic connections but also in connections to other parts of the brain. Experiments using neonatal hypothalamic explants demonstrate that offspring exposed to maternal obesity or HFD feeding show reduced neurite extension *in vitro* (49). Reduced neurite outgrowth may be caused by altered expression or responsiveness to classical axon guidance cues. During normal development, neurites from NPY neurons in the ARC are repelled away from the ARC, in part due to Netrin-1 expression, to head toward their target areas, such as the PVH. In fetuses developing in obese mothers, this repulsive force may not occur because of the overexpression of *Dcc*, which mediates attraction and reduced expression of *Unc5d*, which mediates repulsion. Consequently, NPY neurites may have a reduced ability to leave the ARC, which reduces their ability to reach the PVH (50). Changes in the density of intra-hypothalamic projections may also be related to altered sensitivity to hormones such as leptin, as described below (section 3.2). Consistent with reduced neurite extension, neural projections from the ARC to the PVH are markedly reduced in the offspring of diabetic (51), obese (38) or HFD-fed mothers (46). Furthermore, transcriptome analysis of ARC POMC neurons in offspring after maternal HFD exposure reveals significantly impaired spatial distribution and axonal extension, without altering POMC cell number in the ARC (52).

Changes in feeding-related neurocircuitry in offspring exposed to maternal obesity are not limited to projections between the ARC and PVH. A rat model has demonstrated increased intra-hypothalamic input to the LHA in exposed offspring that was associated with changes in leptin sensitivity (53), and a separate study in mice showed LHA neurons receiving synaptic input from the bed nucleus of the stria terminalis have enhanced excitatory input following exposure to maternal overnutrition. This persisting enhancement of excitatory drive within LHA circuitry could be an underlying cause of increased responsiveness to food cues in offspring of obese mothers (54).

A recent mouse study has demonstrated a previously unknown role for microglia in shaping hypothalamic development. Microglia transiently accumulate in the mediobasal hypothalamus during neonatal life, playing a key role in synaptic pruning and the refinement of perineuronal nets (PNNs) (55). Maternal HFD during lactation enhances microglial activation, leading to prolonged synaptic engulfment and PNN overabundance, which impairs the establishment of brain-to-pancreas circuits critical for insulin secretion in adulthood, whereas depleting microglia during PND6-16 results in an overabundance of PNNs and impaired glucose tolerance in adulthood (55). This shows that microglial sculpting of neuronal circuits during early life is a previously unappreciated critical regulator of metabolic programming, which can be impacted by maternal metabolic state.

In humans, it is not possible to visualise precise connections between different parts of the hypothalamus in living subjects. However, changes in AgRP projections between the ARC and PVH have been observed in offspring in a NHP model of maternal obesity (56). In humans, the only current data on changes in hypothalamic circuitry or anatomy comes from functional Magnetic Resonance Imaging (fMRI) analysis of hypothalamic microstructure. Mean diffusivity (MD) describes the amount of water diffusion within brain tissue and can be used to examine differences in brain structural integrity. In humans, hypothalamic MD in newborns is positively correlated with later childhood adiposity. A prospective longitudinal study in two independent cohorts of mother-infant pairs observed a positive linear association between maternal pre-pregnancy BMI and newborn offspring hypothalamic MD (57). While the prospective study design cannot formally establish causality, it may help disentangle the temporal sequence of effects, as offspring hypothalamic MD was characterized shortly after birth, before significant

exposure to the postnatal environment. Maternal circulating free fatty acid levels during pregnancy are also associated with newborn hypothalamic MD (58), demonstrating that maternal metabolic factors in addition to BMI can alter offspring hypothalamus.

2.3. Blood-brain barrier disruption

In adult mice fed a high-fat diet, one of the hypothalamic abnormalities that is thought to contribute to worsening energy homeostasis is damage to the BBB at the MEE–ARC interface. BBB permeability in the ARC is determined by capillary endothelial cells and tanycytes. Tight junctions between endothelial cells limit paracellular entry of blood-borne molecules into the brain, whereas endothelial cell transporters and fenestrations regulate transcellular entry. Tanycytes form a further barrier that prevents free diffusion of blood-borne molecules. BBB permeability is increased in neonatal offspring of obese dams, as assessed by Evans Blue diffusion into the ARC (59), possibly due to a reduced number of tanycytic processes in the ARC of offspring after exposure to maternal obesity. Interestingly, rodent models suggest that maternal obesity does not alter the total number of blood vessels irrigating the ARC but rather increases the proportion of permeable vessels (59). Obesity and diabetes during pregnancy are both associated with transient neonatal hypoglycemia at birth, and dips in blood glucose levels have been suggested to trigger the release by tanycytes of vascular endothelial growth factor (VEGF)-A, the main growth factor known to promote endothelial cell fenestration (60). Therefore increased tanycytic release of VEGF could explain the changes in BBB permeability seen after birth in offspring from pregnancies complicated by maternal obesity or diabetes. A further cause of disruption to the offspring BBB could be increased circulating fatty acids, which have been shown to promote functional and structural changes in the MEE (61). The consequences of disrupted BBB integrity are long-lasting. Not only might disrupted BBB contribute to the development of resistance to circulating hormone signals such as leptin and insulin (discussed further below), altered integrity of BBB is also associated with long-term metabolic changes. Interestingly, cross-fostering of neonatal offspring of obese mice to a lean dam is able to rescue both the disruption of BBB and some accompanying metabolic phenotypes (62), demonstrating that any programmed damage is reversible.

3. Mechanisms mediating the effects of maternal obesity on offspring hypothalamus

Changes to the normal metabolic milieu of pregnancy can influence offspring hypothalamic development, modulating hypothalamic function and elevating the risk of metabolic disorders in children. In order to stop the inter-generational transmission of obesity risk, researchers must first identify the molecular mechanisms mediating disrupted hypothalamic development and thus changes to long-term hypothalamic function. This section will discuss the potential underlying mechanisms affecting hypothalamic development in offspring due to maternal obesity.

3.1. Epigenetic modifications

Fetal development is a critical window for epigenetic programming. Epigenetic modifications such as DNA methylation, histone modifications, and the activity of small non-coding RNAs have been proposed as a mechanism linking maternal obesity and offspring development, and as a critical mechanism for early neurodevelopmental reprogramming (63). The correction of maternal obesity pre-pregnancy by bariatric surgery results in a change in methylation status of key genes required for glucose homeostasis in offspring (64). Furthermore, evidence is emerging that epigenetic marks can persist over multiple generations (65), thus contributing to trans-generational transmission of obesity risk.

Evidence from both human and animal studies suggests the *Pomc* gene is particularly susceptible to epigenetic regulation in the peri-natal period (66, 67). Exposure to a HFD during gestation can induce permanent hypermethylation in the *Pomc* promoter and enhancer

regions, resulting in altered integration of leptin signalling in the hypothalamus (68) and suppression of satiety response (69). These epigenetic alterations are associated with weight gain, increased adiposity, and insulin resistance. Changes in DNA methylation may also be a source of sex differences in the offspring phenotype in response to maternal obesity (70). A recent study in rats demonstrated that hypothalamic insulin receptor expression and DNA promoter methylation are sex-specifically altered in adult offspring of HFD-fed mothers, with males showing more pronounced changes in methylation that are associated with insulin resistance and metabolic dysfunction (71).

Micro-RNAs (miRNAs) are small non-coding RNAs that post-transcriptionally modify target gene expression. miRNAs are a promising candidate for translating dynamic changes in nutritional state into changes in genomic regulation in offspring. A recent human study has shown significant differences in the expression of candidate miRNAs in circulating blood samples of newborn offspring dependent on maternal BMI (72) or GDM (73). Studies in animals have begun to explore and demonstrate causative roles for altered miRNA expression in mediating some of the effects of maternal obesity on offspring phenotype. Exposure to maternal obesity causes upregulation of miR-505-5p in the fetal hypothalamus, and downregulation of miR-505-5p targets that are related to fatty acid sensing pathways (29). Over-expression of miR-505-5p in the adult mouse brain causes increased intake of a high-fat diet, suggesting that programmed over-expression of this miRNA resulting in altered hypothalamic fatty acid sensing could underlie altered food choices in offspring that lead to obesity. Interestingly, increased expression of miR-505-5p is rescued in a model of maternal exercise that improves insulin sensitivity without weight loss (29), suggesting that targeting maternal and/or fetal insulin levels is a potential intervention strategy to mitigate the long-term health risks faced by offspring.

3.2. Hormonal disruptions: insulin, leptin, and ghrelin

Pregnancy is a time of high energy demand for the mother, and as a result the body makes a series of metabolic adjustments in pregnancy to support the growing fetus. Many metabolic hormones are altered in obesity and thus remain altered- or are further dysregulated- in a pregnancy complicated by obesity or GDM. Some of these key metabolic hormones have dual roles in coordinating neurodevelopment of the hypothalamus. Therefore, changes in hormone levels in the mother and fetus can impact hypothalamic development and result in permanent metabolic dysfunction in offspring.

3.2.1 Leptin

Leptin is a key anorexigenic hormone that plays an integral role in energy balance by acting on the hypothalamus to modulate food intake and energy expenditure. Leptin signalling suppresses NPY/AgRP neurons that stimulate hunger, activates POMC neurons that drive satiety and provides a negative feedback system that balances energy intake and expenditure. Beyond energy homeostasis, leptin is critical for neuronal development, particularly in circuitry related to hypothalamic regulation of feeding behaviour. The formation of projections from the ARC to the PVH during development is a critical process regulated by leptin. In rodents, a post-natal increase in leptin levels is required for the proper development of ARC-PVH projections (32). Modulation of offspring post-natal leptin levels by maternal obesity and/or postnatal overnutrition can impact the proper wiring of the feeding circuits (74, 75). Interestingly, in leptin-deficient ($Lep^{ob/ob}$) mice, exogenous leptin treatment can restore these projections when administered only during a short window of the postnatal developmental period, emphasising the requirement of temporal specificity for leptin action on proper hypothalamic connectivity (76). This crucial window for establishing ARC-PVH projections is defined by the formation of perineuronal nets (77). However, whether the formation of perineuronal nets is altered with exposure to maternal obesity remains to be investigated. Molecular studies have shown that leptin promotes neurite formation by promoting the

formation of axonal growth cones in neurons (78). Leptin regulates the development of feeding circuits in the hypothalamus through a specific leptin receptor long isoform (LepRb), with distinct signalling pathways downstream of the receptor guiding the formation of NPY and POMC projections to different PVH neuronal compartments (79). The involvement of leptin in establishing intra-hypothalamic circuitry is a likely source of nutrition-mediated disruption to these projections, particularly as maternal obesity leads to hypothalamic leptin resistance in postnatal offspring (49). Leptin is also responsible for the formation of external feeding circuits into the hypothalamus, such as the GLP1R positive neurons originating in the NTS that innervate the PVH (80), but whether these extra-hypothalamic circuits are regulated in response to fetal nutrition are unknown.

3.2.2 Insulin

Insulin, like leptin, exhibits both metabolic and neuromodulatory functions. Insulin is produced by the pancreas and in the fetal brain and is vital for neuroplasticity; variations in insulin sensitivity due to age or metabolic conditions result in changes to neuronal structure and function. Insulin plays a crucial role in neuronal differentiation and synapse formation during fetal development. Early research demonstrated that insulin stimulates neurite outgrowth in cultured neurons (81, 82). Insulin receptor activation triggers downstream signalling pathways, including the PI3K-Akt and MAPK pathways, which affect neuronal growth, survival, and energy balance (83). It is therefore significant that human fetuses from pregnancies complicated by maternal GDM or obesity exhibit whole body insulin resistance in utero (84). Rodent models have shown that maternal obesity causes insulin resistance specifically in the fetal hypothalamus. This may be an underlying cause of structural and functional alteration in the hypothalamus (38, 85), as well as in other circuits, such as the dopaminergic system, which regulates energy and glucose homeostasis (86). High insulin levels in the offspring hypothalamus do not only cause problems in the fetal brain, persistent hypothalamic hyperinsulinemia during the neonatal period results in lasting malformations in hypothalamic nuclei, which are associated with alterations in insulin receptor signalling and glucose homeostasis (87). A study by Vogt et al. demonstrated that genetic knock-out of the insulin receptor on POMC neurons rescues the reduced projections of these neurons to the PVH in offspring exposed to maternal over-nutrition, suggesting that insulin signalling in the neonatal period is at least in part responsible for reduced POMC connectivity between the ARC and PVH, and associated dysglycemia (46).

3.2.3 Ghrelin

Ghrelin, secreted from the stomach, is well characterised as a stimulant of appetite and feeding behaviour through its action at growth hormone secretagogue receptors in the ARC. In normal pregnancy, ghrelin levels typically peak during mid-gestation and then decline (88, 89). However, in cases of maternal obesity, these levels may be lower or display varying patterns (90). Previous studies have shown that maternal ghrelin is closely linked to fetal birthweight (91).

Research has indicated that ghrelin is required to restrict the formation of projections from the ARC to the PVH, and therefore loss of ghrelin signalling during the post-natal period in rodents results in increased density of projections between the ARC and PVH and associated metabolic dysfunction (92). Furthermore, the study also showed that direct exposure of postnatal ARC neuronal explants to ghrelin inhibited axonal growth and obstructed the neurotrophic effect of leptin. Interestingly, the effects of neonatal ghrelin on body weight regulation seem to be sex-dependent, as treatment with ghrelin in female rats and anti-ghrelin in female mice does not lead to weight changes. Postnatal ghrelin resistance—characterised by reduced c-fos activation in NPY/AgRP neurons following ghrelin administration, has been

associated with maternal obesity and postnatal overnutrition (93). Given that ghrelin signalling is implicated in the neonatal programming of metabolism, these alterations in the ghrelin system may disrupt the normal regulation of feeding and energy balance in mice subjected to postnatal overnutrition.

3.3. ER stress and unfolded protein response

Metabolic and nutritional factors, such as elevated glucose and lipid levels, can lead to endoplasmic reticulum (ER) stress (94, 95). Recent studies have demonstrated that maternal obesity induces hypothalamic ER stress in offspring (96) and that this is causally associated with disrupted energy homeostasis (49). Studies utilising hypothalamic explants show that saturated fatty acids such as palmitic, lauric, and myristic acids- which are increased in high-fat diets and in animals consuming them- are a key driver of ER stress in neonatal hypothalamic cells. The observation that administration of the ER stress-relieving drug tauroursodeoxycholic acid (TUDCA) rescues the reduction of neurite formation caused by saturated fatty acids (42), suggests that fatty acid-induced ER stress is a key mechanism by which perinatal nutrition impacts on hypothalamic circuitry.

Maternal obesity also disrupts the interaction between two vital stress response pathways: the unfolded protein response (UPR) and the heat shock response (HSR) (97). The UPR is activated by the buildup of misfolded proteins in the ER, where it helps regulate protein homeostasis by enhancing folding capacity and limiting the synthesis of new proteins. Conversely, the HSR serves to protect cells from heat-induced protein denaturation and has a role in numerous neuronal cellular functions, including acting as a mediator in neuronal axon guidance (98). Offspring exposed to maternal obesity have a disruption of these protective cellular responses, resulting in chronic hypothalamic ER stress, which is associated with altered intra-hypothalamic projections and hyperphagia (97). Interestingly, research has shown that administration of 4-phenyl butyrate (PBA), a protein-folding chemical chaperone and UPR activator from PND 4, can rescue metabolic outcomes in the offspring of obese mothers (96). This suggests that modulating either ER stress or the UPR in offspring in the post-natal period could mitigate some of the negative effects of maternal obesity.

3.4. Neuroinflammation

ER stress is a key factor in triggering neuroinflammation, a process that can exacerbate the effects of maternal obesity on the offspring's hypothalamus. The activation of stress pathways, including NF- κ B and JNK, during ER stress, can promote glial cells to release inflammatory mediators such as IL-6 (99) and IKK β /NF- κ B in the hypothalamus, which may be a driver of central insulin and leptin resistance (49, 100, 101). Many studies have shown that higher maternal BMI is linked to increased placental production of pro-inflammatory cytokines, such as IL-6 and TNF- α (102). These cytokines may cross the placental barrier and access the developing fetal brain, activating glial cells, including microglia and astrocytes (103, 104). This activation initiates a cascade of inflammatory responses that may impair neuronal maturation, cause neuronal damage, and modify neural circuitry (102, 105). Gliosis refers to the brain's response to local inflammation, which is characterised by increased proliferation of glial cells. Studies in both humans and animals suggest that hypothalamic gliosis is linked to obesity and related metabolic dysfunction. Children exposed to GDM *in utero* display evidence of hypothalamic gliosis after adjusting for child's age, sex, BMI z score and maternal pre-pregnancy BMI (106) (101). In these studies the evidence of gliosis was limited to the mediobasal hypothalamus, highlighting the vulnerability of the developing hypothalamus, and independent of current child adiposity.

In an NHP model of maternal obesity, an inflammatory milieu has been shown to disrupt the development of hypothalamic melanocortin circuits involved in appetite and energy homeostasis, increasing the offspring's susceptibility to obesity, insulin resistance, and other

metabolic diseases (104). Pro-inflammatory cytokines, such as IL-6, have been shown to play a critical role in hypothalamic development. Sanders et al. (2014) demonstrated that maternal obesity results in increased IL-6 levels, which can deregulate gene expression in the ARC of the hypothalamus, as well as disrupt intra-hypothalamic connections to other hypothalamic regions (50). Furthermore, IL-6 regulates neurite outgrowth, affecting the formation of normal neuronal connectivity in the hypothalamus, which is essential for the proper functioning of feeding circuits.

Dietary factors further exacerbate the impact of maternal obesity on hypothalamic development and inflammation. Pimentel et al. (2015) revealed that maternal consumption of trans fatty acids during pregnancy and lactation stimulates hypothalamic inflammation in offspring through the TLR4/NF κ Bp65 signalling pathway (107). Trans fatty acids, commonly found in industrial food products, activate TLR4 receptors in the hypothalamus, triggering an inflammatory cascade that activates microglia and astrocytes. This inflammatory stimulus alters the development of hypothalamic circuits responsible for feeding behaviour, compromising the offspring's ability to regulate food intake and energy expenditure. These findings underscore the importance of not only maternal weight, but also maternal diet as a key regulator of the inflammatory environment surrounding the developing brain and its long-term metabolic consequences. However, from current studies, it is not yet clear how much neuroinflammation occurs as a direct consequence of maternal factors on the offspring developing hypothalamus, and how much is secondary to other metabolic changes in the offspring.

3.5. Gut microbiota and the gut-brain axis

The gut microbiota, a significant player in the gut-brain axis, is increasingly being recognised as a potential mechanism through which maternal obesity can impact on offspring long-term metabolic control. Recent papers have thus addressed the consequences of changes to the microbiome on the offspring brain. A recent study in rodents demonstrated that microbiota alterations are correlated with brain metabolites, particularly those associated with metabolism commonly found in the hypothalamus (108). Even short-term changes in maternal diet-induced microbiota are linked to metabolic alterations and behavioural disturbances observed in adolescent offspring, suggesting a potential interaction between microbiota and the hypothalamus that leads to enduring changes associated with feeding behaviour and metabolism (108).

Maternal consumption of low-calorie sweeteners (LCS) also leads to gut microbiota alterations that affect hypothalamic melanocortin circuits in offspring (109). Phenylacetylglutamine (PAG), a gut microbial co-metabolite, was identified as a mediator of these effects. Maternal LCS intake resulted in a rewiring of the hypothalamic melanocortin system, characterised by reduced POMC fibre density and increased AgRP fibre density in male offspring. These alterations were associated with increased adiposity, glucose intolerance and disrupted parasympathetic innervation of pancreatic β -cells in adult male offspring. Interestingly, these alterations were not observed in female offspring, indicating sex-specific vulnerabilities to changes in gut microbiota.

Considering the potential significance of maternal microbiota in influencing fetal brain development, the specific category of gut bacteria that impacts brain metabolism necessitates further investigation. Administration of the maternal microbiota *Bifidobacterium breve* to germ-free mice resulted in alterations to fetal brain metabolism and alleviated the detrimental effects of maternal obesity (110). It remains unclear whether this occurs via a direct effect of microbiota supplementation on the fetal brain or via a change to maternal physiology. However, this finding highlighted the importance of recognising potential protective bacteria

within the gut that contribute to the preservation of fetal brain function in the context of maternal obesity.

4. Conclusion and future directions

Strong evidence suggests that maternal obesity both with and without GDM programs lasting changes in metabolic regulation in offspring. As shown in Figure 1 (left panel), the data available from human and animal studies show that maternal obesity in pregnancy is associated with epigenetic modifications, altered hormonal signalling, and ER stress/neuroinflammation and that these factors together cause changes to the development of hypothalamic feeding pathways (Figure 1, right panel) which drive later obesity. The studies presented here together highlight the critical influence of the intrauterine environment on lifelong health outcomes.

Despite significant advancements in understanding these mechanisms in the last decade, key questions remain unanswered. The extent to which these effects can be reversed through interventions targeting the mother or offspring is still unclear, as is the timing that any interventions would need to occur (pre-pregnancy, during pregnancy or in offspring after birth). While emerging research suggests that dietary modifications, physical activity, and microbiome-targeted therapies may mitigate some of these adverse effects, further studies are needed to determine their efficacy, timing, and long-term impact. The DOHaD field has only recently beginning to address sex-specific differences in susceptibility to metabolic programming (70) and further research into the hormonal and genetic influences that may shape differential responses to maternal obesity will be critical in developing effective intervention strategies.

Whilst many studies have focused on feeding behaviour and the projection of AgRP/ NPY and POMC neurons from the ARC to the PVH, relatively few studies have examined the effect of the peri-natal environment on other hypothalamic areas or other energy homeostatic processes, e.g. the circuitry required for hypothalamic glucose sensing. Advanced techniques like single-cell RNA sequencing will help to identify in an unbiased way the non-traditional hypothalamic cell types that are altered by changes in perinatal nutrition. Although we know astrocytes are impacted in addition to neurons (section 2.1.2), limited studies have examined the impact of the peri-natal environment on other glial cell types within the fetal hypothalamus such as oligodendrocytes or tanycytes. Maternal HFD has been shown to reduce both immature and mature oligodendrocytes in the prefrontal cortex in adult offspring (111), but the effects on hypothalamic oligodendrocytes are yet to be examined. The shared developmental origin of neuronal cell types and the emerging essential role of glial cells in maintaining energy homeostasis makes them an important target for future research.

Another critical area for future research is the role of extracellular vesicles (EVs) in mediating maternal-fetal crosstalk. EVs, which carry bioactive molecules such as proteins, lipids, and miRNAs, have emerged as key mediators of intercellular communication. During pregnancy, the placenta is a source of EVs that have been proposed to act on both the maternal (114) and fetal (115) brain. Given the recent report that EVs contain neural components such as potential trophic factors that could have a role in neurodevelopment (116), understanding whether the mechanisms of EV release and cargo of the vesicles are altered in the context of maternal obesity should be a key priority of future research and could reveal both new biomarkers for predicting offspring metabolic risk and inform interventions to block harmful signals.

The potential for trans-generational transmission of metabolic risk through epigenetic inheritance also warrants further exploration. Identifying whether these epigenetic marks can persist across generations- independent of associated phenotypes and gametes that are

exposed due to their development in-utero- and whether targeted interventions in early life can prevent their transmission will be crucial for designing effective prevention strategies.

In conclusion, improving the health of mothers before and during pregnancy is essential not only for improving obstetric outcomes, but also for breaking the intergenerational cycle of obesity and metabolic disorders that contribute to the trapping of families within unhealthy lifestyles. A multidisciplinary approach, integrating insights from neuroscience, endocrinology, and public health, will be vital in developing targeted interventions that optimise fetal hypothalamic development and therefore long-term metabolic health. By bridging the gaps in knowledge and translating mechanistic research from animal models into clinical and public health strategies, we can work toward improving health trajectories for future generations.

References

1. Okunogbe A, Nugent R, Spencer G, Powis J, Ralston J, Wilding J. Economic impacts of overweight and obesity: current and future estimates for 161 countries. *BMJ Glob Health*. 2022;7(9).
2. Hall KD. From dearth to excess: the rise of obesity in an ultra-processed food system. *Philos Trans R Soc Lond B Biol Sci*. 2023;378(1885):20220214.
3. Nettle D, Andrews C, Bateson M. Food insecurity as a driver of obesity in humans: The insurance hypothesis. *Behav Brain Sci*. 2017;40:e105.
4. Bateson M, Pepper GV. Food insecurity as a cause of adiposity: evolutionary and mechanistic hypotheses. *Philos Trans R Soc Lond B Biol Sci*. 2023;378(1888):20220228.
5. Egger GJ, Vogels N, Westerterp KR. Estimating historical changes in physical activity levels. *Med J Aust*. 2001;175(11-12):635-6.
6. Westerterp KR, Speakman JR. Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. *Int J Obes (Lond)*. 2008;32(8):1256-63.
7. Speakman JR, de Jong JMA, Sinha S, Westerterp KR, Yamada Y, Sagayama H, et al. Total daily energy expenditure has declined over the past three decades due to declining basal expenditure, not reduced activity expenditure. *Nat Metab*. 2023;5(4):579-88.
8. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197-206.
9. Loos RJF, Janssens ACJW. Predicting Polygenic Obesity Using Genetic Information. *Cell Metab*. 2017;25(3):535-43.
10. Schrepft S, van Jaarsveld CHM, Fisher A, Herle M, Smith AD, Fildes A, et al. Variation in the Heritability of Child Body Mass Index by Obesogenic Home Environment. *JAMA Pediatr*. 2018;172(12):1153-60.
11. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;303(6809):1019-22.
12. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*. 1993;36(1):62-7.

13. Schulz LC. The Dutch Hunger Winter and the developmental origins of health and disease. *Proc Natl Acad Sci U S A*. 2010;107(39):16757-8.
14. Perng W, Oken E, Dabelea D. Developmental overnutrition and obesity and type 2 diabetes in offspring. *Diabetologia*. 2019;62(10):1779-88.
15. England N. Maternity services dataset 2.0 2024. 2025.
16. Digita N. National Pregnancy in Diabetes Audit. 2025.
17. Abdi F, Alizadeh S, Roozbeh N, Montazeri F, Banaei M, Mehrnoush V, et al. Prepregnancy overweight and obesity and the risk of adverse pregnancy outcomes. *Sci Rep*. 2025;15(1):16846.
18. Digital N. National Child Measurement Programme, England, 2021/22 school year. 2022.
19. Heslehurst N, Vieira R, Akhter Z, Bailey H, Slack E, Ngongalah L, et al. The association between maternal body mass index and child obesity: A systematic review and meta-analysis. *PLoS Med*. 2019;16(6):e1002817.
20. Smith J, Cianflone K, Biron S, Hould FS, Lebel S, Marceau S, et al. Effects of maternal surgical weight loss in mothers on intergenerational transmission of obesity. *J Clin Endocrinol Metab*. 2009;94(11):4275-83.
21. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes*. 2000;49(12):2208-11.
22. Luo S, Angelo B, Chow T, Monterosso JR, Xiang AH, Thompson PM, et al. The role of maternal BMI on brain food cue reactivity in children: a preliminary study. *Brain Imaging Behav*. 2021;15(6):2746-55.
23. Page KA, Luo S, Wang X, Chow T, Alves J, Buchanan TA, et al. Children Exposed to Maternal Obesity or Gestational Diabetes Mellitus During Early Fetal Development Have Hypothalamic Alterations That Predict Future Weight Gain. *Diabetes Care*. 2019;42(8):1473-80.
24. Luo S, Angelo BC, Chow T, Monterosso JR, Thompson PM, Xiang AH, et al. Associations Between Exposure to Gestational Diabetes Mellitus In Utero and Daily Energy Intake, Brain Responses to Food Cues, and Adiposity in Children. *Diabetes Care*. 2021;44(5):1185-93.
25. Purcell RH, Sun B, Pass LL, Power ML, Moran TH, Tamashiro KL. Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. *Physiol Behav*. 2011;104(3):474-9.
26. Kojima S, Catavero C, Rinaman L. Maternal high-fat diet increases independent feeding in pre-weanling rat pups. *Physiol Behav*. 2016;157:237-45.
27. Rivera HM, Kievit P, Kirigiti MA, Bauman LA, Baquero K, Blundell P, et al. Maternal high-fat diet and obesity impact palatable food intake and dopamine signaling in nonhuman primate offspring. *Obesity (Silver Spring)*. 2015;23(11):2157-64.
28. Naef L, Moquin L, Dal Bo G, Giros B, Gratton A, Walker CD. Maternal high-fat intake alters presynaptic regulation of dopamine in the nucleus accumbens and increases motivation for fat rewards in the offspring. *Neuroscience*. 2011;176:225-36.
29. Dearden L, Furigo IC, Pantaleao LC, Wong LWP, Fernandez-Twinn DS, de Almeida-Faria J, et al. Maternal obesity increases hypothalamic miR-505-5p expression in mouse offspring leading to altered fatty acid sensing and increased intake of high-fat food. *PLoS Biol*. 2024;22(6):e3002641.
30. Padilla SL, Carmody JS, Zeltser LM. Pomc-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits. *Nat Med*. 2010;16(4):403-5.
31. Ishii Y, Bouret SG. Embryonic birthdate of hypothalamic leptin-activated neurons in mice. *Endocrinology*. 2012;153(8):3657-67.
32. Bouret SG, Draper SJ, Simerly RB. Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci*. 2004;24(11):2797-805.

33. Grayson BE, Allen SE, Billes SK, Williams SM, Smith MS, Grove KL. Prenatal development of hypothalamic neuropeptide systems in the nonhuman primate. *Neuroscience*. 2006;143(4):975-86.
34. Alcantara IC, Tapia APM, Aponte Y, Krashes MJ. Acts of appetite: neural circuits governing the appetitive, consummatory, and terminating phases of feeding. *Nat Metab*. 2022;4(7):836-47.
35. van der Klaauw AA, Croizier S, Mendes de Oliveira E, Stadler LKJ, Park S, Kong Y, et al. Human Semaphorin 3 Variants Link Melanocortin Circuit Development and Energy Balance. *Cell*. 2019;176(4):729-42.e18.
36. Gervais M, Labouèbe G, Picard A, Thorens B, Croizier S. EphrinB1 modulates glutamatergic inputs into POMC-expressing progenitors and controls glucose homeostasis. *PLoS Biol*. 2020;18(11):e3000680.
37. Alvarez-Bolado G, Paul FA, Blaess S. Sonic hedgehog lineage in the mouse hypothalamus: from progenitor domains to hypothalamic regions. *Neural Dev*. 2012;7:4.
38. Dearden L, Buller S, Furigo IC, Fernandez-Twinn DS, Ozanne SE. Maternal obesity causes fetal hypothalamic insulin resistance and disrupts development of hypothalamic feeding pathways. *Mol Metab*. 2020;42:101079.
39. Lemes SF, de Souza ACP, Payolla TB, Versutti MD, de Fátima da Silva Ramalho A, Mendes-da-Silva C, et al. Maternal Consumption of High-fat Diet in Mice Alters Hypothalamic Notch Pathway, NPY Cell Population and Food Intake in Offspring. *Neuroscience*. 2018;371:1-15.
40. Kokoeva MV, Yin H, Flier JS. Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. *Science*. 2005;310(5748):679-83.
41. Xu Y, Yang D, Wang L, Król E, Mazidi M, Li L, et al. Maternal High Fat Diet in Lactation Impacts Hypothalamic Neurogenesis and Neurotrophic Development, Leading to Later Life Susceptibility to Obesity in Male but Not Female Mice. *Adv Sci (Weinh)*. 2023;10(35):e2305472.
42. Moraes JC, Coope A, Morari J, Cintra DE, Roman EA, Pauli JR, et al. High-fat diet induces apoptosis of hypothalamic neurons. *PLoS One*. 2009;4(4):e5045.
43. de la Pompa JL, Wakeham A, Correia KM, Samper E, Brown S, Aguilera RJ, et al. Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development*. 1997;124(6):1139-48.
44. Aujla PK, Naratadam GT, Xu L, Raetzman LT. Notch/Rbpjk signaling regulates progenitor maintenance and differentiation of hypothalamic arcuate neurons. *Development*. 2013;140(17):3511-21.
45. McNay DE, Pelling M, Claxton S, Guillemot F, Ang SL. Mash1 is required for generic and subtype differentiation of hypothalamic neuroendocrine cells. *Mol Endocrinol*. 2006;20(7):1623-32.
46. Vogt MC, Paeger L, Hess S, Steculorum SM, Awazawa M, Hampel B, et al. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. *Cell*. 2014;156(3):495-509.
47. Kim DW, Glendinning KA, Grattan DR, Jasoni CL. Maternal obesity leads to increased proliferation and numbers of astrocytes in the developing fetal and neonatal mouse hypothalamus. *Int J Dev Neurosci*. 2016;53:18-25.
48. Huang Y, Wang A, Zhou W, Li B, Zhang L, Rudolf AM, et al. Maternal dietary fat during lactation shapes single nucleus transcriptomic profile of postnatal offspring hypothalamus in a sexually dimorphic manner in mice. *Nat Commun*. 2024;15(1):2382.
49. Park S, Jang A, Bouret SG. Maternal obesity-induced endoplasmic reticulum stress causes metabolic alterations and abnormal hypothalamic development in the offspring. *PLoS Biol*. 2020;18(3):e3000296.
50. Sanders TR, Kim DW, Glendinning KA, Jasoni CL. Maternal obesity and IL-6 lead to aberrant developmental gene expression and deregulated neurite growth in the fetal arcuate nucleus. *Endocrinology*. 2014;155(7):2566-77.

51. Steculorum SM, Bouret SG. Maternal diabetes compromises the organization of hypothalamic feeding circuits and impairs leptin sensitivity in offspring. *Endocrinology*. 2011;152(11):4171-9.
52. Haddad-Tóvolli R, Altirriba J, Obri A, Sánchez EE, Chivite I, Milà-Guasch M, et al. Pro-opiomelanocortin (POMC) neuron translational signatures underlying obesogenic gestational malprogramming in mice. *Mol Metab*. 2020;36:100963.
53. Kelley L, Verlezza S, Long H, Loka M, Walker CD. Increased Hypothalamic Projections to the Lateral Hypothalamus and Responses to Leptin in Rat Neonates From High Fat Fed Mothers. *Front Neurosci*. 2019;13:1454.
54. Shrivastava K, Swaminathan T, Barlotta A, Athreya V, Choudhry H, Rossi MA. Maternal overnutrition is associated with altered synaptic input to lateral hypothalamic area. *Mol Metab*. 2023;71:101702.
55. Valdearcos M, McGrath ER, Brown Mayfield SM, Jacuinde MG, Folick A, Cheang RT, et al. Microglia mediate the early-life programming of adult glucose control. *Cell Rep*. 2025;44(3):115409.
56. Sullivan EL, Rivera HM, True CA, Franco JG, Baquero K, Dean TA, et al. Maternal and postnatal high-fat diet consumption programs energy balance and hypothalamic melanocortin signaling in nonhuman primate offspring. *Am J Physiol Regul Integr Comp Physiol*. 2017;313(2):R169-R79.
57. Rasmussen JM, Tuulari JJ, Nölvi S, Thompson PM, Merisaari H, Lavonius M, et al. Maternal pre-pregnancy body mass index is associated with newborn offspring hypothalamic mean diffusivity: a prospective dual-cohort study. *BMC Med*. 2023;21(1):57.
58. Rasmussen JM, Thompson PM, Gyllenhammer LE, Lindsay KL, O'Connor TG, Koletzko B, et al. Maternal free fatty acid concentration during pregnancy is associated with newborn hypothalamic microstructure in humans. *Obesity (Silver Spring)*. 2022;30(7):1462-71.
59. Kim DW, Glendinning KA, Grattan DR, Jasoni CL. Maternal Obesity in the Mouse Compromises the Blood-Brain Barrier in the Arcuate Nucleus of Offspring. *Endocrinology*. 2016;157(6):2229-42.
60. Duquenne M, Dehouck B, Prevot V. Programming the Brain from the Womb: Maternal Obesity Perturbs the Hypothalamic Blood-Brain Barrier. *Endocrinology*. 2016;157(6):2201-3.
61. Ramalho AF, Bombassaro B, Dragano NR, Solon C, Morari J, Fioravante M, et al. Dietary fats promote functional and structural changes in the median eminence blood/spinal fluid interface-the protective role for BDNF. *J Neuroinflammation*. 2018;15(1):10.
62. Haddad-Tóvolli R, Morari J, Barbizan R, Bóbbó VC, Carraro RS, Solon C, et al. Maternal obesity damages the median eminence blood-brain barrier structure and function in the progeny: the beneficial impact of cross-fostering by lean mothers. *Am J Physiol Endocrinol Metab*. 2023;324(2):E154-E66.
63. Bale TL. Epigenetic and transgenerational reprogramming of brain development. *Nat Rev Neurosci*. 2015;16(6):332-44.
64. Guenard F, Deshaies Y, Cianflone K, Kral JG, Marceau P, Vohl MC. Differential methylation in glucoregulatory genes of offspring born before vs. after maternal gastrointestinal bypass surgery. *Proc Natl Acad Sci U S A*. 2013;110(28):11439-44.
65. Tuscher JJ, Day JJ. Multigenerational epigenetic inheritance: One step forward, two generations back. *Neurobiol Dis*. 2019;132:104591.
66. Candler T, Kuhnen P, Prentice AM, Silver M. Epigenetic regulation of POMC; implications for nutritional programming, obesity and metabolic disease. *Front Neuroendocrinol*. 2019;54:100773.
67. Reynolds CM, Segovia SA, Vickers MH. Experimental Models of Maternal Obesity and Neuroendocrine Programming of Metabolic Disorders in Offspring. *Front Endocrinol (Lausanne)*. 2017;8:245.

68. Gali Ramamoorthy T, Allen TJ, Davies A, Harno E, Sefton C, Murgatroyd C, et al. Maternal overnutrition programs epigenetic changes in the regulatory regions of hypothalamic *Pomc* in the offspring of rats. *Int J Obes (Lond)*. 2018;42(8):1431-44.
69. Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, et al. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J Physiol*. 2009;587(Pt 20):4963-76.
70. Dearden L, Bouret SG, Ozanne SE. Sex and gender differences in developmental programming of metabolism. *Mol Metab*. 2018;15:8-19.
71. Schellong K, Melchior K, Ziska T, Ott R, Henrich W, Rancourt RC, et al. Hypothalamic insulin receptor expression and DNA promoter methylation are sex-specifically altered in adult offspring of high-fat diet (HFD)-overfed mother rats. *J Nutr Biochem*. 2019;67:28-35.
72. Sørensen AE, van Poppel MNM, Desoye G, Simmons D, Damm P, Jensen DM, et al. The Temporal Profile of Circulating miRNAs during Gestation in Overweight and Obese Women with or without Gestational Diabetes Mellitus. *Biomedicines*. 2022;10(2).
73. Serati A, Novielli C, Anelli GM, Mandalari M, Parisi F, Cetin I, et al. Characterization of Maternal Circulating MicroRNAs in Obese Pregnancies and Gestational Diabetes Mellitus. *Antioxidants (Basel)*. 2023;12(2).
74. Skowronski AA, Shaulson ED, Leibel RL, LeDuc CA. The postnatal leptin surge in mice is variable in both time and intensity and reflects nutritional status. *Int J Obes (Lond)*. 2022;46(1):39-49.
75. Kirk SL, Samuelsson AM, Argenton M, Dhonye H, Kalamatianos T, Poston L, et al. Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. *PLoS One*. 2009;4(6):e5870.
76. Kamitakahara A, Bouyer K, Wang CH, Simerly R. A critical period for the trophic actions of leptin on AgRP neurons in the arcuate nucleus of the hypothalamus. *J Comp Neurol*. 2018;526(1):133-45.
77. Mirzadeh Z, Alonge KM, Cabrales E, Herranz-Perez V, Scarlett JM, Brown JM, et al. Perineuronal Net Formation during the Critical Period for Neuronal Maturation in the Hypothalamic Arcuate Nucleus. *Nat Metab*. 2019;1(2):212-21.
78. Valerio A, Ghisi V, Dossena M, Tonello C, Giordano A, Frontini A, et al. Leptin increases axonal growth cone size in developing mouse cortical neurons by convergent signals inactivating glycogen synthase kinase-3beta. *J Biol Chem*. 2006;281(18):12950-8.
79. Bouret SG, Bates SH, Chen S, Myers MG, Jr., Simerly RB. Distinct roles for specific leptin receptor signals in the development of hypothalamic feeding circuits. *J Neurosci*. 2012;32(4):1244-52.
80. Biddinger JE, Lazarenko RM, Scott MM, Simerly R. Leptin suppresses development of GLP-1 inputs to the paraventricular nucleus of the hypothalamus. *Elife*. 2020;9.
81. Recio-Pinto E, Rechler MM, Ishii DN. Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J Neurosci*. 1986;6(5):1211-9.
82. Schechter R, Abboud M, Johnson G. Brain endogenous insulin effects on neurite growth within fetal rat neuron cell cultures. *Brain Res Dev Brain Res*. 1999;116(2):159-67.
83. Chiu SL, Cline HT. Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev*. 2010;5:7.
84. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care*. 2009;32(6):1076-80.
85. Gomes RM, Bueno FG, Schamber CR, de Mello JCP, de Oliveira JC, Francisco FA, et al. Maternal diet-induced obesity during suckling period programs offspring obese phenotype and hypothalamic leptin/insulin resistance. *J Nutr Biochem*. 2018;61:24-32.

86. Vogt MC, Bruning JC. CNS insulin signaling in the control of energy homeostasis and glucose metabolism - from embryo to old age. *Trends Endocrinol Metab.* 2013;24(2):76-84.
87. Plagemann A, Harder T, Rake A, Melchior K, Rittel F, Rohde W, et al. Hypothalamic insulin and neuropeptide Y in the offspring of gestational diabetic mother rats. *Neuroreport.* 1998;9(18):4069-73.
88. Mirlesse V, Frankenne F, Alsat E, Poncelet M, Hennen G, Evain-Brion D. Placental growth hormone levels in normal pregnancy and in pregnancies with intrauterine growth retardation. *Pediatr Res.* 1993;34(4):439-42.
89. Fuglsang J, Skjaerbaek C, Espelund U, Frystyk J, Fisker S, Flyvbjerg A, et al. Ghrelin and its relationship to growth hormones during normal pregnancy. *Clin Endocrinol (Oxf).* 2005;62(5):554-9.
90. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes.* 2001;50(4):707-9.
91. Nakahara K, Nakagawa M, Baba Y, Sato M, Toshinai K, Date Y, et al. Maternal ghrelin plays an important role in rat fetal development during pregnancy. *Endocrinology.* 2006;147(3):1333-42.
92. Steculetory SM, Collden G, Coupe B, Croizier S, Lockie S, Andrews ZB, et al. Neonatal ghrelin programs development of hypothalamic feeding circuits. *J Clin Invest.* 2015;125(2):846-58.
93. Collden G, Balland E, Parkash J, Caron E, Langlet F, Prevot V, et al. Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin. *Mol Metab.* 2015;4(1):15-24.
94. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, et al. Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab.* 2009;9(1):35-51.
95. Schneeberger M, Gomez-Valades AG, Altirriba J, Sebastian D, Ramirez S, Garcia A, et al. Reduced alpha-MSH Underlies Hypothalamic ER-Stress-Induced Hepatic Gluconeogenesis. *Cell Rep.* 2015;12(3):361-70.
96. Nguyen LT, Saad S, Tan Y, Pollock C, Chen H. Maternal high-fat diet induces metabolic stress response disorders in offspring hypothalamus. *J Mol Endocrinol.* 2017;59(1):81-92.
97. Chen N, Zhang Y, Wang M, Lin X, Li J, Li J, et al. Maternal obesity interrupts the coordination of the unfolded protein response and heat shock response in the postnatal developing hypothalamus of male offspring in mice. *Mol Cell Endocrinol.* 2021;527:111218.
98. Miller DJ, Fort PE. Heat Shock Proteins Regulatory Role in Neurodevelopment. *Front Neurosci.* 2018;12:821.
99. Hosoi T, Honda M, Oba T, Ozawa K. ER stress upregulated PGE(2)/IFNgamma-induced IL-6 expression and down-regulated iNOS expression in glial cells. *Sci Rep.* 2013;3:3388.
100. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell.* 2008;135(1):61-73.
101. Jais A, Bruning JC. Hypothalamic inflammation in obesity and metabolic disease. *J Clin Invest.* 2017;127(1):24-32.
102. van der Burg JW, Sen S, Chomitz VR, Seidell JC, Leviton A, Dammann O. The role of systemic inflammation linking maternal BMI to neurodevelopment in children. *Pediatr Res.* 2016;79(1-1):3-12.
103. Wu WL, Hsiao EY, Yan Z, Mazmanian SK, Patterson PH. The placental interleukin-6 signaling controls fetal brain development and behavior. *Brain Behav Immun.* 2017;62:11-23.
104. 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):1622-32.
105. Dammann O, O'Shea TM. Cytokines and perinatal brain damage. *Clin Perinatol.* 2008;35(4):643-63, v.

106. Olerich K LW, Sewaybricker LE, Kee S, Melhorn SJ, Chandrasekaran S, Schur EA. In utero exposure to maternal diabetes or hypertension and childhood hypothalamic gliosis. *Int J Obes (Lond)*. 2024;48(4):594-7.
107. Pimentel GD, Lira FS, Rosa JC, Oliveira JL, Losinskas-Hachul AC, Souza GI, et al. Intake of trans fatty acids during gestation and lactation leads to hypothalamic inflammation via TLR4/NFkappaBp65 signaling in adult offspring. *J Nutr Biochem*. 2012;23(3):265-71.
108. Ratsika A, Codagnone MG, Bastiaanssen TFS, Hoffmann Sarda FA, Lynch CMK, Ventura-Silva AP, et al. Maternal high-fat diet-induced microbiota changes are associated with alterations in embryonic brain metabolites and adolescent behaviour. *Brain Behav Immun*. 2024;121:317-30.
109. Park S, Belfoul AM, Rastelli M, Jang A, Monnoye M, Bae H, et al. Maternal low-calorie sweetener consumption rewires hypothalamic melanocortin circuits via a gut microbial co-metabolite pathway. *JCI Insight*. 2023;8(10).
110. Lopez-Tello J, Kiu R, Schofield Z, Zhang CXW, van Sinderen D, Le Gall G, et al. Maternal gut *Bifidobacterium breve* modifies fetal brain metabolism in germ-free mice. *Mol Metab*. 2024;88:102004.
111. Frankowska M, Surowka P, Gawlinska K, Borczyk M, Korostynski M, Filip M, et al. A maternal high-fat diet during pregnancy and lactation induced depression-like behavior in offspring and myelin-related changes in the rat prefrontal cortex. *Front Mol Neurosci*. 2023;16:1303718.
112. Yoo S, Cha D, Kim DW, Hoang TV, Blackshaw S. Tanycyte-Independent Control of Hypothalamic Leptin Signaling. *Front Neurosci*. 2019;13:240.
113. Barahona MJ, Ferrada L, Vera M, Nualart F. Tanycytes release glucose using the glucose-6-phosphatase system during hypoglycemia to control hypothalamic energy balance. *Mol Metab*. 2024;84:101940.
114. Zierden HC, Marx-Rattner R, Rock KD, Montgomery KR, Anastasiadis P, Folts L, et al. Extracellular vesicles are dynamic regulators of maternal glucose homeostasis during pregnancy. *Sci Rep*. 2023;13(1):4568.
115. Kinkade JA, Seetharam AS, Sachdev S, Bivens NJ, Phinney BS, Grigorean G, et al. Extracellular vesicles from mouse trophoblast cells: Effects on neural progenitor cells and potential participants in the placenta-brain axis†. *Biol Reprod*. 2024;110(2):310-28.
116. Herrera Lopez M, Bertone Arolfo M, Remedi M, Gastaldi L, Wilson C, Guendulain GG, et al. Human neural rosettes secrete bioactive extracellular vesicles enriched in neuronal and glial cellular components. *Sci Rep*. 2025;15(1):1987.