Title: Early life origins of metabolic disease: developmental programming of hypothalamic pathways controlling energy homeostasis

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Abstract
A wealth of animal and human studies demonstrate that perinatal exposure to adverse metabolic conditions- be it maternal obesity, diabetes or under-nutrition- results in predisposition of offspring to develop obesity later in life. This mechanism is a contributing factor to the exponential rise in obesity rates. Increased weight gain in offspring exposed to maternal obesity is usually associated with hyperphagia, implicating altered central regulation of energy homeostasis as an underlying cause. Perinatal development of the hypothalamus (a brain region key to metabolic regulation) is plastic and sensitive to metabolic signals during this critical time window. Recent research in non-human primate and rodent models has demonstrated that exposure to adverse maternal environments impairs the development of hypothalamic structure and consequently function, potentially underpinning metabolic phenotypes in later life. This review summarizes our current knowledge of how adverse perinatal environments program hypothalamic development and explores the mechanisms that could mediate these effects.

Keywords
Hypothalamus; maternal programming; energy homeostasis; neurodevelopment; insulin; leptin

1 Introduction
In recent years, global metabolic disease levels have reached epidemic proportions. Worldwide obesity has nearly doubled since 1980; and upwards of 10% of adults worldwide are now classified as diabetic (primarily with type 2 diabetes) (1). Current figures also highlight a worrying rise in adolescent obesity and Type 2 diabetes (T2DM). Given the significant co-morbidities associated with obesity, the increasing incidence of obesity represents an enormous social and financial burden on society.

The recent rise in obesity prevalence cannot be attributed to an individual’s lifestyle and diet alone. A hereditary element to obesity susceptibility is undisputed, but the rapid nature of the world-wide increase in obesity suggests the increased incidence is not solely down to genetic predisposition (2). Indeed only a small proportion of the body mass index (BMI) variation within the population can be explained by known genetic variants (of which there are around 30), suggesting there is an interaction between genetic factors and the environment (3). The current obesogenic environment of high- fat, high- sugar diets and increasingly sedentary lifestyles is undoubtedly fuelling the obesity crisis. However, evidence from numerous clinical and experimental studies show that the occurrence of many non-communicable diseases- including obesity- can be influenced by the early life environment (4). Adverse changes to maternal metabolic phenotype (be it obesity, diabetes or under-nutrition) before, during and after pregnancy compromise offspring development by contributing to a sub-optimal in utero and neonatal environment.

The central nervous system (CNS) is a key player in metabolic regulation, and
receives constant updates on energy status from the periphery, which it integrates in order to coordinate adjustments to appropriate physiological parameters. Over the past two decades the importance of the hypothalamus in regulating whole body energy homeostasis has become increasingly clear (5). The importance of the hypothalamus in maintaining both energy and glucose homeostasis, and the relative plasticity of hypothalamic development suggests disruptions to hypothalamic development- leading to altered hypothalamic function- may underpin increased metabolic disease risk later in life.

The purpose of this review is to summarize our current understanding of how the early life environment influences hypothalamic development, structure and ultimately function in later life. We also discuss the possible mechanisms responsible for mediating the effects of the early life environment on hypothalamic development, and highlight areas of experimental discord and gaps in knowledge within the field.

1.1 Different early life exposures with common long term outcomes on offspring metabolic health

1.1.1 In utero under-nutrition
The link between low birth weight- as a crude measure of restricted fetal growth- and later cardio- metabolic disease risk was first noted in the seminal papers by Hales and Barker (6-8), in which they proposed the “Thrifty Phenotype Hypothesis”, postulating that poor in utero nutrition drives fetal metabolic adaptations that would be beneficial should the baby be born into an environment with limited access to food. However, if the baby is born into an environment where food is plentiful, these fetal adaptations may become detrimental to metabolic health. The original observations made by Hales and Barker in the Hertfordshire birth cohort have since been reproduced in many different populations worldwide (9), and valuable insight into the effects of exposure to under-nutrition have been made in studying individuals exposed to the Dutch Hunger Winter in utero (10). The Thrifty Phenotype Hypothesis has since been encompassed within the broader terms of the “Developmental Origins of Health and Disease” hypothesis, which takes into account fetal adaptations due to a range of maternal environments such as increasingly prevalent obesity and over-nutrition (11).

1.1.2 Maternal obesity
Obesity is rapidly increasing among women of a childbearing age (12, 13), with one in five women obese at the time of conception (14). It has been known for some time that there is an association between maternal BMI and offspring BMI. Analysis of the 1958 British birth cohort revealed that the BMI of offspring increases proportionally with BMI of parents (15). Additionally high maternal BMI before and during pregnancy is a predictor of offspring obesity, adiposity and metabolic syndrome as a young adolescent and as an adult (16-18).

Compared to siblings born before the mother underwent bariatric surgery and subsequent weight loss, children born after surgery display an improved metabolic profile as adolescents. In particular, offspring born post-surgery have decreased
birth weight and macrosomia, decreased obesity incidence, increased insulin sensitivity, lower blood pressure, improved lipid profile and decreased adiposity (19-21). These findings are consistent with the hypothesis that development in utero within an obesogenic environment increases the risk of obesity and metabolic dysfunction. Importantly the decrease in transmission of obesity to offspring after weight loss surgery occurs even in mothers who remain overweight despite their weight loss (21), showing that a complete reduction of body weight is not necessary to see improvements in offspring outcome, and suggesting that body weight per se is not the most important maternal programming factor. This concept is further supported by results from studies in non-human primates, and is discussed later in the review (Section 5.2).

1.1.3 Gestational diabetes mellitus
Maternal glucose levels during pregnancy— independent of diabetes— can impact on offspring metabolic outcome. In non-diabetic mothers, average circulating glucose levels have been shown to positively correlate with offspring body fat percentage and BMI as an infant (22, 23).

Gestational diabetes mellitus (GDM) is diagnosed when glucose intolerance occurs either at the onset of pregnancy, or later during gestation. Maternal GDM is particularly common among overweight and obese mothers, and as such the prevalence is increasing in line with the obesity epidemic (24). Current estimates suggest that 10% of pregnancies are complicated by diabetes (25). Studies of siblings discordant for in utero GDM exposure show that in utero exposure to diabetes programs metabolic disease risk in the offspring. Children born after maternal diabetes diagnosis have a significantly higher risk of T2DM later in life than siblings born before the mother developed diabetes; additionally among non-diabetic offspring of diabetic mothers, the exposed offspring have an increased BMI compared to their unexposed siblings (26).

1.1.4 Post-natal growth as a predictor of metabolic disease risk
It is becoming increasingly apparent that rapid post-natal growth is associated with later life disease risk. One of the biggest risks for later life metabolic disease appears to be crossing growth percentiles during early life. A high weight velocity in the early post-natal period is often found in combination with small for gestational age (SGA) births, and may explain why a low birth weight predisposes offspring to metabolic disease (27). Additionally, studies of the ALSPAC cohort and others have revealed a rapid post-natal weight velocity is linked to insulin resistance and increased T2DM risk (28). The effects of rapid post-natal weight gain are long lasting; it has been demonstrated that peak weight velocity up to 2 years old is positively correlated with blood pressure, waist circumference and BMI at 31 years of age (29).

Infant nutrition during the early post-natal period has a significant impact on early growth and metabolic disease risk in adulthood. Infants fed a nutrient-enriched formula feed display accelerated early growth compared to breast fed infants, and increased overweight and obesity risk as adults (30-32). Thus, developmental programming in humans is not limited to the in utero environment and nutritional
status during the post-natal environment has a considerable impact on later life metabolic disease risk.

2 Insight from animal models of maternal programming

Whilst it is primarily desirable to examine data from human studies when investigating maternal programming of offspring metabolic systems, a greater understanding of the heritability of obesity between mother and offspring is needed to better interpret human studies. Additionally, the majority of human studies are inevitably complicated by confounding factors such as diet and lifestyle. Therefore researchers have utilized animal models with a controlled genetic background, and in which pre- and post-natal diet of both the mother and offspring can be strictly regulated. Recently, the use of genetically altered rodent models has enabled researchers to begin to examine the molecular mechanisms underpinning programming of offspring phenotype. Information obtained from these studies, alongside physiological observations from larger mammalian species such as non-human primates (NHP) and sheep, will be instrumental in understanding how the early life nutritional environment shapes later life metabolic disease risk.

2.1 Maternal Obesity

NHP offspring of mothers fed a high-fat diet (HFD) display increased adiposity (33), non-alcoholic fatty liver disease (NAFLD) (34) and pancreatic inflammation and insulin resistance (35). It has also been reported that the offspring in this model develop a range of neuronal phenotypes, including female specific increased anxiety-like behaviour, circadian disruption and alterations to the fetal thyroid axis (36-39).

One of the most consistent findings in rodent studies of maternal obesity is increased body weight in offspring (40-44). This usually begins during the early post-natal period and continues throughout adult life. Offspring obesity is often accompanied by insulin resistance, progressively disrupted glucose homeostasis and the development of T2DM later in life (40, 42, 45, 46). Pancreatic β-cell dysfunction (47, 48) as well as insulin resistance contributes to the development of T2DM and hypothalamic dysfunction could contribute to both of these parameters.

In order to make translatable observations in rodent models of maternal obesity, it is important to note that in many rodent models the dams display a 10-20% increase in body weight as a result of the HFD consumption. According to many human classifications, this would result in the dams being labeled ‘over-weight’ rather than obese. However, the recent use of highly palatable diets combining high fat and high sucrose content have led to several recent papers in which the dams show up to a 30% increase in body weight, making the metabolic state of dams in these studies more similar to the human classification of obesity.

2.2 Maternal glucose levels

Gestational diabetes is commonly associated with fetal macrosomia (49-51), as a result of increased fetal insulinemia in response to high maternal glucose levels.
Perhaps surprisingly, the fetus can develop insulin resistance whilst still in utero as a result of maternal hyperglycemia (52). Macrosomic GDM offspring display increased body weight, hyperinsulinemia and reduced glucose tolerance as adults (51). Additionally, maternal hyperglycemia has significant effects on placental growth and function (53), which may explain the alterations to birth weight commonly reported in GDM offspring.

2.3 Maternal under-nutrition and Intra-uterine Growth Restriction
NHP models of gestational under-nutrition have reported a wide range of offspring phenotypes, including disrupted cardiac function (54), altered hepatic function leading to glucose intolerance (55-57) and increased activation of the HPA axis (58). A loss of hepatic and pancreatic function resulting ultimately in loss of glucose homeostasis has also been reported in sheep models of under-nutrition and IUGR (59-61). In rodents, both models of total calorie restriction (62), and macro nutrient restriction (most commonly reduced protein in the maternal diet) result in a loss of glucose homeostasis in offspring (63), and this phenotype is worsened with age (64). Mimicking human studies, the effects of exposure to maternal low protein during gestation are exacerbated by rapid post-natal catch up growth, resulting in a strong T2DM-like phenotype in offspring that are cross fostered to a control diet-fed dam in the post-natal period (65, 66).

3 Hypothalamic development

3.1 Development of hypothalamic circuits governing energy homeostasis
Due to the relatively immature state at which rodent offspring are born, full hypothalamic development does not occur during gestation. Roughly speaking, neurogenesis in rodents occurs pre-natally, whilst full circuit formation and connectivity is not achieved until the post-natal period (Figure 1). Therefore both the fetal and neonatal period represent critical periods of vulnerability of the hypothalamus. Early studies of neurogenesis using thymidine labeling suggested the majority of neurons in the murine hypothalamus are formed between E11-14 (67). Recent studies using more sophisticated labeling methods have identified a peak in hypothalamic neurogenesis at E12 and further characterised neurogenesis in individual hypothalamic nuclei. The majority of neurons in the paraventricular nucleus (PVH) and dorsomedial nucleus (DMH) are generated between E12-E14, whereas the arcuate nucleus (ARC) and ventromedial nucleus (VMH) have longer periods of neuronal generation from E12-E16 (68, 69). In contrast to rodents, neurogenesis and circuit formation are both achieved predominantly during the pre-natal period in humans and NHP (70, 71) (Figure 1). In the human fetus hypothalamic nuclei can be characterised as lateral, core or midline structures when grouped by location and timing of development. Lateral hypothalamic structures are the first to develop between 9-14 weeks of gestation, followed by the development of core structures (mainly intra-hypothalamic projections) between 15-23 weeks. Lastly, mid-line structures such as the ARC and PVH develop during the morphogenetic period after 34 weeks of gestation (70).
One of the critical periods of hypothalamic development is the generation of the neuronal projections originating in the ARC that are key components of the energy balance circuitry. In rodents these connections are formed post-natally. Studies by Bouret et al have elegantly demonstrated that projections from the ARC do not represent an adult distribution until P18, with connections specifically between the ARC and PVH forming between P8-10 (72). Further studies in rodents have demonstrated that orexigenic neuropeptide Y (NPY) positive neurons from the ARC innervate the PVH at P10-11, but brainstem NPY positive neuronal fibers arrive at the PVH much earlier and are present from P2 (73). In comparison, in the NHP the development of NPY positive projections from the ARC occurs during the third trimester of gestation, and offspring are born with an abundance of NPY positive fibers originating from the ARC. However, the pattern of ARC projections seen in the NHP in late gestation is less dense than in adults, suggesting further refinement of the connectivity occurs in the post-natal period (71).

In the pre-natal and early post-natal stages of development it is imperative for offspring to maintain a positive energy balance to enable adequate growth, therefore homeostatic feedback control of energy intake doesn’t begin until relatively late in the post-natal period. In rodents, the plasticity of the hypothalamus during early life is reflected in the often paradoxical roles of pathways involved in energy homeostasis during early development. The PVH integrates NPY and pro-opiomelanocortin (POMC) signals from the ARC and regulates downstream parameters of energy homeostasis, and this connectivity is flexible during rodent post-natal development. Melnick et al reported a developmental switch in NPY and melanocortin effects on specific neuronal populations in the PVH during the third post-natal week (i.e. neurons changed from being NPY responsive to melanocortin responsive) (74).

Recently, Baquero et al have published two elegant papers demonstrating the highly plastic nature of the hypothalamus during development. The first of these reported that during the early postnatal period leptin depolarizes NPY neurons, in stark contrast to leptin hyperpolarisation of NPY neurons in adult mice (75). The second publication demonstrates the re-wiring of synaptic input onto NPY neurons that occurs during early post-natal life, in particular the increase in inhibitory GABAergic tone onto arcuate NPY neurons that occurs up until 10 weeks of age in mice (76). This early dominance of orexigenic signals allows the newborn to maintain a positive energy balance for early post-natal growth, before switching to an adult profile after the rapid growth required for early development is achieved.

NPY is also expressed transiently in areas of the rodent CNS outside of the ARC during the early post-natal period. It has been suggested that the novel expression of NPY in neurons in the DMH and peri-fornical region (in addition to the ARC) during development is another mechanism by which the newborn maintains a positive energy balance. The expression of NPY increases in these novel areas between P0-4, and reaches a peak by P16. However by P30 NPY expression reflects that of adult distribution and is limited to the ARC (73). By conducting an extensive
characterization of POMC and NPY neuron development, Padilla et al demonstrated that a subpopulation of POMC precursors give rise to a population of orexigenic NPY neurons (69). This intriguing process likely allows tight coupling of these two opposing neuronal pathways in adulthood, however cell fate decisions such as this that occur during the perinatal period may be vulnerable to programming by maternal and offspring nutrient status.

The data described in this section is essentially limited to rodents, as there is a distinct lack of data on hypothalamic development in relation to NHP and humans. Although the highly conserved functions of hypothalamic regions between rodents and higher organisms suggest that many developmental mechanisms may be shared, our knowledge of NHP and human hypothalamic development is far from complete.

3.2 Programming of hypothalamic development
A common cause of the increased body weight observed in offspring of obese dams is hyperphagia (44, 45), implicating altered CNS regulation of food intake as an underlying cause of the programmed metabolic phenotypes. The plasticity of hypothalamic development during the perinatal period means it is susceptible to disruption by exposure to adverse environments, and represents a mechanism by which changes in metabolic homeostasis are permanently programmed in offspring.

3.2.1 Neurogenesis and cell number
Our knowledge on how the perinatal environment impacts on early neurogenesis in the hypothalamus is limited, but one study has reported that the fetuses of HFD-fed dams display increased neurogenesis around the third ventricle during gestation, and increased neuronal migration from this area to other areas of the hypothalamus where neurons ultimately display an orexigenic phenotype (77). Furthermore, Plagemann and colleagues have demonstrated that offspring exposure to GDM results in the malformation of medio-basal hypothalamic nuclei, which may be secondary to reduced neuron formation (78-80). Whilst this remains the extent of our knowledge of maternal programing of hypothalamic neurogenesis, a recent paper has demonstrated that exposure to maternal obesity alters the expression of genes in the Notch signaling pathway- a key regulator of neural stem cell differentiation- in cerebral neural stem cells of offspring (81), and thus similar mechanisms may act in the hypothalamus.

3.2.2 Intra-Hypothalamic Connectivity
Intra-hypothalamic projections, particularly those originating in the ARC, are particularly susceptible to programming by the perinatal environment (Figure 2). The offspring of GDM mothers display decreased projections of AgRP and POMC neuronal fibers from the ARC to PVH as adults, which is due to disrupted axonal projections rather than decreased neurogenesis in the ARC (82). Furthermore, cross-fostering of control offspring to a GDM dam during the lactation period has been shown to cause perturbations to the development of hypothalamic energy balance circuitry, suggesting exposure to milk from a diabetic mother could cause long term changes to body weight and food intake in offspring (80).
Vogt et al have recently carried out a wider characterisation of ARC connections in the offspring of obese dams, and reported reduced ARC projections to the PVH, DMH and lateral hypothalamus (83). This programming of ARC projections occurs even when offspring exposure to maternal obesity is limited to the suckling period, which corresponds with the reported timing of development of these projections. This suggests that the disrupted circuitry reflects a disruption of axonal projections, rather than a cellular defect. As well as maternal diet, the exact composition of offspring diet during the early post-natal period impacts on hypothalamic development, as neonatal mice fed a diet containing either low omega-6 or high omega-3 fatty acid display reductions in both anorexigenic and orexigenic projections from the ARC to the PVH (84).

Decreased POMC projections from the ARC to the PVH are also reported in offspring exposed to IUGR (85, 86), revealing that opposite nutritional challenges have similar effects on hypothalamic connectivity. Whether the changes in hypothalamic structure reported in these divergent nutritional states are a result of neurodevelopmental adaptation or dysfunction remains to be elucidated. However these common effects on hypothalamic connectivity may explain how these different nutritional challenges in early life have the same effect on regulation of energy balance later on in life.

Recently, Sanders et al have made some interesting progress in uncovering the molecular mechanisms underpinning the reduction in ARC to PVH projections that are commonly reported in offspring exposed to an adverse perinatal environment. This group reported that the classical axon guidance molecule Netrin-1 and its receptors are expressed along the ARC to PVH tract during late gestation, suggesting Netrin signaling may be key to the formation of projections along this route (87). They further demonstrated altered expression of key Netrin receptors on NPY neurons in offspring exposed to maternal obesity, and proposed that disruption of Netrin signaling mediates the decreased NPY projections from the ARC to the PVH in offspring exposed to maternal obesity (87).

A recent publication from the Bouret laboratory has demonstrated a previously unknown role for the metabolic hormone ghrelin in development of projections from the ARC to the PVH (88). This is particularly interesting given the recent finding that neonatal over-nutrition causes central ghrelin resistance (89), and demonstrates that changes to both ghrelin and leptin levels caused by the perinatal nutritional environment can have long term consequences for hypothalamic development (discussed in 4.1).

### 3.2.3 Gene expression and neuropeptide profile

Given the altered hypothalamic structure that has been reported in offspring exposed to an adverse early life environment, it is perhaps not surprising that the perinatal environment can also impact on the expression and regulation of hypothalamic neuropeptides. Such changes in functional pathways within the hypothalamus can perturb the fine balance between orexigenic and anorexigenic...
pathways, thus changing the overall tone of hypothalamic control and underpinning food intake phenotypes commonly observed in offspring.

Offspring exposure to maternal obesity and/or over-nutrition have been shown to cause significant changes to expression of feeding related genes at a basal state in the hypothalamus (77, 90, 91), plus changes to the normal gene regulation in response to metabolic state (92-94). Reported changes in gene expression may change the tone of feeding regulation within the hypothalamus, and reflect an altered body weight set-point. Poon et al identified distinct populations of hypothalamic neurons expressing low- and high- levels of orexigenic neuropeptides, and found that isolated hypothalamic neuronal cultures from E19 offspring exposed to HFD in utero display a shift from low to high orexigenic peptide expressing neuronal populations (95).

GDM offspring are reported to display altered expression of catecholamines in the hypothalamus, in particular increased levels of both noradrenaline and dopamine in the PVH, and an increase in NPY positive neurons in the ARC (50, 96). Plagemann and colleagues have demonstrated that offspring exposure to GDM results in significant disruptions to the expression of neuropeptides in both orexigenic and anorexigenic circuits (78, 79). Interestingly, this phenotype can be rescued by pancreatic islet transplantation in GDM dams, suggesting that either maternal hyperglycemia or hypoinsulinemia is the main contributor to programming of hypothalamic malformations in the offspring. Similar changes in the gene expression of key feeding regulated neuropeptides has been reported in both rodent and NHP models of maternal under-nutrition and IUGR (57, 97-99), again demonstrating that distinct adverse environments result in similar hypothalamic outcomes.

Unfortunately, many of the above-mentioned studies have provided conflicting reports as to whether the expression of anorexigenic and orexigenic genes is increased or decreased in offspring. This may be due to differences in the individual set up of the study (i.e.- mother’s diet and metabolic state, age and sex of offspring) or because some groups have investigated the hypothalamus as a whole and not examined transcription in individual areas. However it is clear the perinatal environment has the potential to program dysfunction in hypothalamic neuropeptide pathways at the level of gene expression, and this may be a molecular mechanism underpinning some of the physiological phenotypes reported in offspring.

Hyperphagia in offspring exposed to maternal obesity and /or GDM is frequently associated with resistance to the anorectic adipokine leptin (100-102). Central leptin resistance has been attributed to changes in the expression and regulation of downstream hypothalamic neuropeptides such as NPY and AgRP (102, 103). An association has also been reported between hyperphagia in offspring and increased expression of the Fat mass and obesity-associated (FTO) gene, variants of which are associated with increased risk of obesity (104).
3.2.4 Food preferences and reward-related feeding
Maternal obesity can also influence offspring feeding behaviour and dietary preferences. Maternal consumption of a junk food or HFD - resulting in maternal obesity - has been reported to increase the preference for fatty and sugary food in offspring, leading to obesity (41, 105). The offspring of obese mothers also display increased frequency of feeding episodes, and a longer duration of feeding during a given episode (106). Interestingly, it has also been reported that the offspring of obese mothers may display alterations to reward systems in the brain that could explain the frequently reported hyperphagia. Several studies have reported programming of the mesolimbic reward system in offspring, resulting in altered activation in response to diverse stimuli including feeding (105, 107). Furthermore it has been demonstrated that the offspring of dams fed a junk food diet display increased hypothalamic levels of serotonin and dopamine (106). Whilst indiscriminate increases in the activity of both the serotonergic and dopaminergic systems do not explain the observed altered feeding behaviour, it demonstrates the widespread effects of maternal obesity on all aspects regulating offspring food intake.

3.2.5 Neuronal nutrient sensing and activation
Although less studied, there is some evidence that exposure to an adverse nutritional state during early life can alter electrophysiological responses of hypothalamic neurons. Whereas distinct subpopulations of PVH neurons from control animals are excited by metabolic signals including melanocortins and NPY in slice preparation, neurons from neonatally over-nourished rats display a switch from activation to inhibition following application of these peptides (108). Unfortunately, indiscriminate changes in PVH neuronal responses to both orexigenic and anorexigenic signals do not explain the obese phenotype observed in offspring in other models of neonatal over-nutrition. However, parvo-cellular PVH neurons that are normally excited by anorexigenic signals of adiposity such as leptin and insulin are inhibited by these same signals in rats exposed to neonatal over-nutrition (109). The rewiring in responses of these neurons is thought to occur partly through inhibitory input from GABA interneurons, as blockade of GABA signaling normalizes neuronal responses in neonatally over-nourished animals. Furthermore, both neonatal under- and over-nutrition alters the basal firing rate of LHA neurons, as well as their electrical response to dopamine and CCK administration (110). These studies demonstrate that perinatal nutrition can have significant effects on neuronal activation, resulting in negative feedback from metabolic signals such as leptin, insulin and melanocortins being replaced by positive feedback signals. This type of adjustment of neuronal response to nutrient status could underpin a difference in body weight set point, causing the hypothalamus to ‘defend’ a higher body weight and result in increased body weight phenotypes in offspring. It is important to note that the neuronal activity changes in neonatally over-nourished rats mentioned above are not independent of offspring body weight. However, alterations in VMH nutrient sensing have been reported in rat offspring exposed to a maternal HFD when the offspring do not themselves display a body weight phenotype, demonstrating that changes to hypothalamic neuronal activity independent of offspring body weight can occur (111).
Recently, Plagemann and colleagues have used a novel system to examine the effects of transient exposure to hyperglycemia independent of other metabolic factors during early life by artificially modulating glucose levels in chick embryos inside eggs. Exposure to hyperglycemia during late gestation results in decreased neuronal glucose sensitivity in hypothalamic brain slices, and altered expression of glucose transporters (112). The authors propose that these changes in hypothalamic glucose sensing—which are independent of changes to the offspring’s body weight—are indicative of pre-natally acquired hypothalamic glucose resistance that could contribute to the development of diabetes later in life.

4 Hunting for the ‘programming factor’

Despite the rapidly increasing number of human and animal studies, the mechanisms underpinning maternal programming of offspring metabolic disease risk are still unclear. Simply put, we still have little idea of the ‘programming factor’ through which maternal nutritional status impacts on offspring in utero development. The identification of programming factor(s) is imperative from an intervention perspective, to know what to target and whether the intervention should target the mother or the fetus. Both obesity and diabetes cause changes to the hormonal milieu, which is of particular significance when these disease states occur during pregnancy as the developing fetus is exposed to altered levels of maternal metabolic hormones.

Recently, attention has focused on the roles of metabolic hormones in hypothalamic development. Although circulating factors such as insulin and leptin are classically thought of as regulating metabolic state, both have roles in neuronal development. The dual roles of these hormones is a powerful mechanism for coupling neuronal development with fetal nutrient status, allowing transmission of signals to the developing organism in response to alterations in the nutritional environment, and enabling neuronal adaptation in line with the Thrifty Phenotype Hypothesis. However, the involvement of metabolic hormones in neuronal development also leaves the brain extremely vulnerable to disrupted development if the maternal metabolic milieu is altered due to e.g. obesity or diabetes, and may cause maladaptive responses.

4.1 Potential mechanisms: leptin

Leptin is most commonly thought of as an adipokine responsible for activating hypothalamic pathways that lower food intake and increase energy expenditure. However, during the early post-natal period in rodents high circulating levels of leptin do not cause a decrease in food intake, an observation that led to the identification of a separate role for leptin in hypothalamic maturation.

The majority of leptin activated neurons in the mouse hypothalamus are generated on E12 (68). Despite this, leptin deficient ob/ob mice do not display a metabolic phenotype as neonates, suggesting a different role for leptin during the early post-natal period (113). Additionally, the expression of leptin receptors within the brain
changes during early development, suggesting a change in the role of leptin in the CNS. At P4, leptin receptor (LepR) expression is restricted to cells lining the third ventricle, but an acute leptin challenge activates intracellular signaling in this region at P4 but not P14- revealing a developmental change in the site of leptin action (114). Further studies have revealed transient expression of the LepR during early neonatal life in areas not associated with energy expenditure, including the cortex, hippocampus and laterodorsal nucleus of thalamus (115).

A series of classical experiments in the ob/ob mice by Bouret have elegantly revealed a neurotrophic role for leptin in the hypothalamus during early post-natal development, as ob/ob mice display a permanent reduction in neural projections from the ARC (116). Importantly, this phenotype can be rescued by exogenous leptin treatment on P12 but not during adulthood, demonstrating that the neurotrophic actions of leptin are limited to a tight developmental window. More recently Bouyer et al have further defined hypothalamic development in the environment of leptin deficiency, demonstrating that ob/ob mice display decreased AgRP and α-MSH projections specifically to neuroendocrine PVH cells, and decreased AgRP projections to pre-autonomic PVH cells (117)(Figure 2). Interestingly, α-MSH projections to pre-autonomic cells appear to be leptin independent. Furthermore, AgRP projections to pre-autonomic but not neuroendocrine PVH cells can be rescued by exogenous leptin administration from P4-P14. Further research by Bouret et al suggests that leptin can only activate individual hypothalamic nuclei after they have been innervated by ARC fibers, thus the neurotrophic role of leptin is responsible for the ability of leptin to then activate other hypothalamic areas (72). Signalling through the LepR is necessary for leptin’s role in neurite outgrowth from the ARC, and distinct signaling pathways downstream of the LepR have differing roles in the formation of energy balance circuitry (118).

In rodents, during the second post-natal week a surge in leptin levels occurs independent of fat mass (119), and is not associated with changes in body weight, glucose or insulin levels. The observation by Bouret et al that defective hypothalamic projections in the ob/ob mice can be rescued by exogenous leptin at a time correlating with the endogenous leptin peak fuelled speculation that the leptin surge is involved in the maturation of neuroendocrine pathways, and that disruption of the leptin surge would have consequences for hypothalamic development. Subsequent research in rodent models has shown this to be true. Sub-cutaneous administration of leptin from P2 (to mimic an early leptin surge) results in a long-term decrease in food intake and decreased ghrelin levels, but no change in body weight. Conversely, ablation of the leptin peak using a leptin antagonist has no effect on food intake but causes decreased adult body weight and sexually dimorphic changes in hypothalamic gene expression (120, 121).

In light of these observations that experimentally altering the leptin surge causes long-term phenotypes, leptin and the leptin surge have attracted a lot of interest as candidates linking defective development to permanent programming of offspring energy homeostasis. Kirk et al have demonstrated that the offspring of obese dams display an amplified and prolonged leptin surge, which is accompanied by decreased
AgRP positive neuronal innervation of the PVH and associated with hyperphagia in adulthood (102). Both Delahaye and Coupe have reported that IUGR results in a reduced post-natal leptin surge in rats, which is associated with decreased hypothalamic POMC projections from the ARC to the PVH in adult animals (85, 86). The observation that both a lack of leptin surge (in ob/ob mice and in IUGR) and an increased or prolonged leptin surge (with maternal obesity) perturb hypothalamic development suggests there is a U-shaped curve in relation to leptin signaling and offspring metabolic disease risk.

It is worth noting that the importance, or indeed presence, of a leptin surge similar to the phenomena noted in rodents in humans and NHP is yet to be confirmed. A post-natal leptin surge has been observed in sheep, although it occurs sooner after birth than in rodents (122). It has been reported that in sheep, maternal obesity abolishes the leptin surge possibly via increased cortisol levels in the post-natal period. This is associated with hyperphagia and increased body weight in offspring in adulthood (122).

In humans, maternal leptin levels increase throughout the first and second trimesters, reaching a peak during the third trimester and returning to pre-pregnancy levels almost immediately at parturition (123). The placenta is a major source of leptin during pregnancy, however fetal adipose tissue is capable of producing leptin as early as 6-10 weeks of gestation (124). Fetal leptin levels are directly correlated with fetal adipose levels (125), suggesting any contribution from maternal and placental leptin is slim. Further supporting this, research suggests that the vast majority of the placental-produced leptin is transported into the maternal circulation (126), suggesting that the main source of fetal leptin is fetal organs (127). However the amount of maternal leptin produced from maternal adipose tissue that is transported to the fetus is unknown.

The common adverse effects of disruption of the leptin surge seem to suggest that the correct regulation of leptin in the post-natal period is critical for development. However it has also been demonstrated that an adverse environment during early life can program offspring metabolism independent of leptin signalling (128). Additionally, Vickers et al found that whilst neonatal leptin treatment rescued the metabolic phenotype in female and male IUGR offspring, the treatment programmed a metabolic phenotype in control male offspring (129, 130). Clearly a better understanding is needed before we can begin to develop effective and translatable intervention strategies based on manipulation of leptin levels in the CNS.

4.2 Potential mechanisms: insulin

It is difficult to study the effects of insulin on hypothalamic development in vivo, as it is often not possible to examine the effects of insulin administration per se independent of hypoglycemia. This has meant that insulin has received less attention as a potential programming factor than leptin. However, early studies suggested that insulin has a neurotrophic function and can promote neurite outgrowth in cultured neuronal cells (131-133). Indeed, insulin signaling is essential for axon guidance in
drosophila (134). It has also been reported that insulin deficiency, rather than hyperglycemia, is responsible for the impaired neurotrophic response to injury observed with T1DM (135). Current knowledge on the molecular mechanisms through which insulin signaling promotes neurite outgrowth is limited, but some groups have suggested a role for insulin signaling in stabilizing microtubule machinery (136, 137).

Maternal hyperinsulinemia and insulin resistance are commonly observed among both obese and gestational diabetic mothers. It has been demonstrated in a rodent model that maternal insulin injections between days 15-20 of gestation cause delayed onset obesity in offspring and increases in both hypothalamic noradrenaline levels and noradrenergic neuron innervation of the PVH (138, 139). However, as only limited amounts of insulin can cross the placenta, it is unlikely that the programming of offspring in this case is due to fetal hyperinsulinemia, and may in fact be a fetal response to maternal hypoglycemia induced by the insulin injections.

Plagemann et al have experimentally modeled neonatal hyperinsulinemia by inserting hypothalamic insulin implants in rat neonates at P2 and P8. This results in increased body weight, hyperinsulinemia and impaired glucose tolerance in adulthood, as well as morphological alterations to hypothalamic nuclei including the ARC and VMH (140, 141). This phenotype is not exclusive to acute hypothalamic administration of insulin, as daily sub-cutaneous insulin injections from P8-P11 also result in increased body weight, hyperinsulinemia and impaired glucose tolerance, as well as a reduced volume of the VMH in adulthood (142). However it should be noted that this latter model fails to control for the effects of insulin on glucose levels separately from the other hormonal actions of insulin. The use of genetically modified mice with defective insulin signaling allows researchers to examine the effects of insulin signaling independent of glucose levels. A recent study by Vogt et al utilized mice lacking the insulin receptor specifically on POMC neurons to demonstrate that insulin signaling in POMC neurons is responsible for the disruption of these projections to pre-autonomic neurons of the PVH in offspring exposed to maternal over-nutrition during the post-natal period (83)(Figure 2).

4.3 Potential mechanisms: epigenetic regulation of the genome
The molecular mechanisms by which changes in the perinatal environment are transmitted to the fetus, and the process by which phenotypes are induced are not yet fully understood. However the stable nature of these phenotypes throughout the lifetime of the exposed offspring, and the recently reported inter-generational transmission of programming effects suggests permanent changes in gene expression. In utero regulation of epigenetic machinery has recently received a lot of interest as a potential mechanism for causing permanent, heritable changes to gene expression.

Maternal environment-mediated alterations to epigenetic markers are a likely source of the transcriptional changes commonly observed in offspring. In a recent study of siblings born before and after maternal gastric bypass surgery, significant differences in the methylation of glucoregulatory genes were observed in blood
samples (20). It has previously been reported in a NHP model that maternal diet modulates SIRT1 histone de-acetylase activity independently of maternal obesity (143), thus dietary programming of epigenetic processes in offspring is certainly possible. It has also been reported that DNA methyl transferase activity is regulated by glucose levels, resulting in changes to global DNA methylation levels, although conflicting reports exist as to whether there is a positive or negative correlation between glucose and methylation state (144, 145). Li et al have recently demonstrated that the major epigenetic modifications distinguishing astrocytes and neurons within the hypothalamus occur post-natally and are nuclei specific, thus changes to offspring nutrient status affecting epigenetic machinery during the early post-natal period could have widespread consequences on cell fate decisions (146).

Tissue specific expression of the insulin gene in pancreatic beta cells in both humans and rodents is associated with hypomethylation of specific CpG sites in the insulin promoter (147), and methylation of the leptin promoter has also been shown to be responsible for tissue specific expression of this locus (148). Thus these genes may be particularly susceptible to epigenetic dysregulation in utero, and changes to the promoter methylation pattern of these genes could cause altered expression and subsequent changes in offspring physiology. Indeed, in humans it has been reported that the methylation state of the leptin promoter on the fetal side of the placenta is positively correlated with circulating maternal glucose levels (149). Thus maternal hyperglycemia results in decreased leptin expression in the placenta, and could therefore affect offspring development. Furthermore, in rodents it has been demonstrated that late gestational HFD exposure causes hypermethylation of the leptin receptor promoter in offspring adipose tissue, which is associated with decreased gene expression (150).

There is emerging evidence that an adverse in utero environment can also cause epigenetic dysregulation in hypothalamic energy homeostasis pathways. Neonatal over-nutrition causes hypermethylation of the POMC promoter in the hypothalamus specifically at CpG dinucleotides within the Specificity Protein 1 (Sp1) binding site, resulting in a lack of POMC mRNA regulation in response to leptin or insulin (151). Similarly, offspring exposed to maternal obesity in utero display hypermethylation of a region 500bp upstream of the ATG site in the POMC gene, which corresponds with decreased pomc expression and increased body weight (152).

Conversely, it has been reported that sheep offspring exposed to IUGR display increased H3K9Ac and decreased H3K27Me3 modifications associated with the POMC promoter, and decreased methylation at a POMC proximal promoter region. These changes are observed specifically in the hypothalamus, although they are not associated with a corresponding change in mRNA or circulating POMC levels (153, 154). Interestingly the latter study also reported reduced DNMT activity in the hypothalamus, suggesting that changes to epigenetic regulation as a result of IUGR may be widespread.
5 Important considerations

5.1 Critical periods of development: gestation vs. the early post-natal period

It is now widely accepted that sub-optimal nutrition during either gestation or the early post-natal period has adverse effects on offspring metabolic outcome, and that the consequences of exposure during these developmental periods can differ. Indeed, the findings from the Dutch Hunger Winter cohort revealed that exposure to famine even during specific periods of gestation had hugely varying outcomes on offspring phenotype (10). In order to develop effective intervention strategies, it is important to understand the programming effects of maternal nutrition during gestation and the post-natal period both separately and combined. Although this is an important issue that needs to be further understood, few studies in rodents have attempted to differentiate the effects of maternal nutrition during gestation and lactation.

Several rodent studies have suggested that post-natal exposure to maternal obesity is as- if not more- important than in utero exposure in programming offspring metabolic phenotype. Obesity- prone rat pups fostered to lean dams at birth remain obese but develop a gradual improvement in insulin sensitivity; whereas lean pups fostered to an obese dam develop increased adiposity and insulin resistance, as well as changes in the expression of hypothalamic neuropeptides (155). Furthermore recent research has demonstrated that exposure to maternal obesity exclusively during the post-natal period is sufficient to disrupt hypothalamic development (83). The mechanism underlying offspring hypothalamic programming during the post-natal period is unknown, but it is likely to involve changes to maternal milk composition that result in neonatal over-nutrition and hormonal changes.

In contrast to development of the rodent brain, the vast majority of human and NHP neuronal development occurs in utero. So how does the post-natal programming observed in rodent models translate to human development? Although the majority of NHP hypothalamic circuit formation occurs in utero, there is some further development of these connections during early neonatal life (71). Furthermore, the increased metabolic disease risk associated with infant formula feeding shows that although the time windows of specifics of development may be different between rodents and humans, nutrition that promotes accelerated growth during this period in both species increases obesity risk. Whether any of these post-natal programming effects are mediated through changes to hypothalamic structure and function after birth in humans and NHP remains to be discovered.

5.2 Maternal diet composition and maternal obesity versus high fat feeding

It is becoming increasingly clear that offspring are extremely sensitive to the exact composition of maternal diet, and differences in the choice of maternal diet are likely to be the cause of conflicting results between studies (see Table 1). This is an issue in the field of maternal programming in general that needs to be resolved in order to make any meaningful comparisons from the research that has been undertaken in rodent models.
It appears that female offspring are particularly vulnerable to programming caused by exposure to a high-sugar environment during gestation. This may explain why many studies using simply high fat, rather than high fat and high sucrose, diets do not report a strong phenotype in female offspring. Interestingly, female susceptibility to high sucrose levels has also been noted in human dietary studies. A study in Finland has found that the consumption of sugar enriched drinks as an adolescent is directly linked to adult BMI in women but not men (156).

In order to develop effective intervention strategies and health guidelines, it is also imperative that the impact of maternal diet vs. maternal obesity per se on offspring phenotype is assessed. In human studies it is hard to examine separately the confounding factors of maternal obesity and maternal diet, as attempts to monitor maternal diet are inherently flawed by the inaccuracy of food intake surveys. Animal models have proved more successful in separating metabolic parameters associated with maternal obesity in order to ascertain which factors have the greatest effect on offspring health. Important observations have been made in a NHP model examining the effects of maternal HFD consumption but not maternal obesity per se by using diet-resistant females who remain lean despite consuming a HFD. These studies have suggested that exposure to maternal HFD alone (without maternal obesity) causes changes in offspring liver function (33, 157). Furthermore, switching the diet of NHP obese females immediately prior to pregnancy reverses the alterations observed in offspring hypothalamic feeding pathways - despite the mothers remaining obese - suggesting that this phenotype is mediated by the maternal diet (33, 157).

Conversely, in a reversed version of this experiment in mice it has been shown that the offspring of both ‘lifetime’ HFD-fed dams and dams fed an HFD only during gestation and lactation display the same phenotype of adult obesity, suggesting that maternal nutrition during gestation and lactation is as important as maternal nutrition and metabolic state pre-conception (158). Other groups have more recently utilized rodent models in which the dam consumes a calorie rich diet but is not overweight to show conclusively that maternal diet alone can program strong metabolic phenotypes in offspring (83, 92, 159, 160). These studies carry an important message for the development of human health guidelines, and suggest that lifestyle intervention alone (i.e. the mother switching to a healthy diet pre-pregnancy) may be sufficient to ameliorate metabolic phenotypes in offspring (Figure 3).

5.3 Sexual dimorphism in hypothalamic programming
Gender differences in developmental programming have so far been largely ignored, with most studies including only male offspring. However, recent studies in which both sexes were included suggest that offspring responses to the early metabolic environment are highly sexually dimorphic (46, 92, 121, 161). This may be due to inherent gender differences in hypothalamic development, or gender specificity of the adaptive response to environmental challenges. Sexual dimorphism in offspring
phenotype has important implications for the development of health guidelines and therapeutic interventions.

The hypothalamic melanocortin system is sexually dimorphic; male mice have less POMC neurons than female mice, which is thought to underlie the hyperphagia observed in male compared to female mice (162). Treatment of neonatal female mice with testosterone decreases POMC neuron number in the ARC and increases food intake (162). It has also been reported that male and female rats differ in their sensitivity to ICV leptin and insulin administration (163). Strikingly, although male rats display a 24-hour reduction in food intake after ICV insulin injection, this response is completely absent in female mice. Given the role of insulin and leptin in hypothalamic development, these inherent differences in sensitivity may confer different risk on offspring exposed to an adverse perinatal environment. Indeed, sexually dimorphic hypothalamic responses to leptin antagonism during the early post-natal period suggest sex differences in the sensitivity to leptin during the perinatal period (121) can ultimately affect hypothalamic development differently in males and females. Whether increased insulin sensitivity would put male offspring at an advantage or disadvantage when exposed to altered hormone levels during the perinatal period remains to be determined, but certainly warrants further investigation.

A recent study by Sun et al revealed that male offspring exposure to a HFD during either gestation or lactation resulted in decreased leptin sensitivity in the medio-basal hypothalamus at P10, whereas in female offspring decreased leptin sensitivity was caused only by in utero exposure to HFD, revealing a sexual dimorphism in programming of leptin sensitivity that may be linked to sex differences in development (159). Furthermore, it has also been shown that male and female offspring have different periods of susceptibility to programming by maternal over-nutrition (92). Studies such as these highlight the importance of including both sexes in programming studies, as programming during the perinatal period clearly differentially affects offspring of each sex.

6 Future work- defining intervention strategies

Maternal programming creates a vicious cycle by which maternal diet, weight or glycemic status can increase offspring susceptibility to metabolic disease. These offspring during their pregnancies have their own children whom are also exposed to an adverse in utero environment and this continues through subsequent generations (Figure 3). Drastic weight loss interventions such as bariatric surgery, whilst effective, are expensive and invasive and thus not practical to control this sequence of events on a large scale. Furthermore, some studies suggest that women are more likely to have SGA births after bariatric surgery, due to nutrient deficiency if the pregnancy occurs too soon after surgery (164, 165). Therefore identification and implementation of more tractable therapies such as lifestyle interventions, potentially during pregnancy, is essential to break the cycle. We are still far from identifying the mechanisms that underlie developmental programming in response to an adverse in utero or early postnatal environment, but this is crucial in order to
develop therapeutic interventions and appropriate guidelines for pregnant women. Furthermore, critical developmental periods need to be defined in order to appropriately time intervention.

Maternal dietary supplementation is an attractive option for therapies targeting fetal development, as indicated by the success of folic acid supplementation in reducing the incidence of neural tube defects. Recent reports from rodent models suggest that maternal diet supplementation with methyl donors blocks some of the adverse effects of maternal obesity on offspring physiology (168), suggesting that changes to global methylation levels contribute to offspring phenotype.

Numerous studies have implicated maternal insulin sensitivity and glucose homeostasis as an underlying cause mediating offspring phenotypes in response to maternal weight. If maternal glucose homeostasis is indeed a key factor, then normalizing maternal glucose tolerance independent of body weight should be an effective intervention. Exercise is an effective way to improve insulin sensitivity and thus glucose homeostasis in obese subjects (169). Small lifestyle changes before and/ or during pregnancy are more likely to be successfully adopted by patients than severe changes and heavy therapeutic regimes. Additionally, pregnancy itself is an ideal opportunity to promote lifestyle changes as women have increased motivation to improve their own health for the benefit of their unborn child. A study promoting lifestyle changes such as moderate exercise and improved dietary choices in overweight pregnant women is currently being carried out in the UK (170). Follow up of both the mother and offspring from this study will be an important indicator of whether lifestyle interventions are sufficient to improve the maternal metabolic milieu and offspring metabolic disease risk, and provide important public healthcare messages. Such information will be critical if we are to break the cycle of obesity transmission from parent to child and halt the increasing prevalence of metabolic disorders.

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Table 1: Comparison of maternal diets used in studies that report hypothalamic programming in offspring
The choice and timing exposure of maternal diet has significant effects on offspring phenotypes, which are often sex specific. In particular, female offspring glucose homeostasis appears to be more susceptible to programming by exposure to a maternal diet including high sucrose. It is also interesting to note the various degrees of increase in maternal body weight caused by different diet options, as even studies with little or even no weight gain in dams that are consuming a calorie- rich diet still result in strong offspring phenotypes.
Figure 1: Comparison between human and rodent neuronal development

During human brain development both neurogenesis and the formation of functional connectivity occur during gestation. However, in rodents full synapse connectivity is not established until the early post-natal period. Of particular interest for hypothalamic development, murine hypothalamic neurons involved in regulation of energy balance appear between embryonic days 12-16 and functional connectivity between different nuclei of the hypothalamus is established during the first four weeks of post-natal life.

Figure 2: Role of metabolic hormones in development of hypothalamic circuitry

Recent papers have shown that the metabolic hormones leptin, ghrelin and insulin are required for the correct formation of projections from the ARC to the PVH. The involvement of metabolic hormones in normal hypothalamic development leaves the hypothalamus vulnerable to disruption in instances where these hormone levels are altered due to the maternal nutritional state. Intra-hypothalamic connections shown in solid lines have been shown to be vulnerable to disruption upon exposure to maternal obesity, GDM or under-nutrition. It is not fully understood whether adverse perinatal nutritional environments impact on the development of the hypothalamic circuitry highlighted in dotted lines, nor whether metabolic hormones are involved in the correct formation of these circuits.
In utero exposure to maternal obesity, diabetes or under-nutrition increases offspring susceptibility to metabolic disease. This creates a vicious cycle by which the next generation is also exposed to adverse nutritional conditions in utero. Recent research from animal models suggests the cycle could be broken in the pre-pregnancy period by non-invasive lifestyle interventions such as a change to the maternal diet, as well as more serious surgical weight loss interventions in the mother. During pregnancy, both lifestyle and therapeutic interventions are being trialed currently in human cohorts. Recent research from animal models highlights the importance of some therapeutic interventions being sex specific.


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