EARLY LIFE PROGRAMMING OF OBESITY

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Abstract
As the prevalence of obesity increases across the globe, vast efforts are being directed towards understanding the origins of obesity and mechanisms underlying this rapid increase. It is well known that the current environment of an individual can affect body weight, however, growing evidence suggests that the environment in very early life may be particularly important in determining long term obesity risk. This was prompted by a series of epidemiological studies demonstrating a relationship between suboptimal early growth and later risk of obesity. Evidence from human studies as well as animal models have shown that alterations in nutrition and growth in utero and during early postnatal life can have permanent effects on systems mediating regulation of energy balance. Rapid postnatal growth in particular has been associated with increased risk of developing obesity while slower postnatal growth lowers this risk. Alterations in pathways mediating energy homeostasis have been associated with both patterns of early growth. These include changes in structure and function of neuronal pathways in the brain which lead to deregulation of pathways mediating energy balance. In addition to the alterations at the central level, early nutrition can have detrimental long-lasting effects on peripheral physiological systems, for example the storage of fat and utilization of nutrients that make an individual more prone to development of obesity. The fundamental mechanisms underlying these programmed changes are still to be fully defined, although epigenetic mechanisms may play an important role.

Key words: animal models, epigenetics, insulin, leptin, maternal diet

INTRODUCTION
As the prevalence of obesity increases across the globe, it is estimated that 400 million adults have a body mass index (BMI) exceeding 30 kg/m² (defining obesity) (1). This number is forecast to rise to 700 million by 2030, with the biggest increases projected to occur in developing countries, such as China and India (1). Of particular concern are rising levels of obesity in children with the pattern mirroring that of the adult population. Understandably, vast efforts of current research are directed towards understanding the origins of obesity and mechanisms underlying such a rapid increase in obesity rates.

It is widely acknowledged that the current environment, including diet, lifestyle choices such as physical activity or substance abuse, can affect the health of an individual. In recent years, however, it has become apparent that the early life environment and in particular early nutrition, can have long-lasting effects on the susceptibility of an individual to develop a wide spectrum of conditions including type 2 diabetes, obesity and cardiovascular disease. Such “programming” occurs when a stimulus or insult experienced during a critical period of development in early life causes permanent alterations in the development and organisation of key tissues and organ systems that lead to irreversible changes in the structure and function of the body.

EPIDEMIOLOGICAL STUDIES
Initial programming studies focused on the effects of intrauterine malnutrition and low birth weight (LBW) on the subsequent risk of developing insulin resistance and obesity. Studies of individuals in utero during period of famines proved particularly useful. The association between intrauterine malnutrition and subsequent development of obesity was found in individuals exposed to the Dutch Hunger Winter and Chinese famine (late 1950s to early 1960s) (2, 3). The effects of the exposure to famine on susceptibility to obesity were dependent on the timing of exposure. Increased obesity rates were observed amongst 19-year old men exposed to the famine in utero during the first half of gestation while exposure to famine during the last trimester of gestation and in early postnatal life was associated with reduced obesity (2). Studies into the effects of poor fetal growth on long term health are clinically important as 23.8% of all the infants have a LBW (4). LBW is not only a problem in the developing world but also in the developed countries. For example, the number of LBW births has increased steadily in the United Kingdom, despite falling birth rates (5). A similar trend has been reported in Canada (6). As LBW infants can be born to both malnourished and over-nourished mothers, rising obesity rates amongst women of reproductive age are likely to contribute to this trend (7).
The increased prevalence of obesity amongst women of reproductive age, and parallel increase in the prevalence of gestational diabetes, has led to a large number of studies assessing the effect of maternal over-nutrition on the development of obesity in the offspring. Maternal body mass index (BMI) of more than 25 and gestational diabetes are both associated with increased offspring adiposity later in adulthood. It could be postulated that higher rates of overweight in the offspring exposed to maternal obesity and/or gestational diabetes arise due to inheritance of a higher number of obesity/diabetes susceptibility genes. A shared current obesogenic environment could also play an important role. However, a direct evidence for a role of the obesogenic/diabetic fetal environment in mediating the effect of maternal body weight on the susceptibility to development of obesity in the offspring has been obtained from sibpair studies. Maternal weight gain and subsequent increase in BMI between pregnancies have been shown to result in increased risk of developing obesity in the child, when compared to its older sibling (8). Similarly, children born to diabetic mothers have higher risk of developing obesity when compared to their siblings born when their mother had normal glucose metabolism. In addition, reduction of maternal BMI using interventional strategies such as bariatric surgery, leads to a decrease in the risk of developing obesity in the offspring, when compared to the sibling(s) born prior to maternal surgery (9). Hence, children born to the same mothers have disproportional risk of developing obesity depending on the intrauterine environment they experienced. This risk is present in addition to and independent of any inherited susceptibility genes. Therefore, both maternal under-nutrition and maternal obesity during pregnancy may increase the risk of developing obesity in the offspring (Fig. 1).

The early postnatal period is also a critical time period for determination of long-term body weight. The risk of developing obesity is the greatest, when conflict occurs between the environment experienced in utero and in early postnatal life. Intrauterine growth restriction (IUGR) infants born into an environment of plentiful or adequate nutrition, grow rapidly and show increased adiposity throughout childhood, adolescence and adulthood. In addition to increased deposition of adipose tissue these individuals have a tendency to store adipose tissue centrally, which has particularly detrimental effects on the metabolic function, including insulin resistance. Postnatal growth rates can also have lasting implications for obesity risk independently of growth in utero. Rapid postnatal growth per se is associated with increased risk of obesity and metabolic abnormalities even in normal birth

![Fig. 1. Proposed model for early programming of obesity. BBB – blood-brain barrier](image)
weight individuals (10). In contrast, a lower risk of developing obesity in childhood and adolescence has been observed in breast-fed infants, who have a slower postnatal growth rate during the first year of life and are leaner than formula-fed children (11). These beneficial effects of breast milk have been attributed to its lower energy and protein content, and better control of the amount of milk consumed.

Recently, a causal relationship was observed between early postnatal nutrition and obesity in children by a number of randomised intervention studies. Koletzko et al. showed that infants fed a protein enriched formula had a higher body weight at two years of age compared to those fed formula milk with a lower protein content (12). Singhal et al. studied the effect of nutrient-enriched formula feeding on obesity risk in older children. In this study, term infants with a lower birth weight were fed either a standard formula or growth-promoting nutrient-enriched formula containing 28-43% more protein and 6-12% more energy (13). At age 5-8 years the children fed the control formula had a lower fat mass than those who received the nutrient enriched formula. Consistent with these observations, infants fed a lower protein diet had slower growth in the first 2 years of life and lower body weight in childhood and adolescence (14, 15). These studies support the link between faster weight gain in infancy and increased risk of developing obesity in later life.

**EVIDENCE FROM ANIMAL MODELS**

Evidence from animal models has supported the observations made in epidemiological studies. Animal models also help in the elucidation of the mechanisms mediating the effects of a suboptimal environment on the risk of developing obesity later in life.

The effects of maternal malnutrition on offspring risk of obesity were initially studied. The outcomes were dependent on when during early life the nutritional challenge was experienced. Maternal calorie restriction (50% of ad libitum) during the first two weeks of gestation led to the development of obesity in male, but not female offspring, shortly after weaning (16). Less severe maternal calorie restriction (30%) throughout pregnancy resulted in male offspring developing hyperphagia and greater accumulation of adipose tissue without any change in body weight (17). Although only male offspring were included in this study, there is now growing evidence that the early nutritional environment affects male and female offspring in different ways. When the whole litter of offspring born to control mothers was cross-fostered to low protein fed dams during the suckling period [postnatal low protein (PLP) offspring], the pups grew slower during the suckling period. This manipulation reduces the available nutrition for each pup and results in permanent down-regulation of appetite and permanent reduction in body weight (18). The reduction in appetite of these mice was powerful enough to prevent excessive weight gain on a highly palatable diet (19).

Several rodent models have been used to study the permanent effects of increased nutrition during early postnatal life. One approach involves manipulation of nutrition during early postnatal life through reduction in litter size (20). This leads to a relative surplus of milk for each pup and changes the composition of maternal milk, leading to increased fat content. This results in hyperphagia, hyperleptinaemia, hyperinsulinaemia and increased growth trajectories leading to permanent overweight and increased fat deposition (21). The second approach involves restricting maternal protein intake during gestation alone and cross-fostering the offspring to control-fed dams for the period of lactation. This results in the birth of IUGR offspring that undergo rapid postnatal growth (recuperated offspring) and gain excess weight post-weaning (18).

Several animal models have been established to study the effects of maternal over-nutrition on energy balance in the offspring. The feeding of obese dam during gestation and lactation that reflects a modern western diet that is high in sugar and fat leads to increased adiposity in both male and female offspring (22). However the timing of obesity development varied between the two genders with female offspring becoming obese earlier than males (22).

**REGULATION OF ENERGY BALANCE**

Energy homeostasis is tightly regulated to maintain a relatively stable body weight over time despite day to day fluctuations in energy intake and expenditure. This is achieved through the interaction of complex peripheral and central mechanisms. A wide range of peripheral factors is involved in conveying information about the current level of adiposity, energy intake and circulating nutrients to the brain. Short-term information related to hunger and satiety is conveyed by signals from the gastrointestinal tract and neuronal afferents, with factors including cholecystokinin, ghrelin and peptide YY (PYY 3-36) mediating the inputs. In addition, two hormones, insulin and leptin, are secreted in proportion to adiposity and are often called the long-term signals. Increases in insulin and leptin concentrations occur during positive energy balance with reductions occurring during negative energy balance. Both hormones enter the central nervous system (CNS) in proportion to their circulating levels using receptor-mediated transport across the blood-brain barrier (BBB) (23). Direct administration of exogenous insulin and leptin into the third ventricle leads to a reduction in food intake and promotion of energy expenditure (24). It should be noted, however, that despite insulin exerting an anorectic effect, it is the key activator of energy storage in adipose tissue, thus prolonged elevation of insulin levels may lead to the development of obesity. In addition to its effect on the hypothalamus to reduce food intake, leptin also reduces storage of fat in non-adipose tissue, therefore playing a role in protecting against lipotoxicity and maintaining glucose sensitivity. A deficiency in leptin or leptin receptors leads to the development of severe early-onset obesity in both humans and rodents (25, 26).

**CENTRAL CONTROL OF ENERGY BALANCE**

The CNS plays a key role in regulating food intake and energy expenditure by integrating signals from circulating nutrient-related factors, gastrointestinal afferents and signals generated within the brain itself to alter neuroendocrine...
function and behaviour. Early lesioning studies showed that the hypothalamus, which encompasses a number of anatomically discrete nuclei, plays a major role in regulation of energy homeostasis. The arcuate nucleus (ARC) located at the base of the third ventricle contains ‘first order neurons’. The nerve endings of these neurons are in direct contact with the microvessels. The ARC contains two distinct neuronal populations: orexigenic NPY and AgRP neurons and anorexigenic POMC and CART neurons. Both insulin and leptin stimulate signal transduction pathways that are projected to other nuclei, containing ‘second order neurons’ within the hypothalamicus [e.g. the paraventricular nucleus (PVN), the lateral hypothalamic area, and the ventro-medial hypothalamic nucleus (VMH)] and to other regions of the CNS. Increased leptin and insulin concentrations stimulate POMC/CART and inhibit NPY/AgRP neurons leading to inhibition of food intake and increased energy expenditure. Upon activation of POMC/CART neurons, melanocyte-stimulating hormone (α-MSH) is released and binds to melanocortin receptors (MCRs) in the PVN, further enhancing catabolic signals. α-MSH action can be inhibited by AgRP via the antagonistic action at MCRs.

In addition to its role in regulation of food intake and body weight, the hypothalamus has also been implicated in playing a key role in peripheral glucose homeostasis. Both insulin and leptin play a role in this process. Administration of insulin into the medio-basal hypothalamus (the region of ARC and VMH) increased hepatic glucosegenesis by enhancing insulin sensitivity, while down-regulation of insulin receptor in this region induced insulin resistance (27). Effects of leptin on glucose homeostasis are also mediated by its effects on the hypothalamus. Improved glucose homeostasis and insulin resistance were observed in the leptin-receptor-deficient animals in which functional leptin receptors were restored in the ARC (28). Other nutrients that target the hypothalamus are free fatty acids, amino acids and gut hormones, such as peptide YY and ghrelin. In addition to the energy regulating pathways in the hypothalamus, other regions (e.g. nucleus tractus solitarius and nucleus accumbens) and pathways within the brain (e.g. the mesolimbic pathway) are also involved in the regulation of body weight.

**EFFECTS OF EARLY SUBOPTIMAL NUTRITION ON REGULATION OF BODY WEIGHT**

Suboptimal nutrition in early life has been shown to lead to impaired body weight regulation that arises as a consequence of alterations in hypothalamic structure, neuronal activity, neuropeptide and receptor levels, as well as hormonal responsiveness in later life. Analysis of the brains of rats raised in small litters revealed increased expression of orexigenic neuropeptides, including NPY and galanin in the ARC and resistance to insulin and leptin within the ARC and PVN (21, 29). These differences could explain their hyperphagia.

The rate of postnatal growth has different effects on the expression of neuropeptides and receptors within the ARC. Rats that underwent slow postnatal growth were hypoglycaemic, hypoinsulinaemic and hypoleptinaemic at 21 days of age and this was associated with increased leptin receptor expression in the ARC (18). At 3 months of age PLP animals remained smaller and leaner than controls. This was associated with increased sensitivity to actions of leptin and melanocortins to reduce food intake and decreased sensitivity to stimulation of food intake by NPY (30).

Re recuperated animals, which were exposed to maternal protein restriction in utero and underwent accelerated postnatal growth were hyperleptinaemic when compared with controls at 21 days of age, but this was not associated with changes in expression of key leptin responsive neuropeptides (18). Consistent with the hypothesis that leptin-independent mechanisms are involved in the programming of obesity in this model, we recently demonstrated that leptin-deficient ob/ob mice could be made fatter by applying the recuperated model of low birth weight and catch up growth (31).

**EFFECT OF SUBOPTIMAL EARLY NUTRITION ON ADIPOSE TISSUE**

Altered adipocyte function had been reported in many models of developmental programming with obesity being developed as a consequence of increasing size and number of adipocytes. In humans, the deposition of adipose tissue starts before birth, unlike in rodents. Therefore, studies in precocial animal models such as the pig and sheep may reflect more closely the human situation. Using sheep studies, it has been shown that offspring exposed to plentiful nutrition in utero have enhanced adipose tissue accumulation that has been associated with upregulation in lipoprotein lipase and leptin (32).

**MECHANISMS OF DEVELOPMENTAL PROGRAMMING OF OBESITY**

Central resistance to the action of leptin

It is well documented that obese individuals have elevated levels of leptin, which implies leptin resistance. Leptin resistance in relation to inhibition of food intake is a common phenotypic outcome in adult offspring of obese and/or diabetic mothers (33). Early over-nutrition has been associated with early-onset ARC leptin resistance and increased weight promoting effects of high-fat diet. Therefore, over-nutrition in early life can permanently alter central regulation of food intake and significantly increase susceptibility to development of obesity. Evidence for such deregulation can be detected very early in life as the ability of leptin to activate intracellular signaling in ARC neurons has been shown to be reduced in the neonatal offspring of diabetic mouse dams (33). It has been proposed that the development of leptin resistance in models of diet-induced obesity is related to the defects of leptin transport across the BBB. One of the factors that may have a direct effect on the BBB is elevation of fasting plasma triglycerides, which often accompany leptin resistance (34).

**Alterations in postnatal leptin**

In addition to its role in energy homeostasis in adults, both leptin and insulin play important roles as neurotrophic factors in early neonatal life in rodents. A surge in circulating
leptin occurs during early postnatal life. The profile of this surge can be altered by maternal and neonatal nutrition. The premature leptin surge observed in the offspring of undernourished dams or a premature leptin surge induced in normally-fed offspring is associated with an increased susceptibility to weight gain when offspring are weaned on a high-fat diet (35). An amplified and prolonged neonatal leptin surge has been reported in the offspring of obese dams (36). As leptin can stimulate neurite outgrowth from ARC explants from day 4 of postnatal life, a disruption in the postnatal leptin surge could alter the formation of neuronal pathways and contribute to the later development of obesity.

The source of leptin in early life is varied and may include leptin transferred across placenta, endogenous leptin and milk leptin. Leptin in maternal milk may play an important role in the maturation of organ systems and feeding pathways in neonates. Breast fed infants have higher levels of circulating leptin than formula fed children and the formula fed infants have elevated risk of developing obesity. Therefore, it is possible that milk leptin is a programming factor. This is supported by observations in experimental animals. Oral intake of physiological doses of leptin during the suckling period in rats has been shown to prevent development of obesity later in life (37). It should be noted, however, that the effects of neonatal leptin treatment on postnatal weight gain depend on the maternal nutritional status during pregnancy. In adulthood, lower body weight was observed in neonatally leptin treated male offspring of undernourished dams, while neonatal leptin treatment of male offspring from normally nourished mothers displayed diet-induced weight gain and increased body adiposity without an increase in food intake (38).

**Modification of circadian rhythms**

Recently it has been reported that maternal nutrition can modify circadian rhythms in the offspring. Adult offspring of low protein dams that was cross fostered to control dams for the suckling period had abnormal circadian rhythms prior to the onset of obesity, and this was evident in brain, liver and muscle. A diurnal increase in NPY mRNA expression was also observed which could contribute to the altered feeding behavior in these animals (39).

**Epigenetic regulation**

There is growing evidence to suggest that epigenetic modifications such as DNA methylation, histone tail modifications, chromatin packaging and microRNAs (miRNAs), which alter gene expression without changing DNA sequence, are important mediators of developmental programming. The epigenome can be altered by early nutrition during specific periods of development and such alterations have been reported across a number of tissues and organs in both humans and animals exposed to suboptimal early nutrition. However, to date there is limited evidence for epigenetic regulation being involved in developmental programming of obesity. Hypermethylation of the hypothalamic POMC gene promoter and insulin receptor promoter were reported in weanling pups raised in small litters, which could contribute to hypothalamic leptin/insulin resistance and obese phenotype observed in this animal model (40).

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**REFERENCES**


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**CONCLUSIONS**

Given that the prevalence of obesity has been increasing at an alarming rate in the last few decades, it is of crucial importance to understand the mechanisms underlying this process. There is now conclusive evidence that the early environment, in particular early nutrition, can have long-lasting effects on metabolic health, including body weight of an individual. It is a major challenge in the developmental programming field to capitalize on the information already gained from mechanistic studies and to design intervention strategies that would confer advantages for long-term health while preventing adverse health outcomes. As research has already identified the early postnatal period as a critical time window for programming of obesity, this time point represents an attractive target for intervention. A major challenge is to identify individuals exposed to suboptimal early environment, who are at increased risk of developing obesity and other obesity related diseases. This ultimately would help not only to reduce the economic burden of obesity on health care but also improve the health outcomes and quality of life of many individuals.
30. Stocker C.J., Wargent E.T., Martin-Gronert M.S. et al.: Leanness in postnatally nutritionally programmed rats is associated with increased sensitivity to leptin and a melanocortin receptor agonist and decreased sensitivity to neuropeptide Y. Int. J. Obes. (Lond.), 2012, 36, 1040-1046.

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