Environment-physiology, diet quality and energy balance: The influence of early life nutrition on future energy balance

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HIGHLIGHTS

• The quality of the early life environment is an important determinant of obesity and metabolic disease risk.

ARTICLE INFO

ABSTRACT

Diseases caused by impaired regulation of energy balance, in particular obesity, represent a major global health burden. Although polymorphisms, lifestyle and dietary choices have been associated with differential risk of obesity and related conditions, a substantial proportion of the variation in disease risk remains unexplained. Evidence from epidemiological studies, natural experiments and from studies in animal models has shown that a poor intra-uterine environment is associated causally with increased risk of obesity and metabolic disease in adulthood. Induction of phenotypes that increase disease risk involves the fetus receiving cues from the mother about the environment which, via developmental plasticity, modify the phenotype of the offspring to match her environment. However, inaccurate information may induce an offspring phenotype that is mismatched to the future environment. Such mismatch has been suggested to underlie increased risk of metabolic disease associated with a poor early life environment. Recent studies have shown that induction of modified phenotypes in the offspring involves altered epigenetic regulation of specific genes. Identification of a central role of epigenetics in the aetiology of obesity and metabolic disease may facilitate the development of novel therapeutic interventions and of biomarkers of disease risk.

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1. Introduction

Diseases associated with impaired regulation of energy balance represent a major health burden of global concern such that over 1 billion adults and 100 million children are over weight or obese [1]. Conditions associated with uncontrolled positive energy balance, including type 2 diabetes mellitus (T2DM) and hypertension, contribute significantly to the global prevalence of cardiovascular disease and so represent a major cause of morbidity and mortality. In developing nations such as India, which has the highest projected increase in T2DM for 2030 [1], there is evidence of a double burden of disease associated with malnutrition, including infectious disease, and of diseases associated with over-nutrition, including obesity. The latter reflects, at least in part, a trend towards changing dietary habits from a rural low energy diet to a Westernised diet that is high in energy, saturated fat and refined carbohydrate due to migration from the countryside to cities [2]. While dietary patterns, in particular those associated with a Westernised diet, together with sedentary lifestyle are major contributory factors to risk of obesity, individuals within populations differ in their susceptibility to such lifestyle challenges. Understanding the factors that underlie differences between individuals in susceptibility to an imprudent diet may have utility in predicting disease risk and facilitating early interventions.

2. Genetics versus environmental factors

Genome-wide association studies and epidemiological studies of candidate genes have identified a number of obesity-associated single nucleotide polymorphisms [3]. However, the proportion of risk that is explained by these alleles is relatively small. For example, for the FTO gene, which exhibits the strongest association with obesity of all the loci identified to date, the risk allele increases body-mass-index by 0.26 to 0.66 kg/m^2 and risk of obesity by an odds ratio of between 1.03 and 1.32 [3]. Furthermore, the association between mutant alleles and risk of obesity differs between ethnic groups. A recent study of the association between *FTO* ($n = 1946$) and *MAF* ($n = 2149$)

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polymorphisms and anthropometric outcomes found no significant association with weight gain, birth weight, BMI or ponderal index in infants [4]. Thus although genetic variation may contribute to risk of obesity, particularly in some individuals, a substantial proportion of the variation of obesity risk within populations remains unexplained.

Variations in the environment during early life acting via developmental plasticity can induce persistent changes in the phenotype of a developing organism. For example, Stockhard has shown that small variations in the oxygen tension of the water in which trout embryos were maintained induced substantial morphological abnormalities in the fry [5]. However, such phenotypic changes were only induced if the oxygen tension was altered during the blastocyst stage of the embryo which indicates that the ability to respond to environmental challenge was restricted to a limited period during development. The morphological changes induced in the fry appear to be detrimental and possibly stochastic. However, the response of other organisms to environmental cues may confer a survival advantage because the induced phenotypic trait represents adaptation to the environment at anticipated birth. For example, exposure to signals from predators during development of Daphnia induces production of a protective helmet [6], while increasing population density in the Desert Locust (*Locusta migratoria*) induces a switch from a solitary phenotype to a morphologically distinct, gregarious migratory form [7]. In the meadow vole, day length at the time of conception modifies the coat of the offspring such that those in autumn have a thicker coat than those born in spring [8]. One possible explanation for the effect of the early life environment on risk of non-communicable diseases in humans may lie in the relationship between the quality of the environment experienced before birth and that into which the fetus is born. Signals from the mother, based on information about her environment, have been suggested to induce in the fetus a phenotype that is suited to the environment that it will encounter after birth [9]. If so, environmental constraint during early life, such as poor nutrition, would induce in the offspring a phenotype that is matched to a postnatal environment in which nutrients are scarce [9]. These offspring, for example, may have an increased tendency to develop nutrient reserves which could confer a survival advantage if indeed the availability of nutrients is limited in the postnatal environment. However, if the signals from the mother provide inaccurate information about the postnatal environment, for example due to constraint in the supply of nutrients across the placenta when in fact nutrients are readily available to the mother, then the fetus would have a phenotype suited to nutrient scarcity but mismatched with a nutrient rich environment. In this case, the composition of the diet of the offspring may exceed the range that it is able to metabolise efficiently or appropriately, which may lead to increased risk of metabolic diseases including obesity [9].

3. Evidence for the role of the early life environment in determining risk of obesity-related diseases

There is now substantial evidence that the quality of the early life environment represents an important influence on future risk of a range of non-communicable diseases including obesity, T2DM, hypertension and cardiovascular disease [10]. Although the nature of the environmental challenge in early life that induced increased risk of disease is not known in most of the epidemiological studies, 'natural experiments' such as the Dutch Hunger Winter provide direct evidence for a causal association between nutrition during development and future risk of disease. For example, adults exposed to famine *in utero* exhibited increased body mass index (BMI) (7.4%) and waist circumference (14.5%) compared to those born before or conceived after the famine [11], although the magnitude of such effects was contingent on the period during development when nutritional constraint occurred [12]. A number of epidemiological studies have demonstrated that birth weight, a proxy marker of the quality of the intra-uterine environment, is associated with subsequent risk of obesity or higher BMI. These have

been reviewed in detail recently by Fall [13]. Overall, these studies had shown that birth weight is associated positively with future BMI [14], although some cohorts have identified an upturn in the lowest birth weight categories [15,16]. Furthermore, the findings of the Raine study cohort have emphasised the importance of postnatal growth trajectories in determining future adiposity [17]. However, more detailed anthropometric analysis has shown an inverse relationship between birth weight and abdominal fat, a risk factor for cardiovascular disease, in some [18–22], but not all [23] studies. Furthermore, the association between lower birth weight and central obesity in girls is modified by ethnic background [24]. Maternal diabetes [25–28], higher maternal blood glucose within the normal range [29] and maternal gestational weight gain have also been associated with increased risk of childhood obesity.

Overall, the findings of these studies point to a significant impact of an unfavourable environment during development on the future risk of obesity and cardio-metabolic disease. However, not surprisingly, such effects are modified by subsequent environmental exposures and by intrinsic factors such as ethnicity.

4. An overview of epigenetics

Epigenetics refers to a group of interrelated processes that regulate transcription without changing the DNA coding sequence. Such processes underpin early development and cell differentiation. The main mechanisms are methylation at the 5′ position of cytosine bases in CpG dinucleotide pairs in promoter regions as well as the gene body, covalent modifications of histones, in particular acetylation and methylation of lysine residues, and non-coding RNA species [30]. These epigenetic marks regulate transcription over periods of time from minutes to the whole life course [31]. Silencing of transcription by DNA methylation involves recruitment of DNA methyltransferases, methyl cytosine binding proteins, histone deacetylases and histone methyltransferases which cause chromatin condensation and inhibition of transcription factor binding [32–36]. However, transcriptional activity can also be regulated by differential methylation of one or more CpG loci within the 5′ regulatory region leading to changes in the binding of individual transcription factors. DNA methylation is primarily a repressive epigenetic mark, although there are exceptions to this rule. Histone acetylation, which is induced by histone acetyltransferases, maintains an open potentially transcriptionally active chromatin structure and is associated with unmethylated DNA sequences.

The amount and timing of plasticity of the DNA methylome are understood poorly, except during gamete maturation and embryogenesis. Szyf has proposed that the prenatal and neonatal periods, puberty and aging are periods of increased epigenetic plasticity [37] and experimental studies have shown marked differences between the DNA methylome of centenarian and a new born [38]. However, there is little information in humans about epigenetic plasticity during childhood or early adulthood, and very few longitudinal studies. Nevertheless, there is increasing evidence that induced changes in the epigenome underpin the early life origins of diseases associated with impaired regulation of energy balance.

5. Epigenetics and the early life origins of metabolic disease

Analysis of the parentally-imprinted insulin-like growth factor (IGF) -2/H19 locus in genomic DNA isolated from whole blood from individuals who were *in utero* during the Dutch Hunger Winter has shown that the level of methylation of exposed individuals was 3% higher than in unexposed same-sex siblings [39]. The same research group also found differences of similar magnitude in the methylation status of non-imprinted genes associated with inflammatory response, regulation of appetite and energy balance [40]. Although these findings demonstrate that epigenetic marks related to nutritional challenges in early life can be detected in adults, such small differences in methylation

are difficult to interpret in terms of gene function. However, such differentially methylated epigenetic marks may have utility as biomarkers of disease risk. This view is supported by the findings of Godfrey et al. who have shown that methylation of one CpG locus in DNA isolated from umbilical cord predicted approximately 25% of the variation in sex-adjusted fat mass in the children at 9 years of age [41].

Much of the current knowledge about the role of induced epigenetic changes in the aetiology of diseases associated with impaired energy balance is based upon studies in animal models. For example, feeding a diet with a moderate reduction in protein content to rats during pregnancy induced hypomethylation of the PPARα and glucocorticoid receptor (GR) promoters and induced higher mRNA expression of these genes and of their respective targets in the liver juvenile [42] and day 80 adult [43] offspring which was consistent with previous reports of higher plasma β-hydroxybutyrate and glucose concentrations in the fasting offspring [44]. These studies showed for the first time in a non-mutant model that changes to the epigenetic regulation of the specific genes involved in energy homeostasis can be induced in the offspring by modest changes to maternal macronutrient intake. The effects of the maternal diet on methylation status of the PPARα promoter were confirmed by sequencing analysis that showed specific CpG loci in putative transcription factor response elements were hypomethylated in offspring of dams fed the protein-restricted (PR) diet compared to controls [45]. Other studies have identified in rats changes in the epigenetic regulation of GR and PPARα induced in the offspring by a maternal PR diet in the heart [46], whole umbilical cord [47] and in the angiotensin receptor 1b promoter in adrenal glands [48]. The changes induced in the methylation of the GR promoter by the maternal PR diet were also associated with an increase in histone modifications which facilitate transcription including acetylation of H3 and H4 and methylation of histone H3 at lysine K4 [49].

Feeding pregnant rats a PR diet induced epigenetic silencing by histone modifications in pancreatic β-cells in the offspring which weakened the interaction between the HNF4α promoter and its downstream enhancer resulting in decreased mRNA expression [50]. Furthermore, age-related decrease in HNF4α activity was greater in the offspring of dams fed the PR diet than controls. HNF4α is involved in glucose sensing and insulin secretion [51] and has been implicated in type 2 diabetes mellitus [52]. Thus epigenetic silencing of HNF4α transcription as a consequence of prenatal nutritional constraint represents one possible mechanism to link the quality of the early life to future risk of diabetes. Furthermore, maternal obesity in sheep was associated with altered expression of specific non-coding RNA species in the liver of the offspring, while maternal weight loss altered the mRNA expression of genes involved in insulin signalling in the liver of the offspring [53]. Increasing sucking due to small litter size induced hypomethylation and increased expression of the proopiomelanocortin promoter in the hypothalamus of the offspring in rats [54].

Reduction of total food intake during pregnancy in rats to 30% of ad libitum induced hypermethylation and lower expression of PPARα and GR in the liver of 170 days old offspring [55] which was associated with obesity and impaired glucose homeostasis [56]. However, such effects on energy balance were reversed by neonatal treatment with leptin [57]. A recent study has shown that treatment with leptin during the neonatal period induces activation of a PPARα isoform that is usually expressed in liver, but not in adipose tissue, through reduction in the methylation status of specific CpG loci in its promoter [58]. Such induction of PPARα in adipocytes by leptin has been shown previously to switch the fat metabolism from storage to catabolism, although such effects were transient in adult animals [59]. Thus the effects of leptin exposure in early life that modulate the epigenetic regulation of genes in peripheral tissues are an important determinant of energy balance throughout the life course.

Together, these findings provide strong evidence that epigenetic changes induced by poor quality nutrition in early life are involved in impaired regulation of energy balance and that such effects involve altered control of key genes in liver and adipose tissue fatty acid metabolism, appetite, and glucose sensing and insulin secretion that persist throughout the life course.

6. The effect of nutritional interventions in juveniles on the epigenetic reregulation of energy balance

The majority of studies of the effect of nutrition in early life on energy balance have focused on the prenatal and neonatal periods. However, the juvenile–pubertal period also appears to exhibit plasticity to nutritional interventions. Supplementing the diet of rats with folic acid during their juvenile–pubertal period induced impaired lipid homeostasis, including down-regulation of hepatic fatty acid β-oxidation, hepatosteatosis and increased weight gain [60]. These changes were associated with hypermethylation of PPARα in the liver and hypomethylation of the insulin receptor in adipose tissue, both accompanied by reciprocal changes in the mRNA expression of the offspring. Although folic acid supplementation has not been shown to alter body composition in children [61], increasing folic acid intake during pregnancy, particularly when combined with low maternal cobalamin status, has been associated with increased risk of insulin resistance in 6 year old children. However, the limited extent to which confounders were taken into account in this study may restrict the extrapolation of the findings to other populations [62].

7. Conclusions

The ability to regulate energy balance appears to be influenced by the quality and quantity of nutrition during early life. Unbalanced nutrition during periods of phenotypic plasticity may induce phenotypes that are poorly suited to the prevailing environment through modifications to the epigenetic regulation of specific genes. Such mismatch between variations in metabolic capacity induced in early life and the abundance of nutrients in the environment may ultimately lead to metabolic disease. However, demonstration that epigenetic and phenotypic plasticity is involved in the aetiology of diseases associated with impaired energy balance may facilitate the development of novel interventions to ameliorate or reverse the effects of a poor environment in early life on future risk of disease.

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