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TOPIC HIGHLIGHT

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Developmental programming of the metabolic syndrome - critical windows for intervention

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Abstract

Metabolic disease results from a complex interaction of many factors, including genetic, physiological, behavioral and environmental influences. The recent rate at which these diseases have increased suggests that environmental and behavioral influences, rather than genetic causes, are fuelling the present epidemic. In this context, the developmental origins of health and disease hypothesis has highlighted the link between the periconceptual, fetal and early infant phases of life and the subsequent development of adult obesity and the metabolic syndrome. Although the mechanisms are yet to be fully elucidated, this programming was generally considered an irreversible change in developmental trajectory. Recent work in animal models suggests that developmental programming of metabolic disorders is potentially reversible by nutritional or targeted therapeutic interventions during the period of developmental plasticity. This review will discuss critical windows of developmental plasticity and possible avenues to ameliorate the development of postnatal metabolic disorders following an adverse early life environment.

Key words: Developmental programming; Metabolic syndrome; Obesity; Type 2 diabetes; Leptin; Animal models; Predictive adaptive responses

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INTRODUCTION

The rates of obesity and the metabolic syndrome are currently at epidemic proportions. Once considered a problem of developed countries, overweight and obesity are now dramatically on the rise in developing economies, particularly in urban settings. Globally, over one billion adults are overweight and with 400 million obese, it has been ranked as a critical public health issue[1-4]. Furthermore, over 20 million children under 5-year old are overweight. This marked increase in childhood obesity and related metabolic disorders will translate to a further increase in adult obesity, predicted to reach 2.3 billion by 2015[4-6]. It is a widely held view that the development of an obesogenic environment, due to ease of access to highly calorific food and reduced energy expenditure in work and leisure activities, is the primary cause of obesity and related metabolic disorders, particularly type 2 diabetes (T2DM)[7]. Metabolic syndrome is a com-

mon complex trait comprising of a set of risk factors for cardiovascular disease and T2DM and is likely the result of complex interactions between genes, dietary intake, physical activity and the environment. Although a number of genes have been identified that are associated with obesity and metabolic syndrome in humans[7,8], the genetic component of this condition cannot account for the dramatic increase in the prevalence of obesity and the metabolic syndrome in recent years. Relevant epidemiological and experimental studies have highlighted a relationship between the periconceptual, fetal and early infant phases of life and the subsequent development of adult metabolic disorders[9-11]. The terms "developmental programming" and the "Developmental Origins of Adult Health and Disease" are preferentially used to describe these associations. The mechanisms underlying developmental programming and the role of genetic *vs* environmental factors remain speculative. One general thesis is that, in response to an adverse intrauterine environment, the fetus adapts its physiological development to maximize its immediate chances for survival. These adaptations may include resetting of set points of metabolic homeostasis and endocrine systems and the down-regulation of growth, commonly reflected in an altered birth phenotype. More recently, the "predictive adaptive response (PARs)" hypothesis proposes that the degree of mismatch between the pre- and postnatal environments is a major determinant of subsequent disease[12,13]. Thus, it is thought that whilst these changes in fetal physiology may be beneficial for short term survival *in utero,* they may be maladaptive in postnatal life, contributing to poor health outcomes when offspring are exposed to catch-up growth, diet-induced obesity and other factors[13,14].

DEVELOPMENTAL PROGRAMMING OF THE METABOLIC SYNDROME - EVIDENCE FROM EPIDEMIOLOGICAL AND CLINICAL STUDIES

Following the initial work of Barker and colleagues that demonstrated a relationship between low birth weight and an increased risk of hypertension, obesity, insulin resistance and dislipidemia[15-17], the importance of maternal nutrition and, in particular, the effect of poor nutrition on birth weight and development of adult disease was addressed in studies of famine exposure. The most widely reported of these being the Dutch Hunger Winter of 1944-1945[11,18-20] where the timing of the exposure was a major determinant in phenotypic outcomes. Whereas famine exposure during early gestation was associated with adult hypertension[18], reduced maternal caloric intake in late gestation was associated with an increased adult adiposity and glucose intolerance[11,21]. Famine exposure in late gestation led to a greater impairment of glucose tolerance than during early or mid-gestation. The rate of obesity was higher in men exposed in the first half of gestation and lower in men exposed in the last trimester of gestation as compared to non-exposed men[11]. However, the data derived from those exposed to famine during the siege of Leningrad did not show any relationship between birth weight and adult metabolic sequelae[22]. Thus, while fetal exposure to a substrate limited environment at most stages of development appears to lead to adult dysregulation of metabolism, the precise mechanisms responsible may vary with the timing of exposure. The disparity between the Dutch and the Leningrad studies may be explained by the PARs hypothesis - in the Dutch offspring, nutrition was plentiful following the famine and thus was mismatched to the predicted environment. In the Leningrad cohort, nutritional status was poor both before and after the period of famine and thus the PAR may have been appropriate for the postnatal environment experienced[23].

In historically undernourished, recently urbanised populations such as India, where individuals of low birth weight are exposed to a high-fat western diet, the incidence of obesity and T2DM is reaching epidemic proportions[24]. Work by Yajnik and colleagues have shown that although Indian babies are born with low birth weight, they exhibit relatively increased visceral adiposity[24]. This is consistent with other studies of small babies, showing a disproportionate abdominal fat mass during adult life, despite a lower body mass index[25]. Although there is considerable debate whether catch-up growth in early postnatal life is beneficial or not, most studies suggested that postnatal "catch-up" growth is associated with adverse outcomes in later life[22,26]. Interestingly, work by Parsons *et al*[27] found that men with a lower birth weight who exhibited catch-up growth and achieved a greater proportion of their adult height by age 7, had a risk of obesity comparable to that of men with higher birth weights.

Being born small for gestational age (SGA) is known to be associated with an increased risk of developing the components of the metabolic syndrome, although the biological mechanisms underlying this association are still unclear. Children born SGA followed by catch-up growth have been shown to have elevated serum leptin concentrations which are significantly correlated with insulin sensitivity parameters[28]. Work by Eriksson *et al*[29] demonstrated that the ponderal index at birth was a reliable predictor of later obesity and they also found that an early adiposity rebound in babies born of low birth weight was associated with obesity in adult life.

Although prenatal growth restriction has clearly demonstrated influences on long term adiposity, it is important to recognise that the relationship between birth weight and later life pathophysiology is not linear. Large for gestational age babies are also at risk of obesity and diabetes, associations that have been supported by a number of studies investigating the long term effects of maternal hypoglycaemia [diabetes or gestational diabetes (GDM)][30-34]. In developed societies and societies transitioning to first world diets and lifestyle habits, caloric and/or fat consumption are generally excessive and therefore, unremarkably, maternal obesity is now a common pregnancy com-

plication[35,36]. Children exposed to maternal obesity are at an increased risk of developing the metabolic syndrome; even though some obese mothers do not fulfil the clinical criteria for GDM, they may still have metabolic factors that affect fetal growth and postnatal outcomes[37]. Maternal obesity is associated with obstetric complications, including fetal and neonatal death and poor lactation outcomes, and is the most significant predictor of childhood obesity[38,39] and metabolic syndrome in offspring[37]. Importantly, these effects may be self-perpetuating, as offspring of obese mothers are themselves prone to obesity, giving rise to transgenerational effects[40,41].

DEVELOPMENTAL PROGRAMMING OF THE METABOLIC SYNDROME - EVIDENCE FROM ANIMAL MODELS

Animal models have been extensively used to study the basic physiological principles of the developmental origins of health and disease (DOHaD) hypothesis and are essential to the search for the mechanistic links between prenatal and postnatal influences and risk for developing the metabolic syndrome in later life. Although epidemiological data suggest that developmental programming occurs within the normal range of birth size[42,43], most experimental work has focused on significant restriction of fetal growth in the assumption that insults impairing fetal growth are likely to be those triggering developmental programming. Several approaches have been adopted to induce early growth restriction in animals. These aim to elucidate the relationship between early growth restriction and adult onset disease and provide a framework for investigating the underlying mechanisms. In the rodent, obesity and metabolic disorders have been induced in offspring by maternal global undernutrition[44-48], a low protein diet[49], maternal uterine artery ligation[50,51], maternal dexamethasone (DEX) treatment[52], maternal anemia[53] or prenatal cytokine exposure[54]. In this context however, intrauterine growth restriction (IUGR) is not essential to developmental programming but is merely a surrogate for evidence that fetal development may be adversely affected.

Epidemiological studies demonstrate that fetal growth restriction correlates with adult disease, implying that fetal nutritional deprivation is a strong stimulus for programming[55]. So, experimental animal models were developed using controlled maternal caloric intake or protein or macronutrient deficiency. However, in many developed societies, maternal and postnatal caloric intake can be excessive. A number of researchers have shown that programmed obesity may represent a U-shaped curve with a higher prevalence of adult obesity occurring in individuals who were on either deprived or excessive planes of maternal nutrition[25,55-58].

MATERNAL UNDERNUTRITION

The early work of Barker and colleagues highlighted the role of fetal nutrition as the primary factor involved in the developmental origins of adult disease. Within the laboratory, early life undernutrition can be achieved through maternal dietary restriction during pregnancy and/or lactation and in some cases during the periconceptional period[59,60]. At present, those investigating the mechanistic links between maternal undernutrition and adult disease generally utilize one of two dietary protocols in the rodent; global undernutrition or isocaloric low protein diets, with the maternal low protein (MLP) diet being the more extensively used[61-65]. The MLP model involves ad-libitum feeding to pregnant rats a low protein diet containing 5%-9% (w/w) protein (casein), generally a little under half the protein content but equivalent in energy of a control diet containing 18%-20% (w/w) protein[61,66]. Offspring from protein restricted mothers are 15%-20% lighter at birth[63] than offspring of control fed mothers. Extending the MLP diet throughout the period of lactation increases the weight difference and permanently limits later growth. If MLP offspring are cross-fostered to mothers fed a control diet, they exhibit rapid catch-up growth[63]. This appears to have a detrimental effect on life span, which results in premature death associated with accelerated loss of kidney telomeric DNA[67]. Altered insulin sensitivity of adipocytes in MLP offspring has also been well documented; the findings of these studies show that enhanced activation of insulin receptor substrate-1 associated phosphatidylinositol 3-kinase (PI3K) activity may be the key to improvements in insulin sensitivity[68]. However, alterations in PI3K subunit expression indicate that the adipocytes of MLP offspring may be resistant to insulin's antilipolytic effects[68].

Experimental observations in the MLP diet model of developmental programming highlight many potential mechanisms that may be involved in the pathogenesis of obesity and T2DM. These mechanisms include both physical and functional changes to various organ and endocrine systems. Gene ontogeny analysis of visceral adipose tissue (VAT) from MLP rat offspring revealed a global up-regulation of genes involved in carbohydrate, lipid and protein metabolism[69]. Thus VAT in the MLP model is marked by dynamic changes in the transcriptional profile of key metabolic genes.

Global undernutrition during pregnancy is a widely used approach to induce nutritional programming of obesity. Various models have been developed with different levels of undernutrition during different periods of pregnancy. In the rat, a moderate nutritional restriction (70% of normal intake) in the first 18 d of pregnancy results in offspring with significant IUGR that catch up in body weight to that of controls by postnatal day 20. These abnormalities increase with age and are most pronounced in male offspring[70].

We have developed rodent models of developmental programming using global maternal undernutrition throughout pregnancy[47,71]. When dams are fed at 30% of ad-libitum intake throughout pregnancy (i.e. a severe level of undernutrition), offspring birth weights and placental

weights are 25%-30% lower than offspring of control fed mothers. These offspring display increased adiposity, hypertension, hyperinsulinemia, hyperleptinemia, reduced locomotor activity, leptin resistance and hyperphagia in adult life[47,72,73]. Severe global undernutrition has also been shown to result in altered neuroendocrine gene expression, including pro-opiomelanocortin (POMC), agouti-related peptide and neuropeptide Y[74,75]. When the degree of undernutrition is reduced to a more moderate level, i.e. 50% of *ad-libitum*, offspring still display a significant level of obesity in postnatal life. Of note, if pre-weaning catch-up growth in offspring is prevented by maintaining the mothers on the restricted diet throughout lactation, offspring do not develop an obese phenotype (authors unpublished observations and[76]). This is similar to reports in the MLP model where continuation of the low protein diet into lactation prevents the development of the metabolic phenotype, once again highlighting the possible adverse consequences of catch-up growth[76].

Although maternal macronutrient malnutrition has been well studied, the role of maternal micronutrient restriction has not been widely investigated. From the limited data available, maternal micronutrient restriction has been directly associated with the developmental programming of several features of the metabolic syndrome. Maternal iron deficiency has been a focus of recent studies with several features of the metabolic syndrome observed in offspring following maternal iron deprivation[36]. Work by Gambling *et al*[77] highlighted that the timing of iron supplementation is critical in reversing the effects of maternal anemia on the developing fetus and postnatal sequelae in offspring. These data correlate well with human studies showing that iron supplementation during pregnancy leads to a higher mean birth weight and reduced incidence of low birth weight infants[78].

Maternal chromium restriction significantly increased body weight and fat percentage, especially central adiposity, in both male and female rat offspring[79,80]. Restricted vitamin intake during pregnancy has been shown to increase the phenotypic expression of obesity and components of the metabolic syndrome in both female and male rats fed a post-weaning obesogenic diet[81]. Maternal and perinatal magnesium restriction has also been shown to predispose rat pups to insulin resistance and glucose intolerance[82,83].

MATERNAL OBESITY

Over recent years there has been an increasing focus on developing models of maternal obesity. Several obesogenic models, primarily in the rodent, show a relatively common phenotype of metabolic disorders in offspring, although the magnitude of effects differs with the timing of the nutritional challenge and diet composition[84].

A maternal cafeteria or high fat diet has been shown to induce obesity, insulin and leptin resistance[58,85,86], hypertension[87-89], hepatic steatosis and non-alcoholic fatty liver disease in offspring[90-92]. Even mild maternal overnutrition has been shown to induce increased adiposity, glucose intolerance and altered brain appetite regulators in offspring[93]. We have recently shown that a moderate maternal high fat diet (HF) results in significant obesity and hyperinsulinemia in male and female offspring, independent of the level of post-weaning diet[56]. Furthermore, in pregnancies which have been complicated by maternal diabetes, GDM or impaired glucose tolerance, offspring have been shown to be at an enhanced risk of developing features of the metabolic syndrome[94-97]. In the sheep, maternal obesity has been shown to accelerate fetal pancreatic β-cell but not α-cell development[98]. Fetuses from obese ewes show increased systemic insulin levels due to increased glucose exposure and/or cortisol-induced acceleration of fetal β-cell maturation, which may contribute to premature β-cell function loss, and lead to a predisposition for obesity and metabolic disease.

The PARs hypothesis suggests that disease only manifests when the actual nutritional environment diverges from that which was predicted. So, it is notable that evidence for the programming of obesity and features of the metabolic syndrome come from both nutrient restriction (caloric, protein, iron) and fat-feeding studies, which suggests a commonality of mechanism[99].

ROLE OF EPIGENETICS

The ability of developmental plasticity to generate biological variation from one genotype is well understood and interest has emerged in the clinical significance of epigenetic processes, particularly those influenced by the external environment[100]. There is increasing evidence that "marked" regions of DNA can become "unmarked" under the influence of dietary nutrients. This gives hope for reversing propensities for metabolic disorders and other diseases that were acquired in the womb[101].

Experimental data in rodents and recent observations in humans suggest that epigenetic changes in regulatory and growth-related genes play a significant role in mediating the patho-physiological phenotypes derived from developmental programming[102,103]. Epigenetic processes lead to heritable changes in gene function by altering DNA chemistry independent of sequence and may be responsible for tissue-specific gene expression during differentiation. These mechanisms may underlie the processes of developmental plasticity[104]. Examples of epigenetic regulation include coordinated changes in the methylation of cytosine in cytosine-guanine (CpG) dinucleotides in the promoter regions of specific genes, changes in chromatin structure through histone modification (acetylation, methylation, *etc.*) and post-transcriptional control by microRNA[104]. Histone modifications in conjunction with DNA methylation regulate chromatin structure and gene expression. However, it is still debated where early life and/or environmental factors can influence the "histone" code in a manner similar to their influence on DNA methylation[105].

Adversity during pregnancy or early neonatal life in ex-

perimental programming models result in changes in promoter methylation, therefore, directly or indirectly, affect gene expression in pathways associated with a range of physiological processes[106]. For example, in the rat, altered promoter methylation and downstream changes in gene expression have been shown for the hepatic glucocorticoid receptor (GR) and the peroxisome proliferator-activated receptor-α (PPAR-α)[107,108], influencing carbohydrate and lipid metabolism[109,110]. Similar epigenetic changes have been observed in *p53* in the kidney[111] and the angiotensin II type 1b receptor in the adrenal gland[112], influencing renal apoptosis and pressor responses, respectively, and in the hypothalamic GR[113,114], influencing stress responses. The phenotypic effects of epigenetic modifications during development may not manifest until later in life, especially if they affect genes modulating responses to later environmental challenges, such as dietary challenges with a high-fat diet. The timing of the developmental windows and the induction of epigenetic changes in key physiological systems are not well characterized, but it appears to extend from the periconceptional period[115] into postnatal life[113,114]. There is also evidence from studies in twins for changes in the human epigenome related to age and the environment[116,117]. Many of the genes regulated by epigenetic change do not appear to be classically imprinted (expressed according to the parental origin of the allele), although some imprinted genes may show altered expression after perturbations during early development, such as if blastocyst culture *in vitro* is prolonged[118].

It is hypothesized that alterations in early life nutrition can influence DNA methylation since one-carbon metabolism is dependent upon dietary methyl donors and cofactors, including folic acid, choline and vitamin B_{12}[102,119]. Maternal dietary manipulations such as low protein exposure result in aberrant changes in DNA methylation in key genes which can be prevented by maternal dietary supplementation with cofactors[107]. Protein restriction in pregnant rats has been shown to induce a significant loss of DNA methylation concomitant with increased expression of key hepatic genes, including the GR and PPAR-α[107]. These epigenetic changes, a result of altered DNA methyltransferase 1 activity[108], were prevented with maternal folate supplementation[107]. Intriguingly, other models of early life adversity, apart from nutrition, have also been shown to influence epigenetic regulation of gene expression. Using a model of maternal uterine artery ligation, a comparison of IUGR *vs* normal rats revealed changes in DNA methylation at a number of novel loci, not limited to canonical CpG islands or promoters. The specific loci affected were in proximity to genes with important roles in β-cell function and development[120]. Also, shown in this model is that after the onset of T2DM in adulthood, the CpG island in the proximal promoter for pancreatic duodenal homeobox (Pdx1) was methylated, resulting in permanent silencing of the Pdx1 locus[121]. Meaney and colleagues have extensively investigated the role of maternal care during neonatal life on epigenetic regulation of gene expression patterns in the brains of offspring born to "low-caring" mothers. In their studies they demonstrate that an increased level of maternal care in the first week of life alters DNA methylation at specific CpGs in the GR gene promoter in the hippocampus of the offspring and in turn leads to a phenotype similar to that of maternal undernutrition models. Reversal of the epigenetic change leads to reversal of the phenotypes. Furthermore, Meaney's team has shown that alterations in offspring behavior may be modified by postnatal environmental enrichment and that these phenotypes can be passed from one generation to the next[122-124]. These results provide evidence for the role of social conditions beyond the postnatal period in altering patterns of maternal care and thus offspring phenotype and illustrate the interaction between the effects of postnatal and post-weaning environments.

Prenatal undernutrition has been shown to induce changes in histone H3 and H4 acetylation, consistent with facilitated transcription, in the GR gene in the liver[125]. From a mechanistic standpoint, studies in humans linking epigenetic change to metabolic disease risk remain very limited although there is some evidence for the inheritance of tissue specific DNA methylation patterns[126]. Differences in environmental exposure lead to different patterns of epigenetic marking in the somatic tissues of individuals. Twin studies show that DNA methylation and histone acetylation patterns diverged more strongly in older twin pairs with more marked life history differences[117].

It has been shown that the promoter in the leptin gene is subject to epigenetic programming and leptin gene expression can be modulated by DNA methylation[127-129]. Recent studies report that impaired glucose tolerance during pregnancy is associated with adaptations in leptin gene DNA methylation although the functional significance of these changes is not yet clear[130]. Yokomori *et al*[131] demonstrated that methylation of specific CpG sites and a methylation-sensitive protein could contribute to changes in leptin gene expression during adipocyte differentiation in 3T3-L1 cells. The same group has also shown that both methylation of specific CpG sites and a methylation-sensitive transcription factor contribute to GLUT4 gene regulation during preadipocyte to adipocyte differentiation[132]. In addition, differential DNA methylation was observed in promoters of genes involved in glucose metabolism including GLUT4[132] and uncoupling protein 2[133], both major contributors to the development of T2DM.

Epigenetic regulators work on the basis that exposure to environmental factors during critical periods of development permanently alters the structure or function of specific metabolic systems. Therefore, developmental epigenetics is believed to establish 'adaptive phenotypes' to meet the demands of the later-life environment[105,134]. Implicit in this concept is an important process of causality on the cellular level, regulating growth and tissue differentiation and involving chemical changes to the DNA or of associated proteins. Once the mechanistic basis of the disease is understood, epigenetic processes are po-

tentially reversible and intervention and strategies aimed at reversal could be devised and implemented. However, there are still many key questions to be answered[105]: How plastic is the system for intervention and reversal and what are the critical windows of development at which strategies should be targeted; how many generations does it take to reverse an epigenetic imprint and can surrogate markers be used for disease prediction?

CRITICAL WINDOWS OF DEVELOPMENT AND AVENUES FOR INTERVENTION

Maternal health and nutrition are key determinants in influencing infant growth but the precise molecular mechanisms underlying this relationship are largely unclear, although it is evident that there are critical windows of plasticity when these effects are important. Evidence from animal studies has shown that nutritional and pharmacological interventions may be able to ameliorate or reverse the consequences associated with developmental programming.

One of the earliest examples of intervention was that of maternal taurine supplementation to MLP dams. Studies have shown that taurine concentrations are low in diabetic and pre-diabetic states and that physiological plasma taurine levels are important for adequate β-cell function and insulin action[135]. In MLP rat offspring, β-cell mass is decreased at birth and metabolic perturbations last through adulthood even though a normal diet is given after birth or after weaning[136]. However, supplementing taurine to MLP dams restored normal release of insulin from MLP fetal islets, demonstrating how important taurine is to the development of normal fetal β-cell function[137].

However, MLP diets of differing composition used in different laboratories have yielded inconsistent data on the relationship between maternal protein intake and offspring blood pressure[66]. A critical role of methionine content in the MLP model was highlighted in work by Langley-Evans *et al*[66] and Rees *et al*[138,139] whereby different levels of methionine resulted in the MLP diets leading to different phenotypic outcomes. Several maternal dietary co-factors have also been shown to prevent the development of hypertension in offspring of MLP dams although the mechanisms are not well established. Maternal supplementation with glycine[140,141], folic acid[142,143] and choline (authors unpublished observations) has been shown to prevent programming-induced elevations in systolic blood pressure in offspring in postnatal life. There is some evidence for an epigenetic basis to these observations utilizing dietary methyl donor and co-factor supplementation; clinically relevant reductions in specific dietary inputs to the methionine/folate cycles during the periconceptional period can lead to widespread epigenetic alterations to DNA methylation in offspring and modify adult health-related phenotypes[107,115]. Moreover, altered methylation of gene promoters induced in the F1 generation by a MLP diet during pregnancy has been shown to be transmitted to the F2 generation, thus representing a mechanism for the transmission of induced phenotypes between generations[110].

There has been a lot of recent focus on the adipokine leptin. It has been proposed that deficiencies in leptin during critical windows of development could lead to a hardwiring of obesity[144]. In adult mammals, leptin acts on the brain to reduce food intake by regulating the activity of neurons in the ARH. Bouret *et al*[145,146] have shown that neural projection pathways from the ARH are permanently disrupted in leptin-deficient (Lep^{ob}/Lep^{ob}) mice. Treatment of Lep^{ob}/Lep^{ob} neonates with exogenous leptin rescues the development of ARH projections and leptin promotes neurite outgrowth from ARH neurons *in vitro*. It is well established that SGA children are hypoleptinemic and cord blood leptin concentrations are significantly diminished[147]. These children go on to develop obesity and leptin resistance in adult life and this can be mimicked experimentally in the rat[47]. Thus, perturbations in perinatal nutrition that alter leptin levels may have enduring consequences for the formation and function of circuits that regulate food intake and body weight[145,146,148,149]. Recent work investigating neonatal systemic leptin treatment in female Wistar rats born following maternal undernutrition has found that leptin prevented the development of diet-induced obesity and associated metabolic sequelae in adult life[150]. Leptin treatment normalised caloric intake, locomotor activity, body weight, fat mass and fasting plasma glucose, insulin, c-peptide and leptin concentrations, suggesting that any effect is not restricted solely to a central mechanism. Moreover, the effects were specific to animals born of low birth weight, with leptin having no effect in animals born to control mothers. The observations of leptin efficacy in the programmed rat have been replicated in the piglet. Work in piglets by Attig *et al*[151] showed that IUGR may be characterized by altered leptin receptor distribution within the hypothalamic structures involved in metabolic regulation and that leptin supplementation partially reversed the IUGR phenotype. The translation of findings across animal models itself bodes well for defining the role of leptin during this critical window of development.

Whether this effect of leptin is central or peripheral is unclear - one possibility is that the period of developmental plasticity is still open and the high leptin levels reverse the cuing effects of prenatal undernutrition[152]. The next piece to the puzzle is the question of the neonatal leptin surge; while the surge is well characterised in normal rodents[153] and may inform a window of intervention, the presence or absence of a leptin surge in humans is uncertain. Although altered maternal nutrition has been shown to alter the timing and duration of the leptin surge, the results are inconsistent across experimental models. Yura *et al*[154] reported a premature onset of the neonatal leptin surge following mild (70% of ad-lib) maternal undernutrition whereas MLP offspring display a delayed leptin surge[155]. Work by Delahaye *et al*[156] showed

that maternal perinatal undernutrition drastically reduced the postnatal leptin surge and altered the development of the POMC neurons in the arucate nucleus of neonatal male offspring. To date, little work has been done in maternal obesogenic models but Kirk *et al*[157] showed a prolonged and amplified leptin surge in neonates following maternal HF feeding.

Recent work in the rodent has shown that both growth hormone (GH) and insulin-like growth factor (IGF)-I can resolve several aspects of the metabolic phenotype in developmentally programmed offspring. Utilizing a model of maternal undernutrition to induce fetal growth restriction, offspring were fed either a chow or high fat diet postnatally. These offspring were hypertensive, obese, hyperphagic, hyperinsulinemic and hyperleptinemic; the effects of which were markedly amplified in the presence of a postnatal high fat diet[47]. Treatment of the adult phenotype with GH normalised systolic blood pressure and reduced fat mass. However, the hyperinsulinemia was exacerbated as a result of the diabetogenic actions of GH[45]. A further study in adult females with IGF-I infusion led to a complete normalisation of adiposity, appetite, fasting plasma insulin and leptin concentrations in developmentally programmed offspring[46]. These studies highlight the role of the somatotropic axis in programmed metabolic disturbances although the longer-term efficacy of such treatments is not known. Trials with GH in small for gestation age children have shown a normalisation in systolic blood pressure which was maintained for the 6 yr duration of treatment[158].

Epidemiological and experimental studies have shown that developmental programming leads to glucose intolerance and an enhanced risk for type 2 diabetes. Work by Park *et al*[121], Raab *et al*[159] and Stoffers *et al*[160] has shown that treatment of neonatal rats with the glucagon-like peptide (GLP)-1 analog Exendin 4 (Ex-4) reverses the adverse consequences of developmental programming and prevents the development of diabetes in adulthood. This occurs because neonatal Ex-4 prevents the progressive reduction in insulin-producing β-cell mass that is observed in IUGR rats over time and expression of Pdx1, a critical regulator of pancreas development and islet differentiation, is restored to normal levels. Although adiposity was not examined in this study, GLPs are known to modify food intake, increase satiety, delay gastric emptying and suppress glucagon release; and therefore further studies are warranted.

The role of possible direct nutritional interventions was highlighted in the work by Wyrwoll *et al*[161]. Pregnant rats were treated with DEX from d13 to term, and offspring were cross-fostered to mothers on either a standard diet or a diet high in omega-3 fatty acids and remained on these diets post-weaning. Maternal DEX reduced birthweight and delayed the onset of puberty in offspring. Hyperleptinemia and increased fat mass developed in offspring by 6-month of age in DEX-exposed animals fed a standard diet but these effects were completely ameliorated by a high omega-3 diet. These results demonstrated for the first time that direct manipulation of postnatal diet can limit adverse outcomes of developmental programming. Furthermore, work by Zambrano *et al*[162] has shown that dietary intervention (changing from an obesogenic HF diet to a normal chow diet) prior to pregnancy and lactation can reverse metabolic programming of male offspring of obese rats.

Although several animal studies have now shown that a range of interventions can reverse or ameliorate programming-induced metabolic disorders, translation to the human setting as regards optimizing maternal health is difficult. Furthermore, some interventions such as leptin have gender-specific effects and may potentiate an adverse metabolic response in normal offspring[150,163]. Some human trials support the initial animal observations. For example, supplementation with iron and folic acid in pregnancy has been shown to increase birthweight but this response was modified by maternal nutritional status, with infants born to women with better short-term nutrition having greater birthweight response[164]. Whether there is an epigenetic basis to these observations similar to those reported for the rat models is not well established although it has been suggested that alterations at the H19 differentially methylated region is a likely mechanism by which folic acid risks and/or benefits are conferred *in utero*[165].

CONCLUSION

Epidemiological, prospective clinical studies and experimental research have clearly shown that the propensity to develop the metabolic syndrome in later life is increased when early life development has been adversely affected. The pathogenesis is not based on genetic defects but on altered genetic expression as a consequence of an adaptation to environmental changes during early life development. However, little is known about the interaction between the pre- and postnatal nutritional environment on either amplification or resolution of the programming phenotype depending on the degree of nutritional match/mismatch. Thus, experiments to examine the PARs hypothesis are required in conjunction with transgenerational work to further the DOHaD paradigm.

The molecular mechanisms underlying developmental programming are only recently beginning to be investigated. Epigenetics has now become a model that is fundamental to research into DOHaD[2]. The two most studied epigenetic mechanisms identified as having a role in the adaptive developmental programming of metabolic disorders are DNA methylation and histone modifications. Availability of dietary methyl donors and cofactors during a critical window of fetal development may influence DNA methylation patterns. Thus, it has been proposed that early methyl donor malnutrition (i.e. excess nutrition or undernutrition) could effectively lead to premature epigenetic aging, thereby conferring an enhanced susceptibility to adult disease in later life[166].

Developmental programming research offers a novel

approach to investigate the mechanistic basis of obesity and related metabolic disorders which in human populations predominantly arise from environmental factors and lifestyle choices. It is notable that the variety of different insults in early life (caloric, protein, iron, fat-fed) produce the same detrimental consequences that occur in adult life, which suggests a common mechanism underlies the developmental early-life programming of adult disease. A recent emerging focus has been on studies aimed at reversing the programmed phenotype; such studies offer an exciting potential for new advances in our understanding of critical windows of developmental plasticity and mechanisms underlying human obesity and related metabolic disorders.

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