

Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome

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Abstract

Fetal programming is gaining momentum as a highly documented phenomenon which links poor early growth to adult disease. It is backed up by large cohorts in epidemiological studies worldwide and has been tested in various animal models. The root causes of programming link closely with maternal condition during pregnancy, and therefore the fetal environment. Suboptimal fetal environments due to poor or inadequate nutrition, infection, anemia, hypertension, inflammation, gestational diabetes or hypoxia in the mother expose the fetus to hormonal, growth factor, cytokine or adipokine cues. These in turn act to alter metabolic, immune system, vascular, hemodynamics, renal, growth and mitochondrial parameters respectively and most evidently in the later stages of life where they impact on the individual as poor glucose homeostasis, insulin resistance, type 2 diabetes, hypertension, cardiovascular disease, obesity and heart disease. These events are compounded by over-nutrition or lifestyle choices which are in conflict with the programming of the fetus. We and others have utilised various species to test the early life programming hypothesis and to identify key molecular mechanisms. With parallel studies of human cohorts, these molecular markers can be validated as realistic targets for intervention.

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1. Introduction

With over 50 references cited in PubMed when “fetal programming” is used as a search descriptor, it is clearly evident that research in this field is burgeoning and the list of pathologies linked to it is ever increasing. The nature of fetal programming is such that it is involved in many disease phenotypes, both for the in-utero affected individual and, as is emerging, that of succeeding generations. Nevertheless, this review will attempt to summarise the extent of the problems encountered when an organism develops in a suboptimal environment, which it perceives it will meet ex-utero, but then goes on to grow in adequate or better conditions.

2. Epidemiological basis of fetal programming effects

Many studies have revealed links between poor early human growth and susceptibility to type 2 diabetes, insulin resistance, cardiovascular disease, obesity and cancer. For a long time, the

markers used to define poor early growth have been relatively crude indices based on birth weight, length, abdominal and head circumference as well as placental weight and the various relative indices. However, there are still no means to measure if a newborn has attained its full growth potential in-utero. Nonetheless, the correlation between low birth weight and increased risk of metabolic disorders is well documented and low birth weight is thought to be due to a range of diverse mechanisms involving adverse maternal exposures. Disease links have been found for low birth weight, including cardiovascular disease [1], type-2 diabetes, abnormal lipid metabolism and hypertension [2]; and increased risk of death from ischemic heart disease in the adult [3–6]. Associations with obesity [7,8] and cancer [9,10] are also apparent. Hypertension and end-stage renal disease also associate with low birth weight and many studies suggest an impaired nephron development in low birth weight infants result in reduced glomerular number and increased glomerular volume [11–13]. The earliest epidemiological study linking poor fetal growth and subsequent development of type-2 diabetes was the observation by Hales et al. [14] which found that among men in their 60s, those who had lower birth weights and weights at 1 year were more likely to develop poor glucose tolerance and type 2 diabetes.

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These findings led to the proposal of the “Thrifty Phenotype” hypothesis [15,16] which suggests that fetal malnutrition may induce physiological and/or metabolic adaptations to ensure nutrient supply to the most vital organs (such as the brain) at the expense of other organs (e.g. pancreas). Such adaptations during critical periods may permanently reset (e.g. reduced β -cell mass) or programme the fetus’ metabolism in order to enhance its chances of survival in what it expects to be a poor nutritional environment (Fig. 1). This type of phenotypic adjustment forms the basis of evolutionary advantage; however, this supposed advantage is quickly turned against the programmed individual/offspring when it encounters nutritional abundance in later life with harmful long-term consequences. Further studies supporting this hypothesis followed. In the Dutch Hunger Winter study, reduced glucose tolerance was observed in adults who were in-utero at the time of strict food rationing towards the end of World War II [17]. This effect was most marked in those who were in their third trimester during the period of the famine, which raised the question as to whether the timing of the insult is critical to the severity of disease. Further evidence for the importance of the fetal environment came from studies of twins [18,19] showing discordance for type 2 diabetes with the diabetic twin having a significantly lower birth weight than the non-diabetic co-twin regardless of zygosity [19]. A recent review conclusively supported the association between low birth weight and adult glucose and insulin metabolism [20]. An extension of the Thrifty Phenotype hypothesis has also emerged as the “predictive adaptive response” hypothesis [21] which states that the fetus predicts

the environment into which it is likely to be born, and adapts in order to gain a competitive advantage when it is born.

An alternative hypothesis termed the Fetal Insulin Hypothesis proposes that genetically determined insulin resistance or defects in insulin secretion result in both impaired fetal growth and susceptibility to diabetes in adulthood. It is supported by human studies which identified mutations in the glucokinase gene in maturity onset diabetes of the young or MODY 2 that cause reduced birth weights (approx 500 g) compared to unaffected siblings [22] and this rare monogenic form of diabetes. However, a study of polymorphisms known to be associated with insulin resistance revealed no association with birth weight [23]. Other studies suggest that there may be genetic polymorphisms [24,25] but these effects are considerably weaker than the effect of birth weight itself or they exert their effects by interaction with the environment.

Various different types of adverse fetal environment have been found to affect birth weight with potential consequence for disease. Pregnancies at high altitude induce fetal hypoxia with consequence for reduced birth weight, increased hemtocrit and erythropoiesis [26]. Offspring of smoking mothers (especially in those smoking >20 cigarettes a day) were found to have a reduced birth weight [27] associated with development of maternal anemia and fetal hypoxia and polyglobulia, as well as post-partum haemorrhaging. In the ALSPAC study, maternal smoking was found to influence fetal size at birth, with infants of maternal smokers being symmetrically small at birth compared to infants of nonsmokers [28]. In the same year, Montgomery and Ekblom [29] showed that maternal smoking

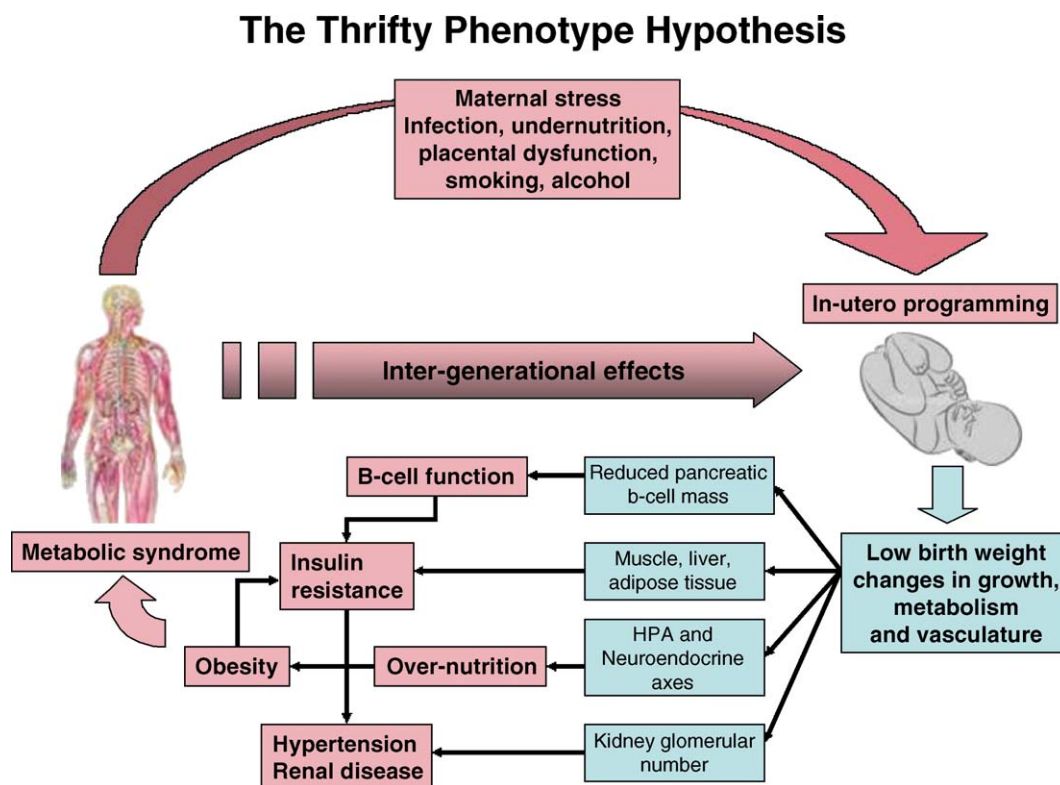


Fig. 1. A schematic representation of the Thrifty Phenotype Hypothesis, illustrating the programming effects of a suboptimal in-utero environment, nutritional or otherwise, on early growth and subsequent development of the metabolic syndrome (adapted from Hales and Barker [16]).

increased type 2 diabetes in the offspring. They also surmised at the time that risk of obesity in the offspring was increased, however, in two subsequent studies, they reported an association with depressed appetite in a cohort of over 10,000 British women aged 42 [30] and more recently, a direct relationship with bulimia [31] in 30 year old women. This suggests possible programming effects via neuropeptide or receptor status in appetite circuits in the brain. Iron deficiency or maternal anaemia in early gestation is also related to low birth weight [32]. Maternal periodontal disease is thought to be responsible for gingival inflammation, which is also a significant risk factor for low birth weight [33]. Placental villous inflammation has also been shown to be an independent risk factor for small for gestational age (SGA) [34] and maternal asthma, especially if unmanaged can lead to increases in maternal morbidity, perinatal mortality, preeclampsia, preterm birth, and low birth weight [35].

3. Animal models

The modeling of fetal growth restriction and adult disease in animals has been employed to study the underlying mechanisms of disease at the molecular level. The majority of these are based on work with rodents, although larger species such as sheep and pigs have also been used. Recent studies in sheep have shown that maternal nutrient restriction over the period of maximal placental growth, i.e. between 28 and 80 days gestation, resulted in offspring with more adipose tissue. In this tissue, mRNA abundance of UCP2 and PPAR α , key components of fetal fat metabolism, was also increased [36]. Perinatal nutrition manipulation also has effects on offspring appetite and obesity [37]. Intrafetal glucose infusion was shown to lead to increased mRNA expression for one of the “appetite regulatory” neuropeptides i.e. pro-opiomelanocortin (POMC), but not for the anorectic neuropeptide cocaine- and amphetamine-regulated transcript (CART), or the orexigenic neuropeptides NPY and agouti-related peptide (AGRP), in the arcuate nucleus of the fetal hypothalamus. This demonstrated that circulating glucose and insulin levels in the fetus may regulate fetal hypothalamic neuropeptide gene expression involved in energy balance in adult life. Glucose–insulin metabolism in offspring has been shown to be affected by maternal under nutrition in sheep [38]. In this study, nutrient restriction of ewes during late pregnancy led to glucose intolerance in year old offspring. This was shown to be associated with reduced adipose, but not muscle, glucose transporter-4 (GLUT4), and increased adipose tissue mass. Offspring of twin sheep pregnancies compared to singletons, were found to exhibit hypertension and reduced glomerular filtration rates [39] mirroring increased blood pressure seen in epidemiological studies of twins.

Cortisol responses to ACTH and insulin-induced hypoglycaemia has been shown to be elevated in low birth weight pigs, consistent with programming of the hippocampal–hypothalamic–pituitary–adrenal (HPA) axis function during impaired early growth [40]. In a study comparing low and high birth weight pigs, low birth weight was shown to be associated with glucose intolerance at 12 months of age although this was not thought to

be due to insulin deficiency or increased hepatic gluconeogenic enzyme activity [41], rather due to insulin resistance [42].

3.1. Rodent models

Rodent models of fetal programming, mainly because of shorter gestations and lifespan have been highly amenable to various forms of insult, and successfully express varying degrees of impairment in insulin signalling/secretion and/or hypertension in the adult, implying nutrient dependency of the 3 target organs, pancreas, muscle and kidney during developmental periods. Other organs have also been shown to be affected. For example, heart weight and cardiomyocyte number have been shown to be reduced in offspring of a low protein pregnancy [43]. As cardiomyocyte number is fixed at birth, it is thought that cardiac function is likely to be compromised in later life.

3.2. Calorie restriction

Total maternal food restriction to 50% of ad-lib in the last week of pregnancy [44] results in impairment of beta cell development. Continued restriction of the mother during suckling results in a permanent reduction in beta-cell mass and number and impaired glucose tolerance [45] in the offspring. A more severe food restriction to 30% of ad lib intake resulted in systolic hypertension and increased fasting insulin concentrations, hyperphagic behaviour and obesity [46]. IGF-I treatment of 6 month old undernourished offspring was found to alleviate hyperphagia, obesity, hyperinsulinemia, hyperleptinemia, and hypertension [47] and neonatal leptin administration from d3–13 of postnatal life normalized caloric intake, locomotor activity, body weight, fat mass, and fasting plasma glucose, insulin, and leptin concentrations in adult life [48]. Postnatal consequences for the HPA axis include reduced adrenal weight, reduced basal corticosterone as well as reduced glucocorticoid receptor (GR) and corticotropin-releasing hormone (CRH) mRNA levels.

3.3. Intrauterine artery ligation

A large proportion of human incidence of intrauterine growth retardation is thought to be due to impaired nutrient perfusion through the placenta [49]. Wigglesworth pioneered the methodology for unilateral and bilateral uterine artery ligation to model placental insufficiency [50] observing reduced offspring birthweight. Later studies extended observations to the development of the pancreas, with offspring at birth having a reduced beta cell mass [51] which persists into adulthood [52]. This leads to the development of type 2 diabetes at 26 weeks of age [53]. Other effects of this model include reduced nephron number and impaired renal function [54] and a 20% decrease in glomerular number with compensatory glomerular enlargement associating with an increased proteinuria [55].

The effects of uterine ligation have been shown to be transmitted to the second generation. Female offspring of uterine ligated dams have reduced beta cell mass and become

diabetic during pregnancy. Their offspring, the second generation are heavier at birth and remain heavy throughout life, develop insulin resistance very early in life, and demonstrate defects in insulin secretion from as early as 5 weeks of age and by 26 weeks of age are overtly diabetic [56]. Gestational diabetes per se is an inducing factor for impaired glucose tolerance and gestational diabetes in the next generation, the altered metabolic milieu of the diabetic pregnancy causing permanent defects in glucose homeostasis in the offspring that leads to the development of diabetes later in life. This forms the basis of a mechanism by which gestational diabetes passes from one generation to the next.

3.4. Gestational diabetes

Gestational diabetes has been achieved by the use of streptozotocin, a chemical which destroys beta cells of the pancreatic islets. Streptozotocin-induced diabetes is dose dependent such that low doses induce mild gestational diabetes resulting in fetal hyperinsulinemia and macrosomia whereas high doses cause severe maternal hyperglycemia resulting in hyperstimulation of fetal pancreatic islets such that their beta cells become degranulated, disorganized and unable to secrete insulin in response to glucose and other secretagogues. The result is a fetus of low birth weight (see Holemans et al. [57] for review). The significance of this finding is that the effects of both extremes of birth weight appear to converge on derangements to the insulin response and impaired glucose metabolism. Epidemiological studies on populations which develop diabetes in the Netherlands [58], Malta [59], and among Pima Indians [60] as well as African-Americans [61], all showed that the relationship with birth weight was U-shaped, which concur with the findings of experimental diabetes. Offspring of both the mild and severely diabetic streptozotocin models subsequently develop gestational diabetes [62–64], which is further transmissible to the third generation. In humans, studies by Dorner et al. described a higher susceptibility for diabetes in descendants from diabetic great grandmothers via the maternal rather than the paternal line [65]. Here again, studies on the Pima Indians, a population with exceptionally high incidence of type-2 diabetes showed that the prevalence of impaired glucose tolerance, type-2 diabetes and gestational diabetes was higher in children whose mothers who had diabetes during that pregnancy [60].

3.5. Maternal hypoxia

There is some epidemiologic evidence to suggest that fetal hypoxia correlates with cardiovascular disease in later adult life: studies on a Bolivian population in the highlands found that high altitude and therefore low oxygen concentrations were associated with low birth weight and altered body shape at birth [66]. Studies in rodents propose a direct link between hypoxia and cardiovascular disease [67,68] with prenatal chronic hypoxia resulting in low birth weight and a significant increase in the susceptibility of the adult heart to ischemia–reperfusion injury by increasing myocardial infarct size and decreasing

post-ischemic recovery of left ventricular function [67]. In another study, perinatal hypoxia induced a reduction in body weight with elevated right and left ventricular weight, reduced pulmonary arterial compliance and reduced maximal pulmonary vasoconstriction to potassium chloride; effects which could predispose to cardiopulmonary diseases in adulthood [69]. The interaction of low birth weight with improved postnatal nutrition and “catch-up” growth in this model is analogous to human studies showing that coronary heart disease both in women and men reflects poor prenatal nutrition, consequent small body size at birth combined with improved postnatal nutrition and “catch-up” growth in childhood. Chronic maternal hypoxia is also linked to suppressed fetal cardiac function, altered cardiac gene expression and increased myocyte apoptosis and myocyte hypertrophy [70].

3.6. Maternal iron restriction

About 20% of women in general and 50% of pregnant women are iron deficient. The main causes of iron deficiency are poor absorption of iron by the body due to insufficient vitamin C levels or inadequate daily intake of iron or high menstrual blood loss. There is ample evidence from observational studies for an association between maternal anemia (defined by hemoglobin concentration) and size at birth. This association is U-shaped, with the proportion of low birth weight infants rising with maternal hemoglobin values at the low or high end of the range (review by Rasmussen [71]). Maternal anaemia in early pregnancy seems to influence the pattern of placental vascularisation. This may alter placental vascular impedance during early fetal life, thereby exerting effects on cardiovascular development [72]. The rodent maternal iron restriction model also results in low birth weight offspring [73] and programs hypertension throughout adult life which is possibly due to a deficit in nephron number [74]. It has also been shown to program lipid metabolism in the liver [75]. Anemia by hypoxia or iron deficiency (which increases serum norepinephrine concentrations) may exert their effects by inducing maternal and fetal stress. This is thought to stimulate CRH synthesis. Raised CRH levels are associated with major risk for preterm labor, pregnancy-induced hypertension and eclampsia, and premature rupture of the membranes, and also increase fetal cortisol production [76]. An alternative mechanism could be that iron deficiency increases oxidative damage to erythrocytes and the fetoplacental unit. Iron deficiency may also increase the risk of maternal infections, which can then stimulate CRH production.

3.7. Maternal endotoxemia

Maternal endotoxemia leads to obesity and insulin resistance in adult male rat offspring [77]. Adult male offspring of *Escherichia coli* lipopolysaccharide-exposed dams have been shown to be heavier and showed increased adipose tissue weights, elevated food intake, increased circulating leptin and insulin resistance. In addition, corticosterone response to stress was blunted and hippocampal glucocorticoid receptor protein

was up-regulated. In females, both testosterone and baseline corticosterone was increased and heart and adrenals were enlarged.

3.8. Maternal glucocorticoid exposure

In humans, glucocorticoids are administered during pregnancy for the treatment of neonatal respiratory morbidity and maternal asthma. Its effects on the fetus are growth retardation and reduced birthweight [78]. It has been hypothesized that prenatal glucocorticoids or stress represents a mechanism linking fetal growth with adult pathophysiology. This has been tested in rats where the treatment of pregnant rats with dexamethasone resulted in reduced birth weight. However, these animals catch up in weight to that of controls by weaning [79]. Other effects include hypertension [80] hyperglycemia in the adult and increased hypothalamic–pituitary–adrenal (HPA) axis activity and behaviour reminiscent of anxiety [81]. The underlying mechanisms directing glucocorticoid excess is thought to be physiological variations in placental 11beta-hydroxysteroid dehydrogenase-2 (11beta-HSD2) activity [82]. In humans, 11beta-HSD2 gene mutations cause low birth weight and low-birth-weight babies have higher plasma cortisol levels throughout adult life, indicative of HPA axis programming. Dexamethasone treatment has been shown to have gender-specific effects as reported by O'Regan et al. [79], such that males exhibit a metabolic phenotype whereas females are hypertensive and have an activated renin–angiotensin system. Glucocorticoid-exposed female offspring exhibit substantial up-regulation of type-2 angiotensin receptor expression, which might account for renal remodelling and hypertension [83]. Mechanisms directing the metabolic phenotype in males remain unclear.

3.9. Maternal high fat feeding

It is well recognised that nutrition in most developed and developing countries is being undermined by western-style diets which contain a high percentage of saturated fats. There is little doubt as to the effects of this kind of nutritional imbalance on the individual's health, however, studies have also shown that high levels of dietary fat intake during pregnancy is also related to an increase in the incidence of cardiovascular risk factors in the offspring [84]. Animal studies have explored the development of such adverse effects. In rats, offspring of dams fed a high level of saturated dietary fats have been found to display abnormal cholesterol metabolism [85] and fetal insulin resistance [86]. Offspring of rats fed a diet rich in animal fat during pregnancy and suckling also develop vascular endothelial dysfunction and gender-specific hypertension [87] as well as increased adiposity and cardiovascular dysfunction [88]. Insulin sensitivity and glucose tolerance in male offspring of dams fed a diet high in omega-6 polyunsaturated fat appeared to be unaffected at 3 months of age, although they were more hyperinsulinemic during an oral glucose challenge. Their liver triglyceride content was elevated and their pattern of insulin signalling protein expression was consistent with reduced

hepatic insulin sensitivity, suggesting a predisposition to metabolic disease later in life [89].

3.10. The low protein model

The low-protein model has emerged as one of the most extensively studied models of maternal dietary manipulation which has been used to test the Thrifty Phenotype Hypothesis and to dissect molecular mechanisms. Initially established by Snoeck and colleagues, neonates of protein-restricted dams were found to have lower birth weights, and there were effects on pancreas development, such that beta cell proliferation, islet size and islet vascularization was reduced [90]. Insulin content was also reduced and insulin secretion in response to arginine and leucine stimulation was impaired [91]. This model consists of feeding dams a diet containing 8% protein throughout pregnancy and lactation and comparing offspring to those of a control dam fed an isocaloric 20% protein diet. This is just under half the protein content of the control diet, and is physiologically relevant to cultures, economies and socio-economic groups for whom protein sources are expensive. The effects on pregnancy outcome are therefore expectedly modest, with no effect on conception rates and litter size, however placental weights and offspring birth weights are consistently and reproducibly reduced [92] and the development of their endocrine pancreas is impaired [90]. All offspring are weaned onto a control diet fed ad-libitum. Young offspring (6 weeks to 3 months) demonstrate an improved glucose tolerance while plasma insulin concentrations are reduced which suggested an improved insulin sensitivity [93–95]. Age reversed this trend such that by 15 months, the offspring had impaired glucose tolerance and by 17 months, frank diabetes with insulin resistance was observed in male low protein offspring [96]. Female offspring only demonstrated hyperglycemia and impaired glucose tolerance and even then only much later in life (21 months) [97]. Insulin-stimulated glucose uptake was reduced in both muscle [98] and adipose tissue [99] of 15 month old males. This was accompanied by a reduction in both GLUT 4 and PKC- ζ in muscle [98].

Adipocytes isolated from 6 week old male low protein offspring displayed increased insulin receptor expression [95], and at 3 months, adipocytes from these males were found to be more responsive to insulin stimulation of glucose uptake [100]. As with muscle, age was found to have a detrimental effect on insulin-stimulated glucose uptake of adipocytes isolated from 15 month old male offspring and on the anti-lipolytic action of insulin [99]. At this age, both muscle and adipocyte insulin receptor expression was similar to that of controls, suggesting that as with human diabetes [101], the molecular basis for insulin resistance lies downstream of the insulin receptor.

3.11. Human studies

In light of the very specific changes in insulin signalling protein expression in the low protein rat offspring, we have carried out parallel human studies to validate these molecular markers. In a Danish cohort of 19 year old men, those with low

birth weights showed increased fasting glucose and increased 2 h glucose levels during an oral glucose challenge [102]. Vastus lateralis muscle biopsies from this cohort were then evaluated for expression of a panel of insulin signalling proteins. Striking similarities were observed in the pattern of expression of insulin signalling components with that of the low protein rat offspring both in terms of specificity and magnitude of effect. GLUT 4, protein kinase C zeta and the p85 regulatory subunit of phosphatidylinositol-3 (PI3)-kinase were all reduced in muscle from both the low birth weight humans [103] and the rat model [98]. Other proteins such as glycogen synthase kinase and Akt were unaffected in either humans or low protein rats.

3.12. Programming of obesity

One concept of the Thrifty Phenotype Hypothesis is that because of nutritional thrift, an undernourished fetus may be more prone to obesity in conditions of adequate or over nutrition. To investigate the effects of fetal programming and post-natal catch-up growth on obesity and longevity, the growth of mice was manipulated by cross-fostering offspring of low protein-fed dams onto mothers receiving control diets (Recuperated group). The body weights of recuperated mice while smaller at birth had caught up and exceeded the weight of control offspring by 7 days of age (i.e. within 4 days of suckling on low-protein mothers' milk) and body weight patterns persisted in adulthood. Recuperated animals also gained more weight than controls when given free access to a highly palatable diet [104]. In a parallel experiment, control-fed offspring were cross-fostered onto mothers receiving reduced protein diets during lactation (Post-natal low protein group or PNLp). In contrast to the recuperated group, PNLp body weights were reduced from Day 7 compared to controls and the PNLps maintained this pattern of body weight in adulthood even when given a highly palatable diet. These results suggest that both the fetal and neonatal time period are critical time windows for long term obesity risk. Whereas poor fetal growth followed by catch-up growth is associated with increased obesity risk, slow growth during lactation is actually protective of diet-induced obesity. Further evidence for the importance of growth during the lactation period has come from studies of litter size manipulation. Postnatally increasing litter size, thereby reducing postnatal nutrition leads to a permanent appetite reduction in the rat [105] whereas the reverse is observed if animals are reared in small litters. These findings have major implications for the drug-free regulation of food intake and obesity.

Data from human studies comparing breast-fed and bottle fed infants also suggest that the lactation period is a critical time window for determination of obesity risk in humans too. They have shown that breast-fed babies are at reduced risk of obesity compared to those who were formula fed [28,106,107]. As bottle fed infants are known to have higher total and protein caloric intake than breast fed infants, the plane of nutrition during lactation may have long term consequences for appetite regulation. The mechanisms for this programming effect are yet to be uncovered; however there are indications that circulating

leptin levels which are higher in breast fed infants during the first 4 months of life may have a role in subsequent obesity risk [108].

Other peripheral factors that have been proposed as links to central appetite control mechanisms include insulin, which along with leptin is associated with long-term appetite control, as well as meal-related peptide hormones such as ghrelin, peptide YY (PYY) and cholecystokinin (CCK), which are produced in the gut and gastrointestinal tract respectively (reviewed by Cripps, Martin-Gronert and Ozanne, 2005) [109]. A dysregulation of neuropeptide action in appetite control centres in the brain have been implicated in the development of obesity (Davidowa et al. [110]). Both alterations in messenger levels of anorectic and orexigenic neuropeptides and disruption of neural projections from the arcuate nucleus (ARC) have been shown in rodent and sheep models (reviewed by McMillen et al. [111]). One important task will be to address the role of energy expenditure in these models to understand how metabolic changes interact with appetite regulation to influence the development of obesity.

4. Common mechanisms

The Thrifty Phenotype hypothesis and the vast experimental evidence supporting it has led to the search for a common mechanism by which all the disparate intrauterine insults go on to exert effects on various different physiological systems in the offspring. This has spawned two main mechanistic theories. Fowden et al. [112] reviewed from the literature a common postnatal outcome for the various experimental intrauterine conditions, i.e. alterations in corticosterone/cortisol and or ACTH levels in response to stress, GR expression within the HPA axis and in peripheral tissues and organs and hypothalamic CRH mRNA. These endocrine changes adapt development of the fetus and slow down its growth to meet with reduced nutrient availability, in each case the nutrient may be general (total calorie restriction, intrauterine artery ligation or placental insufficiency) or more specific (in the case of protein, iron deficiency or hypoxia). The experimental evidence therefore seems to support a hypothesis that adult metabolic disease arises in utero as a result of programming of the HPA axis, at least for a large range of maternal insults.

One other mechanism recently proposed is the "oxidative stress" hypothesis [113]. Reactive oxygen species (ROS) function in normal physiology by regulating enzymes and redox-sensitive gene expression, and these processes are delicately balanced by ROS scavengers (e.g. thiols, vitamin C and E) and detoxifying enzymes (e.g. superoxide dismutase, glutathione reductase). An imbalance results in oxidative stress which is transferable from mother to fetus to either act directly to modulate gene expression or indirectly through the effects of oxidised molecules. Epidemiological support for this hypothesis comes from studies of pregnancies complicated by preclampsia [114] and gestational or type-1 diabetes [115], common causes of poor fetal growth, where maternal plasma and erythrocyte oxidant status was found to be raised. Oxidative stress was also seen in small for gestational age neonates born to

undernourished mothers [116] as well as pre-term infants [117]. These studies suggest that maternal malnutrition may result in protein or micronutrient deficiencies which are involved in antioxidant enzyme synthesis or are antioxidants themselves. Infections and inflammation can also stimulate macrophage activation, another source of oxidative stress. The experimental evidence for a role for oxidative stress in adverse programming is sparse however, two separate groups have demonstrated its role in the in-utero programming of hypertension [118,119]. Furthermore, dietary supplements which support nitric oxide formation and scavenge ROS, administered to spontaneously hypertensive rats during pregnancy and lactation, resulted in a persistent lowering of blood pressure in the offspring [119].

5. Future directions

A range of animal models have been used to dissect (i) the maternal milieu which directs fetal programming and (ii) the molecular mechanisms which are adversely altered in the metabolic syndrome. There is increasing opinion that phenotypic adjustments brought about by fetal programming are inherited. This is supported by inter-generational studies showing that the effects of an adverse fetal environment influence both the offsprings' birthweight and glucose tolerance and that of the next generation(s) [120]. The susceptibility of epigenetic mechanisms controlling gene expression to environmental influences due to their inherent malleability was recently highlighted in a review by Junien et al. [121]. Here, the authors proposed the participation of transposable elements and the potential role of imprinted genes during critical time windows in epigenetic programming, from the very beginning of development, throughout life. In the past few years, a sudden immediacy has developed in the drive for understanding the significance of epigenetic patterns and their role in development, evolution and adaptation. Naturally therefore, immense interest is now centered on identifying the epigenomics of early life-programmed disease.

6. Intervention strategies

Although it is undisputed that a suboptimal maternal diet, smoking and stress have adverse effects for the fetus, these are difficult to address successfully. One of the most immediate and intuitive measures would be to ensure adequate maternal nutrition throughout pregnancy and lactation; however, it is important that any dietary enhancement in the mother should promote lean and not fat mass. Fat intake during pregnancy has been shown to correlate with the incidence of gestational diabetes mellitus and impaired glucose tolerance [122] and infants of women with gestational diabetes have increased fat mass and percentage body fat, which could be a significant risk factor for obesity in early childhood and possibly in later life [123]. Smoking is another lifestyle choice which not only affects the health of the smoker but also the unborn child. Stress and infection as well as maternal hypertension are also factors which like smoking, are not easily controllable and for these suitable therapeutic approaches must apply. One such

approach is based on our identification of molecular markers involved in type-2 diabetes risk. Pharmacological intervention directed at any of the defects identified could potentially improve disease. Identifying markers for potential disease outcome in placental tissue is another approach, which could allow very early preventative strategies. One such study identified a reduction in insulin-like growth factor-1 (IGF-1) receptor content and selective impairment of the insulin receptor substrate-2 (IRS-2)/PI3-kinase pathway and reduced p38 and c-Jun N-terminal kinase activation in placentas of intrauterine growth restricted pregnancies [124]. Obesity risk could be reduced by controlling post-natal nutrition. Encouragement to breast feed should remain a high priority given the benefits of immunity to certain diseases as well as protection against obesity. Breast milk is also rich in long chain polyunsaturated fatty acids (LCPUFAs) which are thought to be protective not only against the development of obesity but also hypertension, insulin resistance and coronary heart disease [125]. Docosahexaenoic acid (DHA) in particular is also known to be important for cognitive, visual and neural structural development and function, which suggest an additional role for LCPUFAs in appetite programming during the development of neural circuits in the early postnatal period. There are several ongoing large randomized trials investigating the efficacy of antioxidant supplementation to counter oxidative stress during pregnancy for the prevention of preeclampsia [126,127], and future follow-up studies of the infants born to these women would be able to test the "oxidative stress" hypothesis. Finally, epigenetic reactivation of genes silenced during fetal programming is also proposed as a pharmaco-nutrient approach [121], albeit in the long-term.

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