Developmental Origins of Adult Disease

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Key Words

Programming • Nutrition • Cardiovascular disease • Metabolic syndrome

Abstract

Variation in the quality or quantity of nutrients consumed during pregnancy can exert permanent and powerful effects upon the developing fetus. This programming of fetal development is emerging as a new risk factor for non-communicable diseases of adulthood, including coronary heart disease and the metabolic syndrome. Epidemiological studies show that indicators of nutritional deficit in pregnancy are associated with greater risk of diabetes and cardiovascular mortality. The study of programming in relation to disease processes has been advanced by the development of animal models, which have utilized both under- and over-feeding of specific nutrients in pregnancy. Studies of this nature support the nutritional programming hypothesis and provide tools with which to examine the mechanisms through which programming may occur. Studies of animals subject to undernutrition in utero generally exhibit changes in the structure of key organs, such as the kidney and pancreas. These effects are consistent with the concept that programming influences remodel the development of organs. The causal pathways which extend from tissue remodelling to disease processes are relatively well characterised. In contrast, the processes which drive disordered organ develop-

ment are poorly understood. It is noteworthy that minor perturbation of maternal nutritional status can programme fetal development. It is suggested therefore that programming is a product of altered expression of key genes. This drives the tissue remodelling response and future disease risk.

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Concept of Programming

During periods of rapid growth, the developing embryo or fetus is highly sensitive to influences of perturbations of the maternal environment. Adverse environmental cues can disturb the processes of cell proliferation and differentiation, leading to changes in the normal developmental pathways for mature organs and tissues. As shown in figure 1, the range of environmental signals which have the capacity to disturb developmental processes during human development may include nutritional factors [1], psychological or physiological stressors [2], and imbalances in the normal endocrine signalling between mother and fetus [3]. A similar range of factors are associated with risk of miscarriage and fetal death [4], so, in a teleological sense, it can be argued that when such disturbances occur, the developing organism must mount adaptive responses to ensure maintenance of critical tissue functions and survival of the insult. As development involves well-ordered formation of key structures, these

Fig. 1. The fetal origins of adult disease hypothesis. Adverse environmental cues from the mother are signalled to the developing fetus. The prevailing conditions may be sufficiently harsh to result in the loss of the pregnancy, or alternatively the fetus may mount adaptive responses to ensure immediate survival. One of these responses may be a slowing of growth that will ultimately result in lower weight at delivery. Other aspects of the adaptive response, which may be localized to specific organs and tissue types, will serve to modify physiology and metabolism, and hence tissue functions. The trade-off for overcoming the challenge in fetal life may be increased risk of disease in later life.

adaptive responses are likely to result in irreversible changes to tissue structure and function (for example, altered cell types, or numbers of cells and functional units) [5]. These will be observable in later life and will have the capacity to modulate physiological function and susceptibility to disease. This process is termed ‘programming’ [5].

The characteristics of programmed responses are dependent upon the nature of the stimulus or insult and upon the timing of the exposure. For example, in pregnant rats, the feeding of a diet that has a lower protein content has been shown to increase blood pressure in the resulting offspring once they attain adulthood [6]. The magnitude of this programmed response is greatest if the nutritional insult is applied in late gestation [7], whilst protein restriction in very early pregnancy has only a limited impact upon the offspring (indicating the importance of timing) [8]. The importance of the precise nature of the insult is amply illustrated by the evidence that programming of blood pressure occurs if the low protein diet is formulated to include complex carbohydrate, but is not observed if the low protein diet delivers carbohydrate as mostly glucose [9].

Epidemiological Evidence of Links between Early Life and Later Disease

Data obtained from historical cohorts of men and women from developed and developing countries is suggestive of associations between early life factors and risk of cardiovascular disease and type 2 diabetes in adulthood [10–14]. Barker et al. reported that among men and women born in Hertfordshire (UK) during the period 1911–1932, coronary heart disease mortality [15], blood pressure [16], occurrence of the metabolic syndrome [17] and type 2 diabetes [18] were all more prevalent in individuals who were of lower weight at birth. The relationship between birth weight and disease risk was graded and linear, and, most importantly, increased risk was noted within the normal range of birth. Similarly Leon et al. [19] reported that among a cohort of 15,000 Swedish men and women born between 1915 and 1929, after adjustment for gestational age, there was a significantly greater risk of death from ischemic heart disease associated with lower birth weight. Compared to individuals in the lowest quartile for birth weight (average weight 2.949 kg), individuals in the upper quartile (average weight 4.113kg) were 33% less likely to die from cardiovascular

Fig. 2. Relationships between birth anthropometry and risk of disease in later life. Relative thinness at birth, as assessed by the ponderal index, is associated with greater plasma glucose concentrations in 7-year-old children at 30 min into an oral glucose tolerance test (a) and increased prevalence of type 2 diabetes in men aged 60 years (b) [data redrawn from references 19 and 22].

disease. A recent systematic review of the literature relating to the birth weight/type 2 diabetes relationship noted that for every 1-kg increase in weight at birth, the risk of diabetes in adulthood decreased by 25% (OR 0.75, 95% CI 0.70–0.81) [20].

In addition to lower birth weight being predictive of later disease risk, anthropometric indicators of disproportionate growth are also associated with certain disease outcomes. Ponderal index at birth ($\text{weight}/\text{length}^3$) is regarded as indicative of the relative fatness of an infant. As shown in figure 2, several cohort studies have shown that individuals who were thin at birth exhibit impaired glucose tolerance during childhood and are more likely to develop type 2 diabetes as adults [21–24].

Studies that consider relationships between birth anthropometry and later disease also suggest that osteoporosis [25], atopic disorders [26] and psychological traits [27] may be subject to early life programming. To some extent, the programming influences of early nutrition are modulated by other factors, including the genotype of the individual. For example, there is evidence that inheritance of the Pro12Pro variant of the Ala12Pro polymorphism of peroxisome-proliferator-activated receptor γ -2 is a risk factor for type 2 diabetes, but only in individuals who are also of lower weight at birth [28]. The early post-natal period is also a critical period during which programming may occur. It is now clear, for example, that infants who are fed formula milk during infancy are at

significantly greater risk of obesity than breast-fed infants [29].

The interpretation of the findings of these epidemiological studies has been somewhat controversial. Lower weight at birth, especially within the normal range for healthy human populations, may arise due to the presence of a number of adverse factors. For example, psychological stress to the mother and maternal smoking are both predictive of lower birth weight [30, 31]. Despite this concern, the general interpretation of the retrospective cohort studies that have linked birth anthropometry to disease in later life, has been that programming of disease risk is a product of maternal undernutrition. Barker et al. [32] advanced the view that undernutrition during specific stages of pregnancy would be predictive of characteristic patterns of growth constraint. Thus, the infant subject to undernutrition in the first trimester would tend to be symmetrically small, whilst undernutrition in the second trimester would result in a smaller and thinner baby at birth [33]. In contrast, undernutrition in the later stages of pregnancy was proposed to produce a normal weight infant, with a short body in proportion to head circumference as brain growth is spared at the expense of truncal growth [33]. The evidence to back up these assertions is largely circumstantial and derived from animal studies, but evidence from other historical cohorts supports the proposed association between maternal undernutrition and later disease.

Where early growth is constrained by nutritional factors, there is a strong tendency for the growth deficit to be recovered once nutritional status improves. This catch-up growth can be shown to be related to programmed disease in humans. Studies of men and women born in Finland in the first half of the 20th century showed that risk of type 2 diabetes was greatest in those who were born small, but who gained weight most rapidly during childhood [34]. Such evidence strengthens the view that undernutrition during fetal life may exert programming effects upon later disease risk, but remains indirect. There is, however, a growing body of literature that considers direct associations between maternal nutritional status during pregnancy and disease risk indicators in the resulting offspring. Godfrey et al. [35], for example, reported that blood pressures in young boys were related to their mother's degree of body fatness and haemoglobin concentrations during pregnancy. Similarly blood pressure in adult men was related to maternal intake of animal protein and inversely related to maternal carbohydrate intake [36]. A prospective cohort, Project Viva, has most recently reported associations between maternal diet and blood pressure, with pressures of 6-month-old babies being inversely related to maternal calcium intake from supplements [37].

The most extensively documented evidence to support a link between maternal nutrition and later disease comes from follow-up studies of the offspring from the Second World War Dutch famine. A Nazi blockade of the western part of Holland resulted in widespread hunger over a period of 6 months. Individuals who were in utero at the time of the famine were subsequently shown to be at greater risk of obesity and glucose intolerance than individuals born before and after the famine. Risk of hypertension, coronary heart disease and renal dysfunction were similarly increased by exposure to maternal undernutrition [38–40].

Criticisms of the Programming Hypothesis

After 2 decades of research in this field, the developmental origins of health and disease (DOHAD) hypothesis remains controversial, particularly as the epidemiological evidence to support the concept is not always consistent. For example, some studies identify increased placental weight at birth as a risk factor for adult hypertension, whilst others show the opposite relationship holds for risk of stroke mortality [16, 41, 42]. There are also some cohort studies that disagree with the DOHAD

hypothesis [43, 44]. Publication bias limits the number of these available in the literature, but, where such studies exist, the discrepancy with the mainstream DOHAD literature does not always have an obvious explanation. Importantly, Huxley et al. [45] performed meta-analyses evaluating the strength of the associations between birth weight and high blood pressure and raised serum cholesterol. These analyses showed that the strongest associations were noted in the smallest populations, and that where very large cohorts had been studied, the associations were weakest. This is the opposite of what might be expected, and could indicate systematic error in measurement and certainly a strong influence of publication bias upon this field of research.

Studies which consider the relationship between anthropometry at birth and disease some 50 years later are easy to criticise on the basis of the potential for missing information to confound the observed associations between birth characteristics and disease outcomes. For example, social class is known to be linked to both low birth weight and cardiovascular risk [46] and may not be adequately adjusted for using statistical modelling. Moreover, most of the epidemiology that links early life exposures to adult disease is based upon anthropometric markers of fetal growth as indirect indicators of exposure to less than optimal maternal nutrition. This assumption may lack validity as consideration of extreme situations, such as the Second World War Dutch famine and siege of Leningrad, indicate that frank starvation has only minor effects on birth weight in human populations. In the case of the Dutch famine, babies exposed to the height of the famine during the first trimester of pregnancy were actually heavier at birth than unexposed individuals [47]. Studies of the Dutch famine are important in that they show that maternal diet can programme fetal development and long-term health, but that it is not a major determinant of fetal growth. This is further demonstrated by other studies of more contemporary European populations. In well-nourished populations, whilst some studies have suggested that intakes of animal protein and sucrose in early gestation may be determinants of birth weight and placental weight [48], most others have found no significant relationships between maternal diet and weights of either baby or placenta [49, 50]. Doyle et al. [51] reported that in socially disadvantaged British populations, nutrient intakes below the dietary reference values were related to lower weights at birth. This may indicate that the constraining effect of undernutrition upon fetal growth only becomes apparent when women have habitually poor nutrient reserves and pregnancy is compromised by poverty.

Fig. 3. An overview of the variety of tools used to modulate maternal and fetal nutrient status during pregnancy in small and large animals.

Such criticisms are inevitable products of the complexity of any relationships between early life nutritional exposures and disease outcomes that do not manifest for several decades after the exposure. Indeed, it is questionable whether epidemiological approaches have the capacity to investigate these questions at all, especially when reliant on indirect anthropometric measures of maternal nutritional status. The cohort studies considering populations born 50–70 years ago, which have been so influential in promoting interest in developmental programming as a risk factor for human disease, may no longer be representative of factors that may drive disease development in contemporary populations. It is critical, therefore, to develop carefully controlled studies that can establish the biological plausibility of nutritional programming as a risk factor for disease. With this aim in mind, a broad array of animal models of both under- and over-nutrition in pregnancy have been developed.

Animal Models as Proof of Principle

When designed within the context of the prevailing evidence from human populations, animal models are able to test specific hypotheses whilst overcoming the major limitations of epidemiological study designs. The use of animal models enables a strong level of control over

confounding factors, the measurement of invasive end-points, and the characterisation of downstream events across the full lifespan and into subsequent generations. Animal models have been instrumental in demonstrating the biological plausibility of the associations observed in human populations, providing proof of principle to the DOHAD theory. A variety of large (e.g. sheep and pig) and small (e.g. mouse, rat and guinea pig) animal models have made important contributions to the field, providing strong evidence of a causal relationship between early life exposures and metabolic risk factors in later life.

Within the context of the developmental origins of chronic disease, studies of nutritional programming using small animal models have been ongoing since the early 1990s. However, the general concept of developmental programming was not new, and the plasticity of tissues during the developmental period had previously been demonstrated using small animal models. Treatment of newborn female rats with testosterone during the first few days of life, for example, had been shown to remodel the regions of the hypothalamus that control reproductive function, permanently rendering the treated animals sterile [52]. Since the human epidemiological evidence of associations between fetal growth restriction and postnatal cardiovascular disease emerged in the late 1980s and early 1990s [15–18], a variety of models have been used to demonstrate that the associations observed in human ep-

idemiological studies could be replicated under experimental conditions (fig. 3). For example, protein restriction [6] and global nutrient restriction [53] in rats and uterine ligation in rats and guinea pigs [54, 55] were shown to restrict fetal growth and induce raised blood pressure in the offspring in postnatal life. More recently, iron restriction [56] and high-fat feeding [57] during pregnancy in rats have also been shown to have similar effects on offspring blood pressure. Other outcomes associated with maternal nutrient restriction include effects on the fetal endocrine pancreas [58], altered muscle development [59], increased propensity for fat deposition during postnatal life [60, 61], altered renal function [62, 63] and altered insulin sensitivity [64, 65]. Importantly, these studies demonstrated a direct effect of maternal nutrition on postnatal physiology and disease risk. Programming events occurring in response to moderate alterations in maternal diet in animal models often occur without impacting on fetal size at birth, indicating that fetal growth restriction is not necessarily a component of the causal pathway between prenatal dietary exposures and postnatal outcomes.

Maternal undernutrition in human populations remains an often ignored global problem, with low intakes of protein and selected micronutrients being commonplace in pregnant women. Changing patterns of diet and lifestyle, however, mean that there is an increasing need to model the impact of maternal overnutrition upon fetal development and long-term health. Work in this area has shown that maternal high-fat feeding during pregnancy can have similar postnatal consequences to maternal nutrient restriction. Offspring from pregnant rodents fed a maternal obesity-inducing hypercaloric diet before and during pregnancy have been shown to exhibit disturbed glucose and lipid homeostasis and greater blood pressure [66, 67]. Cafeteria feeding, whereby rodents are provided with highly palatable human foods to override appetite regulation and induce obesity, has been shown to slow fetal growth [68]. Similar protocols indicate that cafeteria feeding during pregnancy can predispose offspring to adiposity and altered feeding behaviour in the rat [69, 70]. Collectively, these studies demonstrate that feeding high-fat obesity-inducing diets during pregnancy in rodent models can lead to permanent alterations in postnatal physiological function, promoting adiposity and cardiovascular disease risk. The extent to which these are truly a product of maternal obesity or excessive intakes of energy, fat or sugar is unclear. Most of the protocols used to induce hyperphagia and consumption of high-fat diets in rodents may also lead to reduced intakes of micronutri-

ents and protein, establishing commonality with the undernutrition models. Similarly to small animal models, although prohibited somewhat by the cost of long-term follow-up, large animal models have also been used to investigate the long-term consequences of an altered prenatal environment. The offspring of sheep exposed to global nutrient restriction during early to mid pregnancy have been shown to exhibit increased blood pressure in some studies [71–73], but not others [74, 75]. In pigs, blood pressure at 3 months of age has been shown to be negatively associated with birth weight [76], associated with increased responsiveness of the hypothalamic-pituitary-adrenal axis to ACTH and insulin-induced hypoglycaemia [77]. Maternal nutrient restriction or fetal growth restriction have also been shown to be related to postnatal glucose homeostasis and insulin sensitivity in the pig and sheep [78–80].

The experimental studies outlined above, amongst many others, have been critical in providing strong evidence of a causal relationship between maternal diet and postnatal disease risk. Importantly, long-term effects are observed across a number of species and in response to a range of physiologically relevant factors, giving further support to the translation of the findings to the human situation. It must be noted, however, that the evidence of specific associations is not always consistent, perhaps reflecting differences in the species or strains of animals used and the composition of experimental diets [9, 81]. There is also a clear interaction between prenatal exposures and postnatal environment, with some developmentally programmed outcomes not becoming apparent unless the offspring are followed into the ageing period [82, 83] or challenged in postnatal life [84–86], perhaps due to a mismatch between prenatal and postnatal diets [87].

Mechanisms Which Drive Programming of Disease

Small animal models have been used extensively to investigate the mechanisms underlying the DOHAD hypothesis. Their short generation time and relatively inexpensive costs, together with the excellent availability of molecular tools for the mouse and rat, make them useful models for testing specific hypotheses relating to biological mechanisms. Despite the time and cost constraints of large animal models, sheep in particular have also been used to investigate the mechanisms which generate physiological changes in response to an altered maternal environment. Together, these studies have given rise to a

number of general and interrelated mechanistic concepts, which are outlined in the following sections.

Impact of Maternal Diet on Tissue Structure

Adverse environmental factors acting during the developmental period have the potential to disturb the processes of cell proliferation and differentiation. Indeed, a reduction in cell number or a change in the balance of cell types within tissues has been observed in a number of animal models in response to altered maternal diet. Such changes may account for subsequent alterations in gene expression and physiological function. A reduction in nephron number, for example, has been observed in response to a prenatal low-protein diet in the rat [63, 88, 89] and mouse [90], and following uterine ligation in the guinea pig [91] and rabbit [92]. A reduction in nephron number has also been observed in offspring of sheep exposed to reduced nutrition during early to mid gestation [93, 94]. Brenner and Chertow [95] proposed that a nephron deficit may predispose to an accelerated age-related decline in renal function and the onset of hypertension. Similarly, in the pancreas, a low-protein diet in the rat has been shown to reduce total pancreatic weight, islet cell mass and the relative contribution of β -cells to the islets [58, 96, 97], perhaps due to an altered balance of precursor cells that contribute to the α - and β -cell lineages during development [98]. Such structural changes within the pancreas may contribute to the subsequent impaired glucose homeostasis observed [65]. Maternal undernutrition during the critical proliferative period for muscle fibre development has also been shown to affect the numbers of secondary muscle fibres in the young offspring of a variety of species, including rats, guinea pigs, sheep and pigs [99]. However, the long-term consequences of this remain unclear, as studies of adult offspring are limited and suggest that offspring have been able to compensate for the effects observed earlier in life. In the brain, a low-protein diet during pregnancy in the rat results in a reduced density of capillaries within the cerebral cortex [100] and lower densities of neurons expressing appetite regulatory systems [101]. Together these studies demonstrate that maternal diet can impact on the key processes of proliferation and differentiation, in a way which could drive disrupted physiology in later life.

Epigenetic Programming

The term epigenetics has been defined as the study of heritable changes in genome function that occur without alterations to the DNA sequence [102]. At present, the study of epigenetic mechanisms within the DOHAD field

is at an early stage. However, researchers within the field have harnessed the techniques available to determine whether maternal diet can influence the patterns of epigenetic markers within the genome; thus, providing a mechanism by which maternal diet can permanently affect gene expression patterns [103]. Early evidence is emerging from small and large animal models that early life nutrition may impact upon both methylation and histone acetylation. Lillycrop et al. [104] demonstrated hypomethylation of the genes encoding the peroxisome-proliferator-activated receptor α and glucocorticoid receptor (GR) in the livers of rats exposed to a low-protein diet during pregnancy, which was associated with increased expression of both genes. Low-protein-exposed offspring also exhibited changes in histone acetylation of the GR promoter, which would further facilitate transcription. Hypomethylation of the proximal promoter of the type 1b angiotensin receptor (AT1b) has also been observed in the adrenal glands of low-protein rat offspring, associated with a persistent upregulation of AT1b gene expression which may contribute to the development of hypertension [105]. Hypomethylation has also been observed in sheep that were exposed to a diet deficient in methyl donors for a period of 8 weeks prior to conception and for the first 6 days of pregnancy. These sheep exhibit insulin resistance and elevated blood pressure in postnatal life. Restriction landmark genome scanning showed that 4% of 1,400 CpG islands in the fetal liver were differentially methylated in a way which may cause overexpression of certain genes [106]. A recent study of human monozygotic twins challenged the view that methylation patterns remain unchanged after establishment in early life [107]. However, the demonstrated effects of maternal diet on offspring methylation patterns provide a tempting theory to explain how maternal diet may bring about permanent changes in gene expression. In addition, epigenetic marks have been shown to be stably inheritable, and epigenetics therefore offers a plausible explanation of how environmental exposures in a single generation can impact upon more than one subsequent generation [108, 109].

Overexposure to Maternal Glucocorticoids

The mechanisms by which maternal diet acts on the developing tissues to cause long-term changes in tissue structure and gene expression patterns remain poorly understood. However, a large body of evidence suggests that imbalances in the maternal diet are associated with overexposure of the fetal tissues to glucocorticoids. This is thought to occur via reduced activity of placental 11 β -

hydroxysteroid dehydrogenase (11 β -HSD), which acts to convert maternal glucocorticoids to inactive forms, or by increased activity of the GR in fetal tissues. Studies of rodents and sheep have demonstrated that exposure to synthetic glucocorticoids can impact upon renal development and subsequent blood pressure [110–114]. The similarities in phenotype associated with dietary and glucocorticoid exposures have led to suggestions that they may act through a shared mechanism, i.e. that maternal diet may act to increase exposure of the fetus to glucocorticoids. In sheep, alterations in maternal cortisol in response to undernutrition appear to be dependent on the stage of gestation, being elevated or unchanged with late-gestation undernutrition [115, 116] and reduced with early-to-mid gestation undernutrition [117, 118]. However, a reduction in placental 11 β -HSD expression or activity has been observed in response to a low-protein diet in the rat [119, 120] or global undernutrition in the sheep [121, 122]. In addition, increased expression of the GR has been observed in a number of tissues in the neonatal sheep exposed to maternal nutrient restriction [122]. Giving further support to the theory of a common mechanism, inhibition of maternal glucocorticoid synthesis using the pharmacological agent metyrapone has been shown to prevent the nephron deficit and raise blood pressure observed in the low-protein rat model [123], suggesting that these outcomes are dependent on overexposure of the fetus to maternal glucocorticoids. The precise mechanisms and gene targets by which glucocorticoids impact on development and subsequent health remain under investigation. During development, glucocorticoids act through a number of mechanisms to alter the balance between tissue proliferation and differentiation [124], with many genes containing glucocorticoid response elements. Overexposure of the fetus to glucocorticoids may therefore explain some of the effects of maternal diet on tissue structure and subsequent function.

Future Directions

Work with animal models has clearly demonstrated the biological plausibility of the associations observed in the human population, providing strong evidence of a causal relationship between early life exposures and metabolic risk factors in later life. Furthermore, animal models have successfully demonstrated alterations in the expression and activity of the systems regulating postnatal physiological function, identifying pathways which are involved in the pathogenesis of developmentally pro-

grammed disease. However, the true initiating mechanisms by which maternal diet impacts on long-term physiology and health remain poorly understood – how do environmental factors act during development to have such long-term effects on metabolic regulatory systems? Changes in the expression or activity of regulatory systems observed during postnatal life could simply be secondary to the observed programmed phenotype, perhaps mediating its progression. Alternatively, the changes in expression or activity observed may actually be drivers of the programmed phenotype. At present, theory suggests that such drivers could arise from the developmental period as a result of the impact of maternal diet on processes determining tissue structure or the pattern of epigenetic markers set down during the reprogramming of the embryonic genome. Further work is required to fully understand the regulation of such developmental processes and how this might interact with signals of maternal nutritional status acting during development. This requires a focus on the developmental period itself rather than further characterisation of downstream events.

Having identified the contribution that nutritional programming effects make to postnatal disease risk, the development of novel preventative and therapeutic strategies is an important priority. Effective interventions have already been observed in animal studies, demonstrating the plausibility of this approach. For example, folate or glycine supplementation of a low-protein diet during pregnancy has been shown to improve postnatal outcomes in the offspring [125, 126]. Similarly, leptin administration in the early postnatal period in the rat completely normalised the programmed phenotype in offspring exposed to maternal nutrient restriction during pregnancy [127], although these findings have proven difficult to reproduce [128, 129]. Improved understanding of the mechanisms of programming will help to identify potential targets and critical periods for intervention, with the ultimate aim of translation to the human population.

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