Fetal programming of CVD and renal disease: animal models and mechanistic considerations

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The developmental origins of health and disease hypothesis postulates that exposure to a less than optimal maternal environment during fetal development programmes physiological function, and determines risk of disease in adult life. Much evidence of such programming comes from retrospective epidemiological cohorts, which demonstrate associations between birth anthropometry and non-communicable diseases of adulthood. The assertion that variation in maternal nutrition drives these associations is supported by studies using animal models, which demonstrate that maternal under- or over-nutrition during pregnancy can programme offspring development. Typically, the offspring of animals that are undernourished in pregnancy exhibit a relatively narrow range of physiological phenotypes that includes higher blood pressure, glucose intolerance, renal insufficiency and increased adiposity. The observation that common phenotypes arise from very diverse maternal nutritional insults has led to the proposal that programming is driven by a small number of mechanistic processes. The remodelling of tissues during development as a consequence of maternal nutritional status being signalled by endocrine imbalance or key nutrients limiting processes in the fetus may lead to organs having irreversibly altered structures that may limit their function with ageing. It has been proposed that the maternal diet may impact upon epigenetic marks that determine gene expression in fetal tissues, and this may be an important mechanism connecting maternal nutrient intakes to long-term programming of offspring phenotype. The objective for this review is to provide an overview of the mechanistic basis of fetal programming, demonstrating the critical role of animal models as tools for the investigation of programming phenomena.

Fetal programming: Pregnancy: Epigenetics: CVD: Animal models

Early-life programming of non-communicable disease

The developmental origins of health and disease hypothesis

The term 'programming' describes the process through which exposure to environmental stimuli or insults, during critical phases of development, brings about permanent changes to the physiology or metabolism of the organism(123). The capacity for the environment to exert such programming effects is a feature of the plasticity of cell lines during embryonic and fetal life. In most types of cell, plasticity is a short-lived characteristic and is only a feature of the embryonic and fetal stages. These critical developmental phases therefore represent a stage of vulnerability, as signals from the maternal system that indicate adverse environments may induce irreversible adaptations that may limit the fitness of the conceptus for life outside the uterus(3). Effectively, programming is the first modulatory influence upon the genetic profile of the individual and is part of a cumulative series of events that determine health and disease in adulthood.

Abbreviations: DOHaD, Developmental Origins of Health and Disease; MLP, maternal low protein.

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As shown in Fig. 1, the quality of the maternal diet is one of a number of potential stressors that may disturb normal fetal development and programme physiological function. Much of the literature in this field has focused on the impact of maternal undernutrition upon development. What has become known as the Developmental Origins of Health and Disease (DOHaD) hypothesis is based upon the concept that poor nutritional status in pregnancy is the primary programming signal associated with disease processes in the human population\(^3,4\). With current trends in maternal obesity across the developed countries, there is an increasing focus upon the potential of maternal adiposity to also be a programming factor\(^5\).

**Epidemiological evidence to support the hypothesis**

The primary evidence that led to the development of the DOHaD hypothesis was generated by the retrospective studies of a Hertfordshire cohort, conducted by Barker *et al.*\(^6-9\). Follow-ups of men and women born between 1911 and 1930 indicated that individuals who were of lower weight at birth, but still within the normal range of birth weights for the population, were at greater risk of CHD mortality, had higher systolic blood pressure, a greater prevalence of type 2 diabetes and a greater risk of developing the metabolic syndrome\(^6-9\). Further studies observed that in addition to lower birth weight, disproportion at birth characterised by a lower ponderal index, a skewed fetal:placental ratio or a large head circumference was also associated with greater risk of disease in later life\(^6,10-12\).

A series of studies of men and women born in Helsinki, Finland in the 1920s and 1930s added strong support to the epidemiology conducted in the United Kingdom\(^13-15\). These studies confirmed the relationship between low birth weight and CVD and type 2 diabetes, and also demonstrated that rapid catch-up growth in infancy was also a risk factor for later disease. Individuals who went on to develop CHD were of lower weight at birth and remained lighter and of smaller stature until approximately 9 years of age\(^13-15\). Rapid weight but not height gain leading to greater BMI in adolescence was predictive of adult disease. The Helsinki cohorts have also provided critical information about the relationship between fetal growth, the genome and later disease\(^16-18\).

The long-term impact of maternal undernutrition on offspring health was apparent in the follow-ups of the 1944/45 Dutch famine. This relatively short period of undernutrition impacted upon women during discrete periods of pregnancy, enabling the long-term effects of undernutrition experienced in early-, mid-, or late-gestation to be considered alongside cohorts of individuals born...
before or after the famine. These studies have suggested a direct association between periods of food restriction in pregnancy and CVD, metabolic and renal disease in the adults exposed to undernutrition in utero\(^{19–24}\).

**Criticisms of the Developmental Origins of Health and Disease hypothesis**

The reliance of the DOHaD hypothesis on a series of retrospective epidemiological cohorts has provided the basis for criticisms of the hypothesis\(^{25}\). These are largely focused on the fact that studies may struggle to adequately adjust for confounding factors across timespans that are often over 50 years, and that the anthropometric measures used as proxies for maternal nutritional status may in fact be only weakly related to nutrition\(^{26,27}\). For example, birth weight is not strongly influenced by maternal nutrient intakes in well-nourished populations. Although there are a small number of studies which have been able to relate more direct markers of maternal nutritional status during pregnancy to later health in the resulting offspring\(^{26–30}\), these do not provide a convincing canon of evidence. It is also asserted that the relationship between birth anthropometry and later metabolic function may be explained by particular genetic variants with common effects on intrauterine growth and energy metabolism\(^{31}\). To resolve these uncertainties there is a need for robust experimental studies that enable demonstration of the biological plausibility of the DOHaD hypothesis, and provide suitable vehicles for examination of the mechanisms that may drive the association between early diet and later disease.

**Animal models of nutritional programming**

Animal models provide a significant advantage over epidemiological studies in that the majority of model species are relatively short-lived and have short gestation periods, allowing rapid assessment of the impact of variation in maternal nutritional status upon the developing offspring, and for intergenerational studies to be performed\(^{4,32}\). Experiments with animals, which have evaluated the plausibility of the DOHaD hypothesis, have utilised a wide range of species, including rats, mice, guinea pigs, pigs, sheep and, more recently, baboons\(^{4,33}\). A wide range of nutritional insults have been applied during pregnancy in these species, including global nutrient restriction (limiting of fed rations), micronutrient restriction (for example, Zn or Fe), macronutrient restriction (low-protein diets) and ligation of the uterine artery to limit nutrient supply to the fetus\(^{4,34–36}\). More recently, researchers have attempted to model the effects of maternal obesity, largely by feeding high-fat, high-sugar diets to pregnant rodents\(^{37–40}\). In addition to the nutritional insults, experiments have also considered the potential for programming by endocrine factors and the stress of maternal and neonatal handling by human subjects\(^{41}\).

Restricting maternal food intake in pregnancy has a number of programming effects in sheep and rodents and has been a widely used experimental approach as it is typically associated with lower weight at birth, providing comparability with the epidemiological studies. Offspring of nutrient restricted sheep exhibit initially lower blood pressure than offspring of normally fed animals, but with increasing age begin to develop a relative hypertension\(^{42–43}\). There is evidence of predisposition to cardiac failure, impaired renal function and altered sensitivity to a range of endocrine factors.

Food restriction in pregnant rats has effects that vary according to the severity of the nutritional insult. Woodall et al. limited maternal intake to just 30 % of ad libitum, which resulted in a marked decrease in offspring birthweight\(^{44}\). These animals went on to develop raised blood pressure by one year of age and had a tendency to greater fat gain when fed a hyperenergetic diet\(^{36,45}\). Food restriction in pregnancy is also associated with programming of glucose intolerance in the rat.

A large body of evidence has been accumulated in relation to the programming effects of maternal protein restriction during rat and mouse pregnancy. Studies of programming using the maternal low protein (MLP) rat model have demonstrated that this form of undernutrition throughout pregnancy or for specific periods in early-, mid- or late-gestation programmes elevated blood pressure in the offspring\(^{36,46,47}\). Elevation of blood pressure is noted from about the time of weaning (3–4 weeks of age) and persists well into adult life. It is assumed that the hypertensive effect of prenatal protein restriction is permanent and lifelong\(^{34,46,47}\). Lifespan in such animals is significantly reduced and in males this may be a consequence of early renal failure\(^{48,49}\). Several studies have shown that offspring exposed to MLP in utero have fewer nephrons in their kidneys, which promotes an earlier decline in renal function and may be related to raised blood pressure\(^{50}\). Interestingly, when the offspring of rats fed MLP in pregnancy are bred, with no further nutritional insult, their offspring also develop high blood pressure associated with low nephron number. This gives important clues about the possible mechanisms involved\(^{32}\).

Renal and vascular functions are not only physiological processes that are programmed by maternal protein restriction in rodents. Offspring exposed to protein restriction initially appear more sensitive to insulin and clear a glucose load more rapidly than unexposed animals\(^{51}\). There is also evidence of resistance to adiposity during early adult life. However, as the animals age they develop a progressive insulin resistance, and over-expression of lipogenic pathways in the liver results in profound hepatic steatosis when compared with controls of the same age\(^{52,53}\).

Feeding behaviour is programmed by maternal protein restriction, which appears to suggest that undernutrition in fetal life impacts upon the development of the hypothalamus\(^{54,55}\). There is also some evidence that aspects of immune function may be sensitive to the maternal diet, providing an interesting comparison to epidemiological studies which also indicate a sensitivity of immune system development to the maternal environment\(^{56–58}\).

Feeding a low-Fe diet prior to and throughout rat pregnancy depletes maternal Fe stores and leads to a significant reduction in fetal Fe uptake and hepatic Fe status by the time of delivery\(^{59}\). The offspring of Fe-deficient rats are
of low birth weight and during embryonic life are noted to have enlarged hearts. These animals develop raised blood pressure shortly after weaning\(^{33,60}\). As with the protein-restricted rat, the offspring of Fe-deficient animals exhibit a lower nephron number in the kidney\(^{61}\).

The induction of obesity in rats and mice prior to and during pregnancy appears to have similar programming effects to those observed in experiments with undernourished animals. Samuelsson \textit{et al}. fed mice a high-fat, high-sugar diet and noted glucose intolerance and elevated night-time blood pressure in their offspring\(^{59}\). Similarly, cafeteria-diet feeding in pregnant rats programmed feeding behaviour and glucose homoeostasis in their offspring\(^{62,63}\). It is challenging to dissect out the effects of maternal obesity from the maternal diet in these experiments and both factors may have programming effects on specific features of metabolic and physiological function. Cafeteria feeding during lactation also influences offspring behaviours indicating that, in rats at least, the window for programming to occur is wide and that the mechanism may be independent of the intrauterine environment\(^{39,64}\).

It is noteworthy that across all of the species studied and with the tremendous diversity of pregnancy challenges that have been applied, the outcomes for the offspring are relatively conserved. Raised blood pressure and glucose intolerance are universal outcomes, while many of the animal experiments also report effects of maternal nutritional status upon offspring adiposity, renal development and immune function\(^{44}\). The commonality of programmed responses to maternal insult may suggest that there are a relatively small number of common mechanisms that provide the link between the early nutritional environment and long-term physiological function.

**Mechanisms of programming**

Understanding of the mechanisms through which nutritional signals at critical stages in early life exert long-term, or permanent, effects is of major importance. Identification of mechanisms at the molecular level will be a critical step in developing new disease prevention and treatment strategies. Evidence to support a range of proposed mechanisms has been provided by experimental studies, largely performed in rats. Although each of these studies has attempted to address the important aim of identifying how nutritional signals during fetal development permanently alter physiological function and hence promote greater disease risk, all are essentially observational studies. In isolation these experiments cannot explain the commonality between models and hence the common underlying mechanisms and principles that drive programmed disease processes.

The animal studies tend to characterise the downstream phenotypes that are observed in adult offspring and hence will focus on processes that may mediate the exact pathology or metabolic consequences of programming. Many of these processes are likely to be secondary phenomena and do not explain the basis of programming. Broad assumptions have been drawn about observed changes in expression of single genes or specific pathways that have been selected on the basis of their plausible involvement in development of metabolic or vascular phenotypes. Close association with the phenotype increases the likelihood that observed effects are secondary events. Therefore it has to be recognised that the most likely drivers of nutritional programming are still unknown\(^{65}\).

The significant problem of dissociating the primary responses to maternal insult (which could in fact be very short-lived, transient adaptations), from secondary responses to these initial adaptations, is well illustrated by consideration of the programming of lipogenesis by maternal protein restriction in the rat. Offspring of rats fed low-protein diets are initially obesity resistant and only with ageing become insulin resistant and develop hepatic steatosis\(^{65}\). Considering the hepatic expression of sterol response element binding protein 1c, an insulin-sensitive protein that regulates lipogenic pathways, and downstream genes such as fatty acid synthase, at the age when steatosis is present, indicates over-expression in the low-protein-exposed animals\(^{65}\). This establishes a \textit{prima facie} case for changes in expression of sterol response element binding protein 1c as an important mediator of the metabolic programming. This case is reinforced by the fact that in younger MLP animals which are more sensitive to insulin and obesity resistant, expression of sterol response element binding protein 1c and the lipogenic pathway are heavily suppressed. However, sterol response element binding protein 1c expression in the livers of fetal animals (i.e. actually still subjected to the maternal insult) is not responsive to maternal protein restriction, indicating that this gene pathway cannot be a primary driver of metabolic programming and must in fact be responding to some other change in expression of the fetal genome, or alteration to hormone sensitivity or organ structure\(^{4,65,66}\).

**Tissue remodelling as a common mechanism**

Normal growth and development \textit{in utero} is a process dependent upon waves of cell proliferation, apoptosis and differentiation. It is hypothesised that the maternal environment can modify these processes with, perhaps transient, stimulus or inhibition of factors which control the cell cycle, rates of mitosis, cell death and maturation resulting in irreversible changes in tissue structure\(^{4}\). It is clear that remodelling of tissues, such that there are fewer or more cells of specialised types, takes place in most major organs of animals that experience maternal undernutrition during fetal development. Where remodelling of tissue impacts in such a way that the numbers or integrity of key functional units in a tissue are reduced, it is relatively simple to understand how subsequent age-related degradation of physiological function occurs at a faster rate than is observed in individuals not subjected to prenatal insult.

There is evidence of remodelling in the kidneys of human subjects. Several studies have indicated that individuals of lower birth weight have fewer nephrons in the kidney and as nephron number is correlated with blood pressure, it is assumed that the structural deficit associated with impaired fetal growth is a driver of cardiovascular risk with ageing\(^{67}\). Protein restriction and Fe deficiency in rat pregnancy are also associated with a reduced nephron
number in kidneys of the offspring\textsuperscript{(50,61)}. As nephron number is finalised in early postnatal life in the rat (at birth in human subjects), nephron deficits cannot be recovered. While the structural deficit may not have effects in early life, renal function will decline with age and the individual will be at greater risk of chronic kidney disease and associated loss of blood pressure control at an earlier age. Further examples of tissue remodelling also come from studies of rats subjected to maternal protein restriction in utero\textsuperscript{75}. These animals have fewer $\beta$-cells in the pancreas, with smaller and less vascularised islets\textsuperscript{(68)}. There is evidence of programming of muscle fibre type, and also of neuronal densities in hypothalamic centres responsible for appetite regulation\textsuperscript{(69,70)}. 

Drivers of remodelling

The primary responses to programming insults will be the signalling pathways that link maternal nutritional status (intake, stores and body composition) to the tissue remodelling outcome. A number of mechanisms have been postulated to be critical in this signalling, all of which could operate together, at different stages of a pregnancy-long episode of undernutrition\textsuperscript{(65)}.

The simplest explanation of programming is that less than optimal maternal nutrition impacts upon the quality or quantity of nutrients which are available to the developing embryo or fetus. Having nutrients that are limiting or present in excess may directly impact upon gene expression and hence key processes such as cell proliferation. Analyses of the effects of protein and Fe deficiency in day 13 rat embryos suggested that nutrient restriction altered the expression of hundreds of genes across the whole genome\textsuperscript{(61,71)}. Critically, across the two separate insults a limited number of pathways and processes emerged as being differentially expressed in the presence of undernutrition. In the context of the tissue remodelling hypothesis it is important to note that these included DNA synthesis, regulation of the cell cycle and apoptosis\textsuperscript{(61,71)}. The delivery of nutrients to the fetus from the mother is a key function of the placenta. The placenta is also responsible for orchestrating the endocrine milieu in which fetal development takes place. Undernutrition can disturb this endocrine environment and perturb fetal growth and tissue maturation by altering hormonal exposures. In this regard glucocorticoid exposure is of particular importance (Fig. 2). Treatment of pregnant rats with synthetic glucocorticoids replicates the features of nutritional programming in their offspring. Furthermore, programming by maternal protein restriction in rats has been shown to be dependent upon the maternal synthesis of glucocorticoids\textsuperscript{(72,73)}.

![Fig. 2. The glucocorticoid hypothesis](https://www.cambridge.org/core/fig/f2)

(a) Optimal maternal nutrition

(b) Maternal undernutrition

Glucocorticoids

Maternal circulation

Placenta

11$\beta$HSD2

Fetal circulation

Gene expression

Tissue growth and maturation

Glucocorticoids

Maternal circulation

Placenta

11$\beta$HSD2

Fetal circulation

Gene expression

Tissue growth and maturation

Fig. 2. The glucocorticoid hypothesis. (a) Placental 11$\beta$-hydroxysteroid dehydrogenase (11$\beta$HSD2) controls the movement of glucocorticoids from maternal to fetal circulation through the conversion of active glucocorticoids to inactive metabolites. (b) Undernutrition is known to down-regulate 11$\beta$HSD2 and this results in greater transfer of glucocorticoid from maternal to fetal circulation. Exposure to glucocorticoids regulates gene expression in fetal tissues. Adapted from\textsuperscript{(64)}. 

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Programming of cardiovascular, metabolic and immune functions in rodent models of DOHaD have been reported to persist across more than one generation\(^{(32,74)}\). In the study of Harrison et al., the programming of raised blood pressure by an MLP diet in the rat was seen not only in the offspring (F1), but also in the grand-offspring (F2)\(^{(52)}\). This intergenerational transmission of phenotype occurred through both the paternal and maternal lineage indicating that animals must transmit the programming signal through both egg and sperm rather than through dietary effects upon maternal physiology. The most likely hypothesis is that this would be through direct effects on the genome or epigenome. Dramatic reprogramming of DNA methylation happens in the initial phase of gestation, making DNA methylation an obvious candidate for this process. Indeed, the low-protein maternal diet in rat pregnancy has been shown to induce lower methylation in the PPAR\(\alpha\) gene promoter as well as other loci in both F1 and F2 generations\(^{(75–78)}\).

DNA methylation is dependent upon the availability of C\(_1\) donors as substrates. Sinclair et al. reported that periconceptual depletion of methyl donors in sheep impacted upon methylation of approximately 4\% of the genome in affected lambs\(^{(79)}\). Furthermore, the observation that dietary supplementation with C\(_1\) donors, such as folic acid or glycine, can reverse the phenotypic changes associated with undernutrition in rat pregnancy is consistent with the hypothesis that disturbance of DNA methylation is an important mechanistic driver of programming\(^{(80–82)}\). The expression of DNA methyltransferases may be modified by the maternal diet and this may explain how undernutrition can alter methylation, gene expression and hence remodel tissues during development\(^{(83)}\).

Future perspectives

The accumulated evidence from animal studies and epidemiology clearly demonstrates that individual risk of non-communicable diseases of adulthood is the complex product of cumulative factors operating across the lifespan. The earliest modifications to the genetically and epigenetically determined trajectories of growth, development and health would appear to be related to variation in the quality or quantity of nutrients supplied to the fetus. As early-life programming events occur during phases of development where all tissues are rapidly growing and developing, the scale of influence of early-life factors is considerable, is likely to impact upon all aspects of adult physiology and metabolism; and is almost certainly irreversible. Disease states such as CHD or type 2 diabetes represent the end-stage consequences of programming.

As our understanding of the relationships between early diet and later disease increases, the full potential of programming to significantly influence prevalence of non-communicable disease risk at the population level is being realised. The development of personalised nutrition advice is a future goal. The availability of robust high-throughput technologies that are capable of genotyping large numbers of people, for multiple SNP and of obtaining readouts of the epigenome, metabolome or proteome will inevitably lead to the construction of individualised nutrition plans based upon easily collected biomarkers. As nutritional programming is a significant contributor to lifelong metabolic and physiological function, it is important to incorporate an understanding of these phenomena into the design of such personalised nutrition strategies. A greater understanding of the mechanisms which link the maternal diet to fetal development and long-term health is critical for this strategy and may also result in novel drug-based therapeutic approaches. For example, rodent studies have demonstrated that inhibition of the renin–angiotensin system during the suckling period can negate the programming effect of maternal protein restriction upon offspring blood pressure\(^{(83)}\).

Other interventions may be applied to resolve the adverse effects of a poor maternal diet upon the developing infant, with a primary focus upon defining a high-quality diet for pregnancy. In current practice the optimal diet for human pregnancy is poorly defined. Advice to increase intake of folate, control the weight before conception, maintain pregnancy weight gain within specified guidelines, avoid high intakes of retinol and non-consumption of foods likely to be contaminated with gut pathogens represents the current state-of-the-art. Improved understanding of the mechanisms of programming and identification of key nutrients that may constitute a biological stressor to fetal development when present in excess or when limiting will help shape such strategies.

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