Animal models and programming of the metabolic syndrome

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The purpose of this review is to consider how current animal models of fetal programming contribute to knowledge of the metabolic syndrome in adult humans. Low birth weight infants have an increased risk of developing cardiovascular and coronary heart disease, hypertension, diabetes and stroke in adulthood. A number of animal studies confirm the association between events during fetal life and subsequent adult disease. This review considers how these have contributed to our understanding of this relationship, and how they may help to uncover the underlying mechanisms. The importance of dietary, pharmacological, genetic and surgical models is assessed, and their usefulness in the prevention of human disease evaluated. Although progress has been made, further investigations using animals are needed to clarify the mechanisms involved in the programming of adult disease. Once these processes are understood, it may be possible to identify and protect at-risk individuals.

Human epidemiological studies link the incidence of a number of adult diseases, such as type 2 diabetes, cardiovascular disease (CVD) and hypertension with poor prenatal nutrition and low birth weight. This group of diseases is often referred to under the term metabolic syndrome (syndrome X). The data relate low ponderal index or short body length to these diseases, suggesting that a compromised maternal–fetal nutrient supply results in fetal growth retardation. This in turn is linked to altered ‘programming’ of development of fetal organs and of physiological homeostatic control processes. As such, programming occurs during critical periods of development, changes become permanent and lead to later pathophysiological events. The mechanisms underlying the pathophysiology of these associations, however, have yet to be elucidated1.

Despite the substantial epidemiological evidence for fetal origins of adult disease, there are intrinsic limitations in long-term retrospective studies. Some aspects can, however, be focused on by using the controlled conditions afforded by animal models, a number of which have been developed to study this in utero programming phenomenon. The purpose of this review is to compare current models, not only to summarise the
data thus far accumulated, but also to consider future options for this area of research. At present, species studied include the rat, mouse, guinea pig, sheep and non-human primate, and treatments used include dietary, pharmacological, genetic and surgical manipulation.

A key determinant of fetal growth is the availability of protein. Through most of fetal life, amino acids, rather than glucose, determine insulin secretion by \( \beta \)-pancreatic cells, and this control of fetal insulin secretion links the availability of amino acids to support fetal growth with the rate of fetal growth since insulin is an important fetal growth hormone. Other nutrients, including oxygen, are critical for optimum fetal growth, and it is possible that a combination of deficiencies are involved in human fetal growth retardation. Research in many laboratories is directed towards disentangling the many threads in this complicated story. In some, an isocaloric maternal low protein (MLP) diet has been used to explore the mechanisms by which protein metabolism affects developing organs. Other studies have used varying degrees of global reduction in nutrition. Many studies have employed pharmacological methods, genetic manipulation or surgical techniques to produce useful animal models. These will be discussed briefly below. It is noteworthy that, in some of these studies, effects on the development of homeostatic mechanisms were produced even in the absence of body growth restriction – findings analogous to those in humans who were exposed to the Dutch famine (see Hales & Barker, this issue). It is, therefore, possible to envisage a spectrum of health problems in adult life deriving from the influence of the intrauterine environment and maternal/placental/fetal compensatory responses to a diet mildly to severely altered in its composition or its volume. It is also important to note that even if an isocaloric low protein diet does not affect birth weight significantly in the first generation, it may reduce it in subsequent generations.

**Dietary manipulation**

The concept that maternal nutrition can programme adult disease was established in animal experiments. Over 30 years ago, Winick and Noble showed that poor nutrition during gestation led irreversibly to reduced cell number in tissues such as the pancreas. In 1974, Weinkove et al established that permanent impairment of insulin secretion resulted from perinatal protein restriction, and subsequently post-weaning protein depletion was shown to produce the same result. More recently, Snoeck and co-workers demonstrated that maternal protein deprivation caused reduced \( \beta \)-cell proliferation and islet size in the offspring.

Two different dietary strategies are used at present – global nutritional restriction, and isocaloric low protein manipulation – and these are discussed below.
Global undernutrition

A number of studies using different levels of global dietary restriction have been reported. In New Zealand, Gluckman’s laboratory developed a rat model of IUGR using severe maternal dietary restriction (30% of ad libitum) throughout gestation and examined the effects of fetal growth retardation on the endocrine and metabolic status during the perinatal period. Previous work had supplied evidence that there is a central role for fetal IGF-1 in the regulation of fetal growth, and that both maternal and fetal IGF-I are regulated by nutrient availability. In the sheep, maternal administration of IGF-I has been shown to enhance amino acid and glucose uptake by the placenta and to promote glucose delivery to the fetus. In rat studies, maternal plasma IGF-I levels were significantly reduced throughout gestation, but not postnatally, and both the mean body weights of late gestation fetuses and the placental weights were significantly lower than controls. Taken together, these studies in the sheep and rat establish a link between IGF-1 and fetal growth. Interestingly, while plasma IGF-1 and plasma insulin levels in the pups were significantly reduced from birth to postnatal day 9, this did not persist into adulthood.

A less severe global restriction model was used by Holemans and co-workers who studied blood pressure (using an implanted femoral artery catheter) in female rats whose dams had received a 50% reduction in food intake in the second half of pregnancy. No significant difference in blood pressure was found, though small mesenteric arteries had reduced endothelium-dependent relaxation (to acetylcholine and bradykinin) but enhanced sensitivity to exogenous nitric oxide (sodium nitroprusside). This indicated that while reduced synthesis of nitric oxide and prostacyclin altered vascular function, it was insufficient to result in a change in blood pressure.

Even mild global restriction has been shown to produce alterations in both metabolism and the HPA axis. Studies in guinea pigs fed an 85% of ad libitum diet throughout pregnancy showed that maternal undernutrition and small size at birth permanently alter postnatal cholesterol homeostasis in the male offspring. This is interesting because a number of investigations into CVD susceptibility indicate that prenatal and early postnatal manipulation of cholesterol affect adult cholesterol metabolism in rodents and baboons. In the sheep, a 15% global reduction of total diet during the first half of pregnancy resulted in blunted ACTH and cortisol responses to exogenous CRH and AVP administration, and also a reduced cortisol response to ACTH. When maternal food reduction was followed by acute hypoxemic challenges between days 114–129, plasma ACTH and cortisol responses were also reduced relative to controls.
basal plasma and cortisol responses were unaltered\textsuperscript{14}, suggesting that the effects are predominantly manifest on the response of the HPA axis to endogenous and exogenous stimuli. It is interesting to note that even the mildest maternal global undernutrition has significant effects at two or more levels of the axis.

\textit{Maternal low protein (MLP)}

Maternal low protein models of fetal programming have been extensively used to study the mechanisms that link maternal nutrition with impaired fetal growth and later cardiovascular disease and diabetes. Interestingly, the differing composition of the low protein diets used in individual laboratories, while generally causing low birth weight, appear to predispose the offspring to different pathophysiological effects in adulthood. A direct comparison of two of these diets in relation to their ability to cause hypertension in adults was recently undertaken by Langley-Evans\textsuperscript{15}, viz the diet used at Southampton and the Hope Farm diet used by Hales and colleagues in Cambridge and by others\textsuperscript{16}. While the diets differed in their overall fat content, fatty acid composition, methionine content and the source of carbohydrate, the protocols used were identical. It was found that offspring of rats fed the Southampton diet, but not those on the Hope Farm diet, became hypertensive. Langley-Evans concluded that different low protein diet manipulations (at 8–9\%) in rat pregnancy elicit different programming effects, and that the balance of protein with other nutrients may be a critical determinant of the long-term health effects of maternal undernutrition in pregnancy.

It is not clear how restricted maternal protein supply perturbs fetal growth. In the rat there are two phases of protein metabolism during pregnancy. During the anabolic phase, maternal body protein is accumulated which can then be used to support fetal growth during the catabolic phase. However, it is not yet known whether this mechanism can compensate for reduced dietary protein intake and thus maintain the supply of free amino acids to the fetus. Analysis of free amino acids in fetuses and mothers fed 9\% casein diets showed a reduction in maternal threonine which was not evident either in control rats or in non-pregnant rats fed the low protein diet\textsuperscript{17}. Rees \textit{et al} suggest that the threonine-methionine-homocysteine group of amino acids may lead to adverse effects in the protein-deficient mother and her offspring. However, although the 9\% casein diet supplies insufficient protein for a pregnant rat, this percentage lies within normal nutritional parameters for a non-pregnant rat. While Rees’s conclusions may be valid, the results should be tested against a nutritionally challenged non-pregnant control.
Maternal protein restriction affects islet cells, as well as insulin-sensitive tissues such as liver, muscle, adipocytes, kidney and brain in the offspring. Protein availability appears to have a specific role in the development of fetal β-cells. *In vitro*, increased essential amino acid concentrations amplify fetal β-cell differentiation, multiplication and insulin secretion more efficiently than increased glucose concentration. Abnormal features thus acquired by the developing β-cell may then lead to postnatal pathological events. Feeding an isocaloric low protein diet during gestation alters the profile of amino acids in maternal and fetal plasma as well as in amniotic fluid, though neither the total essential and non-essential amino acid concentrations nor the glucose and insulin levels are modified. α-Aminobutyric acid, phosphoserine, taurine and valine are reduced in maternal as well as in fetal plasma. Kwong et al investigated the effect of mild protein malnourishment on pre-implantation embryos, and found that feeding 9% protein to mothers from conception to implantation only, followed by standard chow, resulted in offspring which developed hypertension as adults. These embryos displayed significantly reduced cell numbers, induced by a slower rate of cellular proliferation rather than by increased apoptosis. At day 4, insulin and essential amino acids in maternal serum were reduced, compared to controls, whilst glucose levels were increased. These changes in maternal values may provide one or more of the mechanisms by which altered maternal diet produces signals for the early embryo.

Maternal protein deprivation studies have also shown uneven distribution of islet cell proliferation in the endocrine pancreas, and islet cell size, pancreatic insulin content and islet vascular density were all reduced at birth. Additionally, when the low protein diet was maintained during weaning, the ontogeny of the endocrine pancreas of offspring was disturbed in that there were more apoptotic cells in islets while the number of cells positive for IGF-II, a survival factor preventing apoptosis, was decreased. In fetal β-cells, insulin secretion was halved *in vitro*, while *in vivo* offspring with normal glucose, insulin and amino acid profiles reacted abnormally to a glucose challenge. Insulin levels were low before and during pregnancy, but plasma glucose levels were higher than normal.

When the adult offspring were maintained on a low protein diet postnatally, they had an abnormal amino acid profile, a smaller endocrine pancreas and reduced pancreatic insulin content. Additionally, there was a reduction in islet blood vessel density together with pancreatic and islet blood flow. Interestingly, the islet mitochondria had reduced glycerolphosphate dehydrogenase (mGPDH) activity. This reduction is also observed in islet cells of human subjects with type 2 diabetes.

Intergenerational studies show that reduced birth weight continues through subsequent generations. After an oral glucose challenge, pregnant offspring had low plasma insulin and high plasma glucose levels.
and their pups had more marked differences in plasma insulin, insulin content and endocrine pancreas density than the previous generation\textsuperscript{23}. A similar intergenerational effect of a protein restricted diet has already been observed on brain development and tryptophan metabolism\textsuperscript{24}.

In the liver, the MLP diet causes changes in zonation and enzyme activity, including a reduction in glucokinase and an increase in PEPCK activity, and altered regulation of hepatic glucose output\textsuperscript{25}, which are not restored at adulthood even when the animals are fed a normal diet\textsuperscript{26}. Insulin receptor number was increased in the liver, skeletal muscle and white fat adipocytes\textsuperscript{27}. Additionally, the adipocytes were smaller and did not show changes in GLUT4 expression, although this was increased in the plasma membrane of skeletal muscle\textsuperscript{28}. The adipocytes of adults had a greater glucose uptake and a higher phosphatidylinositol 3-kinase activity\textsuperscript{29}. Adipose tissue of offspring was also affected by global dietary restriction, which comprises low protein availability in the dams. In this instance, white adipose tissue increased and brown adipose tissue decreased in the adult, possibly indicating lower sympathetic activity. Rats whose mothers had restricted food during the first 2 weeks of pregnancy indeed became obese; but, depending on the strain and the diet used, it was either the males or the females which were affected\textsuperscript{30,31}.

Hoet and colleagues in Louvain demonstrated that protein plays a key role in development of the islets of Langerhans \textit{in utero}. Offspring of rats fed a diet containing 50\% less protein during pregnancy had poor \(\beta\)-cell proliferative capacity and islet size at birth, as well as reduced blood vessel density in the islets\textsuperscript{32}. Rats which were weaned on a low protein diet for 3 weeks also had a permanently altered insulin response to glucose. MLP offspring fed a low protein diet into adulthood were subsequently shown to have reduced glucose tolerance associated with reduced insulin secretion. Interestingly, the MLP group fed normal chow after birth had an intermediate response\textsuperscript{33}. This suggests that exposure to poor nutrition over brief periods during development, even when followed by normal food intake, can lead to irreversible changes. Thus, the developing pancreas appears to be sensitive to amino acid availability, and its endocrine function may also be regulated by nutritional elements. Consistent with the above findings are Hales’ observations on pancreatic glucokinase, an enzyme which plays a central role in the regulation of glucose-stimulated insulin release from the \(\beta\)-cell (glucose enters \(\beta\)-cell via GLUT2 transporter protein and is then phosphorylated by glucokinase). Glucokinase activity was measured in whole pancreatic extracts, and compared to offspring of control and MLP dams which were cross-fostered. MLP offspring were found to have significantly lower pancreatic glucokinase activity compared with controls and crossover groups. Using the same model, Hales and colleagues looked at glucose tolerance. All rats became less
glucose tolerant with age, but the worsening of glucose tolerance was more profound in the MLP group. Males tended to have higher plasma insulin concentrations, suggesting that, as in the human condition, the glucose intolerance was due mainly to insulin resistance. In females the situation appeared to be reversed, suggesting that their lower glucose tolerance was due mainly to insulin deficiency.

**High fat diet in pregnancy**

In addition to the phenomena obtained by undernutrition, studies of Pima Indians suggest that prenatal overnutrition can also programme later susceptibility to type 2 diabetes, and there is evidence that dietary fat intake during pregnancy increases the prevalence of cardiovascular risk factors in children. Offspring of rats fed high saturated fats during pregnancy have fetal insulin resistance, abnormal cholesterol metabolism and raised adult blood pressure. These symptoms would certainly predispose offspring to obesity in adulthood, and it is possible that a high fat diet in childhood or adulthood would amplify their effects.

Eriksson and colleagues conducted a longitudinal study of catch-up growth and death from CVD in Helsinki, and found that the highest death rates occurred in men who were thin at birth, but whose weight caught up, so that they had an average or above average body mass from the age of 7 years. They concluded that death from CVD might be a consequence of poor prenatal nutrition followed by improved postnatal nutrition. In consequence, a number of laboratories are now investigating the effect of cafeteria diets on offspring which were malnourished in utero and the results are awaited with interest.

**Blood pressure measurement**

There has been much debate recently regarding the measurement of blood pressure in the rat and this merits discussion here. Blood pressure can be measured using one of three methods:

1. Many studies, including those performed at Southampton and Cambridge use the indirect tail-cuff plethysmography method, which involves restraint and mild heat stress to vasodilate the tail bed. Only systolic pressure can be reliably measured and the method gives a single point measurement.

2. An in-dwelling carotid or iliac artery catheter allows continuous measurements of diastolic, systolic and pulse pressures to be taken with minimal disturbance, though the fact that the animal is continuously attached to a cable is inherently stressful. The catheter must be implanted under general anaesthesia, and the time taken to recover cannot be clearly established.
Most recently, it has been possible to use radiotelemetric methods which utilise an in-dwelling catheter in the descending aorta coupled to an intraperitoneal transmitter. This method has the benefit of a stress-free environment and the opportunity for 24 h monitoring of diastolic, systolic and pulse pressures.

The hypertensive model produced by mild protein malnutrition developed at Southampton has provided consistent data from a number of different laboratories, using tail cuff plethysmography. Experience has shown that providing the rat is trained with this equipment, stress can be kept to a minimum. These data have been reproduced when blood pressure was measured by cannulation in anaesthetized rats\textsuperscript{40}, but it is important now to validate these results using the latest telemetric methods, and this is presently being undertaken. Using telemetry, Tonkiss\textit{et al} found only small baseline changes in diastolic pressure, with an augmented elevation of systolic and diastolic pressures when the animal was subjected to stress, and they questioned the validity of the larger elevations in blood pressure in MLP offspring observed using the tail-cuff procedure\textsuperscript{41}. However, this study utilised a diet which differed not only in protein content (6\%) but also in fat source, mineral balance and methionine levels. Since it has been demonstrated that different diets produce differing effects on blood pressure (see above)\textsuperscript{15}, a similar study using the Southampton diet is underway to clarify the situation. Additionally, the stress used by Tonkiss was olfactory (ammonia) and this may elicit a different response to that caused by restraint stress. The topic of the stress response is discussed later.

**Pharmacological manipulation**

**Streptozotocin**

The most common pharmacological model for diabetes in rodents and sheep is the administration of streptozotocin (STZ). It is mostly used as a tool for elucidating mechanisms which result from induction of the disease directly in the experimental animal. However, some studies have employed STZ to investigate programming of diabetes, and as such it has a place in this review (see also van Assche\textit{et al}, this issue). Mild, maternal diabetes induced by STZ increased birth weight, produced β-cell hyperplasia in pancreatic endocrine tissue and an increase in the number of degranulated cells. However, when severe diabetes was induced, the fetal weight, islet size and β-cell mass were decreased. Additionally, in STZ-induced maternal diabetes, the pups of a second generation showed a reduction in birth weight together with permanent changes in endocrine pancreatic structure and function. Pups from both groups became diabetic at adulthood\textsuperscript{42}. Pancreatic insulin depletion in pups from moderately diabetic dams and insulin resistance in pups from
severely diabetic mothers were causal in initiating the diabetes in the offspring postnatally\textsuperscript{43}. Additionally, maternal STZ-induced diabetes affected the responses of small resistance arteries in the pups\textsuperscript{44}.

**Pharmacological disruption of the glucocorticoid pathway**

A great deal of work has been targeted at glucocorticoid exposure in the fetus and at glucocorticoid receptor (GR) status both \textit{in utero} and in adult life. Goland \textit{et al} found 5-fold higher corticotrophin releasing hormone (CRH) concentrations in cord blood plasma of growth-retarded babies, suggesting an overactive fetal HPA axis in pregnancies associated with growth retardation. Furthermore, this increased activity appears to be permanent as adult hypertensive patients demonstrated increased sensitivity to cortisol in dermal vasoconstriction tests\textsuperscript{45}. Many of the phenotypic features of the metabolic syndrome are also seen in Cushing’s disease, suggesting that antagonism of the effects of insulin by increased glucocorticoid hormone action may occur in patients with the syndrome. However, increasing evidence suggests that the HPA axis is not grossly altered in these individuals. For example, cortisol concentrations are usually increased in patients with Cushing’s disease but are decreased in patients with the metabolic syndrome\textsuperscript{46,47}. Interestingly, Phillips \textit{et al} found that plasma cortisol concentrations were increased (although still in the normal range) in men whose birth weights were low (<5.5 kg) compared with those who weighed >9.5 kg, a trend that was independent of age and body mass index. This suggests that plasma concentrations of cortisol within the normal range could have an important effect on blood pressure and glucose tolerance and provides evidence that intra-uterine programming of the HPA axis may be a mechanism underlying the association between low birth weight and the insulin resistance syndrome in adult life\textsuperscript{48}.

There is some evidence that HPA axis function in MLP offspring is modified resulting in hypersensitivity to glucocorticoid actions. While plasma corticosterone concentrations are similar at all points of the light cycle in rats exposed to control or MLP diets \textit{in utero}, the normal diurnal variation in ACTH secretion was absent in the rats exposed to low protein, suggesting the adrenal response to ACTH is altered. The increased sensitivity to glucocorticoids (GC) action in the absence of elevated hormone concentrations may be mediated at the level of receptor binding. MLP rats have increased GR numbers in a variety of tissues\textsuperscript{49,50}, and the up-regulation of receptors in MLP animals may increase sensitivity and amplify the actions of circulating and tissue corticosterone.

Glucocorticoids induce the expression of numerous genes. During fetal life, the expression of many of these genes at the correct developmental...
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...stage is essential for optimal maturation of tissues. Normally, exposure to glucocorticoid does not occur until late gestation when cortisol (human, ovine) or corticosterone (rat) is produced by the fetal adrenal. It has been shown in the rat that if placental 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) activity is reduced, maternal glucocorticoid can cross the placenta inappropriately, and modify the HPA axis\(^56\). We have shown recently that the MLP rat offspring have higher levels of glucocorticoid receptor in adulthood and that this increase is tissue specific\(^52\). Langley-Evans showed that the MLP rat model has reduced placental 11β-HSD2, suggesting that excessive maternal glucocorticoids are transferred to the fetus, possibly permanently altering the set point of the HPA axis\(^56\). McMillen and co-workers found that while 11β-HSD2 was differentially regulated in the fetal adrenal and kidney in the sheep fetus during late gestation, expression was not affected by placental growth restriction. Expression of hepatic 11β-HSD1 mRNA, which is normally increased in the second half of pregnancy was enhanced (2-fold) by placental growth restriction\(^53\), suggesting that there is increased hepatic exposure to cortisol in the growth-restricted fetus, which may be important in the reprogramming of hepatic physiology that occurs after growth restriction \textit{in utero}. Strong evidence for this comes from pharmacological studies. Studies using the synthetic glucocorticoid dexamethasone (DEX), and the 11β-HSD inhibitor carbenoxolone (CBX) demonstrated that failure to inactivate maternal glucocorticoid by fetal and placental 11β-HSD2 gives rise to rats with features of the metabolic syndrome\(^54,55\). This effect could be prevented by administration of metyrapone (11β-hydroxylase inhibitor) which blocked the synthesis of corticosteroids by both mother and fetus. Expression of glucocorticoid-sensitive genes, such as phosphoenolpyruvate carboxykinase (PEPCK) is also altered in the offspring, suggesting that the changes made to GC and GR \textit{in utero} have effects on the metabolic efficiency of the offspring, leading to type 2 diabetes and other symptoms of the metabolic syndrome. Work undertaken in Jonathan Seckl’s laboratory has focused on the HPA axis, using DEX or CBX. Administration of i.v. CBX during pregnancy led to hyperglycaemia in offspring, suggesting that inappropriate maternal glucocorticoid in the fetal circulation may be a factor in the programming of type 2 diabetes. 11β-HSD2 is normally co-located with the mineralocorticoid receptor (MR), and confers selectivity by inactivation of glucocorticoids which would otherwise bind to the MR. Interestingly, in sheep, CBX is a less potent inhibitor of 11β-HSD2 than in other species\(^58\).

When rats were exposed to the DEX in late gestation, hepatic PEPCK and GR expression were permanently altered, causing glucose intolerance in adult offspring\(^55\). Unlike endogenous glucocorticoid, DEX is able to cross the placenta without being inactivated by placental 11β-HSD2\(^59\). When fetal sheep were exposed to DEX for 2 days at d27/150
they became hypertensive in adulthood, while those exposed at d64/150 did not. This programming was independent of insulin resistance or amino acid metabolism, but the DEX exposure did lead to increased insulin sensitivity of the inhibition of lipolysis, which may increase susceptibility to the development of obesity postnatally. Clearly glucocorticoids exert powerful effects on development at specific times, and these merit further investigation.

Genetic models

Recently, it has become apparent that the spontaneously hypertensive rat (SHR) strain is somewhat more than a purely genetic model of hypertension. Like the infants identified in a number of studies of human populations in the UK and world-wide, SHR pups are born small with a large associated placenta. A series of studies by McCarty and colleagues has demonstrated that cross-fostering of SHR offspring with normotensive dams prevents development of hypertension. This effect has a clear critical window in the early postnatal period and appears to involve a nutritional, rather than a behavioural stimulus, since SHR milk has an altered electrolyte balance, is low in protein and has a different fatty acid profile when compared to Wistar Kyoto rat milk.

The recent development of a maternal low protein mouse model (personal communication by CB Whorwood) will allow mice to be used in studies focusing on particular genes of interest. For example, IGF-I knockout mice, IGF-II transgenic mice, IRS-1 disrupted mice, and tissue-specific insulin receptor knockout mice could be used to pinpoint the mechanisms involved in programming of insulin resistance/glucose intolerance. Equally, mice with disruptions of the HPA axis such as CRH-deficiency could be used to test the hypotheses implicating changes in the fetal HPA axis set-point.

Surgical models

Surgical interventions have been used primarily to provide further evidence of phenomena found in dietary or pharmacological models of programming. For example, adrenalectomy in the rat was used in conjunction with synthetic glucocorticoid administration to confirm that maternal glucocorticoids are involved in the programming of hypertension in the offspring: adult female rats that had been adrenalectomized prior to mating and fed low protein diets during pregnancy gave birth to normotensive offspring, whilst administration of DEX to adrenalectomized rats re-instated the hypertensive phenotype.
Disruption to fetal nutrition via the placenta can be achieved using a number of procedures, such as carunclectomy, placental embolisation and uterine artery ligation. Persson and Jansson ligated the arterial supply to one uterine horn in guinea pigs, which produced growth retardation of the pups in that horn whilst pups from the untreated horn were unaffected. The pups from the ligated horn had higher blood pressure than their litter-mates, although very severe reductions of birth weight (50–60%) were required to produce 10 mmHg increase in blood pressure. This work showed that nutritional deprivation during fetal life led to a reduction in tissue cell number which could not be reversed by adequate postnatal nutrition. In a more recent study using telemetry, however, they found no correlation between blood pressure and birth weight. In the sheep, it is relatively easy to undertake fetal as well as maternal manipulations. Removal of endometrial caruncles prior to conception (carunclectomy) has been used to manipulate fetal and placental growth rate. This procedure produces fetuses that are up to half normal size, albeit with a high incidence of mortality/fetal re-absorption, and the reduced placental size causes a fall in placental transport capacity which has significant consequences for the fetus. Decreased fetal growth velocity, soft tissue wasting and low birth weight are seen, with increased morbidity and mortality perinatally, and increased risk of pathophysiological effects on the cardiovascular system as well as type 2 diabetes in adulthood.

Murotsuki and colleagues embolised the fetal side of the placenta and measured cardiovascular and hormonal changes in fetal heart. They concluded that, as a result of fetal hypoxaemia and increased umbilical artery resistance caused by chronic placental damage, fetuses developed arterial hypertension and asymmetrical growth restriction, as well as myocardial hypertrophy due to increased afterload to the heart and raised plasma noradrenaline. Using the same technique, this group also found that chronic hypoxaemia selectively inhibited renal 11β-HSD2 mRNA expression and enzyme activity in the ovine fetus, contributing, at least in part, to the mechanisms leading to fetal hypertension.

**Which models relate to human disease?**

Animal models allow study of the pathophysiology of disease, and afford a means to study the underlying biochemical and molecular biological mechanisms. Whilst they cannot be used entirely as a substitute for the study of human diabetes, they allow exploration of aspects which cannot ethically be considered in the patient. A drawback is that the dietary model does not cause frank diabetes in the rat, although it does cause symptoms which are consistent with future manifestation of the disease.
All the approaches discussed provide methods for the elucidation of aspects of the programming phenomenon. Possibly, however, pharmacological, genetic and surgical manipulations should be regarded as tools for further understanding of the metabolic syndrome in the nutritional model.

On the face of it, global dietary restriction appears to have more relevance to the human condition than isocaloric low protein diets. Although both regimens produce effects equivalent to symptoms of the metabolic syndrome, an isocaloric diet is easier to manipulate in order to tease out underlying mechanisms \textsuperscript{15}. However, it will also be important to study a diet that more closely mimics sub-optimal human nutrition to identify possible interventions. Equally important is the study of postnatal diet on a system which is already compromised by intra-uterine nutritional restriction. If a balanced diet during pregnancy is not achieved, a central question to be answered is whether postnatal nutritional or pharmacological intervention can prevent the adult disease. As alterations to the HPA axis are one of the most important features in programming of adult disease, research using the protein restricted model backed up by pharmacological work, best serves the purpose. This would permit interference with the stress axis at a number of levels and thus enable isolation of the relevant nutritional deficit. Equally, surgical intervention which reduces fetal nutrition can be used in conjunction with, or instead of, dietary manipulation in order to replicate the metabolic syndrome in offspring. It is particularly useful in larger animals, such as the sheep, since the outcome of any manipulation can be directly monitored in a viable fetus.

Though animal models manifest most symptoms associated with the metabolic syndrome, frank disease is not easily reproducible, and this could be a criticism of this \textit{in vivo} research. However, a combination of dietary, pharmacological, genetic and surgical manipulations provide indicators of the disorders involved and as such are valuable tools in the elucidation of the role of programming \textit{in utero} as a cause of adult disease.

\textbf{Do the various models give a clue to finding a common path?}

In this review, we have shown that the effects of fetal undernutrition are manifest at various levels, as summarized in Figure 1.

\textit{Epigenetic}

Recent studies on imprinting give new insights into how changes in gene expression can determine the trajectory of fetal growth\textsuperscript{73} and environmental conditions can produce effects on the early embryo\textsuperscript{20}. The effect of diet on expression of some GC-sensitive proteins has been
established. For example, placental activity of $11\beta$-HSD2 is reduced by maternal protein restriction. This enzyme plays a crucial role in the development of the fetal adrenal and hence may determine patterns of glucocorticoid secretion throughout life, and may alter fetal blood pressure through increased exposure to maternal glucocorticoids. Hypertension induced by reduced maternal protein is abolished by inhibition of cortisol synthesis, leading to the hypothesis that maternal protein restriction programmes life-long changes in the fetal HPA axis and resets homeostatic mechanisms controlling blood pressure.
Limited protein intake during gestation leads to alterations in glucose output by the liver as well as in the sensitivity of tissues to insulin. Glucose transporters in muscles and the expression of key components of insulin signalling pathways in adipocytes are also altered. In addition, studies have suggested that maternal dietary restriction during gestation and lactation and transient dietary protein restriction after weaning may permanently alter growth hormone secretion in offspring. In the sheep, the pituitary response to a CRH challenge and the adrenal cortical response to an ACTH challenge are reduced. It is proposed that the suppression of the HPA axis function relates to prior exposure to elevated corticosteroids and it is already known that DEX administration suppresses ovine HPA axis function.

**Nutrient demand/nutrient supply**

Both the experimental conditions of maternal protein restriction and diabetes stress the impact of maternal nutritional limitation or poor health in producing effects on the offspring. An isocaloric low protein diet during pregnancy reduces the fetal:placental weight ratio, produces elevated systolic blood pressure in the offspring and produces changes in glutathione metabolism and glucose tolerance as well as insulin secretion. Several tissues are affected, including the vasculature, endocrine pancreas, insulin sensitive tissues, kidney and brain.

Some of the pathological changes can develop after a delay and there are intergenerational effects. The effects may cause degenerative diseases in adults and they can occur without major changes in birth weight, suggesting that birth weight on its own may be a poor proxy for intra-uterine events.

**Postnatal diet**

Epidemiological evidence from the Helsinki study and others, suggest that postnatal diet influences the incidence of adult disease originally programmed by poor nutrition in utero. In the rat, manipulation of dietary carbohydrate to protein ratio from weaning until adulthood following low protein in utero alters gene expression of hepatic fibrinogen genes.

**Conclusions**

We suggest that extensive animal studies are now needed to uncover the specific mechanisms by which altered fetomaternal nutrition and amino acid metabolism lead to degenerative diseases in the offspring. Only
when the perinatal origins of these human diseases are established, will it be possible to devise methods for their primary prevention.

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