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REVIEW

The nutritional basis of the fetal origins of adult disease

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It is now widely accepted that the risks of a number of chronic diseases in adulthood may have their origins before birth. Such diseases include non insulin-dependent diabetes mellitus, hypertension and coronary heart disease. Professor David Barker and colleagues in Southampton have produced a large proportion of the data in this field over the last decade,[1] although the relationship between early life events and adult disease had been raised many years earlier.[2] Most of this work has been based on epidemiological studies wherein cohorts of subjects whose birth records were available have been traced into adulthood. They have shown that measurements made on babies at birth, including birthweight, length, body proportions and placental weight, are strongly related to either later disease incidence (coronary heart disease mortality, non-insulin-dependent diabetes)[3,4] or risk factors for those diseases (hypertension, glucose intolerance, hyperlipidaemia).[1,5,6] Such relationships have been shown to hold in many different populations and are apparent from early childhood.[7,8]

The basis of these epidemiological observations is proposed to be that of programming. That is, an event operating at a critical or sensitive period results in a long-term change in the structure or function of the organism. Programming is a well-established biological phenomenon, and there are many common and well-known examples. Female rats given testosterone during the first 4 days of life develop a male pattern of gonatotropin secretion after puberty, and despite normal ovarian and pituitary function, fail to develop normal patterns of female sexual behaviour.[9] Administration of androgen at 10 days of age has no such effect. Similarly, transient immunization of neonatal rats against growth hormone releasing factor results in permanent impairment of pituitary growth hormone secretion and permanent impairment of growth rate.[10] Exposure of neonatal rats to short periods of stimulation, handling or various stressors results in permanent changes in hypothalamic structure and systemic responses to stress.[11,12] Since most examples of programming involve a critical period early in life, this phenomenon as the proposed basis of the epidemiological observations is both epidemiologically and biologically plausible. Thus a programming stimulus in fetal life is proposed to lead both to changes in size at birth and also to altered homeostatic mechanisms such as regulation of blood pressure or insulin sensitivity, which in turn result in susceptibility to disease in later life.[13]

The question which then arises concerns the nature of the programming stimulus. Undernutrition was proposed early as a likely programming stimulus, although others such as excessive fetal exposure to glucocorticoids have also been proposed.[14–16] This review will focus on nutrition as the hypothesized primary programming stimulus. It will examine the experimental basis for this hypothesis, a number of assumptions and misconceptions surrounding the hypothesis, and the need for caution in applying the results of animal experiments to the human situation.

Why nutrition?

The proposal that nutrition in fetal life is a central stimulus for programming of susceptibility to adult disease is now supported by three main sets of evidence. The first is that manipulation of nutrition during pregnancy in animals can be shown to produce many of the phenomena observed in the epidemiological studies. Publication of the early epidemiological studies led us and many others to attempt to verify experimentally the link between reduced birth size and later disease risk. Size at birth is readily manipulated in experimental animals by altering maternal nutrition in pregnancy. Experimental scientists thus began by using these approaches to investigate the consequences of size at birth for postnatal physiology, producing a rapidly burgeoning literature in the area over recent years.

For example, reducing the proportion of protein in the diet of pregnant rats results in offspring which have reduced size at birth and also elevated blood pressure[17] and glucose intolerance[18–20] in adult life. A variety of experimental approaches to reduce maternal nutrition in pregnancy have lead to similar observations in rats,[21] guinea pigs[22] and sheep.[23] Thus there can no longer be any doubt that changes in maternal nutrition in animals can both change size at birth and also permanently alter (programme) aspects of the physiology of the offspring in a way that is consistent with the disease susceptibility observed in human studies.

The second line of evidence is based on 'pseudo experiments' of maternal nutritional manipulation in human pregnancy, most notably that of the Dutch Hunger Winter. These studies have shown that women exposed during pregnancy to the nutritional limitation imposed by severe famine have offspring with reduced birth size[24] and an increased risk of glucose

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intolerance[25] and obesity in adult life.[26,27] Thus there is a small but growing set of data providing direct evidence in human populations that maternal nutrition in pregnancy can influence both size at birth and disease susceptibility in the offspring.

The third line of evidence supporting nutrition as a likely programming stimulus is essentially that of biological plausibility, based on current knowledge of the regulation of mammalian fetal growth. There is ample evidence from cross breeding and embryo transplant experiments that size at birth is largely determined by the maternal uterine environment, with relatively little influence of parental genotype.[28] Fetal growth in late gestation is normally limited by maternal size and her capacity to supply nutrients to her fetus, a phenomenon known as maternal constraint. Thus fetal growth in late gestation is normally regulated by fetal nutrient supply.[29] In addition, it is now known that the major hormonal mediators of fetal growth are insulin and the insulin-like growth factors (IGF).[30,31] These in turn are regulated by fetal nutrient supply.[32,33] Thus reduced glucose supply to the fetus results in reduced circulating insulin and IGF concentrations and in reduced fetal growth. Since nutrition has such a central role in the regulation of fetal growth, it is a good candidate for a programming stimulus, holding a central role in the link between size at birth and subsequent disease risk.

Distinguishing fetal from maternal nutrition

At first glance it is difficult to reconcile this central role of nutrition in the regulation of fetal growth with the traditional teaching that a woman's diet in pregnancy has little influence on the size of her baby at birth. Meta-analysis of the randomized controlled trials of maternal dietary supplements in pregnancy shows relatively small effects on birthweight (weighted mean difference +32 g).[34] Even in the situation of balanced protein/calorie supplements to undernourished women, the situation expected to show the greatest effect, the increment in birthweight is still very small (weighted mean difference +24 g).[34] Similarly, severe famine in previously well-nourished women, such as that seen in the Dutch Hunger Winter, caused only a relatively small reduction in birthweight.[24]

This apparent paradox is readily reconciled if careful distinction is drawn between maternal nutrition and fetal nutrition; a term used here to describe the net supply of metabolic substrates to the fetus. The mammalian fetus grows at the end of a long and sometimes precarious 'supply line', which links maternal diet at one end with fetal tissue uptake at the other.[35] It includes maternal nutrient uptake, maternal metabolism and endocrine milieu, uterine and umbilical blood flows and placental transfer and metabolism (Figure 1). Relatively large changes in maternal diet may have little impact on fetal nutrition if the capacity of the fetal supply line allows a large margin of safety for fetal growth. Conversely, common clinical causes of impaired fetal growth such as maternal hypertension associated with reduced uterine blood flow, or placental infarcts resulting in reduced placental transfer capacity, may severely limit fetal nutrient supply without a corresponding change in maternal nutrition. Much confusion and debate in the literature about the relevance of nutrition to human fetal growth has arisen from failure to make this distinction between maternal nutrition (relatively easy to measure but relatively less important) and fetal nutrition (very difficult to measure but very important).

Much of our knowledge of fetal nutrition comes from studies in sheep, which are widely used in studies of fetal physiology because of their large size, long gestation and relative ease of surgical manipulation. These characteristics allow chronic fetal catheterization. Vascular catheters and other monitoring devices such as electrodes and growth measuring devices can be surgically placed in the ewe and fetus, the fetus returned to the uterus and the ewe allowed to recover. Fetal blood can then be sampled and the fetus studied over many days or weeks in the relatively undisturbed conditions of intrauterine life *in vivo*. Although there are important interspecies differences which must be considered, there are also remarkable similarities in the physiology of fetuses of different species. The fetal 'diet' of the late gestation mammalian fetus is remarkably consistent in different species, comprising glucose, lactate and amino acids as the major fuels for oxidative metabolism. However, the relative proportions of these fuels varies with the species and with the time in gestation (Table 1). For example, the human fetus is very dependent on glucose as a major oxidative substrate, while the sheep fetus derives an increasing proportion of its carbon requirements from lactate as gestation proceeds.[36]

The placenta has multiple roles as an important component of the fetal supply line, and hence of the potential dissociation between maternal and fetal nutrition. Perhaps the most obvious placental influence on fetal nutrition is via its capacity to transport nutrients from the maternal to the fetal circulation. This transfer capacity is influenced by such factors as placental surface area and availability of specific nutrient transporters on the membranes. Recent evidence suggests that these may be influenced in turn by the maternal nutritional environment.[37,38]

The placenta will also influence fetal nutrition via its role in the metabolism of key nutrients. In sheep, for example, the placenta converts glucose to lactate which is then released into the fetal circulation where it provides an important fetal oxidative fuel.[36] This may also be true in the human placenta. Similarly, placental metabolism is important in fetal amino acid supply, with virtually all fetal glycine requirements synthesized in the placenta rather than taken up from the maternal circulation in sheep[39] and probably in human pregnancy.[40] Serine is also taken up by the placenta from the fetus, converted to glycine

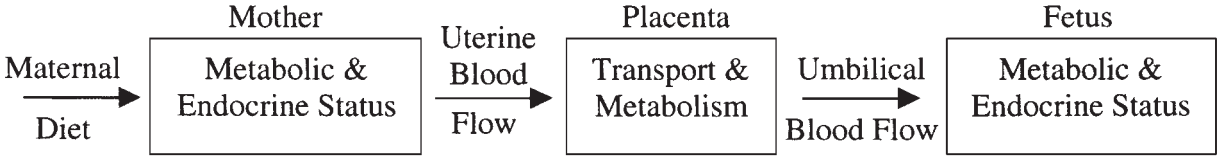

Figure 1 The fetal supply line

Factors along the fetal 'supply line' which can mediate the differences between maternal nutrition and fetal nutrition.

Table 1 The 'fetal diet'

	Cow	Sheep	Human
Glucose	50	50	75
Lactate	40	25	10
Amino acids	10	25	15

Estimated percentage of total oxidative metabolic requirements obtained from each substrate in different species in late gestation. The major substrates are the same for each species, although their proportions differ.[36]

Table 2 Factors influencing the effects of fetal undernutrition in different species

	Guinea pig	Sheep	Human
Birthweight (kg)	0.09	3.0	3.5
% Fat at birth	11.7	2.0	16.0
Growth rate (g/kg/d)	68	36	15
% Energy consumption directed to growth	76	37	43

Species with a high growth rate and a high energy requirement for fat deposition in late gestation direct a large proportion of their total energy consumption to growth.[36] This may make them more susceptible to growth restriction if substrate supply is limited.

and released back to the fetus.[41] Thus placental metabolism contributes to important qualitative as well a quantitative differences between fetal nutrition and maternal nutrition.

The placenta also influences fetal nutrition via its own metabolic demand for nutrients. In sheep the placenta consumes some 60% of the glucose and oxygen taken up from the uterine circulation in late gestation.[42] If uterine glucose supply falls, the placenta consumes an increasing amount of glucose from the fetal circulation to maintain its own metabolic demands.[43] Hence the placenta competes directly with the fetus for available nutrients. Similarly the fetus has been shown to export amino acids back to the placenta when supply is limited.[42] This may underlie clinical observations of fetal wasting.

Finally, the placenta will influence fetal nutrition because it produces hormones which in turn may influence fetal and maternal nutritional supply. Both placental lactogen and growth hormone are produced by the placenta in large amounts. They contribute to maternal insulin resistance, increasing the availability of glucose and other nutrients in the maternal circulation for transfer to the fetus.[44]

The principles governing fetal nutrition and the fetal supply line outlined above apply in all mammalian species studied to date. However, there are important species differences, particularly in maternal metabolism and placental structure and function, which will determine the effects on the fetus of a given nutritional insult to the mother. The most thoroughly studied animal, the sheep, is a ruminant. One result of this digestive arrangement is that circulating blood glucose is produced endogenously by the maternal liver via gluconeogenesis rather than being derived directly from the products of digestion in the gut. Reduced maternal dietary intake thus leads to reduced production of gluconeogenic substrates from the rumen, largely short chain fatty acids and amino acids. This results in a prompt large fall in maternal blood glucose concentrations. For example, the maternal undernutrition protocols used in our own experiments in sheep have resulted in a decrease of maternal blood glucose concentrations by approximately 40%, and this decrease has been maintained over several weeks.[45,46] Although fasting in pregnant women has been claimed to lead to 'accelerated starvation', maternal blood glucose is nevertheless much better maintained during undernutrition in women than in ruminants. For example, overnight fasting in healthy pregnant women caused only approximately a 10% fall in blood glucose concentrations,[47,48] and complete starvation for 3–4 days is required to reduce maternal blood glucose concentrations by 30%.[49,50] Since fetal glucose supply is directly related to uterine glucose supply from the mother, and glucose is the major fetal substrate, maternal undernutrition in sheep leads directly to fetal undernutrition. This is less likely in human pregnancy as maternal undernutrition leads to relatively smaller changes in circulating maternal blood glucose concentrations and hence fetal glucose supply.

The effect of a change in maternal nutrition will also vary between species according to the growth rate and body composition of the offspring. Thus a very small animal with a short gestation, such as the guinea pig, has fetuses with a relatively high growth rate in late gestation (Table 2). Such fetuses must allocate a large proportion of total nutrient supply for growth. Limitations on nutrient supply will therefore have a much bigger effect on growth rate than they would in a larger species with a smaller relative growth rate. Similarly, in species with a large proportion of body fat at birth such as the guinea pig or human infant, a given rate of tissue acquisition will require a higher energy input because of the high energy density of the fat. Once again, this will increase the proportion of available energy supply which must be directed towards tissue growth. Such species would therefore be more vulnerable than other species to restrictions in energy supply in terms of their effects on fetal growth.[36]

There are also important species differences in placental structure and function.[51–53] Species which are relatively fat at birth such as the guinea pig and human have placentas which are relatively permeable to fatty acids and related molecules in late gestation. Fatty acids may thus form a small but important component of the fetal 'diet' in these species at the end of pregnancy.[36] Maternal fasting in both sheep and women results in increased lipolysis and increased circulating ketone and fatty acid concentrations. In the sheep, ketones cross the placenta in only small amounts,[54] but are oxidized by the placenta[55] resulting in increased lactate production, apparently sparing what glucose is available for fetal use. However, the human placenta is permeable to ketones and fatty acids[47,56] and fetal tissues may directly oxidize ketones as a substitute for glucose, particularly in organs such as the brain.[57] Thus maternal fasting may lead to very different effects on fetal nutrition in the two species. In sheep maternal fasting reduces fetal glucose supply but lactate supply is relatively maintained, whereas fasting in pregnant women leads to relative maintenance of fetal glucose supply but increased availability of ketones and fatty acids. Species differences in maternal/fetal amino acid interactions are likely to be even more complex but have been little studied to date. However, these examples serve to illustrate how fetal nutrition may be critical in the regulation of fetal growth, while maternal nutrition may have very variable effects depending on such factors as species differences in metabolism and placental function.

Distinguishing birthweight from fetal growth

The initial epidemiological studies linked birthweight to subsequent disease risk. Later studies examined these risks in relationship to various body proportions at birth such as ponderal index (thinness), abdominal circumference, etc.[58,59] This appears to have occurred because in many cases these measures are more closely related to disease risk than is birthweight itself. Such apparent *post hoc* analysis is in practice an attempt to get closer to the origin of the association; that fetal nutrition as a programming stimulus affects fetal growth rather than birthweight.[60] This distinction between fetal growth and birthweight is difficult to make in human pregnancy, although repeated ultrasound measures of fetal size during pregnancy are beginning to assist here. However, the distinction can be readily demonstrated in animals. Fetal sheep growing rapidly in late gestation slow their growth promptly in response to 10 days of maternal undernutrition and resume growth on maternal (and hence fetal) refeeding. When examined after 10 days of refeeding, these fetuses have the same birthweight and length as control fetuses of well-fed ewes, but have increased heart and kidney size and increased blood pressure.[45,61] In this case fetal weight does not reflect the direct causal relationship between fetal nutrition, fetal growth and altered physiology. A similar situation can be imagined in human pregnancy where fetuses of similar birthweight may arrive at that point via very different growth trajectories (Figure 2). It seems likely that these trajectories would be associated with different patterns of physiological function and likely programming and thus disease risk, although this remains to be demonstrated.

If fetal growth is poorly reflected in birthweight, then it seems likely that body proportions would be more informative. Although this seems a reasonable hypothesis, there are few data to assist, and many common assumptions in this area are excessively simplistic.

One common assumption is that body proportions provide information about the timing of nutritional insults leading to the limitation of fetal growth. Thus a baby which is proportionately small in weight, length and head circumference at birth is presumed to have suffered from nutrient limitation in early pregnancy, while a baby of similarly low birthweight who is relatively long and thin is presumed to have suffered nutrient limitation in late pregnancy.[62–64] These two patterns are commonly referred to as symmetrical and asymmetrical growth restriction, respectively, and are described in the clinical literature as having different origins and different clinical problems. However, careful examination of large human data sets have failed to find any evidence of two distinct populations in this regard.[65] Instead, a continuum of changes in body proportions has been demonstrated. Furthermore, measurement of fetal growth by ultrasound showed no clear differences in timing or pattern of growth changes in babies found at birth to be either symmetrically or asymmetrically growth restricted.[66] Such findings are consistent with a continuum of nutritional limitation affecting fetal growth, but not with the assumptions about distinct timing. Indeed, studies of maternal undernutrition in sheep have shown that reduced ponderal index (thinness) is seen in fetuses exposed to undernutrition from early or mid gestation through to term, but not in fetuses exposed only in late gestation. Contrary to expectation, exposure only in early or mid gestation results in increased ponderal index.[67]

Another common assumption is that nutrient limitation to the fetus at a given stage of development is likely to have maximum effect on organs growing rapidly at that stage. However, simple limitation of substrates to growing organs leading to reduced size of that organ does not explain the complex effects observed. Maternal protein restriction in pigs results in reduced fetal weight and length at mid-gestation at a time when the fetus is extremely small and fetal protein requirements for growth are most unlikely to have been limiting by this time.[68] Similarly, maternal undernutrition in either early or late gestation in sheep, leading to fetal undernutrition and limiting nutrient supply to growing organs, does not explain the observed increase rather than decrease in size of the heart and kidneys (Table 3).[69]

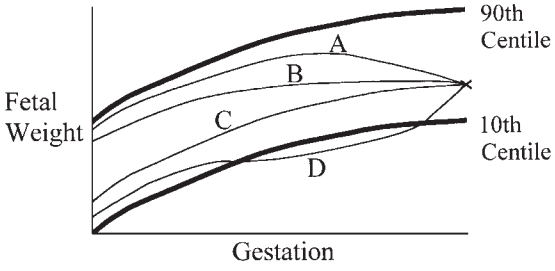

Figure 2 Possible fetal growth trajectories

A fetus can reach a given birthweight via a variety of possible different growth trajectories. These fetuses may be of different body composition, and may have differing disease risk in adulthood.

A Late growth restriction and fetal wasting.

B Early growth restriction.

C Normal growth.

D Late growth restriction followed by catch-up growth.

Table 3 Effect of maternal undernutrition on fetal body proportions in sheep

	Control	Undernourished
Periconceptual undernutrition[a]		
Weight (kg)	4.3	4.0
Length (cm)	50.4	50.1
Heart (% BW[b])	0.64	0.74**
Liver (% BW)	2.55	3.28**
Kidneys (% BW)	0.57	0.65
Late gestation undernutrition[c]		
Weight (kg)	3.1	3.2
Length (cm)	42.5	41.8
Heart (% BW)	0.75	1.10***
Liver (% BW)	3.71	5.10**
Kidneys (% BW)	0.72	1.32**

[a] Maternal undernutrition from 61 days before until 30 days after mating. Fetuses examined at 131 days gestation (term = 145 days).[69]

[b] Body weight.

[c] Maternal undernutrition from 105 to 115 days gestation. Fetuses examined at 125 days.[45]

** $P < 0.01$, *** $P < 0.0001$ for comparison between control and undernourished groups.

Further assumptions have been made in the literature regarding the significance of altered body proportions at birth. Reduced abdominal circumference has been assumed to reflect reduced liver size[63] and this has been used as a possible explanation of the relationship observed between abdominal circumference at birth and lipid metabolism in adulthood. This apparently reasonable assumption has recently been questioned by findings that ultrasound measurements of growth restricted human fetuses show little relationship between liver size and abdominal circumference.[70] We have found in fetal sheep, using direct measures of organ weight, that abdominal circumference is strongly related to weight of the fetal gut as well as that of the liver. There is a need for more extensive pathological studies to determine the true relationship between birth measurements and organ size in human infants.

In a similar vein, relative preservation of head circumference at birth ('head sparing') is commonly assumed to occur as a consequence of blood flow redistribution in fetal life. There is certainly good evidence of redistribution of cardiac output in fetuses exposed to hypoxia, with maintenance of blood flow to essential organs such as the brain and heart at the expense of other organs such as the gut and skin.[71] There is also ultrasound evidence that such redistribution does occur in chronically hypoxaemic intrauterine growth restricted (IUGR) human fetuses, and can be partly reversed by administration of oxygen.[72,73] However, this is not the only mechanism by which brain growth may be maintained during periods of fetal substrate limitation, and indeed may not be the most common. Hypoxaemia appears to occur late in the process of growth restriction in human fetuses, and many IUGR fetuses are not hypoxaemic on direct measurement *in utero* although head sparing can be demonstrated in these fetuses.[74] Indeed, if IUGR is induced by feed restriction in sheep, fetal growth is impaired and relative head sparing is observed with no evidence of hypoxia.[46]

Other nutritional mechanisms may allow relative preservation of brain growth in the substrate limited fetus. Glucose uptake into many tissues is mediated by insulin, and fetal insulin secretion is regulated by glucose and amino acid supply. However glucose uptake into the brain does not require insulin. Thus limitation of glucose and amino acid supply to the fetus will reduce circulating insulin concentrations and glucose uptake into peripheral tissues such as muscle, sparing the available glucose for uptake into the brain which is insulin independent.

In addition, as described above, fasting in women increases the supply of ketones to the fetus[47] and the fetal brain has been shown to preferentially take up and oxidize ketones.[57,75] Similarly, maintenance of fetal lactate supply by the placenta of the undernourished pregnant sheep may allow continued growth of the fetal heart which will preferentially utilize lactate as an oxidative fuel.[76] Thus altered body proportions at birth, and particularly relative preservation of brain and heart size, may reflect altered distribution of cardiac output *in utero*. However, it is likely to reflect also complex metabolic adaptations to limitations in fetal nutrient supply including an altered hormone environment and altered substrate availability.

Timing and balance of nutrients

Much of the discussion so far has addressed nutrition in the general terms of overall macro-nutrient supply. However, it is increasingly apparent that the balance of macro- and micronutrients reaching the fetus and the timing of any changes in their supply is likely to be important in determining the effects on fetal growth and later physiology. Most information on the balance of nutrients has so far come from human studies. Although the randomized controlled trials of maternal dietary supplements show relatively little effect on birthweight overall, supplements with a relatively high proportion of calories provided as protein actually resulted in reduced mean birthweight.[77,78] The mechanisms of this effect are not known. However, amino acids are transported across the placenta to the fetus by a number of amino acid transporters. Increasing availability of some amino acids may therefore result in competition for transporters and reduce the availability of other amino acids to the fetus, potentially limiting growth.[79] Similarly, in the extreme example of maternal phenylketonuria high maternal circulating concentrations of phenylalanine may be toxic to the developing fetus both directly, and also by limiting fetal supply of tyrosine and tryptophan due to competition for placental amino acid transporters.[80] Godfrey *et al.* have shown in a relatively well-nourished population that a combination of high carbohydrate in early pregnancy and low protein intake in late pregnancy was associated with reduced birthweight, low ponderal index and reduced placental weight.[81,82] The proportions of protein and carbohydrate in women's diets during pregnancy have also been shown to influence placental size and the blood pressure of their adult offspring.[83]

So far there is little experimental work exploring the effects of different specific nutrients and the balance of these on fetal growth and later effects, with the exception of reduced maternal protein intake which has been widely used. Individual amino acids may also have critical roles. Rats fed an isocaloric low protein diet have offspring whose pancreatic islet cells have impaired insulin release. However, supplementation of the maternal low protein diet with taurine alone restored the insulin secretion of the fetal islets.[84] It seems likely that other specific amino acids are important in particular phases of organ development in different tissues, but this has not yet been explored in detail. Glycine, for example, appears to be a conditionally essential amino acid for a number of important metabolic compounds including nucleic acids, collagen, haeme and keratin.[85] Marginal glycine insufficiency appears to be common even in pregnant women with otherwise adequate diets, so that in some pregnancies the ability of the mother to provide glycine for herself and the developing fetus is marginal or inadequate.[86] This marginal insufficiency can be exacerbated if the dietary methionine content is increased, apparently because glycine is consumed in detoxification of the excess of methionine.[87] From these examples it is possible to see that the balance of protein and carbohydrate in the diet, and the balance of different amino acids, can have critical effects on fetal growth and thus may be important nutritional programming influences which have yet to be explored in detail.

Fluctuations in nutrient supply to the fetus may also have important effects on fetal growth and the programming of later disease risk. Maternal diabetes in pregnancy is a relatively common cause of altered fetal nutrient supply, with increased glucose availability resulting in increased fetal growth and impaired pancreatic function which persists over more than one generation (see below). However, it is not the total supply

of glucose to the fetus but the pattern of that supply that appears to determine the extent of this effect. Fetal sheep exposed to constant maternal hyperglycaemia in late gestation downregulated their insulin secretion. However, if the equivalent amount of glucose was given to the mother as intermittent boluses, fetal hyperinsulinaemia was demonstrated.[88] An association has also been reported between the incidence of ketosis during pregnancy in diabetic women and the outcome of her offspring.[89,90] This may reflect a similar mechanism, with intermittent exposure of the fetus to altered nutrient balance such as decreased glucose and increased ketone supply leading to permanent changes in the growth of the brain and potentially of other organs.

The role of a variety of micronutrients in the regulation of fetal growth and their potential as programming stimuli has yet to be explored in detail. However, there is already some evidence of their potential importance. The discovery of a variety of polymorphisms for homocysteine metabolism has revealed an association between increased homocysteine levels and not only coronary heart disease but also a variety of complications in pregnancy, including intrauterine growth restriction and pre-eclampsia.[91,92] Folate supplementation in pregnancy is commonly recommended because it reduces the incidence of birth defects. It appears that at least part of this effect is via effects on homocysteine levels.[92,93] One role of folate is its involvement in donation of methyl groups in the placenta when serine is converted to glycine with the generation of single carbon methyl groups.[41] These methyl groups in turn can be used for the generation of nucleotides and phospholipids, essential for cell division and cell membranes. Thus folate availability may be critical in fetal and placental growth as well as adult disease risk.

Multigenerational effects

Much debate has surrounded the role of genetic influences in the relationship between size at birth and later disease risk. The associations between maternal hypertension, reduced birth size and hypertension in the next generation, for example, could be at least partly of genetic origin. While the details of this debate are outside the scope of this review, it is appropriate here to recall that nutritional influences, too, may have effects on more than one generation. This makes the distinction between genetic and intrauterine environmental influences even more problematic.

It is now 20 years since Aerts and Van Assche published their experiments under the provocative title 'Is gestational diabetes an acquired condition?'[94] In it they described the experimental induction of glucose intolerance in pregnant rats. The offspring had impaired pancreatic function, made inadequate adaptation to the demands of pregnancy in adult life, were glucose intolerant in pregnancy and gave birth themselves to offspring with impaired pancreatic function. Thus altered nutrient availability in the first generation fetus resulted in a similar alteration in the fetal environment for the next two generations. Glucose tolerance was inherited, but not by genetic mechanisms.

A similar salutary lesson in the heritability of programming events was provided by studies of nutritional rehabilitation in a rat colony marginally protein malnourished for 12 generations.[95] Refeeding during pregnancy resulted in offspring with obesity in adulthood. Three generations of refeeding were required before adult size or behaviour was equivalent to that of the control colony.

The molecular basis of heritability of traits by non-genetic mechanisms is beginning to be revealed. Such epigenetic phenomena may involve altered packaging and activation of a variety of genes. Imprinting is one such example, where gene activation will be influenced by the parent of origin. Another example has recently been reported in mice.[96] Genetically identical mice may have different coat colour depending on the coat colour of the mother. The mechanism appears to be that of differing methylation and hence activation of the relevant controlling genes regulating expression of coat colour. Most importantly from the perspective of nutritional programming, the degree of methylation, and hence the gene expression and coat colour, can be altered by feeding the mother a diet high in methyl donors during pregnancy.[97]

Ultimately all programming phenomena must have their basis in altered expression of genes. Further understanding of how fetal nutrition may alter gene expression by this and presumably other mechanisms will be helpful in clarifying the nutritional basis of the link between size at birth and adult disease risk.

Summary

Epidemiological observations linking size at birth with the risk of adult disease have now been extensively replicated and are widely accepted. It is hypothesized that such observations are the result of programming events in fetal life leading both to altered birth size and to permanent changes in structure and function which predispose to disease in adult life. Programming is a well-established biological phenomenon and there is good experimental evidence that nutrition can be an important and probably central programming stimulus. However, clear distinctions need to be drawn between maternal nutrition and size at birth on the one hand, and between fetal nutrition and fetal growth on the other. Maternal nutrition may bear little or no relationship to size at birth, but fetal nutrition is critically important in fetal growth. Many common assumptions about the relationship between body proportions and prenatal physiological events lack a sound experimental basis. Furthermore, important species differences in physiology, metabolism, placental structure and function necessitate cautious interpretation of animal experiments in their application to human situations. Details of these nutritional influences are likely to be very species dependent. Despite these caveats, it is clear that altered fetal nutrition can influence both fetal growth and later disease risk. There is indeed a nutritional basis for the fetal origins of adult disease.

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