Metabolic programming in animals

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A large number of epidemiological studies have revealed that there is a relationship between early growth restriction and the subsequent development of type 2 diabetes or the metabolic syndrome. The mechanistic basis of this relationship and the relative roles played by genes and the environment remains the subject of much current debate. Animal models of early growth restriction have been developed in an attempt to understand its relationship with adult disease and to provide insight into the underlying molecular mechanisms. These models show many features of the metabolic syndrome. In the maternal protein restriction model, insulin resistance and hypertension is observed. The uterine artery ligation model shows obesity in adulthood. This provides strong evidence that alterations in the fetal environment can lead to diabetes in adult life.

The term programming has been used to describe the process whereby a stimulus or insult when applied at a critical or sensitive period of development results in a long-term or permanent effect on the structure or function of the organism[1]. The long-term effects of an insult during a critical period of development has been recognised for many years. However, over the last decade, immense interest in programming has been prompted by the results of a large number of epidemiological studies which have shown that there is a relationship between early growth restriction and the subsequent development of adult degenerative diseases such as type 2 diabetes, ischaemic heart disease and hypertension[2]. Little is known about the mechanistic basis of this relationship or the relative role of genetic and environmental factors. Extensive genomic scans have been unsuccessful in identifying universal diabetes susceptibility genes/polymorphisms. However, recent studies have identified rare mutations in the glucokinase gene which are associated with a reduced birth weight and the development of maturity-onset diabetes of the young (*see* Frayling & Hattersley, this issue)[3]. The importance of environmental factors has been demonstrated by a number of human studies. A study of twins in Denmark revealed that, in monozygotic twin pairs who were discordant for diabetes, the diabetic twin had a significantly lower birth weight than the normoglycaemic twin[4]. In addition, studies of individuals exposed *in utero* to famine during the Dutch hunger winter have revealed that poor maternal

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nutrition, especially during the last trimester of pregnancy, leads to growth restriction of the fetus and is associated with poor glucose tolerance and insulin resistance[5].

In an attempt to understand the molecular basis of the relationship between early growth restriction and development of subsequent disease, animal models have been developed. There are a number of insults during pregnancy that have been shown to result in growth restriction in various species. These include both nutritional and hormonal insults as well as surgical interventions.

Nutritional models of early growth restriction

Maternal low protein

The maternal low protein model is one of the most extensively studied models of early growth restriction[6]. There are a striking number of parallels between findings from this model and those from studies of individuals with type 2 diabetes and/or the metabolic syndrome (Table 1). This low protein rat model has been used for a number of years and involves feeding rats a low (5–8%) protein diet during pregnancy which results in growth restriction of the offspring[7]. If such offspring are cross-fostered to mothers being fed a control (20%) protein diet during lactation, they rapidly gain weight such that by weaning (21 days of age) these recuperated offspring have similar body weights to controls. This catch-up growth appears to have a detrimental effect on longevity, resulting in premature death which is associated with accelerated loss of kidney telomeric DNA[8]. The detrimental effect of catch-up growth has also been reported in human populations. In Sweden, it has been shown that men who were born small but who grew to above average height have raised blood pressure[9]. More recently it has been shown that catch up growth in a Finnish cohort is associated with increased death from cardiovascular disease[10].

Table 1 Similarities between the low protein rat model and human metabolic syndrome

Physical features	Low birth weight Short stature
Whole body characteristics	Diabetes Insulin resistance Hypertension
Tissue characteristics	Altered regulation of hepatic glucose output Depot-selective adipocyte insulin resistance

Permanent growth restriction results if maternal protein restriction is continued during lactation even when the offspring themselves are weaned onto a control diet[7]. In young adult life, these offspring have a significantly better glucose tolerance than controls[11]. However, early growth restricted offspring undergo a greater age-dependent loss of glucose tolerance such that by 15 months of age glucose tolerance is significantly worse than that of controls[12]. This is associated with insulin resistance. In addition to an age-dependent loss of glucose tolerance, maternal protein restriction has been shown to be associated with hypertension[13]. It has been suggested that this hypertension may be related to changes in both kidney structure and the activity of the renin-angiotensin system[13]. Obesity and maternal protein restriction when combined have an independent and additive effect on blood pressure[14].

The long-term effects of maternal protein restriction on the structure and function of individual organs have also been investigated. Most of the early studies focused on the effect of early protein restriction on the development of the endocrine pancreas (see Bertram & Hanson, this issue). β-Cell proliferation and islet size was shown to be significantly reduced in the head of the pancreas of neonates from dams fed a low protein diet during pregnancy[15]. Apoptosis of β-cells was also shown to be increased in 14-day-old low protein neonates[16] and islet vascularization[15] was significantly reduced in the head and tail of the pancreas of these offspring. The studies were extended to determine if these structural changes were associated with any changes in insulin secretion. No differences in basal secretion were observed. However, islets from 21.5-day-old fetuses of low protein mothers had a reduced secretory response to both leucine and arginine *in vitro*[17]. Subsequently, it was shown that a defect in glucose-stimulated insulin secretion from islets of adult low protein offspring is only observed when an additional dietary insult such as high fat or sucrose feeding is introduced postnatally[18]. This supports the hypothesis that it is an imbalance between the environment in early and adult life that may lead to diseases such as type 2 diabetes and thus make nutritional intervention programmes a realistic possibility. Indeed, recent studies have demonstrated that supplementing the mothers' diet with taurine prevents the impaired insulin secretion normally observed in fetuses of low protein-fed dams[19]. The precise time window available for such an effect of taurine requires further investigation.

Maternal protein restriction has also been shown to have long-term effects on insulin sensitive tissues. In the liver, this includes both structural and functional changes. It has been observed that low protein offspring have larger hepatic lobules compared to controls[20]. *Ex vivo* liver perfusions of 3-month-old male animals have shown that low protein offspring are relatively resistant to the ability of glucagon to stimulate hepatic glucose output compared to controls[21]. This glucagon

resistance is related to a reduction in expression of glucagon receptors[21]. These studies also revealed that livers of low protein offspring exhibited an anomalous response to insulin, with the hormone initially stimulating hepatic glucose output[21]. A similar paradoxical response to insulin has been reported in subjects with type 2 diabetes[22] and in young Aborigines[23] (a population where a large number of individuals develop diabetes).

In young adult life, skeletal muscle from low protein offspring is more sensitive to insulin in terms of its ability to stimulate glucose uptake[24]. This increased sensitivity is related to increased expression of insulin receptors and presumably, at least in part, contributes to their better glucose tolerance at this age compared to controls.

Detailed analysis of adipocytes from low protein and control offspring have identified potential markers of early growth restriction[25]. Adipocytes isolated from young adult, low protein offspring have an elevated basal and insulin-stimulated glucose uptake and increased levels of insulin receptor[25]. Over the last decade, our understanding of the way in which insulin signals to metabolic actions has increased enormously[26]. Following insulin binding, the insulin receptor becomes autophosphorylated on tyrosine residues and subsequently phosphorylates a number of insulin receptor substrates including insulin receptor substrate (IRS)-1. A number of downstream signalling elements are able to bind to phosphorylated insulin receptor substrates and become activated. One such enzyme is phosphatidylinositide (PI)-3-kinase which has been shown by inhibitor studies to be necessary for both the action of insulin to stimulate glucose uptake and to inhibit lipolysis[27]. Consistent with the observed changes in glucose uptake, adipocytes from 3-month-old low protein offspring have an elevated basal and insulin-stimulated IRS-1 associated PI-3-kinase activity. However, despite having elevated levels of PI-3-kinase activity, these adipocytes are resistant to the antilipolytic action of insulin[28]. This observation was at first surprising, but a more detailed analysis of PI-3-kinase has suggested a potential mechanistic basis of these findings. PI-3-kinase is a heterodimeric enzyme which consists of a regulatory subunit (p85) and a catalytic subunit (p110)[27]. Two isoforms (p110α and p110β) of the catalytic subunit are present in adipocytes[27]. Early protein restriction leads to a dramatic reduction in expression of p110β while expression of p110α remains unchanged[25]. Little is known about the functional differences between these two isoforms. However, the existence of differentially regulated isoforms with divergent signalling roles would allow the cell to adjust its metabolic status in response to its environment. Measurement of the relative expression levels of these two isoforms of p110 could provide important information on the success of a fetus at achieving its growth potential. However, data on the expression of these proteins in human growth restriction are not currently available. This information may prove to be difficult to obtain, as studies in the low

protein offspring[28] and in humans[29] with metabolic syndrome have both suggested that resistance to the antilipolytic action of insulin is depot specific with intra-abdominal fat being resistant and subcutaneous fat (the most available depot for biopsy) remaining relatively insulin sensitive.

Maternal calorie restriction

The effects of various regimens of total food restriction have been studied by a number of investigators. Early studies focused on the long-term effects of short-term food restriction in early postnatal life[30]. It was shown that feeding rats a calorie restricted diet between 3–6 weeks of age caused an impairment of insulin secretory response that was still evident at 12 weeks of age[30]. More recently, the focus of studies has been on the long-term effects of maternal calorie restriction. Severe food restriction (to only 30% of *ad libitum* intake) during pregnancy has been shown to induce severe growth restriction in the fetus[31]. In adulthood, these offspring have slightly elevated systolic blood pressures[31] and increased fasting plasma insulin concentrations[32] compared to control offspring. These offspring have also been shown to have increased food intakes compared to control offspring. This hyperphagia was shown to increase with age and could be amplified by hypercaloric nutrition. This finding is consistent with findings in humans which suggest that early growth restriction is associated with adult central obesity[33]. Less severe food restriction (to 50% of *ad libitum* intake) from day 15 of pregnancy to weaning has been shown to result in insulinopaenia and an age-dependent loss of glucose tolerance which is apparent in 12-month-old male offspring[34].

Maternal iron restriction

Iron deficiency is a common nutritional problem in humans and is especially prevalent in pregnant women. It has been shown that feeding rats an iron-deficient diet during pregnancy leads to anaemia and growth restriction of the fetus[35]. The long-term effects of such maternal iron-deficient anaemia are not well documented. A number of studies have shown that the offspring have decreased iron concentrations in brain tissue which can not be normalised by iron treatment after weaning[36]. In addition, behavioural differences have been noted in these offspring[36]. In early postnatal life (day 20), heart weights of the offspring of anaemic dams have been shown to be increased suggesting an alteration in their cardiovascular development[37]. This, however, is paradoxically associated with decreased systolic blood pressure

compared to control pups at this age[37]. Chronic fetal anaemia in the sheep has been shown to be associated with similar cardiac hypertrophy and a lowering of mean arterial pressure in ovine fetuses around day 133 of gestation[38]. This is suggested to be related to a decrease in total peripheral resistance. In the rat model, changes in blood pressure have been reported to be age-dependent. Despite having lower systolic blood pressure on day 20 of postnatal life, by day 40 the pressures of offspring of iron-deficient dams were reported to be significantly elevated compared to controls[37]. The mechanistic basis of this elevation of blood pressure is not known.

Hormonal insults

Glucocorticoid exposure

It has been known for over 20 years that glucocorticoid treatment during pregnancy in both humans and animals causes a reduction in birth weight[39]. However, it is only in the last decade, in light of the epidemiological data linking low birth weight to adult disease, that the long-term consequences of prenatal glucocorticoid exposure have been investigated[40]. Offspring that have been exposed to excess prenatal glucocorticoids undergo catch-up growth postnatally and it has been shown that body weights have normalised by weaning (3 weeks of age) in the rat. Findings in such adult offspring are consistent with the hypothesis that rapid postnatal catch-up growth is deleterious to adult health. Fetal glucocorticoid overexposure in rats has been shown to be associated with elevated blood pressure[41] and raised blood glucose levels[42] in adulthood. This phenotypic outcome is similar to that of the low protein model and it has been suggested that fetal glucocorticoid overexposure may be a common mechanism linking maternal environmental factors with fetal growth and programming[40]. This suggestion in based on the observation that dietary protein restriction during rat pregnancy reduces 11β-hydroxysteroid dehydrogenase 2 activity[40]. This enzyme forms a placental 'barrier' which catalyses the rapid metabolism of active physiological glucocorticoids to inert 11-keto forms, thus minimising fetal exposure to glucocorticoids[40].

Surgical intervention

Uterine artery ligation

Impaired utero–placental perfusion with an associated reduction in placental transport of nutrients is thought to be responsible for a large number of cases of intra-uterine growth restriction in humans[43]. Reduction

in placental blood flow and transport and a consequent restriction of fetal growth can be produced in the rat by uterine artery ligation in late gestation[44]. Most studies on this model to date have focused on the offspring during fetal and early postnatal life. It has been shown that at birth and at 2 weeks of age, growth-retarded offspring have reduced nephron number[45]. This nephron deficit was associated with impaired renal function at 2 weeks of age despite an apparent large compensatory hypertrophy of nephrons in these animals[45]. A more molecular analysis of skeletal muscle from fetuses and 21-day-old offspring following uterine artery ligation has revealed that this mode of growth restriction is associated with changes in both mitochondrial gene expression and function. In fetal life, mRNA levels of the mitochondrial proteins NADH-ubiquinone-oxidoreductase subunit 4L, subunit C of the F_1F_0 ATP synthase and adenine nucleotide translocator 1 were all reduced[46]. In contrast, by day 21 postnatally, mRNA levels of all three proteins were reduced compared to controls[46]. This was associated with a reduced skeletal muscle mitochondrial NAD^+/NADH ratio, indicative of an alteration in mitochondrial function[46].

One study of adult offspring (3–4 months of age) has suggested that this method of early growth restriction is not associated with hypertension[47]. This contrasts with the data obtained from offspring which were growth restricted by maternal protein restriction, maternal calorie restriction, maternal iron restriction or maternal dexamethasone treatment. This suggests that intra-uterine growth restriction *per se* is not sufficient to cause elevated blood pressure in adulthood. Subtle differences such as the timing of the insult during pregnancy and the composition of the adult diet may also be important. In terms of glucose tolerance, effects of such placental insufficiency appear to be sex specific[47]. Young adult male offspring were shown to have similar fasting blood glucose and plasma insulin levels compared to controls. In addition, glucose tolerance was not related to birth weight[47]. In contrast, in female offspring, growth restriction was associated with increased fasting blood glucose levels. Fasting plasma insulin levels were unaltered by growth restriction suggesting a degree of insulin resistance in growth restricted animals[47]. Early growth restriction in females was also associated with impaired glucose tolerance and lower insulin secretion during a glucose tolerance test. This suggests that, in female rats, intra-uterine growth restriction caused by uterine artery ligation is associated with an impaired regulation of insulin secretion by glucose.

Future prospects

One of the major problems in applying the data obtained from the epidemiological studies to clinical practice is in identifying individuals

who have been growth restricted *in utero*. Birth weight is only a crude index of early growth (*see* Barker, this issue) and reveals nothing about the success of a fetus at achieving its growth potential. In addition, animal and human studies have shown that certain insults during pregnancy can have long-term effects on the metabolism of the offspring in the absence of an effect on birth weight. A key area of future research will thus be to identify markers of early growth restriction which may be of future diagnostic use as early predictors of adult disease. It is not clear if these markers will be specific to individual causes of growth restriction or if these markers will be shared between numerous forms of growth restriction.

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