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Fetal programming of postnatal phenotype and obesity - experimental evidence from biomedical research

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ABSTRACT

The evidence for the fetal origins of adult disease (diabetes, cardiovascular) is reviewed. There is evidence of a disturbed endocrine communication between the hypothalamus, adipose tissue and the endocrine pancreas in the pathogenesis of programming-induced obesity. Hypercaloric nutrition during postnatal life greatly amplifies prenatal effects on metabolic abnormalities, obesity, overeating and diminished exercise behaviour. Fetal programming research offers a novel approach to investigate the mechanistic basis of metabolic disorders.

Keywords: fetal programming, appetite, obesity, animal models.

INTRODUCTION

Obesity and its related metabolic disorders have become a major health issue for modern society in the 21st century. It is a widely held view that dietary and lifestyle factors are the primary cause of these diseases in the general population. The mechanisms by which diet and other environmental factors influence physiological systems that control appetite, weight regulation, behaviour and ultimately the aetiology of metabolic disease are poorly understood.

Increasing evidence suggests that chronic metabolic and cardiovascular diseases which commonly manifest in adult life have their roots before birth. The ‘fetal origins of adult disease’ (FOAD) or ‘fetal programming’ paradigm is based on the observation that adverse stimuli during the prenatal period can alter the developmental path resulting in an increased susceptibility to obesity and cardiovascular and metabolic disorders later in life. This pathogenesis is not based on genetic defects but on altered genetic expression, which occurs as a result of fetal adaptations to adverse intrauterine influences. After initial controversy when these relationships were first suggested, both prospective clinical and experimental studies have clearly shown that the propensity to develop abnormalities of cardiovascular, endocrine and metabolic homeostasis in adulthood are increased when fetal development has been adversely affected (Godfrey & Barker 2000; Barker 1998).

The mechanisms underlying the relationship between prenatal influences and postnatal outcome are relatively unknown and remain speculative, as are the interactions between genetic and environmental factors. One general thesis is that in response to an adverse intrauterine stimulus, the fetus adapts its physiological development to maximise its immediate chances for survival. These adaptations may include resetting of metabolic homeostasis and endocrine systems and the down-regulation of growth, commonly reflected in altered birth phenotype. This prenatal plasticity of the fetus may allow environmental factors to alter the physiological function of the conceptus in preparation for sub-optimal environmental conditions after birth (Gluckman 2001). It is thought that whilst these changes in fetal physiology may be beneficial for short term survival in utero they may be maladaptive in postnatal life, contributing to poor health outcomes when offspring are exposed to catch up growth, diet-induced obesity and other factors.

EXPERIMENTAL EVIDENCE FOR FETAL PROGRAMMING OF OBESITY

Animal models have been used extensively to investigate the basic physiological principles of the FOAD hypothesis. The variety of models that have been developed are essential to the search for the mechanistic links between prenatal influences and postnatal pathophysiological outcomes. An example is the diabetic pregnant rat, in which the long-term effects on offspring following diabetic pregnancy can be investigated, a study which would not be possible in humans as treatment is ethically proscribed. Although epidemiological data suggest that fetal programming occurs within the normal range of birth size (Barker 1998), most experimental work has tended to focus on significant restriction of fetal growth in the assumption that the nature of the insults that impair fetal growth are likely to be those that trigger fetal programming. Alterations in maternal nutrition are commonly used experimentally to induce intrauterine growth retardation (IUGR), as it is an experimentally practical and reproducible way to induce nutrient limitation to the fetus and thus change its developmental trajectory. In this context IUGR is not essential to fetal
programming, but is merely a surrogate for evidence that fetal development has been affected.

Several animal models of early growth restriction have been developed in an attempt to elucidate its relationship with adult onset of disease and provide a framework for investigating the underlying mechanisms. Animal studies have clearly shown that prenatal undernutrition programs not only postnatal cardiovascular dysfunction but also obesity, elevated plasma leptin concentrations, glucose intolerance, and even activity levels and dietary preferences. In rats hypertension, insulin resistance and obesity have been induced in offspring by maternal undernutrition (Woodall et al. 1996a; Woodall et al. 1996b; Vickers et al. 2000), a low protein diet (Langley-Evans et al. 1996), maternal uterine artery ligation (Rajakumar et al. 1998), maternal dexamethasone (DEX) treatment (Nyirenda et al. 1998) or prenatal exposure to the cytokines interleukin (IL)-6 and tumour necrosis factor (TNF)-alpha (Dahlgren et al. 2001).

There are also increasing experimental data in other species. In guinea pigs IUGR caused by uterine artery ligation or maternal undernutrition results in reduced glucose tolerance, increased sensitivity to cholesterol loading (Kind et al. 1999) and elevated blood pressure in offspring (Persson & Jansson 1992). DEX treatment of pregnant ewes in early gestation results in elevated blood pressure (Dodic et al. 2000) and altered regulation of lipolysis (Gatford et al. 2000) in the adult offspring. Placental restriction by carunclectomy has also been associated with adiposity and increased insulin sensitivity during postnatal life (Owens 2003). Work by Bispham et al has shown that, independent of maternal nutrition in late gestation, fetuses sampled from ewes with nutrient restriction in early gestation possessed more adipose tissue, whereas when ewes were fed to appetite throughout gestation, fetal adipose tissue deposition and leptin mRNA abundance were both reduced. These changes suggested that offspring of nutrient restricted mothers were at increased risk of developing obesity in later life (Bispham et al. 2003). This study also suggested that the increased incidence of obesity in adults born to mothers exposed to the Dutch famine during early pregnancy (Roseboom et al. 2000) may be a direct consequence of adaptations in the endocrine sensitivity of fetal adipose tissue.

NUTRITIONAL PROGRAMMING

Fetal undernutrition has been highlighted as a primary factor involved in the early life origins of adult disease. Within the laboratory, fetal undernutrition can most commonly be achieved through maternal dietary restriction during pregnancy. Manipulation of maternal nutrition during pregnancy has been known to alter fetal growth and development for some time (Dobbing 1981). At present, rodent models investigating the mechanistic links between maternal undernutrition and adult disease generally utilise one of two dietary protocols; global undernutrition or isocaloric low protein diets. The maternal low protein (MLP) diet during pregnancy and lactation is one of the most extensively utilised models of nutritional programming (Snoeck et al. 1990; Langley & Jackson 1994; Desai et al. 1996; Hales et al. 1996; Petry et al. 2001). This model involves ad libitum feeding to pregnant rats a low protein diet containing 5-8% (w/w) protein (casein), generally a little under half the protein content but equivalent in energy of a control diet containing 18-20% (w/w) protein (Snoeck et al. 1990; Langley-Evans 2000). Offspring from protein restricted mothers are around 15-20% lighter at birth (Desai et al. 1996). Maintenance of a MLP diet during lactation enhances this weight difference and permanently restricts later growth. If restricted offspring are cross-fostered to mothers fed a control diet, offspring exhibit rapid catch-up growth (Desai et al. 1996). This catch-up growth appears to have a detrimental effect on life span, resulting in premature death which is associated with accelerated loss of kidney telomeric DNA (Jennings et al. 1999).

Restricted protein offspring exhibit significantly elevated blood pressure at an early age in comparison to controls (Langley & Jackson 1994). However this finding has not been consistent and is related to differences in the composition of the low protein diet (Langley-Evans 2000). The diet used by Langley-Evans supplemented with methionine has been consistent in programming hypertension; low protein diets without methionine supplementation report either no change or a slight depression in blood pressure (Langley-Evans 2000). These results highlight the importance that the balance of micronutrients plays in determining the long-term health effects of maternal nutrition during pregnancy. Investigations into fetal programming of hypertension in offspring consistently reveal a reduction in renal mass, increased apoptosis without a balancing increase in cell proliferation and reductions in glomeruli number (Langley-Evans et al. 1999b). Changes in kidney structure and development are associated with enhanced activity of the renin-angiotensin-system (RAS), (Langley-Evans et al. 1999a). Increased expression of glucocorticoid receptors and reduced expression of 11β-HSD-2 in the kidney is associated with an enhancement of glucocorticoid mediated increases in blood pressure (Bertram et al. 2001).

Carbohydrate metabolism in offspring is also altered by a MLP diet during pregnancy. Fasting plasma insulin and glucose levels are lower in MLP offspring and are associated with improved insulin sensitivity in early adulthood. However programmed offspring exhibit a greater age dependent loss of glucose tolerance (Hales et al. 1996). By 15 months of age glucose tolerance in MLP offspring is significantly diminished compared to that of controls, and is associated with hyperinsulinemia in males and hypoinsulinemia in females (Hales et al. 1996). The mechanisms behind these phenotypic observations include altered development of the pancreas and insulin signalling.

The MLP diet programs pancreatic function in restricted offspring through a reduction in \( \beta \)-cell proliferation, islet size and vascularity coupled with an enhanced coupling of \( \beta \)-cell apoptosis (Snoeck et al. 1990). Subsequently, it has been shown that a defect in glucose-mediated insulin secretion from islets of adult MLP offspring only manifest when an additional dietary insult such as high fat feeding is introduced postnatally (Ozanne 2001).

Global undernutrition at various times during pregnancy is another widely used approach to induce nutritional programming. Various models have been developed with different levels of undernutrition during different periods of pregnancy. A mild nutritional restriction to 70% of normal intake in the first 18 days of pregnancy in the rat, results in offspring with significant growth retardation at birth that catch up to controls at postnatal day 20 (Ozaki et al. 2001). Restricted offspring exhibit elevated blood pressure in adult life with an increased vasoconstriction response to potassium and thromboxane A\(_2\) mimetics. These abnormalities increase with age and are most pronounced in male offspring (Ozaki et al. 2001). Another model using a nutritional restriction to 50% of standard \textit{ad libitum} intake in the second half of gestation had no effect on blood pressure, with only subtle alterations in vasoconstrictive ability being observed (Holemans et al. 1999). In another study a 50% restriction of a normal diet from day 15 of pregnancy showed that 21-day-old rat fetuses had significantly decreased pancreatic insulin content (Blondeau et al. 2001).

We have developed a rodent model of fetal programming using maternal undernutrition throughout pregnancy. On day one of pregnancy animals are randomly assigned to a standard rat diet \textit{ad libitum} throughout pregnancy (\textit{ad libitum} (AD) group) or 30% of the AD group intake of the standard diet throughout gestation (undernourished, UN group). After birth, litter size and birth weights are recorded and litter size is adjusted to 8 pups per litter. The number of pups per litter from UN and AD mothers is identical in this experimental paradigm; it is not affected by maternal undernutrition. The UN offspring are cross-fostered within 24 hours of birth onto AD dams to assure adequate and standardised nutrition from birth until weaning. At birth offspring of UN mothers had fetal and placental weights that were 25–30% lower than offspring of AD mothers. A lack of catch-up growth despite a standard postnatal diet (Woodall et al. 1996a) was accompanied by a transient reduction in circulating IGF-I and hepatic IGF-I mRNA expression which normalised at weaning. Consistent with this observation, we also showed that UN offspring had a reduced responsiveness to growth hormone (GH) during the neonatal period, possibly reflecting delayed maturation of the somatotrophic axis, which was fully restored before puberty (Woodall et al. 1996a; Woodall et al. 1998). In addition, UN offspring developed elevated blood pressure in adult life (Weder & Schork 1994; Woodall et al. 1999).

**POSTNATAL NUTRITION**

We can distinguish two conceptually different types of interactions between prenatal influences and postnatal nutrition in the pathogenesis of metabolic disorders and obesity. Diet-induced obesity during postnatal life can amplify pathogenic mechanisms established by adverse prenatal influences (Vickers et al. 2000). Alternatively, changes caused by prenatal influences can facilitate a disease process that is induced by postnatal environmental factors such as nutrition. An example is the development of obesity and insulin resistance in individuals who are exposed to a high fat diet during postnatal life. The direction of the interactions between prenatal and postnatal influences is most likely dependent on timing and severity of each factor.

In historically undernourished, recently urbanised populations such as India, where individuals of low birth weight are exposed to a high-fat Western diet, the incidence of obesity and type 2 diabetes is reaching epidemic proportions (Yajnik 2000). Work by Yajnik and colleagues has shown that although Indian babies are born of low birth weight, they exhibit relatively increased visceral adiposity and hyperinsulinemia at birth (Yajnik et al. 2002). Such observations have been explained by the “thrifty-phenotype” hypothesis proposed by Hales and Barker (Hales & Barker 1992) and may illustrate the long-term disadvantage of postnatal “catch-up” growth. Although there is considerable debate whether catch-up growth in early postnatal life is beneficial or not, most studies suggested that postnatal “catch-up” growth is associated with adverse outcomes in later life (Ong & Dunger 2000; Bonora et al. 1994; Ong et al. 2000; Eriksson et al. 1999).

Epidemiological studies have shown that the greatest insulin resistance is observed in people of low birth weight who develop obesity as adults (Phillips 1998). In rats, the combination of prenatal undernutrition with retarded fetal growth, and good postnatal nutrition with accelerated growth, leads to a striking reduction in life span (Hales et al. 1996; Jennings et al. 1999). The well-established concept that diets high in saturated fats play a key role in the development of insulin resistance and obesity has recently been extended to the frequency of food intake. Zammit et al. (2001) suggested that the pathogenesis of insulin resistance may be related to a pattern of frequent snacking which results in a continuous post-prandial state for most of the day. This prevents the attainment of low basal inter-prandial insulin levels even in normal individuals. Prolonged exposure of the liver to high basal insulin, through its stimulatory effect on hepatic triglycerides and very-low-density lipoprotein secretion,
may contribute to the initial induction of muscle insulin resistance (Zammit et al. 2001).

In our studies, we introduced offspring of undernourished rats to a hypercaloric (high fat / high protein) diet after weaning to investigate whether enhanced nutritional supply would facilitate postnatal catch-up growth. This led to development of obesity during adulthood (Vickers et al. 2000; Vickers et al. 2001a). UN offspring also developed hypertension, hyperinsulinemia, hyperleptinemia and hyperphagia independent of postnatal diet. Postnatal hypercaloric nutrition amplified the existing cardiovascular, metabolic and endocrine abnormalities of UN offspring (Vickers et al. 2000). Interestingly, hyperphagia was established before puberty independent of caloric content of the diet and increased with advancing age (Vickers et al. 2000). The increased plasma insulin and leptin concentrations were paralleled by altered pancreatic histology (Vickers et al. 2001b). The hyperleptinemia and hyperinsulinemia seen in UN offspring may be a mechanism induced by a nutrient-deprived fetal environment to store large quantities of triglycerides when food is plentiful, thus representing a competitive advantage ("thrifty phenotype") in preparation for a nutrient-deprived postnatal environment (Hales & Barker 1992). However, when hypercaloric nutrition persists for long periods of time, adipogenic diabetes may develop. Our work to date cannot resolve whether the primary defect in this cascade is in appetite regulation, peripheral metabolism, or altered leptin or insulin action. We have shown that therapy with insulin-like growth factor-1 or growth hormone can ameliorate obesity, hyperphagia and hypertension induced by fetal programming and high fat nutrition, but the precise mechanisms underlying these effects are yet to be resolved (Vickers et al. 2001a; Vickers et al. 2002). We have recently reported that maternal undernutrition can induce sedentary behaviour in offspring (Vickers et al. 2003). Hyperphagia and concomitant obesity in offspring are amplified by hypercaloric nutrition. In the course of these studies we noted that the onset of the abnormal eating behaviour occurred prior to puberty, thus preceding the development of obesity.

**SUMMARY AND CONCLUSIONS**

Numerous epidemiological studies have shown that perturbations in early life can have persistence consequences for health in later life. Both prospective clinical studies and experimental research have clearly shown that the propensity to develop abnormalities of cardiovascular, endocrine and metabolic homeostasis in adulthood is increased when fetal development has been adversely affected. The pathogenesis is not based on genetic defects but on altered genetic expression as a consequence of an adaptation to environmental changes during fetal development.

Studies of the interaction between maternal undernutrition throughout pregnancy followed by postnatal hypercaloric nutrition in the rat have shown that offspring from undernourished mothers are growth retarded at birth and develop obesity, hypertension, hyperleptinemia, hyperinsulinemia and hyperphagia during postnatal life. Close associations between a major rise in circulating insulin and leptin concentrations and a large increase in appetite and fat mass provide evidence for disturbed endocrine communication between the hypothalamus, adipose tissue and the endocrine pancreas in the pathogenesis of programming-induced obesity. Hypercaloric nutrition during postnatal life greatly amplifies prenatal effects on metabolic abnormalities, obesity, overeating and diminished exercise behaviour.

Fetal programming research offers a novel approach to investigate the mechanistic basis of metabolic disorders, hyperphagia and diminished exercise behaviour which in human populations predominantly arise from environmental factors and lifestyle choices. The use of animal models can establish model conditions that will reliably provide high contrasts of phenotypes. Such studies offer an exciting potential for advances in our understanding of critical determinants and mechanisms for human obesity and metabolic disorders.

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