

Fetal origins of insulin resistance and obesity

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A number of epidemiological studies worldwide have demonstrated a relationship between poor early growth and an increased susceptibility to insulin resistance, visceral obesity, type 2 diabetes and other features of the metabolic syndrome in adulthood. However, the mechanistic basis of this relationship and the relative roles of genes and the environment remain a subject of debate. The 'thrifty phenotype' hypothesis proposes that poor fetal nutrition leads to programming of metabolism and an adult phenotype that is adapted to poor but not plentiful nutrition. The maternal reduced-protein rat model has been used to examine the importance of the maternal environment in determining susceptibility to adult disease. Pregnant and lactating rat dams are fed a diet containing 80 g protein/kg as compared with 200 g protein/kg, which leads to growth restriction *in utero*. Offspring of low-protein dams have increased susceptibility to diabetes, insulin resistance and hypertension when fed a palatable high-fat diet that promotes obesity. Administration of leptin during pregnancy and lactation to these protein-restricted dams produces offspring that have increased metabolic rate and do not become obese or insulin resistant when fed on a high-fat diet. Increased glucocorticoid exposure, particularly during late gestation, has been linked with insulin resistance in adulthood. High levels of fetal glucocorticoids may result from a decreased activity of placental 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 2, which normally protects the fetus from high maternal glucocorticoid levels. Leptin administration to protein-restricted dams inhibits the suppression of 11 β -HSD-2 and may be one mechanism by which the metabolic syndrome is prevented.

Intrauterine growth restriction: Low birth weight: Obesity: Insulin resistance

Evidence for the early origins of adult disease

Low birth weight remains an important public health problem in many countries. It may be a consequence of premature birth, genetic programming or intrauterine growth restriction. Intrauterine growth restriction is predominantly affected by the maternal diet, placental function, infection and underlying social factors. The focus of the present article is on low birth weight resulting from intrauterine growth restriction and the relationship between being small-for-gestational age and the susceptibility for adult disease.

The relationship between early growth and the later occurrence of insulin resistance, type 2 diabetes, hypertension, hyperlipidaemia and CVD has been documented in studies of both men and women in distinct populations in the UK, mainland Europe, Asia, Africa and the USA (Newsome *et al.* 2003). In one early study it was found that men who are the smallest at birth (<2.5 kg) are almost

seven times more likely to be glucose intolerant or type 2 diabetic than those who are the heaviest at birth (>4.3 kg; Hales *et al.* 1991). An even stronger relationship has been observed between birth weight and the presence of the metabolic syndrome (glucose intolerance, hypertension and elevated triacylglycerol content), with those men who are smallest at birth being eighteen times more likely to have the syndrome at the time of study than those who are the heaviest at birth (Barker *et al.* 1993). The combination of low birth weight followed by obesity in later life appears to carry the highest risk of insulin resistance. The persisting high incidence of intrauterine growth restriction and a worldwide increase in obesity probably contributes to the rise in CVD and type 2 diabetes in developing countries (Fall, 2001). However, the molecular basis of this association is largely unknown, and the relative roles played by both genetic and environmental factors are still a subject of intense debate.

Abbreviation: 11 β -HSD, 11 β -hydroxysteroid dehydrogenase.

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Genes *v.* the environment

There is evidence that paternal factors or genes influence fetal growth and later susceptibility to diabetes. The 'fetal insulin' hypothesis suggests that low birth weight and type 2 diabetes are two independent consequences of the same insulin-resistant genotype (Frayling & Hattersley, 2001). One cause of insulin resistance might be from mutations in the gene encoding glucokinase, which have been shown to be associated with reduced birth weight and the appearance of maturity-onset type 2 diabetes of the young (Hattersley *et al.* 1998; Njolstad *et al.* 2003); however, these mutations are rare. Currently, extensive genomic scans have not identified universal polymorphisms for diabetes susceptibility (i.e. polymorphisms present with sufficient frequency to be considered causative).

Susceptibility to developing an adult phenotype such as type 2 diabetes is probably a result of both genetic and early environmental factors, with different factors having predominance in different individuals. The 'thrifty phenotype' hypothesis (Hales & Barker, 1992) proposes that the components of the metabolic syndrome originate from environmental influences and that both fetal and early nutrition are important factors. Adaptations associated with fetal malnutrition become detrimental to the health of the offspring if they experience a period of adequate or plentiful nutrition leading to postnatal obesity (Jaquet *et al.* 2000). Such observations may explain why the prevalence of the metabolic syndrome is low in regions of Africa where poor fetal nutrition is followed by poor postnatal nutrition and high physical activity, but it is markedly higher in populations moving rapidly from poor to increased nutrition and low physical exercise.

There is direct evidence from both human and animal studies to support the 'thrifty phenotype' hypothesis. First, men and women who were *in utero* during the 'Dutch Hunger Winter' at the end of the Second World War have been shown to have a worse glucose tolerance than those who were not exposed to the famine (Ravelli *et al.* 1998). Second, among homozygotic twins it is the twin with the lower mean birth weight that is more susceptible to diabetes (Poulsen *et al.* 1997). Third, the offspring of mothers who smoke during pregnancy (a known cause of low birth weight) are more likely to become obese (Toschke *et al.* 2002) and develop type 2 diabetes (Montgomery & Ekblom, 2002).

Experimental models of early growth restriction

In an attempt to understand the molecular basis of the relationship between early growth restriction and the development of adult disease, several animal models have been developed. These models employ uterine artery ligation, nutritional interventions (e.g. protein or energy restriction, Fe deficiency) and hormonal insults, including overexposure to glucocorticoids.

The maternal low-protein model is one of the most-extensively-studied models (Ozanne & Hales, 1999). Pregnant rat dams are fed a diet containing 80 g protein/kg during pregnancy and lactation that has no observable detrimental effect on litter size or viability. The resulting

pups from these litters have a reduced birth weight of approximately 15%. At 21 d of age both control and low-protein offspring are weaned onto standard chow containing 200 g protein/kg, yet the reduction in body weight of the low-protein offspring is maintained throughout life (Desai *et al.* 1996).

In early adult life the low-protein offspring are more glucose tolerant and insulin sensitive than the controls (Langley *et al.* 1994; Hales *et al.* 1996). There is, however, an age-dependent impairment in glucose tolerance; by 15 months of age there is markedly worsened glucose tolerance and by 17 months the male low-protein offspring are diabetic with elevated insulin levels. The deterioration can be accelerated by high-fat feeding (Wilson & Hughes, 1997; Stocker *et al.* 2004). Blood pressure is also raised (Langley-Evans *et al.* 1996; Petry *et al.* 1997; CJ Stocker, unpublished results).

Absolute organ growth in growth-restricted low-protein offspring is reduced, but not uniformly (Desai *et al.* 1996). Brain growth is spared and as a proportion of body weight it is greater in the low-protein offspring. This factor may not reflect brain function, as vascularisation of the developing brain is permanently reduced in these animals (Bennis-Taleb *et al.* 1999). By contrast, the pancreas shows reduced β -cell proliferation, islet size, vascularisation (Snoeck *et al.* 1990) and a reduction in islet cell function (Cherif *et al.* 1998). In the adult impairment of glucose-stimulated insulin secretion is observed in response to a dietary insult such as high-fat feeding (Wilson & Hughes, 1997).

The impact of maternal protein restriction on metabolic programming is most pronounced in the insulin-sensitive tissues. The liver from 3-month-old low-protein male rats is resistant to the stimulatory effect of glucagon on glucose output (Ozanne *et al.* 1996) and has increased phosphoenolpyruvate carboxykinase activity (Desai *et al.* 1997), both indicative of reduced gluconeogenic activity. Glucose uptake by skeletal muscle is more sensitive to insulin. These findings are consistent with improved glucose tolerance at this age. The age-dependent loss in glucose tolerance is reflected in isolated muscle studies and is thought to be through a defect in insulin action downstream of the receptor (Ozanne *et al.* 2003). It may in part be a result of permanent changes in mitochondrial function in both the liver and muscle (Park *et al.* 2003).

Glucose uptake in adipocytes is also more sensitive to insulin in 3-month-old offspring of low-protein dams (Ozanne *et al.* 1997). This finding is consistent with increased insulin receptor substrate 1-associated phosphatidylinositol 3-kinase activity. However, intra-abdominal adipocytes are resistant to the anti-lipolytic action of insulin (Ozanne & Hales, 1999) and more sensitive to the lipolytic actions of catecholamines. Plasma catecholamine levels and NEFA levels are raised (Petry *et al.* 2000). Thus, this phenotype of low-protein offspring is like that of normal fasted rats and may aid survival in animals exposed to intermittent or poor nutrition (Hales & Ozanne, 2003). As with muscle there is an age-dependent loss in adipocyte function by 15 months of age (Ozanne, 2001) as a result of a defect in insulin signalling and reduced phosphatidylinositol 3-kinase activation.

The molecular mechanisms underlying these programming events are under investigation. Proteome analysis of isolated fetal islets from maternal protein-restricted pregnancies has highlighted a complex pattern of changes in islets (Sparre *et al.* 2003). In the liver and kidney of young adult low-protein rats, the binding activity of the nuclear transcription factor HNF1 α is reduced, as is the expression of the downstream signals (Hales & Ozanne, 2003). Epigenetic mechanisms that regulate chromatin conformation and imprinted genes regulated by cytosine methylation are sensitive to nutritional influences (Waterland & Jirtle, 2003) and may lead to programmed alterations in metabolism.

Glucocorticoid programming of the fetus

Fetal overexposure to maternal glucocorticoids in both human and animal models triggers programming events *in utero* (Langley-Evans *et al.* 1996; Nyirenda *et al.* 1998) that establish increases in glucocorticoid action throughout life (Nyirenda *et al.* 1998; Lesage *et al.* 2001) and result in a metabolic syndrome. Moreover, exogenous glucocorticoids also retard fetal and placental growth and lower birth weight in both animal models and in man (Hofmann *et al.* 2001). These effects of the glucocorticoids appear to be relevant to epidemiology because there are strong correlations between birth weight, plasma cortisol concentrations and the development of hypertension and type 2 diabetes (Phillips *et al.* 2000). Fetal cortisol levels are increased in human fetuses following intrauterine growth-restriction and in pregnancies complicated by pre-eclampsia (Goland *et al.* 1995), and low birth weight has been shown to predict increased plasma cortisol levels in independent adult populations (Phillips *et al.* 2000). Since perinatal exposure to glucocorticoids increases the activity of the hypothalamic–pituitary–adrenal axis (Holmes *et al.* 2003), it has been proposed (for review, see Matthews, 2002) that the programming of this axis *in utero* is linked to modifications in not only behavioural and neurological responses, but also endocrine and cardiovascular function, regardless of the environment into which the fetus will be born.

Glucocorticoid action within cells is regulated by the expression of the glucocorticoid receptor and two isoforms of 11 β -hydroxysteroid dehydrogenase (11 β -HSD), 1 and 2. 11 β -HSD-1 acts *in vivo* primarily as a reductase, catalysing the conversion of cortisone to bioactive cortisol (11-dehydrocorticosterone to corticosterone in rats) and amplifying glucocorticoid action at its receptor. The 11 β -HSD-2 isoform acts as a dehydrogenase and catalyses the inactivation of cortisol to corticosterone (Brown *et al.* 1996). Fetal glucocorticoid levels are lower than maternal levels and this differential is ensured by the high fetoplacental expression of 11 β -HSD-2 (Condon *et al.* 1998), although this enzyme is not a complete barrier to maternal glucocorticoids (Benediktsson *et al.* 1997). It has therefore been proposed that a relative deficiency of placental 11 β -HSD-2 will allow increased exposure of the fetus to maternal glucocorticoid levels, retard growth and programme responses that lead to later adult disease. In support of this

proposal deleterious mutations of the 11 β -HSD-2 gene in man are associated with reduced birth weight (Dave-Sharma *et al.* 1998), and in rats the offspring of dams given the non-selective 11 β -HSD inhibitor carbenoxolone in pregnancy have reduced birth weight, hypertension and hyperinsulinaemia (Lindsay *et al.* 1996). Dietary protein restriction attenuates 11 β -HSD-2 in the placenta (Langley-Evans *et al.* 1996), and this response may be one mechanism linking the maternal environment with fetal programming. In this model administration of glucocorticoid-synthesis inhibitors to the pregnant dam prevents hypertension in the female offspring (Langley-Evans, 1997).

Energy balance

Neonatal nutrition appears to have long-term effects on appetite (Vickers *et al.* 2000), but how it influences central mechanisms that regulate food intake and energy balance is ill-defined. There is evidence in rodents, however, for a role of leptin in the development of the brain, particularly within specific hypothalamic regions associated with energy homeostasis (Bouret *et al.* 2004). The postnatal surge in leptin may serve as a key developmental signal to the hypothalamus and influence subsequent food intake and body weight throughout later life (Ahima *et al.* 1998; Proulx *et al.* 2002).

As well as increasing appetite, maternal protein restriction, at least in rodents, programmes the expression of lipogenic genes in the offspring (Maloney *et al.* 2003). In sheep enhanced fat deposition is seen in late gestation in fetuses that have experienced a period of nutrient restriction (Symonds *et al.* 2003). Not only is adipogenesis increased, but both rodent and human low-birth-weight offspring have reduced lean mass (Gale *et al.* 2001).

In addition to nutrition *in utero* the level of nutrition during lactation correlates with appetite in later life (Vickers *et al.* 2000; Ozanne *et al.* 2004). In studies of small-for-gestational-age infants an increased rate of postnatal weight gain ('catch-up growth') is associated with reduced satiety (Ounsted & Sleight, 1975). Offspring of malnourished mice cross-fostered onto normally-fed animals rapidly gain weight, possibly as a result of suppressed thermogenesis (Crescenzo *et al.* 2003), and at weaning catch up with the growth of control litters. These animals are more susceptible to obesity and have reduced longevity (Ozanne & Hales, 2004). In human populations a period of catch-up growth is associated with an increased risk of elevated blood pressure (Leon *et al.* 1996), CVD (Eriksson *et al.* 1999) and type 2 diabetes (Soto *et al.* 2003), as well as a disproportionately high rate of fat deposition.

Of intense debate is the question of whether bottle-feeding or breast-feeding is more beneficial to the long-term health of the infant. Many studies have reported that breast-fed infants show a different growth pattern from formula-fed infants (Agostoni *et al.* 1999). Breast-fed infants are leaner and are protected from becoming obese when older (for review, see Locke, 2002). These differences may in part be a result of a different endocrine

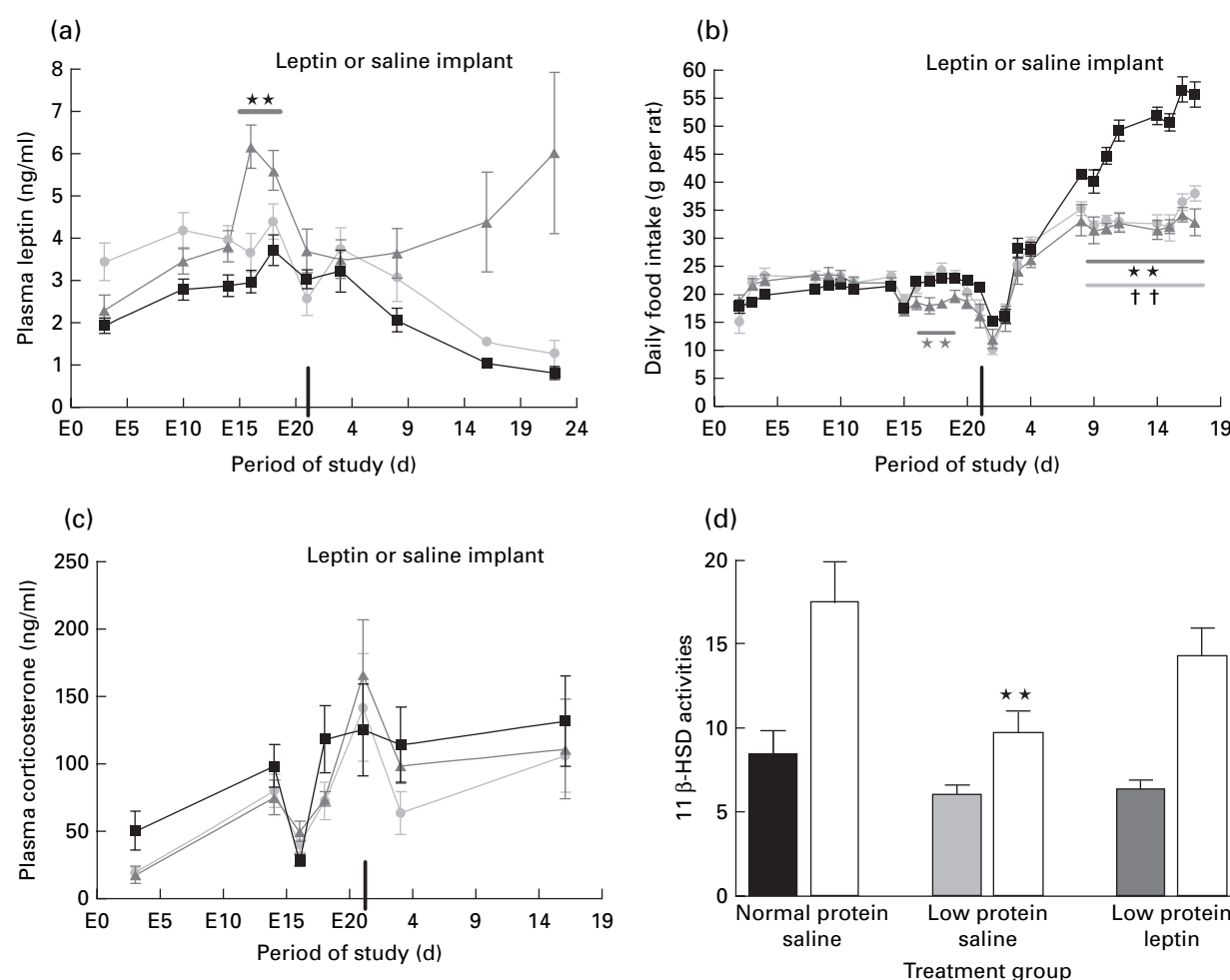


Fig. 1. Plasma leptin levels (a) and daily food intake (b) and plasma corticosterone levels (c) for rat dams fed on a normal-protein diet (200 g/kg) and infused with saline (9 g sodium chloride/l; ■), or fed on a low-protein diet (80 g/kg) and infused with either leptin (▲) or saline (●) during pregnancy (E) and lactation. (†), Parturition. Values are means with their standard errors for twelve pregnant dams per group. Mean values for low-protein leptin-infused dams were significantly different from those for normal-protein dams: ** $P < 0.01$. Mean values for low-protein saline-infused dams were significantly different from those for normal-protein dams: †† $P < 0.01$. (d) Placental 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 1 (■) and type 2 (□) activities expressed as the percentage conversion of corticosterone to dehydrocorticosterone. Values are means with their standard errors for six to eight placentas per group from mixed litters. Mean value for 11 β -HSD type 2 for low-protein saline-infused dams was significantly different from that for normal-protein dams: ** $P < 0.01$.

response to feeding. Leptin is present in breast milk, but not infant formula (Savino *et al.* 2002). Breast-fed infants appear to have a different ability to regulate food intake both when breast-feeding (Dewey *et al.* 1993) and when eating solid food (Perez-Escamilla *et al.* 1995). This difference raises the possibility that the presence of leptin in breast milk has a positive effect on satiety and the regulation of energy balance (Dessolin *et al.* 1997).

Leptin's role in breast milk may not be limited to energy regulation and metabolism. It may be important in the continued development of the organ systems, e.g. the gastrointestinal tract and the immune system. The level of cord blood leptin is inversely related to rates of growth during infancy in human studies (Ong *et al.* 1999), and low-birth-weight infants have reduced leptin levels. Early growth rates are strongly influenced by a drive to compensate for earlier intrauterine growth restriction or

antenatal restraints, which may involve the programming of appetite (Ong *et al.* 2002).

Leptin in pregnancy

Maternal leptin is produced from adipose stores and the placenta. During pregnancy and lactation it modulates energy expenditure and metabolism, as well as lactation itself. It also affects placental growth, nutrient transfer (Jansson *et al.* 2003), angiogenesis and trophoblast invasion (for review, see Sagawa *et al.* 2002). Placental function has been shown to be impaired in pregnancies in which the fetus is growth-retarded through the down-regulation of specific amino acid transport proteins (Glazier *et al.* 1997). Consistent with these observations, transplacental passage of leptin is reduced (Smith &

Waddell, 2003). Fetal leptin produced from both fetal adipocytes and the placenta is secreted into the amniotic fluid and stimulates fetal growth and development, fetal angiogenesis and haematopoiesis. Leptin levels increase throughout gestation, peak in the second trimester and remain high until parturition. These elevated levels are not associated with negative energy balance as might be expected, perhaps because leptin resistance in pregnancy sustains maternal food intake for fetal growth (Seeber *et al.* 2002).

The role of leptin in fetal growth and development

There is accumulating evidence to suggest that leptin production from the placenta and fetal adipose tissue, and in rodents from the maternal circulation (Smith & Waddell, 2003), acts through fetal leptin receptors to regulate fetal growth and development. In part, this effect of leptin is a result of a direct influence on the insulin and the insulin-like growth factor–growth hormone systems. Leptin levels in umbilical cord blood are positively correlated with birth weight and neonatal adiposity. Leptin may play a role in the control of substrate utilisation and in the maintenance and functional characteristics of fat mass before birth (Bispham *et al.* 2003; Yuen *et al.* 2003), producing permanent effects on adiposity and body composition in adult life (Nilsson *et al.* 2003). Postnatal leptin levels, which are predominantly regulated by nutrition, also play a key role (Ehrhardt *et al.* 2003). For example, suboptimal nutrition leads to a delay in the onset of puberty, and it is likely that leptin may contribute to this effect (Léonhardt *et al.* 2003). Leptin levels in neonates decrease rapidly following birth and this rapid decline may be an important stimulus of feeding behaviour in early life. This process may be one explanation for why growth-restricted babies exhibiting low leptin levels at birth have increased growth rates in early neonatal life.

A role for leptin in the programming of metabolic disease?

An aim has been to address whether leptin is involved in the programming of adult metabolic disease in low-birth-weight rodents, either by acting directly on fetal leptin receptors, or indirectly via maternal or placental receptors. Reports that administration of leptin prevents the endocrine response to fasting (Ahima *et al.* 1996) and potently suppresses the secretion of glucocorticoids (Proulx *et al.* 2001) have been encouraging. To investigate the role of leptin in fetal programming the maternal protein-restricted rat model of intrauterine growth restriction has been used.

Pregnant rat dams were fed either an 80 g protein/kg diet or control chow throughout pregnancy and lactation. At the beginning of the third trimester a continuous infusion of either saline (9 g NaCl/l) or leptin was given utilising a subcutaneously-implanted osmotic mini-pump. Following infusion of leptin, leptin levels were found to be elevated fivefold in the low-protein-fed dams as compared with both low-protein and control-protein saline (9 g NaCl/l)-treated groups. Despite late pregnancy being a state of apparent leptin resistance in rodents, food intake is reduced

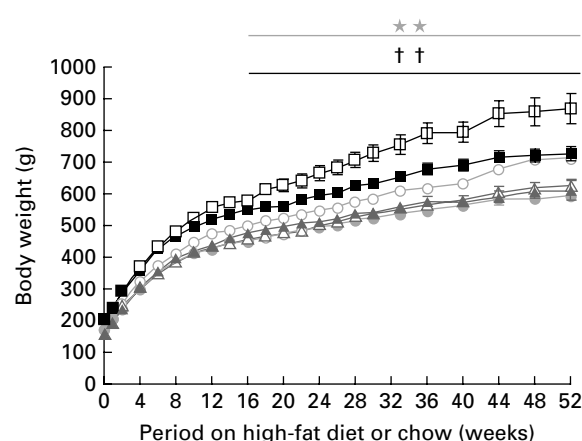


Fig. 2. Effect of maternal leptin treatment on body weight for chow-fed (■, ▲, ●) and high-fat-fed (68% fat as metabolisable energy; □, △, ○) offspring of rat dams fed on a normal-protein (200 g/kg) diet and infused with saline (9 g NaCl/l; ■, □) or fed on a low-protein (80 g/kg) diet and infused with either leptin (▲, △) or saline (●, ○) during pregnancy and lactation. Values are means with their standard errors for sixteen offspring per group. There was a significant increase in the body weights of offspring fed on the high-fat diet compared with offspring fed on chow for offspring of dams fed on a low-protein diet and treated with saline during pregnancy and lactation: ** $P < 0.01$. There was a significant increase in the body weights of offspring fed on the high-fat diet compared with offspring fed on chow for offspring of dams fed on a normal-protein diet and treated with saline during pregnancy and lactation: †† $P < 0.01$.

in the low-protein leptin-treated group. Following parturition, absolute food intake is suppressed in both the saline- and leptin-treated low-protein groups compared with the normal-protein group (Fig. 1a,b).

Fetal weights are reduced in both the low-protein groups. Placental weights are also lower in the saline-treated low-protein diet group as compared with those in the normal-protein diet group, which results in an increased fetal:placental weight. Maternal leptin administration reduces fetal:placental weight to that of the control group, suggesting that elevating leptin levels in the low-protein dams may have prevented any placental insufficiency as a result of placental size. Levels of circulating maternal glucocorticoids are not significantly affected by dietary intervention or leptin administration. However, while the activity of placental 11 β -HSD-1 is similar in all groups, that of 11 β -HSD-2 is significantly reduced in the saline-treated low-protein dams ($P < 0.01$) but not in those treated with leptin (Fig. 1c,d). This finding is consistent with a role for glucocorticoids in programming the fetus to increased susceptibility to adult disease, and may be an important mechanism by which in early life leptin can modulate the susceptibility of the offspring to later adult disease (Stocker *et al.* 2004).

Evidence is now available to suggest that leptin may directly influence fetal growth and development through the insulin-like growth factor–growth hormone axis (CJ Stocker, unpublished results). Leptin administration to low-protein dams partially reverses the reduction in fetal insulin-like growth factor 1 levels in the intrauterine

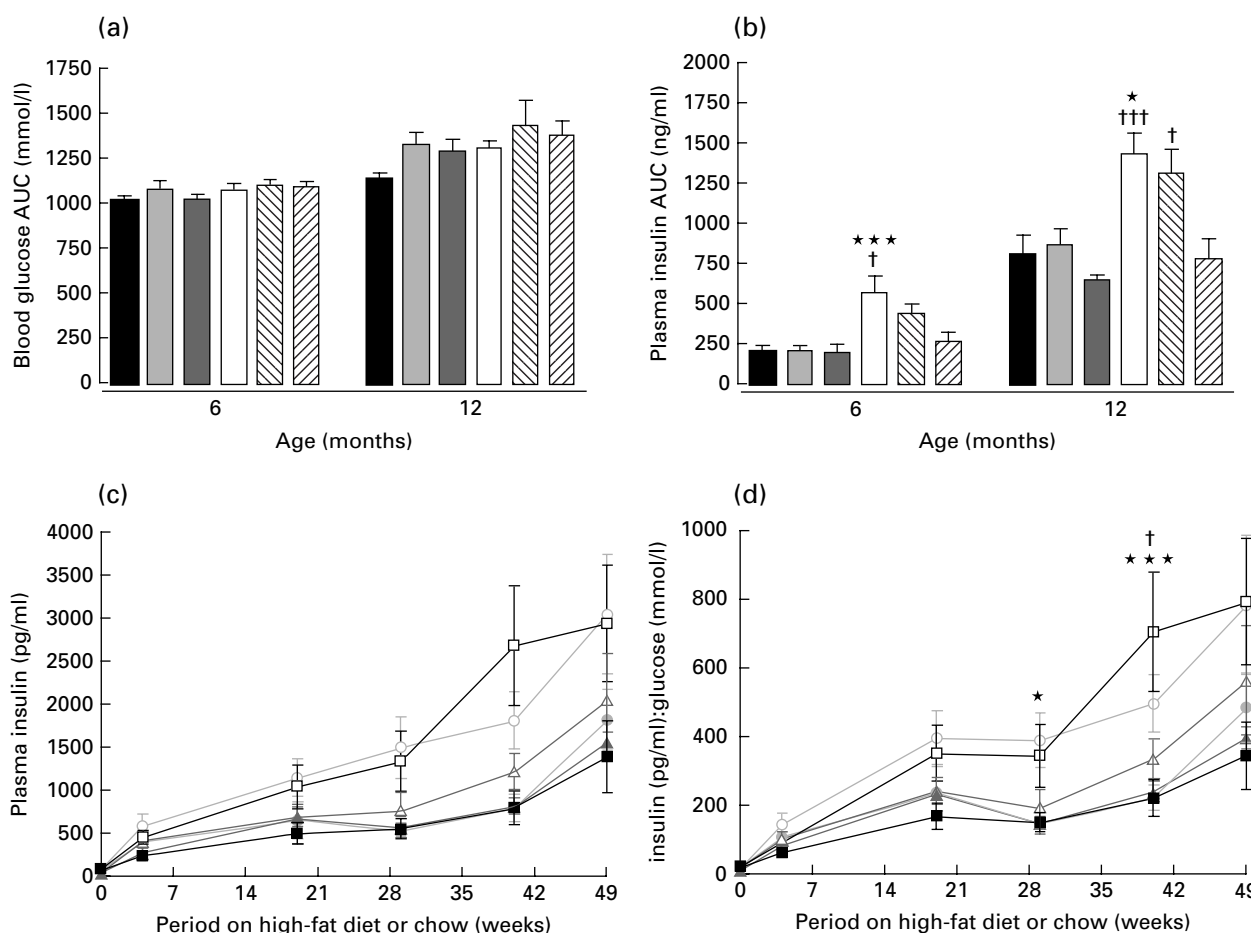


Fig. 3. Area under the curve (AUC) for blood glucose (a) and plasma insulin (b) concentrations during a glucose tolerance test for chow-fed (■, ■, ■) and high-fat-fed (□, □, □) 6- and 12-month-old male offspring of rat dams fed on a normal-protein (200 g/kg) diet infused with saline (9 g NaCl/l; ■, □) or a low-protein (80 g/kg) diet infused with either leptin (■, □) or saline (□, □) during pregnancy and lactation. Fasting insulin (c) and fasting insulin:fasting glucose (d) for chow-fed (■, ▲, ●) and high-fat-fed (□, △, ○) male offspring of dams fed on a normal-protein diet and infused with saline (■, □) or fed on a low-protein diet and infused with either leptin (▲, △) or saline (●, ○) during pregnancy and lactation. For a–d, values are means with their standard errors for six to eight offspring per group. Mean values were significantly different from those for chow-fed offspring of dams receiving the same treatment during pregnancy and lactation: * $P < 0.05$, *** $P < 0.001$. Mean values were significantly different from those for high-fat-fed offspring of dams fed on a low-protein diet and infused with leptin during pregnancy and lactation: † $P < 0.05$, ††† $P < 0.001$.

growth restriction offspring and significantly elevates both insulin-like growth factor 2 and fetal leptin levels. These elevated maternal and/or fetal leptin and insulin-like growth factor levels affect the development of key endocrine organs, e.g. the pancreas. Maternal leptin administration results in alterations of pancreatic hormone levels, including an increase in pancreatic insulin content to a level comparable with those in offspring from normal-protein dams.

The birth weights of the leptin-treated low-protein-diet group are lower than those for both the saline-treated low-protein and the normal-protein groups (Stocker *et al.* 2001). This difference in body weight continues throughout lactation and into adulthood in both the male and female offspring. At weaning this reduction in body weight in the offspring from the leptin-treated low-protein-diet groups is reflected by a decrease in adipose tissue in the males but not females. This finding suggests a role for

leptin in the programming of adipose development. In support of this role, it has been shown that when these offspring are placed on a highly-palatable high-fat diet (68% fat as metabolisable energy) from 6 weeks of age the offspring of the leptin-treated mothers are completely resistant to the weight gain associated with this feeding regimen (Fig. 2). This resistance to high-fat-diet-induced weight gain may in part be a result of the elevated metabolic rate in the young adult offspring from leptin-treated dams (CJ Stocker, unpublished results). These offspring are also more insulin sensitive following an intraperitoneal glucose tolerance test (Fig. 3). Thus, the glucose tolerance profile is similar in all groups of 6- and 12-month-old male rats irrespective of dietary treatment or maternal group. However, with the exception of those offspring from mothers receiving leptin, rats fed on a high-fat diet are hyperinsulinaemic both in the fasted state and during the glucose tolerance test.

An individual's susceptibility to later metabolic disease is probably influenced by poor nutrition at particular times during early development and confounded by later post-natal nutritional excess, interacting with genetics. Data from investigations into whether leptin might play a role in this process collectively suggest that leptin influences the early-life metabolic programming events occurring in the offspring of an intrauterine growth-restricted pregnancy, but the precise timing and relative contribution of the effects are likely to be complex. Leptin administration in both pregnancy and lactation has been shown to provide long-term protection from type 2 diabetes and obesity. The clinical relevance of these observations has yet to be established.

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