REVIEW ARTICLE

# Early-life programming of susceptibility to dysregulation of glucose metabolism and the development of Type 2 diabetes mellitus

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There is increasing epidemiological evidence in humans which associates low birthweight with later metabolic disorders, including insulin resistance and glucose intolerance. There is evidence that nutritional and hormonal factors (e.g. maternal protein restriction, exposure to excess maternal glucocorticoids) markedly influence intra-uterine growth and development. A picture is also emerging of the biochemical and physiological mechanisms that may underlie these effects. This review focuses on recent research directed towards understanding the molecular basis of the relationship between indices of poor early growth and the subsequent development of glucose intolerance and Type 2 diabetes mellitus using animal models that attempt to recreate the process of programming via an adverse intra-uterine or neonatal environment. Emphasis is on the chain of events and potential mechanisms by which adverse adaptations affect pancreatic-$\beta$-cell insulin secretion and the sensitivity to insulin of key metabolic processes, including hepatic glucose production, skeletal-muscle glucose disposal and adipose-tissue lipolysis. Unravelling the molecular details involved in metabolic programming may provide new insights into the pathogenesis of impaired glucoregulation and Type 2 diabetes.

Key words: fetal development, insulin resistance, obesity, pancreatic $\beta$-cell.

## INTRODUCTION

The biochemical origins of major non-communicable metabolic diseases in the adult, including Type 2 diabetes mellitus and cardiovascular disease, are becoming increasingly understood. It is well established that key determinants of the onset of these metabolic diseases include genetic predisposition, together with adult lifestyle risk factors. Genetic predisposition has been assumed to play the major role in determining susceptibility to these diseases; nevertheless, genetically susceptible individuals may not develop these diseases if adult lifestyle risk factors are avoided [1,2]. The ability to cope with the demands placed on metabolic systems by environmental factors varies between individuals, although how this is determined remains unclear. Epidemiological studies have raised the possibility that 'early lifestyle' factors, which are not determined by the individual but by the intra-uterine or neonatal environment, are critically important.

This review will focus on the modulation of the prenatal (intra-uterine) and neonatal environment that occurs in response to particular features of the maternal diet and hormonal status that appear to programme an increased risk of subsequent development of certain non-communicable metabolic diseases, in particular dysregulation of glucose metabolism and diabetes mellitus. We emphasize studies using animal models that have proved useful to explore the molecular mechanisms that underlie both the programming events themselves and the metabolic consequences of such programming events (see Table 1).

## METABOLIC PROGRAMMING

### Epidemiological evidence for programming of metabolic disorders

A geographical correlation has been identified between prevalence of, and mortality from, adult cardiovascular disease and early growth (as reflected by birthweight and early postnatal growth) [3–5]. A graded, inverse relationship was observed between birthweight (and weight at 1 year) and death rates from ischaemic heart disease in adult men [3]. Thus, at 1 year of age a weight of 8.2 kg compared with 12.3 kg corresponded to a 3-fold greater death rate from ischaemic heart disease [3]. Similarly, men and women who were smallest at birth had the highest blood pressure in adulthood [6]. The associations between hypertension and Type 2 diabetes, linked possibly by insulin resistance [7,8], led to a subsequent examination of the possible connection between fetal growth and glucose tolerance. It was found that the risk of developing glucose intolerance and the insulin resistance syndrome (IRS) in later life was 2-fold greater among men who weighed less than 8.2 kg at 1 year of age than those who weighed 12.3 kg or more [9]. This study also showed a link between reduced early growth and 32,33-split proinsulin concentrations, indicating that pancreatic $\beta$-cell function might be impaired. The term 'programming' was employed to define collectively the molecular mechanisms underlying the impact of early interventions on an individual's susceptibility to later metabolic diseases.

Abbreviations used: GLUT4, insulin-regulatable glucose transporter; GK, glucokinase; HPA, hypothalamic–pituitary–adrenal; 11$\beta$-HSD, 11$\beta$-hydroxysteroid dehydrogenase; IGF, insulin-like growth factor; IRS, insulin resistance syndrome; PDE, phosphodiesterase; PEPCK, phospho*enol*pyruvate carboxykinase; PI 3-kinase, phosphatidylinositol 3-kinase.

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**Table 1 Summary of the effects of maternal protein restriction or exposure to excess maternal glucocorticoids on glucose and lipid metabolism in the adult offspring**

| Effect | Reference(s) |
|---|---|
| Decreased whole-body glucose disposal in the post-absorptive state in 3-month-old female offspring | [38] |
| Enhanced whole-body glucose disposal during euglycaemic-hyperinsulinaemia in 3-month-old female offspring | [38] |
| Enhanced glucose clearance after intraperitoneal glucose administration in 6-week-old male offspring | [126] |
| Enhanced rates of glucose disappearance after intravenous glucose challenge in 3-month-old female offspring | [38] |
| Glucose intolerance after oral glucose challenge | [117] |
| Fasting hyperglycaemia, reactive hyperglycaemia and hyperinsulinaemia in adulthood | [116,117] |
| Stimulation of hepatic glucose production by insulin in perfused livers | [122] |
| Decreased hepatic glucokinase activity | [122] |
| Increased hepatic PEPCK activity | [117,122] |
| Altered hepatic zonation with loss of perivenous cells | [45] |
| Altered ketone-body production and/or utilization | [124] |
| Decreased skeletal-muscle glucose utilization in the post-absorptive state, but not during hyperinsulinaemia | [38] |
| Increased insulin receptor number and GLUT4 glucose transporters in skeletal-muscle plasma membranes | [127] |
| Impaired suppression of isoprenaline-stimulated rates of lipolysis by insulin in isolated adipocytes | [128] |
| Increased insulin receptors in isolated adipocytes | [97,126] |
| Increased basal and insulin-stimulated insulin-receptor-substrate-1-associated PI 3-kinase activity and Akt/protein kinase B activity in adipocytes | [97,128] |

### Programming of metabolic disorders: the 'thrifty phenotype' hypothesis

On the basis of epidemiological studies, the concept was developed that the early life environment influences both growth of the fetus and the subsequent development of adult disease. The 'thrifty phenotype' hypothesis [10] specifically proposed that those of low birthweight are characterized by early adaptations to a potentially adverse intra-uterine environment that optimize the use of a restricted nutrient supply to ensure survival but, by favouring the development of certain organs over that of others, lead to persistent alterations in the physiology and metabolism of developing tissues. There are critical specific and restricted periods during development, often coincident with periods of rapid cell division, during which individual tissues and organs differentiate and mature in preparation for survival after birth. Either a stimulus or insult during such critical periods may have long-lasting consequences on tissue or organ function postnatally. In particular, impaired growth during these periods caused by maternal malnutrition is thought to result in an irrecoverable deficit in cell number, the tissue affected being dependent on the timing of the insult [11,12].

### Programming of Type 2 diabetes mellitus

In the case of Type 2 diabetes, the less favourable early-life adaptations to adverse environmental influences may include altered structure and function of the endocrine pancreas and/or insulin-sensitive target tissues that persist into adult life and predispose to the development of the disease (Figure 1). Insulin-sensitivity (the ability of insulin to evoke responses in its target tissues, including liver, muscle and adipose tissue) varies between individuals. In some individuals, an impaired ability of insulin to maintain glucose homoeostasis can be compensated for by enhanced insulin secretion by pancreatic $\beta$-cells. Thus about one quarter of individuals with normal glucose tolerance are insulin-resistant to the same extent as those with Type 2 diabetes, but can compensate for this with enhanced insulin secretion [7,8]. In others, insulin secretion is inadequate to compensate for insulin resistance, and glucose intolerance develops. Several studies have suggested that the connection between poor early growth and Type 2 diabetes may take the form of impaired pancreatic $\beta$-cell function [9,13], while others support the concept that the link exists due to impaired insulin action [14]. Abnormal early growth, as indicated by thinness at birth, has been shown to predict impaired insulin action in adult men and women [14–16], the greatest insulin resistance being observed in the individuals who were thin at birth but obese as adults [14]. There is thus increasing evidence for a link between poor fetal and infant growth and development and glucose intolerance and features of the IRS in adulthood. Since the original 'thrifty phenotype' postulate, links have been demonstrated to exist between low birthweight and deficient insulin release and/or the IRS in several populations, including men and women from the U.K. [17–19], the U.S.A. [20–22] and Sweden [23,24], and other epigenetic influences have been identified, including maternal diabetes during pregnancy and neonatal hyperinsulinization [25,26].

## FACTORS INFLUENCING FETAL GROWTH AND DEVELOPMENT

### Impact of protein restriction on intra-uterine growth and development

Although many factors may influence fetal growth [27], maternal hormonal and nutritional status may be particularly important [28,29]. A high carbohydrate intake in early pregnancy and low protein intake later in pregnancy are both associated with small placentae [30], low birthweight [30] and thinness at birth [31] in human infants. In humans, undernutrition early in pregnancy has been suggested to produce small, but normally proportioned, babies, while undernutrition later in pregnancy may have lesser effects on weight, but profound effects on the proportions of the baby [32]. Birthweight in humans is positively related to plasma glucose levels in normal [33] as well as in diabetic [34] pregnancies. Amino acids are also major determinants of fetal growth [34,35], indicated by the observation that the concentrations of certain fetal plasma amino acids are reduced in small-for-gestational-age

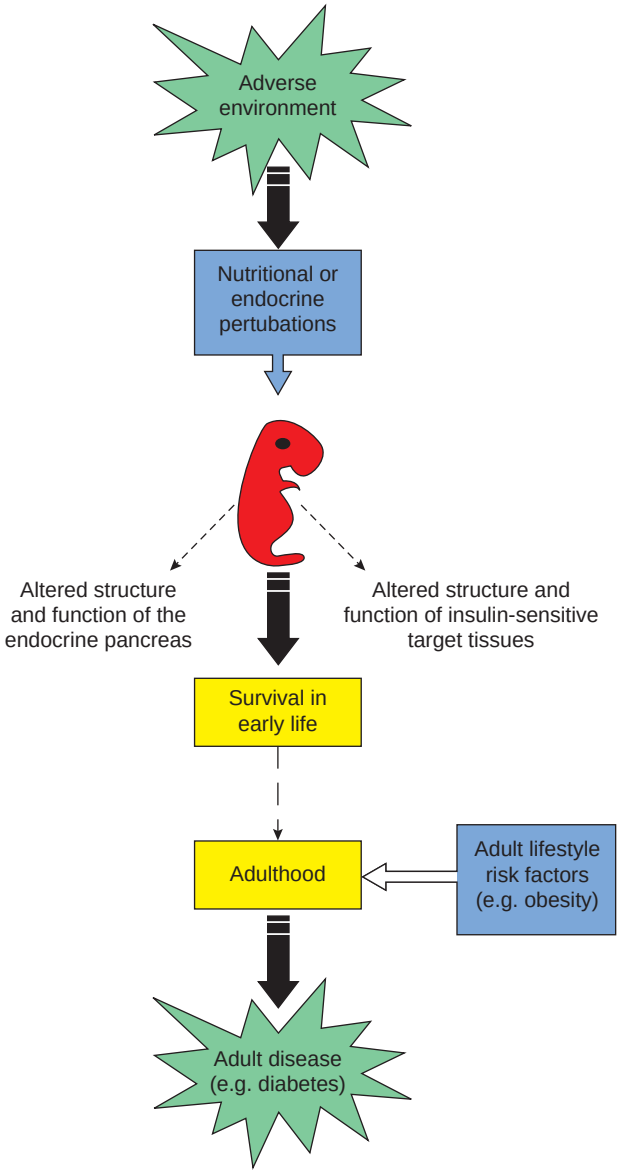


**Figure 1 Schematic representation of the influence of early-life events on the progression to adult-onset metabolic disease**

infants [36]. In particular, the essential amino acids are prerequisite for normal fetal growth.

In the rat, relatively modest maternal dietary protein restriction throughout pregnancy, in the absence of a decline in energy intake, has been reported to cause both altered fuel homoeostasis and hypertension in the adult offspring [37–46]. Offspring from rats malnourished during pregnancy and lactation (either by reducing the intake of normal diet, or by feeding a diet low in protein) remain smaller than normal, even after a period of recuperation [38,39,44]. In offspring from protein-malnourished rats, this reduction in body size is not associated with a linear reduction in the weight of internal organs – the relative weights of the pancreas, muscle and liver at weaning are reduced, whereas the brain and lungs are relatively protected [47]. This pattern is consistent with channelling of nutrients to the essential organs, although the mechanisms behind this remain unclear.

## Impact of excessive exposure to glucocorticoids on intra-uterine growth and development

A current theory is that metabolic programming may arise from defects in the materno-placental support of the fetus. A key factor may be the control exerted by the placenta on the influence of the maternal glucocorticoid environment on the fetus [48]. Indeed, some nutritional influences – including maternal protein restriction – may operate through this mechanism [46]. The fetal adrenal gland becomes active in late gestation [49], but the developing fetus is normally protected from excess exposure to glucocorticoids of maternal origin by the presence in the placenta of the type 2 isoform of 11$\beta$-hydroxysteroid dehydrogenase (11$\beta$-HSD2) [50]. 11$\beta$-HSD2 converts the active, receptor-binding physiological glucocorticoids (cortisol in humans, corticosterone in rodents) to inert 11-oxo derivatives (cortisone or 11-dehydrocorticosterone respectively). *In situ* hybridization has shown that this enzyme is highly expressed in the syncytiotrophoblast of the rat [51], and it is believed to maintain a gradient of cortisol from the maternal to the fetal circulation. The very high affinity of the enzyme for its substrate is believed to ensure that fetal tissues are fully protected from fluctuations in maternal glucocorticoid status through inactivating maternal cortisol or corticosterone. In late gestation, when the fetal adrenal gland itself becomes active, separation from maternal adrenal influences is particularly important to enable independent function of the fetal hypothalamic–pituitary–adrenal (HPA) axis [52]. The immediately pre-partum glucocorticoid surge is a signal for maturation of organ systems crucial for the transition from intra- to extra-uterine life. The glucocorticoids promote the differentiation and maturation of key tissues (including lung, gut, liver and brain) (see, e.g., [53,54]; reviewed in [55]). Intracellular glucocorticoid receptors are expressed in most fetal tissues from mid-gestation onwards [56–58], including the major systems and tissues – including liver and adipose tissue – whose structure and function in adult life is affected by early-life programming events [59]. Raising glucocorticoid levels in experimental animals earlier in gestation has been shown to accelerate the maturation of those tissues essential for survival immediately at birth, and also organs that are involved in the more long-term adaptation to extra-uterine life [60]. This effect has been exploited therapeutically in that synthetic glucocorticoids are administered to women in threatened premature labour to improve neonatal viability. Although the immediate outcome is beneficial – there is an improvement in neonatal mortality and morbidity – birthweight is reduced by maternal glucocorticoid treatment in humans and in animal models [61–64]. Levels of corticotrophin-releasing factor and cortisol levels are increased in growth-retarded human fetuses [65]. Growth retardation due to increased exposure to glucocorticoids during early development in part reflects a stimulation of tissue differentiation at the expense of proliferation. The resultant permanent alteration in cell number and function has potentially adverse consequences in later life.

The activity of 11$\beta$-HSD in human and rat placentae is found to be inversely proportional to placental size and proportional to fetal weight [48,66]. Furthermore, patients bearing mutations of the gene encoding 11$\beta$-HSD2 have low birthweights [67]. These observations have led to the suggestion that impaired protection by the placenta may result in overexposure of the fetus to maternal glucocorticoids, which is manifested as growth retardation and later disease. In support of this, inhibition of placental 11$\beta$-HSD2 in rat dams by the specific inhibitor carbenoxolone reduces birthweight and leads to impaired glucose tolerance in maturity [68]. These effects are dependent on the resence of maternal adrenals, suggesting that they result directly

from increased exposure to maternal glucocorticoids as a consequence of inhibition of placental 11$\beta$-HSD2. Furthermore, the ratio of radiolabelled corticosterone to 11-dehydrocorticosterone in fetuses of carbenoxolone-treated mothers infused with radiolabelled corticosterone is increased [68], indicative of a reduction in the placental glucocorticoid barrier. Maternal protein deficiency in the rat also reduces the activity of 11$\beta$-HSD2 [46]. Thus increased prenatal exposure to excessive amounts of glucocorticoids may provide one link between fetal nutrient environment and adult metabolism. It may be relevant that dexamethasone (a synthetic glucocorticoid) inhibits glucose-stimulated insulin secretion by pancreatic islets [69], confirming the endocrine pancreas as a target for glucocorticoid action.

The studies reported above clearly demonstrate that perturbations in the nutritional and/or hormonal status of the mother can have a serious impact on the growth and development of the fetus. There is evidence that available nutrients are directed to essential organs at the expense of others to ensure survival, and the severity of the impact of an adverse environment may be critically dependent on its timing during fetal and neonatal development.

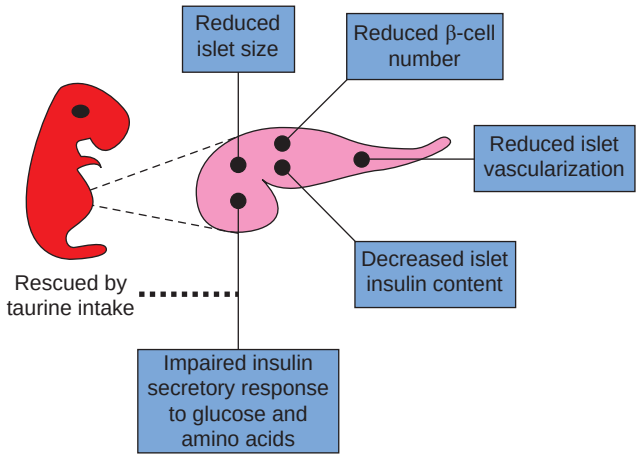


**Figure 2 The influence of low-protein feeding during gestation on the structural and functional development of the fetal endocrine pancreas**

## PROGRAMMING OF PANCREATIC $\beta$-CELL STRUCTURE AND FUNCTION

### Pancreatic $\beta$-cell structure and function

As maturation of pancreatic $\beta$-cell function takes place during early development, the pancreatic $\beta$ cells may be particularly susceptible to the effects of poor maternal nutrition. In humans, glucose-responsiveness appears in the first to second trimester, but the typical adult biphasic secretory response does not appear until after birth [70,71]. In rats, the insulin secretory response to glucose appears in the last 4–5 days of gestation (term = 22–23 days) [72,73]. Glucose promotes maturation of glucose-stimulated insulin secretion in rat islets in culture [74], raising the possibility that malnutrition may retard islet functional maturation. Most islet growth (in both humans and rats) also takes place during the fetal-neonatal period. The cell cycle of proliferative pancreatic $\beta$-cells is believed to be regulated through the recruitment of cells into a proliferative compartment [75,76]. The proliferation of islet cells is most rapid between 20 and 22 days of gestation in the rat (term is 22–23 days) [77] and pancreatic $\beta$-cell mass increases by 100% in the 48 h before birth [78]. Pancreatic $\beta$-cell replication is also higher during late gestation and the neonatal period than after weaning [79]. Thus the regulation of pancreatic $\beta$-cell replication is such that some 10% of $\beta$-cells may enter the proliferative compartment in fetal rats, compared with less than 3% in young adult rats [80]. Recruitment for proliferation is controlled by nutrient supply, and is therefore likely to be sensitive to maternal malnutrition.

In humans, growth *in utero* correlates with fetal insulin levels [81]. Small-for-gestational-age infants have reduced plasma insulin concentrations [82] and pancreatic $\beta$-cell numbers [81]. In humans, a high carbohydrate intake early in pregnancy and a low-protein intake late in pregnancy (associated with reduced birthweight) is associated with reduced concentrations of insulin, split proinsulin and C-peptide in umbilical-cord plasma [83], indicating reduced fetal $\beta$-cell function. Insulin has an important growth-supporting activity on fetal rat tissue (skeletal and connective tissues) in a transplant system (paws from 15-day-old fetal rats transplanted under the kidney capsule of 1-month-old syngeneic hosts) [84], and the injection of streptozotocin to specifically destroy pancreatic $\beta$-cells in lamb fetuses causes fetal hypoinsulinaemia and a 20% reduction in body weight [85]. In culture, the differentiation and multiplication of the pancreatic $\beta$-cells is increased to a greater extent by an increase in an essential amino acid than by an increase in glucose concentration [72]. Furthermore, amino acid stimulation of replication of rat islet cells [72,86] is effective in fetal pancreatic $\beta$-cells before glucose-responsiveness develops in late gestation [87]. Thus the phenotype of reduced birthweight (or otherwise impaired *in utero* growth) secondary to maternal protein restriction may reflect reduced stimulation of pancreatic $\beta$-cell replication and/or maturation.

### Programming of pancreatic $\beta$-cell structure and function by protein malnutrition

A specific limitation of protein in a well-tolerated isocaloric diet (which prevents any constraint on total energy intake) has been widely studied within the context of the functional development of the endocrine pancreas in rats (Figure 2). Studies of fetuses of rat dams fed a diet containing $\approx$ 40% of the normal amount of protein throughout gestation demonstrate that fetal pancreatic $\beta$-cell function is affected, with a 50% suppression of insulin secretion [88]. The functional changes are associated with abnormal islet-cell proliferation, islet-cell size, pancreatic insulin content and islet vascularization, all of which are reduced at birth [44,89]. The postnatal development of the pancreas is also modified if the dam continues to be maintained on a low-protein diet during lactation. Morphometric analyses demonstrate a reduction in $\beta$-cell proliferation and islet size in the head of the pancreas (and to a lesser extent in the pancreatic tail, a glucagon-rich and pancreatic-polypeptide-poor region) in neonatal rats whose mothers have been subjected to protein restriction [89]. Islet vascularization in the neonatal animal is also dramatically reduced [89].

In rats, protein malnutrition affects maternal plasma amino acid concentrations [90] and, in pregnancy, this impairs placental transfer of amino acids to the fetus [91]. The plasma amino acid profile of not only the mother, but also the fetus, is modified by the provision of an isocaloric 8% protein diet during gestation [88]. Although the total essential and non-essential amino acid concentration is unchanged, there are specific reductions in the plasma concentrations of $\alpha$-aminobutyric acid, phosphoserine, valine and taurine. Taurine is an indispensable amino acid during fetal development in rats, cats and baboons [92]. Taurine functions as an insulin secretory stimulus for fetal islets and also

enhances insulin secretion in response to other insulin secretagogues [93]. Islets of fetuses from rat dams maintained on a low-protein diet do not secrete insulin in response to taurine, nor does taurine restore a normal insulin secretory response to other secretagogues. However, insulin secretion can be rescued by the inclusion of supplementary taurine in the drinking water [93]. Thus current observations identify taurine as a necessary amino acid for the normal functional development of the fetal pancreatic $\beta$-cells.

### Role for apoptosis in islet remodelling during early postnatal development

Decreased replication and an increased incidence of apoptosis in pancreatic $\beta$ cells is observed between 1 and 2 weeks of postnatal life in normal rats, and it has been suggested that apoptosis may be an important mechanism in remodelling of the $\beta$-cell mass during early postnatal development [94]. Circumstantial evidence links the insulin-like-growth-factor (IGF) axis to the regulation of apoptosis *in vivo* in pancreatic islets during this period. The timing of the transient wave of apoptosis observed after 1–2 weeks of postnatal life in the rat is associated with a loss of expression of IGF-II in the pancreatic islets [95] (at this time, the pancreatic expression of IGF-I has not yet reached adult values [96]). Furthermore, overexpression of IGF-II in transgenic mice is associated with pancreatic-islet-cell hyperplasia and reduced attrition of islet cells by apoptosis in late gestation [97]. The number of apoptotic cells in islets from protein-restricted animals is increased at each fetal and postnatal day analysed, while the number of cells positive for IGF-II is decreased [67]. Thus it is possible that poor nutrition in early life may programme the insulin-secretory capacity of the pancreas by influencing islet remodelling at the level of apoptosis through a mechanism linked to modulation of the IGF axis.

### Impact of protein restriction on insulin secretion in adulthood

While channelling of nutrients may represent a survival advantage during malnutrition, impaired pancreatic $\beta$-cell development may cause a lasting reduction in the insulin-secretory response. In turn, this could lead to an impaired insulin-secretory response to glucose in adulthood [98], a risk factor for the development of diabetes [99], particularly if the ability of the pancreatic $\beta$-cells to undergo compensatory replication during a nutritional challenge is reduced. The limited capacity of pancreatic $\beta$-cells to regenerate after the first few years of life means that such an affected individual is left with a suboptimal complement of pancreatic $\beta$-cells. Provided this individual remains thin and relatively insulin-sensitive, normal glucose homoeostasis can be maintained; however, insulin resistance consequent on advancing age, obesity or pregnancy may render this pancreatic $\beta$-cell insufficiency manifest as glucose intolerance and, in the worst instance, Type 2 diabetes.

Maternal malnutrition during pregnancy and lactation in the rat has lasting effects on glucose homoeostatic systems. Adult rats previously exposed to protein restriction are characterized by decreased insulin content of isolated pancreatic islets [100,101], an impaired secretory response of isolated pancreatic islets to glucose and arginine [100,101] and moderately impaired glucose-stimulated insulin secretion *in vivo* [38]. A multifactorial perturbation of nutrient metabolism in the pancreatic islets of protein-restricted rats may account for the decreased insulin content and secretory response to glucose and amino acids [100]. One metabolic anomaly suggested to account, at least in part, for the impairment of insulin release elicited by protein restriction is an imbalance between oxidative and anaerobic glycolysis in the islets of protein-restricted rats. This coincides with a decreased circulation in the glycerol phosphate shuttle, and is probably attributable to the deficiency of mitochondrial FAD-linked glycerophosphate dehydrogenase previously documented in islet homogenates of the protein-restricted rats [102]. Islet blood-vessel density [88], as well as pancreatic and islet blood flow [103], are diminished as a consequence of early protein restriction. A more severe maternal protein restriction (5%) during both pregnancy and lactation causes demonstrable impairments in glucose-stimulated insulin secretion (*in vitro*) in adult offspring fed a normal diet from weaning, and this is worsened when the diet is changed to one designed to cause insulin resistance [104].

## PROGRAMMING OF GLUCOSE METABOLISM AND INSULIN ACTION IN ADULTHOOD

### Mechanisms underlying the programming of liver development and function in response to excessive exposure to glucocorticoids

In the fetal liver, cortisol increases the synthesis of a range of proteins, including all of the key gluconeogenic enzymes [105,106]. At the same time, the expression of other proteins – including angiotensinogen, IGF-II and certain of the IGF-binding proteins – is suppressed [107–109]. In addition, cortisol increases the deposition of glycogen in the fetal liver and other tissues, including heart and skeletal muscle [105,106,110,111]. These adaptations are predicted to facilitate the maintenance of blood glucose levels immediately after delivery. Enzymes induced in the final stages of liver development [112] have been grouped into three clusters: the late-fetal cluster, the neonatal cluster and the late-suckling cluster. The glucocorticoids are particularly important for the induction of the late-fetal and -suckling clusters [113,114], although studies of transgenic mice with a glucocorticoid-responsive unit fused to a member of the neonatal cluster demonstrate that glucocorticoids alone are unable to induce neonatal gene expression [115]. It therefore appears that the prenatal glucocorticoid surge 'primes' the liver for the induction of the neonatal cluster in response to a further stimulus.

The synthetic glucocorticoid dexamethasone is a poor substrate for placental 11$\beta$-HSD2. The administration of dexamethasone during the last third of pregnancy in the rat impairs fetal growth, while the offspring exhibit fasting hyperglycaemia, reactive hyperglycaemia and hyperinsulinaemia in adulthood [116,117]. The gluconeogenic enzyme phospho*enol*pyruvate carboxykinase (PEPCK) is an important target gene under potent glucocorticoid regulation [118]. Over-expression of PEPCK in a rat hepatoma cell line impairs suppression of gluconeogenesis by insulin [119] and transgenic mice with over-expression of hepatic PEPCK have impaired glucose tolerance [120]. Changes in glucose homoeostasis seen in adult offspring of dexamethasone-treated dams are associated with increased hepatic PEPCK expression [117], raising the possibility that increases in PEPCK activity elicited by early glucocorticoid exposure contribute to the programmed changes in the ability of insulin to regulate hepatic glucose production.

### Effect of maternal protein malnutrition on hepatic metabolism in adulthood

Liver perfusion studies in adult rats exposed to protein restriction in early life indicated that hepatic glucose production is favoured [43]. This response is observed concomitantly with decreased hepatic glucagon receptor number, together with decreased hepatic glucokinase (GK) and increased hepatic PEPCK activity [122]. On the basis of studies of marker enzymes

it has been suggested that these effects may be a consequence of altered zonation of the liver favouring expansion of periportal and contraction of perivenous liver cell populations [45,123]. Adult rats exposed to protein restriction in early life also exhibit significantly lower plasma $\beta$-hydroxybutyrate concentrations both in the fed state and after 24 and 48 h starvation [124]. Although this latter study did not determine whether low plasma $\beta$-hydroxybutyrate concentrations were the consequence of decreased ketone-body production or increased ketone-body utilization, it was suggested that glucagon resistance in the liver of early-protein-restricted rats may be a contributing factor [124]. If this is the case, it can be concluded that the up-regulation of PEPCK activity is unlikely to be a consequence of hypersensitivity to glucagon.

### Effects of early protein restriction on peripheral glucose metabolism and insulin action in adulthood

The skeletal-muscle mass is a major site at which insulin action is impaired in insulin-resistant individuals [125]. Offspring of rats subjected to moderate protein restriction during gestation and lactation exhibit decreased glucose turnover and glucose utilization by oxidative skeletal muscles *in vivo* in the post-absorptive state [38]. These observations probably reflect an increased reliance on lipid rather than glucose as an energy fuel. The observations of lower plasma $\beta$-hydroxybutyrate concentrations in early-protein-restricted rats [124] would be consistent with increased lipid fuel utilization. The finding of suppressed glucose turnover at the low insulin concentrations that pertain in the post-absorptive state also suggests that an inability to respond to modest (submaximal) increases in insulin might trigger a transition to overt diabetes. However, offspring exposed to protein restriction in early life exhibit enhanced rates of glucose disappearance after intravenous [38] or intraperitoneal [126] glucose challenge in early adulthood. Furthermore, there is a greater increment in the stimulation of whole-body glucose clearance in response to euglycaemic hyperinsulinaemia in protein-restricted offspring at this time point [38]. The enhanced sensitivity of whole-body glucose disposal to euglycaemic hyperinsulinemia in protein-restricted rats is associated with increased *in vivo* glucose utilization by insulin-sensitive peripheral tissues (muscle and adipose tissue) [38]. Early-protein-restricted offspring have also been demonstrated to exhibit an increased insulin receptor number and an increased number of glucose transporters (GLUT4) in the plasma membrane [127], both of which would be consistent with increased insulin-stimulated glucose uptake.

Adipocytes isolated from early-protein-restricted rats have increased insulin-receptor levels, increased basal and insulin-stimulated levels of insulin-receptor-substrate-1-associated phosphatidylinositol 3-kinase (PI 3-kinase) activities and increased Akt/protein kinase B activities [97,126,128]. These alterations in insulin signalling are associated with increased basal and insulin-stimulated glucose uptake by adipocytes *in vitro* [97] and *in vivo* during euglycaemic hyperinsulinaemia [38]. Adipocytes isolated from early-protein-restricted rats also exhibit an impaired ability of insulin to suppress isoprenaline-stimulated rates of lipolysis [128], an effect most likely due to insensitivity to insulin in the low physiological range [41]. Adipocytes from early-protein-restricted rats have relatively low levels of the p110$\beta$ catalytic subunit of PI 3-kinase, whereas levels of p110$\alpha$ are unaffected [128]. It has therefore been suggested [128] that the molecular mechanism underlying the disparate acute effects of insulin on glucose uptake and lipolysis may reflect selective activation of the isoforms of the catalytic subunit of PI 3-kinase. Insulin and noradrenaline have a common target, namely a specific phosphodiesterase (PDE), PDE3B. PDE3B is phosphorylated in response to both insulin and isoprenaline (which, like noradrenaline, stimulates lipolysis through activation of cAMP-dependent protein kinase) [129,130]. The effect of adrenaline is perceived to represent a feedback mechanism to prevent excessive lipolysis. Activation of PDE by insulin has recently been demonstrated to occur through Akt [129,131,132]. Thus evidence to date suggests that Akt may occupy a pivotal role co-ordinating the effects of insulin on lipolysis. Since Akt activity is increased in early-protein-restricted rats, enhanced noradrenaline-stimulated lipolysis implies that a component downstream from Akt – possibly PDE3B – is negatively affected.

In summary, early protein restriction elicits a number of disparate effects on glucose metabolism and insulin action, including altered regulation of hepatic glucose production and impaired suppression of adipose-tissue lipolysis, that are consistent with a state of insulin resistance. However, it would appear that insulin-stimulation of whole-body glucose disposal is unaffected or enhanced in early-protein-restricted offspring in early adulthood, probably as a consequence of enhanced insulin action in skeletal muscles.

### Influence of lifestyle factors on glucose homoeostasis in programmed animals

Although glucose tolerance in early adulthood appears to be unimpaired by exposure to protein restriction during fetal and early postnatal development, the possibility nevertheless exists that long-term perturbations introduced by early protein restriction may impair the ability to respond to pathophysiological challenges associated with the development of insulin resistance. Aging in early-protein-restricted rats is associated with worsening of glucose tolerance [133]. Interestingly, this response appears to be sex-dependent. Glucose intolerance is associated with impaired insulin secretion in female offspring, but is associated with greater insulin resistance in male offspring [134]. Antecedent protein restriction also accelerates and augments the development of impaired glucoregulation and insulin resistance after the provision of a diet high in saturated fat [40]. This latter effect is observed in conjunction with a specific impairment of insulin's anti-lipolytic response in isolated adipocytes [40]. A further persistent effect of early protein restriction is suppression of whole-body glucose turnover in the post-absorptive state during late pregnancy, a state associated with insulin resistance [37]. This effect is a consequence of reduced glucose utilization (transport/phosphorylation) both by maternal tissues (including fast-twitch skeletal muscle and adipose tissue) and by the fetus itself. However, there is no overall impairment in insulin's ability to stimulate maternal glucose disposal as a consequence of early protein restriction [37]. This finding may indicate that the hormones of pregnancy offer protection against pancreatic $\beta$-cell malfunction and/or promote recuperative growth of the endocrine pancreas, and highlight the capacity of the endocrine pancreas for recuperative growth.

## CONCLUSIONS

In the present review we have highlighted a number of interventions during fetal and neonatal life that elicit persistent effects on both insulin secretion and action and that may, under certain circumstances, lead to the development of glucose intolerance and Type 2 diabetes. It is clear from studies to date that the nature and timing of insults to the fetus and neonate may be critical factors determining the long-term changes in the structure and function of various tissues and organs. In addition, it is

becoming apparent that, although early-life interventions may programme deleterious changes in the individual, it is likely that these changes do not manifest themselves in the form of adult-onset diseases unless subsequent events in adulthood exacerbate the defect. However, possible biochemical and molecular mechanisms of prenatal programming are of considerable interest, both for scientific understanding and to help develop strategies to minimize the occurrence of metabolic diseases (such as diabetes mellitus) in adulthood, and this area is clearly one that will elicit a great deal of future interest. Specific areas for future research include the influence of glucocorticoids on tissue development, the potential role for apoptosis in islet remodelling during early postnatal development which could impair the insulin secretory capacity of the pancreas, and the influence of early-life interventions of the intracellular signalling in the adipocytes (e.g. the control of PDE3B) which could alter control of adipocyte lipolysis and favour lipid utilization in programmed offspring, leading to impaired peripheral glucose metabolism and Type 2 diabetes.

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