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The impact of dietary protein restriction on insulin secretion and action

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The goal of this review is to develop the hypothesis, and review the evidence, that protein restriction, through synergistic effects on multiple organ systems predisposes to loss of normal regulation of fuel homeostasis that plays the central role in the development of type 2 (non-insulin-dependent) diabetes mellitus. The ability of insulin to regulate glucose production and disposal varies between individuals. These differences, together with the various compensatory mechanisms that are invoked to attempt to normalize fuel homeostasis, are of fundamental importance in the development and clinical course of type 2 diabetes mellitus. Protein deprivation impacts on both insulin secretion and insulin action. These effects may persist even when a diet containing adequate protein is presented subsequently. Data are presented that suggest that protein restriction results in an impaired ability of pancreatic β-cells to compensate adequately for the defect in insulin action in insulin-resistant individuals. This persistent impairment of insulin secretion resulting from protein restriction predisposes to loss of glucoregulatory control and impaired insulin action after the subsequent imposition of a diabetogenic challenge. This inability to maintain the degree of compensatory hyperinsulinaemia necessary to prevent loss of glucose tolerance may have relevance to the increased incidence of diabetes on changing from a nutritionally-poor diet to a Western diet, and to the hypothesis that some cases of type 2 diabetes in adulthood may be related to poor early nutrition.

Protein restriction: Fetal programming: Non-insulin-dependent diabetes mellitus: Insulin resistance

Malnutrition-related syndromes of diabetes mellitus have been identified as widespread in developing countries (World Health Organization, 1985). Glucose intolerance is characteristic of protein–energy malnutrition, particularly in severely-protein-deficient children (Bowie, 1964; Milner, 1971; Becker, 1983; Rao, 1988). Impaired glucose tolerance in response to dietary protein deficiency has been proposed to arise as a consequence of impaired pancreatic β-cell function (Rao, 1988), and numerous studies have demonstrated that protein–energy malnutrition leads to an impaired insulin secretory response of the pancreatic β-cell (Becker *et al*. 1971; Levine *et al*. 1983; Dollet *et al*. 1985; Okitolonda *et al*. 1987; Swenne *et al*. 1987, 1992; Picarel-Blanchot *et al*. 1995). The study of the metabolic mechanisms underlying changes in insulin secretion and action evoked by protein restriction are thus of great practical importance.

There has been much interest in the proposition that altered nutrition during early life can influence the normal metabolic control mechanisms in adulthood. Epidemiological studies have identified an association between low birth weight and weight at age 1 year and the subsequent development of type 2 (non-insulin-dependent) diabetes (Hales *et al*. 1991). There is also an association between the increased incidence of the insulin resistance syndrome (syndrome X) and decreasing birth weight (Barker *et al*. 1993). It has been proposed that an adverse intrauterine environment 'programmes' the development of fetal organs, producing permanent changes in tissue responses that cause or predispose to impaired insulin action (Hales & Barker, 1992). As small-for-gestational age babies are born to mothers whose diets during pregnancy are energy or protein deficient (Habicht *et al*. 1974), the possible relationships

Abbreviation: TAG, triacylglycerol.

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between protein deficiency during intrauterine growth and development, and changes in insulin secretion and action in later life has been explored using a rat model, with a view to elucidating the mechanisms by which changes are induced. Again, precise knowledge of the mechanisms underlying fetal programming is important, since this information may allow the development of focused preventative and therapeutic measures.

The aim of the present review is to discuss the interplay between impaired insulin secretion and altered insulin action introduced as a consequence of inadequate dietary protein either during early development or in adulthood, drawing analogies with the aetiology of type 2 diabetes, where the deterioration of glucose tolerance depends on the magnitude of the extent to which enhanced insulin secretion can compensate for the defect in insulin action (Reaven, 1995*a*,*b*). The review also describes the implications of protein restriction to the maternal adaptations to pregnancy within the context of impaired fetal growth.

The maternal response to dietary protein restriction during pregnancy

The potential influence of the widespread protein malnutrition existing in developing countries on the health of the mother and fetus during pregnancy has prompted studies examining the maternal and fetal responses to protein restriction during gestation. Several studies have utilized a rat model involving the administration of diet containing just under half (40 %) the standard amount of protein, but normal energy density. The administration of a low-protein (80 g/kg) diet throughout pregnancy in rats (term 22–23 d) does not affect maternal energy intake and body-weight gain or fertility (as assessed by fetal number at term; Sugden & Holness, 1995). Placental weight during pregnancy is also unaffected, although fetal growth is retarded (Dahri *et al*. 1991; Sugden & Holness, 1995) and development of the fetal endocrine pancreas is impaired (Dahri *et al*. 1991).

Moderate protein restriction throughout pregnancy profoundly influences maternal homeostatic mechanisms in late gestation (Sugden & Holness, 1995). Abnormalities in maternal fuel homeostasis introduced as a consequence of moderate protein restriction include dysregulation of insulin's actions to promote glucose disposal and suppress lipid mobilization. Rates of endogenous glucose production and glucose utilization by maternal skeletal muscles in the post-absorptive state are not significantly affected by the reduction in dietary protein content. However, by maintaining insulin concentrations in the high physiological range using the euglycaemic-hyperinsulinaemic clamp, it is possible to demonstrate a decrease in the glucose infusion rate required to maintain glycaemia (an index of whole-body insulin action). Maternal skeletal muscle is the major site at which peripheral glucose utilization (uptake and phosphorylation, assessed *in vivo* using the 2-deoxy[^{3}H] glucose technique) during hyperinsulinaemia is impaired by protein restriction. This effect is not trivial; the overall mean increment in muscle glucose utilization elicited by a physiological rise in insulin is almost halved as a consequence of moderate protein restriction during pregnancy. By contrast, the ability of insulin to suppress endogenous glucose production is unimpaired.

Protein-restricted dams exhibit a severely impaired anti-lipolytic response to insulin at day 19 of pregnancy (Holness *et al*. 1998). This effect, manifest in the intact animal as incomplete suppression by insulin of plasma non-esterified fatty acid concentrations, is observed in conjunction with dysregulation of insulin's anti-lipolytic action and an enhanced response of adipose tissue to lipolytic agonists. Dysregulation of adipose tissue lipolysis can be demonstrated *ex vivo* using adipocytes prepared from two distinct abdominal white adipose tissue depots (including the important mesenteric depot). Protein restriction during pregnancy also leads to relative hypertriacylglycerolaemia. This effect is observed in the post-absorptive state and during hyperinsulinaemia. Not only is whole-body triacylglycerol (TAG) turnover modified, but there is an additional effect of protein restriction to enhance TAG secretion rates (estimated *in vivo* using Triton WR 1339) in the post-absorptive state. Whereas hyperinsulinaemia (2 h) suppresses plasma TAG concentrations by two-thirds in dams maintained on a diet containing an adequate amount of protein, the same procedure fails to completely suppress plasma TAG concentrations in protein-restricted dams. The rate of decline of plasma TAG concentrations is greater in protein-restricted pregnant rats, despite the intrinsically higher rates of TAG secretion. This finding strongly implies that TAG utilization during hyperinsulinaemia is enhanced by dietary protein restriction during pregnancy. There is no evidence for altered intra-hepatic regulation of fatty acid disposal (esterification *v*. oxidation).

During pregnancy, additional dietary protein is required to subserve fetal requirements. To use dietary protein efficiently and to reduce requirements to a minimum, it is necessary to ensure that there is adequate provision of energy from non-protein sources, e.g. to spare protein-derived amino acids from use in gluconeogenesis. When dietary protein intake is limited, the increased production of non-carbohydrate energy substrates (non-esterified fatty acids and TAG) together with the substitution of lipid for glucose as a maternal energy substrate may be viewed as part of a strategy to reduce the maternal requirement for amino acids. This process optimizes the supply of amino acids to the fetus and thereby minimizes growth retardation secondary to dietary protein (amino acid) deficiency. Thus, during periods of protein deprivation, the maternal adaptation to pregnancy is modified to optimize the provision of nutrients required for fetal survival and growth (Fig. 1).

The influence of early dietary protein restriction on pancreatic development and function

Nutrition during intrauterine growth is of major importance for proper tissue development and function. Low-birth-weight infants born to diabetic and non-diabetic mothers exhibit reduced pancreatic islet cell mass, reduced pancreatic β-cell number and low insulin content in the pancreas and in cord blood (Van Assche, 1975). Experimental models based on *in utero* protein malnutrition provide evidence that the structural and functional development of the fetal endo-

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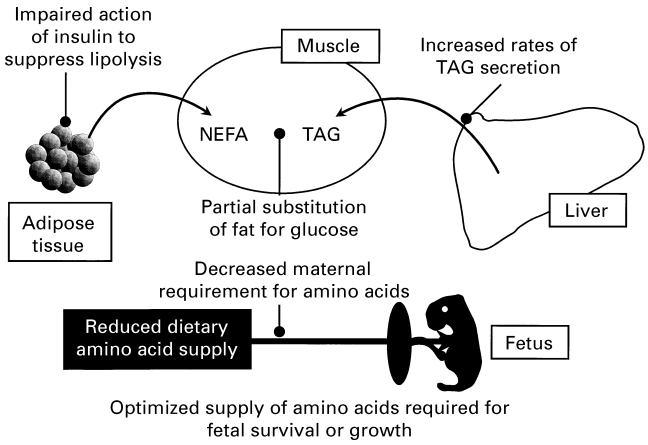

Fig. 1. Increased production of lipid substrates optimizes the fetal amino acid supply when maternal protein intake is restricted. The increased production of the non-carbohydrate energy substrates, non-esterified fatty acids (NEFA) and triacylglycerol (TAG), together with the substitution of lipid for glucose as an energy substrate for maternal muscle, reduces the maternal requirement for amino acids for use as gluconeogenic precursors. This strategy optimizes the supply of amino acids to the fetus, thereby enhancing fetal survival and minimizing the impairment in fetal growth that might be occasioned by protein restriction.

crine pancreas is severely compromised (Dahri *et al*. 1991). Reduced β-cell proliferation, islet size, islet vascularization and pancreatic insulin content are observed in 21-d-old fetuses of protein-restricted dams. In response to stimulation with amino acid secretogogues (arginine and leucine) insulin secretion by isolated islets prepared from 21-d-old fetuses of protein-restricted dams is impaired (Dahri *et al*. 1991). The potential importance of taurine during the normal development of fetal β-cell function has recently been highlighted (Cherif *et al*. 1998). Protein restriction during gestation lowers plasma taurine concentrations both in pregnant rats and in their fetuses. Direct stimulation with taurine, methionine or leucine increases insulin release from islets prepared from fetuses of dams provided with adequate dietary protein, but no response to either taurine or methionine is observed in islets prepared from fetuses of protein-restricted dams. The supplementation of drinking water with taurine throughout gestation restores the response of fetal insulin secretion to stimulation with taurine, methionine, leucine or arginine. Morphometric analyses (Snoeck *et al*. 1990) have demonstrated a reduction in β-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. Islet vascularization in the neonates is also dramatically reduced.

Adult rats exposed to protein restriction from conception to adulthood are characterized by decreased insulin content of isolated pancreatic islets (Rasschaert *et al*. 1995), an impaired secretory response of isolated pancreatic islets to glucose (Rasschaert *et al*. 1995), and impaired glucose-stimulated insulin secretion *in vivo* (Holness, 1996*a*). 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 (Rasschaert *et al*. 1995). 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 imbalance coincides with a decreased circulation in the glycerolphosphate shuttle, and is probably attributable to the deficiency of mitochondrial FAD-linked glycerol-3-phosphate dehydrogenase (*EC* 1.1.99.5) previously documented in islet homogenates of the protein-restricted rats (Sener *et al*. 1996).

Influence of dietary protein restriction on fuel homeostasis during adulthood

The blunted response of glucose-stimulated insulin secretion often observed in rats subjected to protein restriction is frequently associated with normal or enhanced glucose tolerance and insulin action (Levine *et al*. 1983; Okitolonda *et al*. 1987; Escriva *et al*. 1991; Picarel-Blanchot *et al*. 1995; Holness, 1996*b*; Wilson & Hughes, 1997). For example, protein–energy restriction (50 g protein/kg, 35 % restriction) of 4-week-old female rats for 4 weeks leads to impaired glucose-stimulated insulin secretion, but enhanced insulin-mediated glucose uptake (Picarel-Blanchot *et al*. 1995). Similarly, providing male rats with a low-protein (50 g/kg) diet from 4 to 15 weeks of age results in slightly enhanced glucose tolerance, despite a blunted insulin secretory response (Okitolonda *et al*. 1987). In a further study, providing 4-week-old rats with an isoenergetic 50 g protein/kg diet for 4 weeks enhanced their tolerance to intravenous glucose and improved insulin action to stimulate peripheral glucose uptake and suppress hepatic glucose output, while glucose-stimulated insulin secretion remained normal (Escriva *et al*. 1991). In rats provided with a 60 g protein/kg diet for 14 weeks, normal glucose tolerance (despite a failure to release insulin following intravenous glucose) has been suggested to indicate increased insulin sensitivity (Levine *et al*. 1983). The glucose disappearance rate after intravenous glucose and the glucose infusion rate required to maintain euglycaemia during a euglycaemic–hyperinsulinaemic clamp (physiological hyperinsulinaemia) are both modestly increased in adult female rats subjected to moderate protein restriction from conception to adulthood (Holness & Sugden, 1999). Thus, in general, the evidence to date suggests that protein restriction does not elicit impaired glucoregulatory control, and an enhanced action of insulin limits the deterioration of glucose tolerance when insulin secretion is impaired (see Picarel-Blanchot *et al*. 1995; Fig. 2).

The influence of dietary protein restriction on the response to a diabetogenic diet

Adult rats previously maintained on a standard diet respond to transfer to a diet containing an increased fat:carbohydrate value with dramatically enhanced glucose-stimulated insulin secretion (Holness, 1996*a*). This response, evident after 4 weeks, compensates for an impaired whole-body insulin action introduced as a result of the increased lipid content of the diet (Sugden *et al*. 1996). As a consequence, normal

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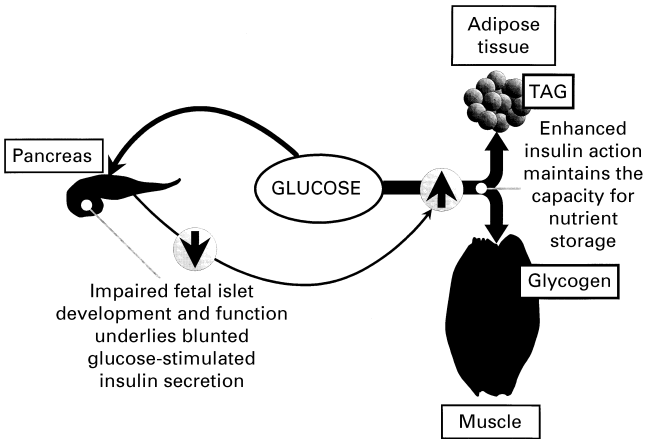

Fig. 2. Adaptations to protein restriction in early life may operate to enhance survival. Impaired fetal islet development and function may be countered by an enhanced insulin action. This process maintains the capacity for glucose storage, as well as normalizing glucose tolerance. TAG, triacylglycerol.

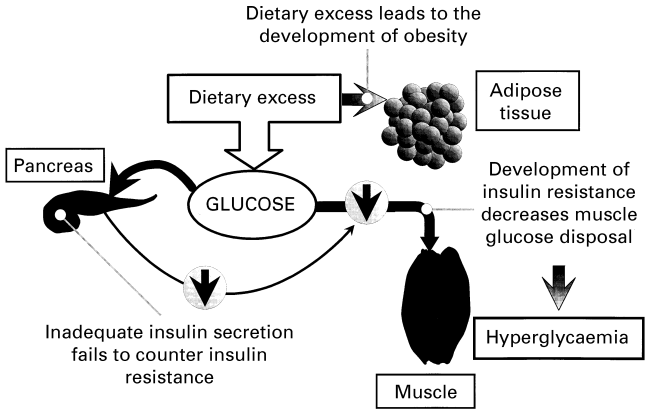

Fig. 3. Adaptations to inadequate nutrition that enhance survival in early life may increase the susceptibility to obesity and type 2 (non-insulin-dependent) diabetes as a consequence of dietary excess in adulthood. Compensatory enhancement of insulin action may assist survival in the face of blunted glucose-stimulated insulin secretion. This adaptation may be detrimental in dietary excess. Since the glycogen storage capacity is limited, excess nutrients are stored in adipose tissue, leading to obesity. The development of obesity may underlie the subsequent development of insulin resistance, impaired glucose tolerance and type 2 diabetes.

glucose tolerance is maintained (Holness, 1996*a*). This response is analogous to that seen in human subjects, where some insulin-resistant individuals exhibit an enhanced insulin secretory response to maintain normal glucose tolerance (Reaven, 1995*a*). Although numerous studies indicate that the adverse impact of protein restriction on insulin secretion is insufficient *per se* to elicit impaired glucose tolerance, the possibility exists that subsequent dietary interventions may precipitate glucose intolerance due to an inability of the compromised β-cell to respond appropriately to the increased insulin secretory demand. Recent work (Holness & Sugden, 1999) clearly demonstrates that the effect of high-fat feeding to impair insulin action is exaggerated by antecedent protein restriction. Thus, protein restriction from conception to adulthood followed by the ingestion of excessive dietary lipid act synergistically to evoke a marked impairment in peripheral insulin action in combination with a blunted insulin secretory response (Fig. 3). As a consequence, there is a greater deterioration in glucose tolerance when high-fat feeding is preceded by protein restriction. The exaggerated response to high-fat feeding observed in rats that have been subjected previously to protein restriction occurs in conjunction with a specific impairment of insulin's anti-lipolytic action in adipose tissue. We have shown previously that an increased plasma fatty acid supply leads to suppression of skeletal muscle glucose utilization *in vivo* (Holness & Sugden, 1990). It is likely, therefore, that increased mobilization and utilization of endogenous TAG stores in adipose tissue underlies the further deterioration of peripheral insulin action. This particular study has relevance to the interpretation of studies in human subjects that demonstrate an increased incidence of adult-onset diabetes on changing from a nutritionally-poor diet to a high-energy diet containing a relatively high proportion of lipid, characteristic of Western affluent societies (Cohen *et al*. 1988; Dowse *et al*. 1992; Collins *et al*. 1994).

Insulin action in adult rats subjected to protein restriction during early life (fetal development and sucking) only

Epidemiological studies have identified an association between low birth weight and weight at age 1 year and the subsequent development of type 2 diabetes mellitus (Hales *et al*. 1991) and the insulin resistance syndrome (Barker *et al*. 1993). It has been suggested that smallness at birth is related to fetal malnutrition, either arising from maternal dietary insufficiency or perhaps from defects in the materno-placental support of the fetus (Hales & Barker, 1992; Phillips, 1996; Hales, 1997). From this information, it has been proposed that those environmental events in early life that retard fetal development may have a long-term impact on health in adulthood (Hales & Barker, 1992; Hales, 1997). Less than optimum nutrition is known to retard fetal growth in human pregnancy (Lumey, 1992). Protein restriction during pregnancy and lactation in the rat has been used, therefore, as a model to investigate the influence of impaired growth in early (fetal and neonatal) life on insulin action in adulthood (Desai *et al*. 1995; Holness, 1996*b*; Holness & Sugden, 1996; Petry *et al*. 1997).

Female offspring of dams provided with an 80 g protein/kg diet during gestation and lactation were studied in adulthood, having been transferred to a diet containing the standard (200 g/kg) amount of protein at weaning (Holness, 1996*b*). These progeny exhibit decreased glucose turnover and glucose utilization by oxidative skeletal muscle in the post-absorptive state when studied at 3 months of age. This persistent effect of early (intrauterine and early neonatal) protein restriction is specific for skeletal muscles containing mainly oxidative fibres. The finding of suppressed glucose

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turnover at the low insulin concentrations that pertain in the post-absorptive state raised the possibility that an inability to respond to modest (submaximal) increases in insulin might trigger a transition to overt diabetes. However, these female rats exhibited both a normal insulin secretory response and enhanced rates of glucose disappearance after intravenous glucose challenge. Enhanced glucose clearance after intraperitoneal glucose administration is also observed in 6-week-old male offspring of dams subjected to protein restriction during pregnancy and lactation (Shepherd *et al*. 1997). During euglycaemic–hyperinsulinaemic clamps conducted with insulin concentrations in the high physiological range, insulin action to suppress endogenous glucose production was enhanced (Holness, 1996*b*). Furthermore, there was a greater increment in the stimulation of whole-body glucose clearance rate induced by insulin. Insulin-stimulated glucose utilization by peripheral tissues (including skeletal muscle and brown and white adipose tissue) was unaffected. Confirming these *in vivo* studies, basal and insulin-stimulated rates of [^{3}H]methylglucose transport by muscle preparations (pre-incubated *tibialis anterior* strips) prepared from offspring of protein-malnourished dams were similar to those of controls (Ozanne *et al*. 1996). However, membranes prepared from muscles sampled from the early protein-restricted rats showed a 2-fold increase in insulin receptors. Similarly, adipocytes isolated from 3-month-old male early protein-restricted rats exhibited a 3-fold increase in insulin receptor number compared with controls (Ozanne *et al*. 1997). Basal and insulin-stimulated insulin receptor substrate-1-associated 1-phosphatidylinositol 3-kinase (*EC* 2.7.1.137) activities were also higher in adipocytes prepared from early protein-restricted rats.

Although insulin secretion and action in young rats appear to be unimpaired by exposure to protein restriction only during early life, nevertheless the possibility exists that long-term perturbations introduced by early protein restriction could impair the ability to respond to pathophysiological challenges associated with the development of insulin resistance (e.g. ageing). For example, one study has shown inappropriately elevated blood glucose concentrations at 15 and 30 min after an intraperitoneal glucose load in aged (15-month-old) rats subjected to early protein restriction (Hales *et al*. 1996). Interestingly, this effect was limited to male offspring only, a sex-specific distinction attributed to the faster growth rates that characterize males. An alternative argument is that the pancreases from females have an intrinsically higher capacity for recuperation from early nutritional insults, perhaps related to the necessary adaptation of the endocrine pancreas to pregnancy. During pregnancy, islets undergo a number of upregulatory changes to meet the increased demand for insulin during pregnancy, including an increase in glucose-stimulated insulin secretion, with a reduction in the stimulation threshold (Nolan & Proietto, 1994, 1996). These effects are associated with increased β-cell proliferation and islet volume and increased gap-junctional coupling among β-cells (Sorenson & Brelje, 1997). It has been demonstrated that early protein restriction has a persistent effect on glucose homeostasis, which is revealed during a subsequent pregnancy. Whole-body glucose turnover in the post-absorptive state is reduced (Holness & Sugden, 1996). This finding appears to be a consequence of reduced glucose utilization (transport and phosphorylation) both by maternal tissues (including fast-twitch skeletal muscle and adipose tissue) and by the fetus itself. A modest (27 %), but nonetheless significant ($P < 0{\cdot}01$), reduction in basal glucose utilization by third-generation fetuses of the early-protein-restricted offspring (Holness & Sugden, 1996) suggests that defects of glucoregulatory control may be transmitted to further generations never exposed to protein restriction. During hyperinsulinaemia, the early-protein-restricted pregnant rats showed no impairment of glucose utilization by adipose tissue and oxidative muscles, but fast-twitch skeletal muscles were specifically targeted. However, despite this finding, there was no overall impairment in the ability of insulin to stimulate whole-body glucose disposal or to suppress endogenous glucose production as a consequence of early protein restriction. Recovery of insulin secretory capacity was also observed in early-protein-restricted pregnant rats. Thus, the increased insulin secretory demand imposed by pregnancy does not expose an impaired insulin secretory response to glucose in adulthood. This finding may indicate that pregnancy hormones may offer protection against β-cell malfunction or promote recuperative growth of the endocrine pancreas, and highlight the capacity of the endocrine pancreas for recuperative development.

Conclusions

The present review has described how biological events in the mother, exemplified by poor nutrition, not only affect the maternal adaptation to the physiological stress of pregnancy, but also transmit signals to the intrauterine milieu that affect the programme of cell differentiation, tissue development and maturation of the fetus. The present review has focused on experimental findings in the rat. However, there is ample evidence for widespread chronic protein malnutrition, particularly in less-privileged societies. The studies described in the present review show that protein malnutrition, even over the rather limited timescale of pregnancy in the rat, leads to widespread changes in maternal fuel homeostasis. These changes arise as a consequence of modification of multiple organ and tissue systems, with inter-tissue collusion to optimize the supply of nutrients to the fetus. Despite this damage limitation, there are nevertheless persistent effects of maternal nutritional insufficiencies on the offspring. These persistent alterations have considerable implications for the long-term health status of the individual.

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