The long-term consequences of intra-uterine protein malnutrition for glucose metabolism

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Our initial observations, in epidemiological studies, linking indices of poor early (fetal and infant) growth to the subsequent development of poor glucose tolerance and the insulin resistance syndrome in adult life, have been confirmed in studies in a wide variety of populations around the world. These findings led us 5 years ago to propose the 'thrifty phenotype' hypothesis. Tests of this hypothesis in an animal model in which the pregnant and/or lactating rat dams are fed on an isoenergetic diet containing just under half the normal protein content are consistent with the ideas put forward. They have also allowed us to refine the hypothesis in the light of the new data as follows: (1) the growth of the fetus (and possibly infant) is quantitatively and qualitatively altered by its nutritional environment (which may include maternal diet-dependent changes in maternal hormones); (2) these changes serve to select between the growth rates of different tissues according to priorities which differ between males and females (nutritional thrift) and to alter organ function to constitute a thrifty offspring adapted to survival in poor nutritional circumstances (thrifty phenotype); (3) an individual so constituted suffers adverse consequences in adult life if he/she experiences good or supranormal nutrition; (4) both poor insulin secretion and insulin resistance can result from these adaptive processes; (5) the adverse consequences include loss of glucose tolerance and hypertension. The precise outcome of growth retardation during early life may vary according to the type and timing of the factors responsible for the retardation. It remains to be determined to what extent these potentially adverse effects can be delayed or prevented by a suitable postnatal diet. Experiments in animal models are largely consistent with the concepts proposed from human epidemiological studies. They show that the metabolism of the liver, muscle and adipose tissue may be programmed by maternal nutrition during gestation and lactation. The combination of early growth restriction and subsequent adult obesity reproduced in the rat are the main features of the insulin resistance syndrome.

Fetal growth: Non-insulin-dependent diabetes mellitus: Insulin resistance: Hypertension: 'Thrifty phenotype' hypothesis

The research which we review was carried out in order to test in an animal model the 'thrifty phenotype' hypothesis of the aetiology of non-insulin-dependent (type 2) diabetes and the insulin resistance syndrome (Hales & Barker, 1992). The 'thrifty phenotype' hypothesis in turn was proposed as an explanation of epidemiological findings linking poor early (fetal and possibly postnatal) growth to an increased risk of loss of glucose tolerance in adult life (Hales *et al*. 1991). We subsequently extended these studies to show that thinness at birth was linked to insulin resistance in adult life (Phillips *et al*. 1994). The strongest relationship between poor early growth and adult metabolic disorders was with the insulin resistance syndrome (poor glucose tolerance, hypertension and a raised plasma triacylglycerol concentration). In men there was an 18-fold increased risk of developing this syndrome in going from those heaviest to those lightest at birth (Barker *et al*. 1993). These observations have been reproduced in a variety of studies and populations worldwide (for review, see Phillips & Hales, 1996). Since shortness is related to glucose intolerance (Brown *et al*. 1991) and changes linking insulin resistance to size at birth can be detected during childhood (Law *et al*. 1995; Yajnik *et al*. 1995), there is further evidence that the processes leading to altered adult metabolism are initiated early in life. In all these processes it is clear that adult obesity strongly increases the risks posed by poor early growth. Studies

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of twins have shown also that the effects of poor early growth can be independent of any genetic effect. Identical twins who are discordant for non-insulin-dependent diabetes showed a lower birth weight in those twins which were diabetic (Poulson *et al*. 1997). Most recently, in studies of adults who were *in utero* at the time of the Dutch Famine we have shown that a poor maternal diet, particularly during the last trimester of pregnancy, leads to a deterioration of glucose tolerance during adult life (Ravelli *et al*. 1998). This finding is the first direct demonstration in human subjects that the maternal diet can alter the glucose tolerance of offspring in adult life.

Since our original proposal, our and other's research has led us to be able to refine and be more specific concerning the concepts originally outlined. A more up-to-date version of the hypothesis contains the following elements:

(1) the growth of the fetus (and possibly infant) is quantitatively and qualitatively altered by its nutritional environment (which may include maternal diet-dependent changes in maternal and placental hormones);
(2) these changes serve a number of biologically beneficial purposes including (a) the (thrifty) ability to alter selectively the relative growth rates of different organs according to priorities which differ between males and females, and (b) to alter the metabolic setting of various tissues including liver, muscle and adipose tissue postnatally in such a way that the offspring is programmed to survive under conditions of poor nutrition (the thrifty phenotype). We believe that the latter may well be a general feature of vertebrate adaptability to enhance the survival of species at times when the nutritional circumstances are particularly unfavourable;
(3) whilst the thrifty phenotype is beneficial under such circumstances it becomes detrimental when adult nutrition is abundant or over abundant;
(4) the detrimental consequences are partly mediated by both poor insulin secretion and insulin resistance;
(5) further adverse consequences include loss of glucose tolerance, hypertension and possibly other features of the so-called adult degenerative conditions.

In formulating the 'thrifty phenotype' hypothesis, attention was drawn to the evidence in the literature over many years that in both human subjects and experimental animals exposure to a low-protein diet both pre- and postnatally could lead to a permanent reduction of insulin secretion and loss of glucose tolerance (Hales & Barker, 1992). As a result we and a number of other groups have explored the metabolic consequences for offspring of rat dams fed on a reduced-protein diet during pregnancy and/or lactation. In most of our experiments animals have been weaned onto a normal diet.

Studies *in vivo*

Glucose tolerance in these animals has been studied by the intraperitoneal injection of glucose. These tests have been carried out either early in adult life (6 weeks and 3 months) or in old age (over the age of 1 year). Two changes have been observed. Young offspring of reduced-protein pregnancies show increased glucose tolerance (Hales *et al*. 1996; Shepherd *et al*. 1997) and insulin sensitivity (as judged by the plasma insulin concentration measured in the fasting state compared with control animals). Conversely, old reduced-protein offspring have a worse glucose tolerance (Hales *et al*. 1996). In females this loss of glucose tolerance tends to be associated with a poorer insulin secretion, whereas in males it is more suggestive of insulin resistance. Thus, the late consequence for glucose tolerance of growth retardation induced by reducing the maternal dietary protein content is a much greater age-related deterioration of glucose tolerance. The mechanism of this effect is not yet clear and may well differ between males and females (Hales *et al*. 1996).

In order to test our proposal that it is the combination of early growth retardation with adult obesity that is most detrimental to adult metabolism we have studied the effects of adding diet-induced obesity to early growth retardation. In these experiments we also extended the period of reduced-protein diet beyond weaning and up to 70 d of age. This extended period of protein restriction was used to maximize the reduction of β cell function and make it irreversible, as shown by Snoeck *et al*. (1990). Some animals were transferred to a highly-palatable ('cafeteria') diet, consumed approximately twice as much food as those on a control pellet diet and rapidly gained weight (Petry *et al*. 1997*a*). As before the reduced-protein animals on the pellet diet had a worse glucose tolerance than controls in old age (CJ Petry and CN Hales, unpublished results). Obesity reduced the glucose tolerance of both control and reduced-protein animals such that the glucose tolerance of the two groups were very similar (i.e. reduction of glucose tolerance by obesity or reduced protein was not additive when the two were combined). Both obesity and the reduced-protein regimen increased blood pressure, and when the two regimens were combined there was an additive effect (Petry *et al*. 1997*b*). Obesity was associated with an increased plasma triacylglycerol concentration, but again in this situation the combination of obesity with the reduced-protein regimen had no additional effect. All obese animals were insulin resistant, as judged by the fasting plasma insulin concentration, but the combination of the two regimens was again not additive. Thus, in summary (Table 1) all obese rats had poor glucose tolerance, insulin resistance, hypertension and hypertriacylglycerolaemia (i.e. features of the insulin resistance syndrome). Those animals from the reduced-protein regimen who were made obese also had these features, but in addition had a further elevation of blood pressure. We were therefore able to replicate the features of the insulin resistance syndrome in this model, predominantly as a consequence of obesity itself.

Table 1. Characteristics of obese reduced-protein offspring*

Short	Glucose intolerant
Fat	Insulin resistant
Hypertensive	Hypertriacylglycerolaemic

* Offspring of rat dams fed on a reduced-protein diet during pregnancy and/or lactation.

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Studies *in vitro*

We have carried out studies of glucose metabolism and its control by insulin in the three key organs involved in glucose homeostasis: liver, muscle, adipose tissue.

Liver

Perfused livers were used to study the control of glucose output as stimulated by glucagon and as thereafter inhibited by insulin. Livers from male reduced-protein offspring at 3 months of age showed a reduced glucose output response to the action of glucagon. When insulin was added in addition to glucagon, contrary to the normal suppression of glucose output, an initial transient increased glucose output was observed (Ozanne *et al*. 1996*a*). Measurements of the receptors concerned showed that those for glucagon were considerably reduced, whilst those for insulin were increased. Thus, whilst the reduced effect of glucagon may have been due to a reduced number of receptors, this was not the case with respect to insulin. The mechanism causing the anomalous insulin response has not been defined, but it is of interest that a similar effect has been observed during oral glucose tolerance tests of prediabetic Aborigines (O'Dea, 1991).

Non-insulin-dependent diabetics in India have been found to be resistant to developing ketoacidosis (World Health Organization, 1985). Glucagon is an important hormone in the control of the production of ketone bodies (Keller *et al*. 1983). In view of the glucagon resistance of the reduced-protein offspring, we decided to investigate whether they were less susceptible to increased ketone-body production during starvation (Ozanne *et al*. 1998). Rats were starved for 48 h and the plasma concentrations of glucose, insulin, non-esterified fatty acids, glycerol, β-hydroxybutyrate and acetoacetate were measured. Reduced-protein animals when fed and after 24 h starvation had lower plasma insulin concentrations but higher plasma non-esterified fatty acid concentrations, all consistent with an adaptation to starvation. In contrast to this finding, they had a lower concentration of β-hydroxybutyrate. After 48 h starvation the reduced-protein and control offspring had virtually identical concentrations of all these metabolites apart from a lower β-hydroxybutyrate concentration. Thus, reduced-protein offspring had apparently 'anticipated' the metabolic adjustments required in starvation, but achieved this adjustment without increasing the production of keto-acids. We suggest that these changes are consistent with the proposal that they have undergone changes beneficial to withstanding periods of food deprivation.

Overall, our studies of the structural and functional changes which we have observed in the livers of our reduced-protein offspring are summarized in Table 2. Key enzymes of glycolysis and gluconeogenesis are altered in a gluconeogenic direction (Desai *et al*. 1996). In studies of perfused livers we have found increased gluconeogenesis from lactate (Burns *et al*. 1997). Alongside these functional changes, we have observed structural changes in terms of an increase in lobule size but decrease in number of hepatic lobules (Burns *et al*. 1997). It is not yet clear whether these changes represent changes on a cellular basis, suggestive of an enhancement of those functions mainly expressed in the periportal zone against the perivenous zone of hepatic metabolism, or whether they reflect changes at the level of the expression of individual genes irrespective of their anatomical location.

Table 2. Findings in liver of reduced-protein offspring*

1. Changes in enzymes of carbohydrate metabolism
2. Increased gluconeogenesis
3. Insulin and glucagon resistance
4. Larger lobule size
5.? General change in zoned functions

* Offspring of rat dams fed on a reduced-protein diet during pregnancy and/or lactation.

Skeletal muscle

Isolated muscle strips have been used to study glucose uptake and its control by insulin (Ozanne *et al*. 1996*b*). Reduced-protein offspring have enhanced basal glucose uptake. Maximal insulin stimulation of these strips produced the same glucose uptake as it did in control muscle strips, with a consequent large reduction in the fold stimulation over basal glucose uptake. The number of insulin receptors expressed in the muscle of the reduced-protein offspring was very considerably increased. In order to determine whether the enhanced basal glucose uptake reflected the persistent effect of *in vivo* insulin in the presence of increased insulin receptors, we pre-incubated muscle strips for 1 h *in vitro*. The basal glucose uptake of muscle strips from reduced-protein offspring declined to that of the control. Stimulation by a maximal insulin concentration in terms of fold effect and absolute maximum was also equal to that of controls. However, the effect of a submaximum insulin concentration was enhanced in the muscle strips from reduced-protein offspring, consistent with their having increased numbers of insulin receptors (Ozanne *et al*. 1996*b*). Since these 3-month-old animals showed increased glucose tolerance and insulin sensitivity, it seems likely that the previously described changes in muscle glucose uptake could at least in part account for their glucose metabolism *in vivo*.

Adipose tissue

Similar studies to those described previously in muscle were carried out in adipocytes prepared from animals aged 3 months (Ozanne *et al*. 1997). Again enhanced basal glucose uptake was observed. However, in this tissue maximum insulin-stimulated glucose uptake was increased in comparison with the controls. The number of insulin receptors was increased (Ozanne *et al*. 1997; Shepherd *et al*. 1997). However, since it takes approximately 1 h to prepare adipocytes after removal of the tissue, the increased basal glucose uptake was unlikely to be caused by exposure to endogenous insulin *in vivo*. Studies of the intermediates of the insulin signalling pathway involved in the stimulation of glucose uptake were also consistent with them being

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activated in the basal state (Ozanne *et al*. 1997). The most striking change in the latter was an 80 % reduction in the expression of the catalytic subunit of phosphatidylinositol 3-kinase (*EC* 2.7.1.137) β isoform.

Studies of the anti-lipolytic effect of insulin on these fat cells revealed similar rates of basal lipolysis in the cells from reduced-protein offspring, and an increased maximal stimulation by isoproterenol, with a marked reduction in the anti-lipolytic effect of insulin (Ozanne *et al*. 1998). Thus, in these fat cells we have the apparent anomaly of increased glucose uptake in the presence and absence of insulin, together with a decreased effect of insulin to inhibit lipolysis.

It is known from studies using the phosphatidylinositol 3-kinase inhibitor wortmannin that both insulin-stimulated glucose uptake and insulin-induced inhibition of lipolysis are mediated through the action of this enzyme (Okada *et al*. 1994). Two isoforms of the catalytic subunit of this enzyme exist in adipose tissue, termed p110α and p110β (for review, see Shepherd *et al*. 1998) The reasons for the existence of two catalytic isoforms of this enzyme in adipose tissue are not understood. However, the β isoform is specifically activated by the βγ subunits of the G-protein which is activated when isoproterenol activates lipolysis (Kurosu *et al*. 1997). It is tempting, therefore, to speculate that the β isoform of the catalytic subunit of phosphatidylinositol 3-kinase mediates the anti-lipolytic effect of insulin, whereas the α isoform mediates insulin-stimulated glucose uptake (Fig. 1). A possible advantage of this proposed and highly speculative dichotomy is that separate programming of the expression of the two isoforms could permanently shift the emphasis of metabolism in adipose tissue. Under our proposal the changes in isoform expression which we have observed could enhance the ability of the tissue to take up glucose and store it as triacylglycerol, whilst at the same time enhancing the outflow of fatty acids in the absence and presence of insulin. The overall effect of this shift in metabolic emphasis would be to enhance the ability to store carbohydrate as fat and increase the use of fat as an energy store for the maintenance of metabolism. This change in balance would seem to be advantageous to an animal exposed to extremes of the intermittent availability of nutrition.

We recognize that this proposal is highly speculative, but intend to investigate further the role of the enzyme isoforms involved in signalling pathways, including their possible involvement in adjusting signalling pathways during the fetal programming of adult glucose metabolism.

Conclusions

(1) Nutrition during pregnancy and lactation can programme the metabolism of offspring with regard to glucose and lipid homeostasis.

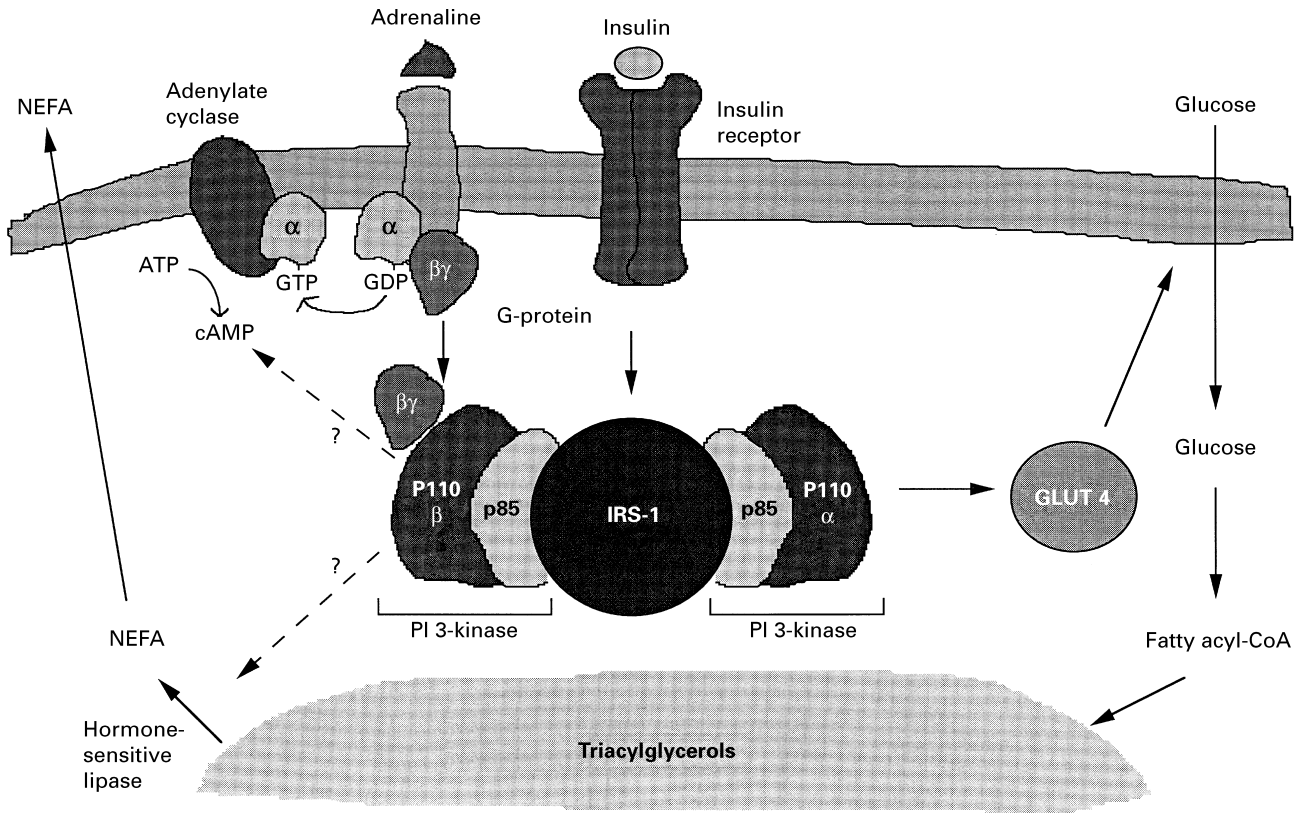

Fig. 1. Diagram showing the possible signalling roles of catalytic subunit isoforms of phosphatidylinositol 3-kinase (*EC* 2.7.1.137; PI 3-kinase). NEFA, non-esterified fatty acids; GLUT 4, insulin-sensitive glucose transporter; adenylate cyclase, *EC* 4.6.1.1.

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(2) The metabolic adaptations thus produced are such that they appear likely to enhance the ability to survive inadequate nutrition.
(3) Obesity itself can lead to the features of the insulin resistance syndrome, and with respect to hypertension this is enhanced by poor early growth in the rat.
(4) These findings in experimental animal models are consistent with the concepts proposed in the 'thrifty phenotype' hypothesis.

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