A little over 6 years ago, Hales and Barker [1] proposed the Thrifty Phenotype Hypothesis to account for results from a series of epidemiological studies which found associations between markers of fetal and early growth retardation and the subsequent development of ischaemic heart disease, hypertension and Type 2 (non-insulin-dependent) diabetes mellitus (NIDDM). These studies showed that people with ischaemic heart disease [2], hypertension [3], impaired glucose tolerance or Type 2 diabetes [4] had significantly lower birthweights or weight at 1 year than people without these degenerative diseases. The strongest association of all between birthweight and adult degenerative diseases has been reported for the insulin resistance syndrome (when diabetes, hypertension and hyperlipidaemia occur together) [4]. In a study of 64-year-old men in Hertfordshire, U.K., the odds ratio of those born lightest for the presence of the insulin resistance syndrome was 18 times that of those born heaviest [5]. Since these initial studies, many other studies in a large number of different populations have shown links between poor fetal and early growth and either loss of glucose tolerance or markers of the insulin resistance syndrome (reviewed in [6]).

Perhaps one of the most significant studies relating low birthweight to loss of glucose tolerance which has been published since the Thrifty Phenotype Hypothesis is that of twins in Denmark [7]. This study found that in both monozygotic twins and dizygotic twins where only one twin had Type 2 diabetes, the birthweight of the diabetic twin was significantly lower than that of the non-diabetic twin. The authors of this paper conclude that ‘the study supports the role of non-genetic (environmental) intrauterine component, such as intrauterine malnutrition for the development of NIDDM much later in life’. These epidemiological studies have led to much speculation as to the mechanistic basis of the relationship between birthweight and the insulin resistance syndrome.

The Thrifty Phenotype Hypothesis suggested that, during times of nutritional deprivation, a growing fetus adopts two main strategies to ensure survival. First, it diverts nutrients to the brain at the expense of other organs such as the liver, pancreas and muscle. Second, it is proposed that metabolic programming occurs which is beneficial to survival under conditions of poor postnatal nutrition. However, if the organism moves into conditions of adequate or overnutrition then this will conflict with the previous programming and Type 2 diabetes, ischaemic heart disease and hypertension may result. In the Thrifty Phenotype Hypothesis particular attention is paid to the importance of protein to the growing fetus. Amino acids are thought to be major factors controlling β-cell growth and development and insulin secretion until late fetal life (reviewed in [1]). This has led to a number of studies in the rat using a maternal low (5–8%) protein diet which produces early growth retardation.

The low protein rat as a model of early growth retardation
A large number of studies have shown that when rats are fed a low (5–8%) protein diet during pregnancy the offspring are significantly smaller than the offspring of rat dams fed a control (18–20% protein) diet during pregnancy [8–10]. If low protein offspring are cross-fostered to mothers being fed a control (20%) protein diet during lactation, they rapidly gain weight such that by weaning (21 days of age) these recuperated offspring have similar body weights to control rats [19]. This catch-up growth appears to have a detrimental effect on longevity, especially in male rats where a 20% reduction in lifespan was observed [11]. Permanent growth retardation in the offspring results if the low protein diet is fed to rat dams during pregnancy and lactation or lactation alone. Such offspring remain smaller than control offspring permanently, even after they have been weaned on to a control diet. This suggests that nutrition during the lactation period in the rat (a total of 21 days) can set the growth trajectory of the offspring for the remainder of their lives.

Abbreviations used: IRS, insulin receptor substrate; PI, phosphatidylinositol.
Effects on glucose tolerance
A number of studies have shown that in young adult life (6–12 weeks) the offspring of rat dams fed a low (6–8%) protein diet either during pregnancy alone or during pregnancy and lactation have a better glucose tolerance than controls [11–14]. However, there are age-dependent changes such that by 44 weeks of age low protein offspring have similar glucose tolerances to controls [12] and by 15 months of age low protein offspring have a significantly worse glucose tolerance than controls [11]. This glucose intolerance in males appears to be related to insulin resistance. The mechanistic basis of the age-dependent loss of glucose is not clear but there is a suggestion that it could be accelerated by high fat feeding [13,15]. These results suggest that in the rat early growth retardation produced by maternal protein restriction alone is not sufficient to produce frank diabetes. This suggests that an added factor (of unknown identity/identities) is required in addition to early growth retardation to induce the development of frank diabetes. The identification of these factors remains the subject of much research. One possible candidate is adult obesity, but data from rat studies again suggest that obesity alone is not sufficient to produce diabetes in early growth-retarded rats [16].

Effects on the pancreas
Initial studies by Snoeck et al. [9] revealed that $\beta$-cell proliferation and islet size were significantly reduced in the head of the pancreas of neonates who were the offspring of mothers fed a low protein diet during pregnancy. In addition, islet vascularization was significantly reduced in both the head and tail of the pancreas from such offspring. This group then extended these studies to determine if structural changes were associated with any changes in insulin secretion. Although there were no differences in basal levels of secretion, islets from 21.5-day-old fetuses of low protein mothers had a reduced secretory response to both leucine and arginine [17].

Effects on the liver
Ex vivo perfusion studies have shown that the livers of 3-month-old offspring of mothers fed a low protein diet during pregnancy and lactation are resistant to glucagon in terms of its ability to stimulate hepatic glucose output [18]. This appears to be related to a 5-fold reduction in the expression of hepatic glucagon receptors. Hepatic insulin receptor expression is increased 2–3-fold in low protein offspring. This is accompanied by increased internalization of insulin into endosomes and release of degraded insulin. Livers of low protein offspring have an altered biphasic response to insulin [18], causing an initial increase in hepatic glucose output before the expected reduction in output. The mechanism of this altered response is not known. However, a similar response to insulin has been seen in subjects with Type 2 diabetes [19] and in young Aborigines (a population where a large number of individuals develop Type 2 diabetes) [20]. Thus, regardless of mechanism, an early low protein diet in the rat appears to produce a hepatic alteration similar to that observed in human diabetes. In addition to the biochemical changes mentioned, a maternal low protein diet in the rat has also been shown to result in structural changes in the liver. It has been observed that low protein offspring have larger hepatic lobules [21]. The functional consequences of these structural changes remain to be fully investigated.

Effects of muscle
Studies of glucose uptake by isolated muscle strips have revealed that tissue from 3-month-old male low protein offspring is more sensitive to insulin [22]. This is consistent with the observation that young adult low protein offspring have a better glucose tolerance than controls (see above). The mechanistic basis of the enhanced sensitivity is not completely clear but, in part, it may relate to an observed 2–3-fold increase in insulin receptor expression. There is also an altered distribution of the GLUT 4 protein, with increased levels present in the plasma membrane under fasting conditions. This leads to an increased basal glucose uptake into freshly isolated muscle strips.

Similar studies on 15-month-old male low protein offspring are currently in progress to investigate possible molecular changes that occur in skeletal muscle in parallel with the deterioration of glucose tolerance.

Effects on adipose tissue
Isolated cell studies have revealed that adipocytes from 3-month-old male low protein offspring have an increased basal and insulin-stimulated glucose uptake [23] which is again consistent with the improved glucose uptake at this age. Greater glucose uptake is paralleled by an
increased basal and insulin-stimulated insulin receptor substrate (IRS) 1-associated phosphatidylinositol (PI) 3-kinase activity. As with skeletal muscle, these observations can at least in part be explained by increased expression of insulin receptors [14,23] with no difference in total expression of GLUT 4. In contrast to the apparent increased sensitivity to insulin of glucose uptake by adipocytes, low protein offspring adipocytes are relatively resistant to the anti-lipolytic action of insulin is known to be sensitive to the PI 3-kinase inhibitor wortmannin [25]. A more detailed analysis of the adipocytes reveals that, although the low protein offspring have elevated levels of basal and insulin-stimulated IRS 1-associated PI 3-kinase activity, there is altered isoform expression of the enzyme [23]. PI 3-kinase is a heterodimeric enzyme which consists of a regulatory subunit (termed p85) and a catalytic subunit (termed p110) (reviewed in [26]). In adipocytes there are two main isoforms of insulin regulated catalytic subunits (termed p110α and p110β) whose individual functions and subcellular distribution are not known. However, adipocytes from 3-month-old male low protein offspring have a striking 6-fold reduction in expression of p110β but similar levels of p110α [23]. It is therefore possible that p110β is required for the anti-lipolytic action of insulin whereas the p110α isoform is the catalytic subunit that signals to glucose transport. Interestingly, recent studies have shown that a PI 3-kinase consisting of p110β (catalytic subunit) and p85 (regulatory subunit) can be activated by the βγ subunit of G-proteins as well as tyrosine phosphopeptides [27]. No activation by the βγ subunit was observed with a PI 3-kinase consisting of p110α and p85. This suggests that p110α/p85 PI 3-kinase has the potential to be regulated in a co-operative manner by insulin and hormones, such as catecholamines, which act via G-protein-linked receptors.

Summary
In recent years a great deal of research effort has been directed towards understanding the molecular basis of the relationship between markers of fetal and early growth retardation and the subsequent development of Type 2 diabetes. A lot of this work has focused on the maternal low protein rat model. Like many animal models, it does not perfectly represent the human situation and frank diabetes has not yet been produced. However, this model has yielded much information on potential mechanisms by which changes in insulin sensitivity can occur and has helped in understanding the role of the molecular machinery involved in the signalling of the metabolic actions of insulin.

Maternal nutrition and endocrine programming of fetal adipose tissue development

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Introduction

Fetal adipose tissue development occurs within a metabolic and hormonal environment in which the fetus aims to maximize its growth potential depending on the nutritional, genetic and spatial constraints to which it is subjected. Adipose tissue is one of the last fetal tissues to be deposited and its quantity is highly sensitive to nutrient restriction in late gestation [1]. After birth, adipose tissue not only functions as an endogenous energy store as white adipose tissue, but also provides an essential source of heat in the form of brown adipose tissue (BAT) that can be rapidly activated. For precocial species with a mature hypothalamic-pituitary axis at birth, adipose tissue development is unique in that sufficient tissue must be deposited in order to meet the very high thermal demands associated with cold exposure to the extrauterine environment.

At the same time, it must possess the capacity to rapidly generate large amounts of heat as a result of the rapid appearance of a unique uncoupling protein-1 (UCP1) that is only detected in BAT [2].

UCP1 is located on the inner mitochondrial membrane and has the capacity to generate 300 W of energy per gram of tissue compared with 1 W per gram for the majority of other tissues [3]. This is due to the proton potential generated by respiration across the inner mitochondrial membrane of the brown adipocyte being directly transferred as heat [4]. It is essential that UCP1 is not activated prematurely in utero as this will place both the fetus and mother at risk from hyperthermia. Conversely, if UCP1 is not present in sufficient quantities, or rapidly activated immediately after birth, the newborn is at substantial risk from hypothermia. This risk is most apparent for lambs who may be subjected to a severe cold challenge after birth, but even infants can be exposed to a drop in ambient temperature of at least 10°C ([5]; Table 1).

Fetal adipose tissue growth and maturation

During fetal development there appear to be three distinct phases in the growth of adipose tissue which culminates in the rapid appearance of UCP1 immediately after birth [8]: (1) tissue growth due to hyperplasia and hypertrophy; (2) endocrine development and appearance of specific receptor populations; and (3) expression of UCP1. These are shown in Figure 1.

Tissue growth occurs as a result of hyperplasia and hypertrophy from mid gestation. In fetal lambs approximately 0.9 g of adipose tissue can be dissected from around the perirenal region at 80 days of gestation which constitutes the major site of adipose tissue deposition in the lamb [1]. The amount of adipose tissue then increases to 25 g by 140 days of gestation, with little further change in deposition until after birth [10]. During late gestation the amount of perirenal adipose tissue remains at 4–5% of

Abbreviations used: BAT, brown adipose tissue; UCP1, uncoupling protein-1.

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