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R Huw Jones, Susan E Ozanne

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Fetal Programming of Glucose-Insulin Metabolism

R Huw Jones[1] and Susan E Ozanne[1]

[1]Institute of Metabolic Science, Metabolic Research Laboratories, Addenbrookes Hospital, University of Cambridge, Cambridge, CB2 0QQ.

Abstract

Epidemiological studies have shown a link between poor fetal growth and increased risk of developing type 2 diabetes. These observations are highly reproducible in many populations worldwide although the mechanisms behind them remain elusive. The 'Thrifty Phenotype Hypothesis' was proposed to explain the underlying causes of these relationships. Animal models of poor intrauterine nutrition have been utilised to help to define the causal factors and identify the molecular mechanisms. Programmed changes in beta cell function and insulin action have been a common feature of animal models of poor intrauterine nutrition. Fundamental underlying mechanisms are starting to emerge, including changes in the epigenotype and mitochondrial function.

Introduction

Links between poor fetal growth and ensuing development of the metabolic syndrome have been reported in many epidemiological studies. The first hypothesis concerning

the "fetal origins" of adult disease was put forward by Barker et al. (1), following their observations of an inverse relationship between systolic blood pressure and death rates from cardiovascular disease with birth weight. This same Hertfordshire cohort, of 64 year old men in the UK, was also used to establish an inverse link between birth weight and glucose intolerance/insulin resistance. Hales, along with Barker, established that subjects who were smaller at birth had a 6 fold increased risk of suffering from type 2 diabetes than those who were heaviest at birth (2) and also more strikingly an 18 fold increased risk of presenting with features of the metabolic syndrome. These relationships are now widely accepted as the outcomes of these original studies have been reproduced in numerous cohorts and many populations worldwide (3) although the molecular mechanisms behind them remain to be fully understood. The concept of metabolic programming attempts to describe the links observed between epidemiological findings and later development of the metabolic syndrome.

Epidemiological Findings

The "Thrifty Phenotype Hypothesis" (Figure 1) was put forward by Hales and Barker (3) to explain the links between low birth weight and features of the metabolic syndrome. It proposed that a fetus, which endures poor nutrition during gestation, would spare the growth of vital organs such as the brain at the expense of tissues such as the muscle and the endocrine pancreas and the fetus would adapt its metabolism to these given conditions of limited nutrition. A fetus that could program its metabolism in such a manner would hold a significant advantage were it to encounter similar

conditions in postnatal life (4). This has been expanded on by the "Predictive Adaptive Response" hypothesis (5) which proposes that the fetus dynamically interacts and reads the environment which it will be born into and adapts to gain a future survival advantage.

Although Hales *et al*. elucidated strong links between low birth weight and poor glucose tolerance in the Hertfordshire cohort, as well as links to the metabolic syndrome, it was also noticed that those who were obese at the time of the study who had a low birth weight had the worst glucose tolerance of all (2). This led to the suggestion that metabolic "programming" to a low plain of nutrition could become detrimental should the fetus find itself in an environment of excessive nutrition postnatally.

Although not mutually exclusive with the "Thrifty Phenotype Hypothesis", the "fetal insulin hypothesis" is an alternative hypothesis that seeks to explain the link between low birth weight and type 2 diabetes (Figure2). Insulin plays an important role in fetal growth (6). Genetic pancreatic beta cell dysfunction can lead to defects in glucose stimulated insulin secretion, which in turn would lead to reduced insulin mediated fetal growth as well as a low birth weight. In adulthood these beta cell defects, along with decreased insulin sensitivity would manifest itself in such a way that whole body glucose metabolism was affected. Individuals who have a rare monogenic form of diabetes known as 'maturity onset diabetes of the young' are proof of principle for this theory (7,8). The mutations in these individuals of the glucokinase gene leads to reduced birth weight and development of type 2 diabetes in early adulthood (9) caused by defective glucose sensing in the pancreatic beta cell

Evidence from human studies

There is strong evidence to suggest that the Thrifty Phenotype Hypothesis is indeed an accurate description of the observed epidemiological studies especially in relation to type 2 diabetes. Studies conducted in Denmark with both monozygotic and dizygotic twins in their sixties who were discordant for type 2 diabetes showed that the twin diagnosed with type 2 diabetes had the lower birth weight (10). This study suggests that the increased risk of low birth weight and, as such, diabetes is independent of genotype as the monozygotic twins are genetically identical. This rules out both gender and gestational length as confounding variables and also supports the hypothesis that "programming" and the fetal environment and not genotype is responsible for the links between low birth weight and type 2 diabetes. More direct evidence supporting the "Thrifty Phenotype Hypothesis" and the intrauterine environment as a predictor for the possible consequences on development of type 2 diabetes comes from the study of individuals exposed to the Dutch Hunger Winter. This was a period in late World War II where daily caloric intake was restricted to 400-800kcal. Individuals who were *in utero* during this defined period of maternal under nutrition had both a low birth weight and impaired glucose tolerance at age 50 y, compared to individuals who were *in utero* either the year before or after the famine (11). Expanding on this, the last trimester of gestation proved to be critical in the relationship, with individuals malnourished in this period having the worst glucose tolerance of all, especially those who became obese as adults (11).

Based on these low birth weight observational studies, the role of poor fetal growth followed by catch up growth was examined. A study of South African children who were born with a low birth weight but who then underwent a rapid weight gain in childhood, showed the worst glucose tolerance at 7 years of age (12). It was proposed that these children had the greatest risk of developing type 2 diabetes as an adult. In India it was observed that children who had a low birth weight and were heaviest at 8 years were the most insulin resistant (13). More recently a study of small for gestational age infants (SGA) and appropriate for gestational age infants (AGA) showed increased insulin sensitivity at birth in (SGA) infants, which could potentially drive their rapid postnatal catch up growth. By 3 years of age (SGA) offspring were more insulin resistant than (AGA) offspring however (14).

More recent studies have focused on maternal age as factor contributing to low birth weight. Mothers under the age of 24 have shown an increased risk of delivering low birth weight offspring (15) compared to mothers over 24 years of age. At the other end of the curve it has been demonstrated that advanced maternal age is also a causal factor in low birth weight (16). In the contemporary population there is an increasing prevalence of teenage and older mothers, which may contribute to the increasing prevalence of type 2 diabetes.

A reduction in birth weight is not always the best predictor of a poor fetal environment, as shown in a number of studies (reviewed here by Wallace *et al.*) (17), which demonstrate that low birth weight can be a crude predictor of *in utero* experiences. In addition diabetic mothers can give birth to large macrosomic offspring and this intrauterine exposure to diabetes is often a prelude to risk factors

for insulin resistance and type 2 diabetes (18,19). This exposure to maternal diabetes also affects the development of the endocrine pancreas (20) and can manifest itself with elevated levels of insulin in the amniotic fluid during pregnancy (21). Macrosomic offspring have an increased prevalence of impaired glucose tolerance at 10-16 years, are more obese and at a higher risk of developing diabetes in later life (22). The Pima Indian population has a very high prevalence of diabetes as well as maternal obesity. This is thought to correlate with an increased risk of diabetes in the macrosomic offspring of mothers with gestational diabetes. Diabetes was more prevalent in offspring of mothers who were diabetic during pregnancy, than in siblings born before the onset of maternal diabetes (23). Maternal obesity independent of diabetes is currently the focus of intense study. Currently, only few studies have shown links between maternal obesity and development of the metabolic syndrome (24) and obesity in adulthood (25,26). However the growing prevalence of obesity amongst women of childbearing age means these links are of growing concern.

Evidence from Animal Models

There are a large number of well-established animal models that have been studied in order to try and elucidate the physiological and molecular relationships between type 2 diabetes and environmentally derived intra uterine growth retardation.

Maternal iron restriction has been documented to cause increased systolic blood pressure and low birth weight, although no significant changes in glucose tolerance or insulin sensitivity were seen (27). In rodents maternal hypoxia has also been linked to

low birth weight offspring and changes in cardiac gene expression (28). Human utero-placental insufficiency, which is the most common cause of poor fetal growth in humans, is examined using the uterine artery ligation rat model. Blood flow to the fetus is restricted to similar levels to those seen in the human situation and the rodent fetus has a similar metabolic profile to that of its human counterparts. This model displays growth restriction *in utero* (29), reduced beta cell mass (30) and glucose intolerance, insulin resistance and decreased levels of IGF-1 in postnatal life (31). By 6 months of age it shows a marked reduction in beta cell mass and significantly raised glucose levels (31). Maternal calorie restriction in the rat to 50% *ad libitum* during pregnancy leads to low birth weight, beta cell dysfunction and to an age dependent loss of glucose tolerance (32). Severe calorie restriction in the pregnant rat to 30% *ad libitum* has been shown to lead to severe growth retardation of the fetus, along with hypertension, hyperinsulinaemia and hyperphagia in adulthood (33). Interestingly, leptin treatment during the first two weeks of life completely reverses the detrimental effects of such severe maternal calorie restriction (34).

The low protein model of growth restriction is one of the most extensively studied rodent models. The isocaloric diet consists of 8% protein compared to the control 20% diet and so naturally is higher in carbohydrates than the control as well as lower in protein. Low protein offspring are born 15% lighter than controls (35) and when suckled by low protein dams during lactation they remain permanently growth retarded throughout life, even when fed the control diet *ad libitum* from weaning (36). Early studies in the low protein model examined the development of the endocrine pancreas. Restricted dams produced offspring with decreased islet mass, less vascularisation and lower insulin content (37-39). These islets also show defective

secretion in response to glucose and amino acids in fetal life (40) as well as impaired insulin secretion in the adult offspring (41). It appears that the offspring of low protein dams spare the growth of the brain at the expense of other tissues such as the kidney and the pancreas. Low protein offspring show enhanced glucose tolerance and increased insulin sensitivity during early life (42,43). In fact, whole body insulin sensitivity is improved during early life in low protein offspring, as is muscle (44) and adipose (45) insulin sensitivity. However, by 15 months of age they have impaired glucose tolerance (46) and at 17 months of age the offspring have frank diabetes (47). The age dependant insulin resistance seen in low protein offspring leads to long term changes in the structure and function of insulin sensitive tissues. In adipose tissue, expression of p110 beta (a catalytic subunit of PI 3-Kinase) is reduced whereas in muscle there is a reduction in PKC zeta, both associated with defects in insulin signalling action (46,48). When insulin resistance develops there is no change in insulin receptor expression confirming a post-receptor defect. More recent work has demonstrated that insulin signalling profiles in muscle biopsies from low birth weight men show striking resemblances to the defects seen in the low protein model (49). Not only does this lend strong support for the model, it also defines the importance of the fetal environment in linking low birth weight to an increased risk of diabetes.

Studies in several models have recently shown that the link between low birth weight and metabolic disorders may be caused by excessive exposure to glucocorticoids *in utero*. Mothers treated with dexamethasone during pregnancy give birth to offspring with a reduced birth weight, who subsequently suffer long term hypertension, hyperglycaemia and hypothalamic-pituitary-adrenal (HPA) axis hyperactivity (50). Recent studies in non-human primates have shown that dose dependant

dexamethasone treatment can induce impaired glucose tolerance, hyperinsulinaemia and a 25% decrease in pancreatic beta cell number (51). Prenatal dexamethasone exposure in humans leads to hypertension (52) and hyperinsulinaemia (53). Excessive dexamethasone exposure in rodents *in utero* also leads to low birth weight and permanent hypertension and hyperglycaemia in adult offspring (reviewed by Nyirenda and Seckl) (54), as well as having a negative effect on fetal beta cell development (55). Low birth weight is not seen in the offspring of dams fed a high fat diet, which is another example of where low birth weight can be a somewhat crude method of measuring fetal nutritional status. A maternal high fat diet leads to an increased risk of cardiovascular disease and type 2 diabetes (56). It also increases the risk of developing hyperinsulinaemia and increased adiposity (57). Maternal diabetes can lead to insulin resistance and ultimately diabetes in two different ways. Mildly diabetic mothers give rise to macrosomic babies with enhanced development of their endocrine pancreas, with hypertrophy and hyperplasia of fetal islets (58), as well as increased proliferation (59), and subsequent increases in beta cell mass, as well as islet vascularisation (60). These offspring also have increased pancreatic insulin content and enhanced insulin secretion (61). However, in adulthood these offspring have a deficit in insulin secretion and impaired glucose tolerance. Permanently high levels of maternal glucose (above 20mM) during gestation give rise to SGA infants. The beta cells in these offspring are almost completely degranulated with lower pancreatic insulin and also reduced plasma insulin levels (61) and the offspring develop insulin resistance in adulthood.

As we have seen in the above animal models there are a large number of phenotypes that bear striking resemblances, not just to other animal models, but to human cohorts as well.

The role of the pancreatic beta cell in glucose/insulin metabolism

The beta cell is critically important for correct glucose/insulin metabolism, both in the fetus and in the adult. It senses glucose levels present in the body and initiates a proportional glucose mediated insulin release. The development of the pancreas is a tightly regulated process involving a complicated network of transcription factors, many of which are active at more than one time point during pancreas development, often carrying out different roles. Pdx1 has been shown to be involved at numerous stages of pancreatic development. Early in development Pdx1 expression is coupled with differentiation into endocrine cells present in islets, whereas in adulthood Pdx1 is vital for transcriptional regulation of the insulin and somatostatin genes (62,63). In a uterine artery ligation model of intrauterine growth restriction (IUGR) Pdx1 expression is reduced by 60% at 14 days compared to controls and this reduction persists to 3 months of age. This decrease correlates with reduced beta cell mass by 80% in IUGR animals at 3 months. Treatment of the IUGR animals with exendin-4 (Ex-4) in the early postnatal period completely rescues Pdx1 expression, as well as rescuing beta cell mass (64). In late gestation the beta cell population roughly doubles on a daily basis (65) which coincides with islet vascularisation (66). It has been demonstrated in the low protein model that islet vascularisation was dramatically reduced in neonates of low protein dams (37) as was the number and volume density

(38). Shortly after birth a wave of apoptosis and remodelling of the beta cells occurs (67,68). It has now been shown that new beta cells arise from the proliferation of existing beta cells (69) as well as differentiating acinar cells (70). It is apparent that this time window of late gestation and early postnatal life is essential for laying down a beta cell mass sufficient for the lifespan of the offspring. Any insults to the tightly controlled process of pancreatic development along with beta cell function could have dire consequences for the organism and correct insulin/glucose metabolism.

Mechanisms of Intrauterine Programming

As we have seen, a suboptimal nutritional environment can reflect maternal under nutrition, over nutrition as well as placental insufficiency and any programming effects can be influenced by the length and timing of these insults as well as the level to which they occur. The mechanisms behind theses manifestations of *in utero* nutritional insults have been elusive. Recently, two potential explanations have been suggested, neither mutually exclusive, which try to unravel the molecular mechanisms behind the phenotypes seen in models of intrauterine growth retardation. The mitochondria play a vitally important role in cell metabolism, even more so in cells with high oxidative energy requirements. The pancreatic beta cells require large amounts of ATP to allow glucose stimulated insulin secretion to take place. Studies in humans have shown that intrauterine growth retardation can lead to increased production of reactive oxygen species (ROS) in the fetus (71-73) along with low levels of oxygen which lead to impaired function of the electron transport chain, which can then increase ROS production further (74,75). The oxidative damage

caused by ROS is not limited to the mitochondria - proteins, lipids and nucleic acids within the cell can also be damaged. Pancreatic beta cells are at a further disadvantage, with their low levels of antioxidant defences (76,77) unable to cope with the increased ROS levels. The resulting oxidative stress leads to multiple problems within the beta cell. Under normal physiological conditions, nutrient stimulated insulin release would be highly dependant on ATP production within the beta cell (78-85). Any defects in ATP production within the beta cell would therefore have large detrimental effects on the function of the beta cell. Indeed, increased levels of ROS lead to decreased levels of glucose mediated insulin secretion (86-88), decreased beta cell proliferation (86) and decreased expression of genes vital for beta cell function, including insulin (89-95). Ultimately the viability of beta cells is at risk (96,97). Uterine artery ligation in the rat has been shown to elicit similar metabolic effects in the muscle as uteroplacental insufficiency does in humans. Decreases in ATP production lead to impaired glucose transporter 4 activity, contributing to insulin resistance and hyperglycaemia (98). Studies have shown that a high fat diet during gestation and lactation leads to a reduced mitochondrial DNA copy number in kidney and aorta (99). Dams fed a low protein diet during pregnancy and lactation give rise to offspring with reduced mitochondrial gene expression in the liver, as well as in the pancreas (100). Low protein fetal islets have been shown to have altered gene expression of proteins involved with glucose metabolism, mitochondrial energy transfer and DNA and RNA metabolism (101).

The study of epigenetics has been around for 20 years and recently the role of these modifications, such as DNA methylation, have been a major focus of studies of nutritional programming. Epigenetic mechanisms include the methylation of DNA

(usually in promoter regions of genes), modification of histones including methylation, acetylation and phosphoryaltion and also RNA silencing. It has been demonstrated that altered intrauterine milieu along with amount and type of nutrients can influence epigenetic programming (102) and these alterations can be passed from one generation to the other in humans (103) and animals (104) Study of the imprinted genes *igf2* and *igf2r* has shown that a methyl deficient diet leads to a loss of imprinting of the *igf2* gene. This demonstrates that diet can affect expression of genes and could contribute to permanent *igf2* imprinting modification (105).

Conclusions and Future Directions

Numerous studies have now shown the relevance of the intrauterine environment in establishing a metabolic phenotype. These links are well established in animal models, yet it is only now that we are starting to uncover the molecular mechanisms behind these observations. It is clear that the endocrine pancreas and insulin responsive tissues play an important role in the programming of the metabolic state, and elucidating the defects manifested in beta cell function and insulin action of growth restricted individuals will be key to furthering our knowledge of the programming of glucose-insulin metabolism. Further study of the role of mitochondrial programming and the mechanisms behind it, as well as the role of epigenetics will also be vital in allowing intervention strategies to be developed.

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Figure 1: The Thrifty Phenotype Hypothesis

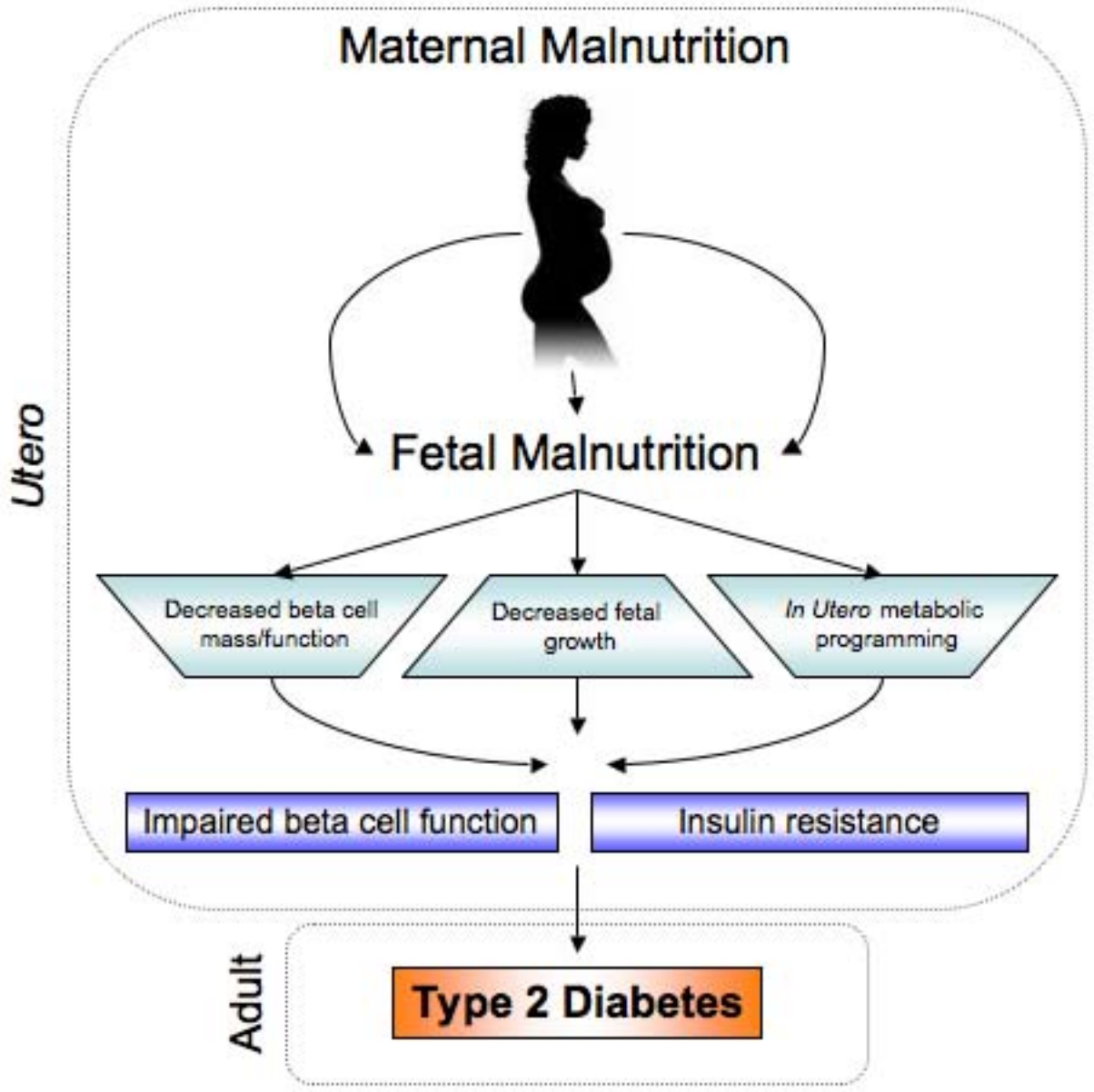

Figure 2: The Fetal Insulin Hypothesis