

## Early and late nutritional windows for diabetes susceptibility

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Our epidemiological studies have led us to propose that events in early (fetal and infant) life are major factors determining susceptibility to non-insulin-dependent diabetes (NIDDM) in adult life (Brown *et al.* 1991; Hales *et al.* 1991; Robinson *et al.* 1992; Barker *et al.* 1993; Phillips *et al.* 1994; Law *et al.* 1995; Yajnik *et al.* 1995). It has been suggested that fetal growth restriction due to poor nutrition (either maternal or fetal or both) is environmentally determined and has at least two major consequences: (1) selective growth of major organs such as the brain at the expense of others such as the liver and kidney, (2) a permanent change in the endocrine and/or metabolic setting of the offspring in a direction likely to aid survival in a nutrient-deficient environment. The conflict between this setting and exposure to good or supra-normal nutrition was envisaged as a major route leading to NIDDM (Hales & Barker, 1992). It has been objected that whilst it is conceivable that nutritional deprivation might have an impact in populations with clear problems of obtaining adequate nutrition, such as the under-developed countries (and which also have severe problems with NIDDM emerging in 'epidemic' proportions; Zimmet & McCarty, 1995), such a mechanism is very unlikely to be of any importance in the developed Western world. It has also been thought highly improbable that processes occurring so early in life could determine aspects of health 50–70 years later. Therefore, in addressing the issue of what life-time 'windows' are important in producing NIDDM we shall first consider whether nutrition during pregnancy could be an issue in the Western world and whether processes at this early stage could be of long-term importance.

### IS NUTRITION DURING PREGNANCY A MATTER OF ANY CONCERN IN THE WESTERN WORLD?

There is abundant evidence that a substantial proportion of the populations of countries such as the USA and the UK suffer from inadequate nutrition during pregnancy. This problem has been most dramatically highlighted in recent years by the recognition of the role of folate deficiency in the aetiology of neural-tube defects in the newborn. In a review of the subject, Rosenberg (1992) concluded, 'The observations on the efficacy of folic acid in preventing birth defects establish nutritional needs at a new moment in the life cycle, the periconceptional period. Our concept of nutritional requirements, obstetrical practice, and public health targeting may never be the same.' One may wonder, therefore, why, if it is so manifest that a nutritional deficiency during pregnancy in susceptible women can lead to a gross visible physical defect at birth, it is so hard to accept that more subtle nutritional deficiencies during pregnancy can determine the emergence of disease 50 years later.

There are many well-documented examples of poor or borderline nutrition in the USA and the UK during pregnancy. A recent international conference on maternal nutrition and

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the outcome of pregnancy concluded that in addition to folate deficiency, deficiencies of Fe, Zn, Se, vitamins E and B<sub>6</sub>, protein and iodine were matters of concern during pregnancy in different populations in developed countries worldwide (Keen *et al.* 1993). For example, the officially recommended adequate intake of Zn in pregnancy is 15 mg in Sweden. However, the calculated mean dietary Zn intake in young first-time pregnant women in Sweden was 9.4 mg. Zn supplementation during pregnancy has been reported to have beneficial effects on the outcome, such as a reduction in premature births, intrauterine growth retardation and neonatal morbidity. When diets are low in protein the Zn intake is also considerably reduced. Protein deficiency in the diet of poor pregnant women is widespread (Gonzalez-Cossio & Delgado, 1991). It was a consensus view of the conference that pre-pregnancy nutritional status significantly affects pregnancy outcome. The problem of adequate nutrition before and during pregnancy is, therefore, by no means restricted to the underdeveloped countries. In 1972 the US Congress initiated a Special Supplemental Food Program for Women, Infants and Children (WIC) following the finding that (amongst others) pregnant women were at special risk by reason of inadequate nutrition. In the fiscal year 1992 funding for this programme was \$2.6 billion. In 1990, WIC served 612 000 pregnant women. It was generally concluded that WIC improved pregnancy outcome with respect to a reduction in the incidence of low birth weight, very-low birth weight and preterm delivery. It appears that the earlier in pregnancy a woman received WIC benefits the greater the positive effects. It was also apparent that even if only the immediate short-term benefits of the outcome of pregnancy were considered, the programme was highly cost efficient. It was estimated that for every dollar spent on WIC there was an associated overall average saving of \$2.91 (Abrams, 1993).

In Britain, steps are at last being taken to address the issue of diet and low-income and related health consequences. The government has set up a Nutrition Task Force which recently issued a final report (Department of Health, 1996a). Also the Low Income Project Team set up by the Task Force has reported and highlighted (amongst other problems) the existence of poor nutrition during pregnancy as being of concern. It concludes that, 'substantial improvements in the diet of low income families throughout Britain will only be achieved through a nationally co-ordinated strategy.' (Department of Health, 1996b).

#### WHAT ARE THE CRITICAL WINDOWS FOR PERMANENT CHANGES ('PROGRAMMING')?

##### *Pregnancy and lactation*

It has been known for more than a decade that permanent changes in behaviour can be induced by the administration of a sex hormone in a narrow early time window. Exposure of female rats to a single exogenous dose of testosterone in fetal life permanently reoriented their sexual behaviour. A similar dose of testosterone in 20-day-old females had no effect (Angelbeck & Du Brul, 1983). Similarly, exposure of animals to an excess of thyroxine in the neonatal period changed the pituitary-hypothalamic responses linked to the secretion of thyroid-stimulating hormone in later life (Besa & Pascula-Leone, 1984). Extensive studies by McCance & Widdowson (1966, 1974) and Winick & Nobel (1966) and Winick *et al.* (1968) have demonstrated that interference with the animal's growth during critical stages of development could have lasting effects. Essentially, malnutrition during early fetal and neonatal life would affect cell division, organ growth and differentiation which may be irrecoverable. Later in life, it would lead to changes in cell composition and cell size.

It is also beginning to be recognized that inter-generational effects due to nutrition can occur, thereby giving the false impression that they are genetic effects. Females in the uterus in the first two trimesters of pregnancy during the Dutch Famine, when they subsequently gave birth to their children, produced newborns with significantly lower birth weights than females not so exposed during fetal life (Lumey, 1992). Thus, the nutrition of the grandmother can affect the growth of the grandchild and, hence, we would suggest the susceptibility of the latter to certain diseases.

More recent studies have shown that pregnant rats fed on a diet containing a little under half the normal protein content produced pups with reduced neonatal  $\beta$ -cell proliferation, islet size and vascularization (Snoeck *et al.* 1990). Other studies have shown that prenatal exposure to maternal low protein diet induced hypertension in the young adult offspring (Langley & Jackson, 1994).

We have studied growth and hepatic metabolism in the offspring of protein-restricted rats during pregnancy and/or lactation. If the offspring of mothers fed on an adequate-protein (200 g/kg) diet during pregnancy were nursed by mothers fed on a low-protein (80 g/kg) diet, permanent growth retardation was observed. This was despite the fact that these animals were weaned onto a normal 200 g protein/kg diet. Conversely, the offspring of mothers fed on a low-protein diet during pregnancy when nursed by mothers fed on a 200 g protein/kg diet, demonstrated a complete catch-up in growth (Desai *et al.* 1996). Therefore, when a rat dam was fed on a low-protein diet, the critical window for the overall growth of the offspring appeared to be the nursing period. Growth retardation was also accompanied by sex-dependent, selective and permanent changes in organ weights. For example, compared with the body weights, organs such as the brain and lung experienced a smaller decrease in weight, the heart, kidney and thymus decreased proportionately, whereas the pancreas, spleen, muscle and liver showed a greater reduction in weight in these offspring at 21 d of age (i.e. following exposure to maternal protein-restricted diet during pregnancy and lactation). As adults, the muscle weight in relation to the body weight was significantly lower in the male rats, whereas the weight of pancreas in relation to the body weight was significantly increased in the female rats (Desai *et al.* 1996).

Changes in liver enzyme activities associated with glucose metabolism were seen in the offspring exposed to maternal low-protein diet during pregnancy. Glucokinase (EC 2.7.1.1) was decreased by about 50% and phosphoenolpyruvate carboxykinase (EC 4.1.1.32) was increased by about 100% in the offspring. These changes were still apparent in the adult offspring despite the fact that they had been nursed by mothers fed on an adequate-protein diet and had remained on a 200 g protein/kg diet for almost 10 months (Desai *et al.* 1995). This indicates that the maternal protein diet during pregnancy can permanently alter the enzyme activities in the offspring in a direction where glucose production rather than utilization would be expected to be predominant. These results are quite striking given that neither glucokinase nor phosphoenolpyruvate carboxykinase are expressed until after birth. Thus, events during fetal life can programme what will happen during postnatal life.

In recent studies, we have also looked at the effects of a low-protein diet during pregnancy and lactation on hormonal regulation of hepatic glucose output in the offspring. These experiments revealed that at 3 months of age the male offspring of protein-malnourished rats had significantly glucagon-resistant livers. In addition, these livers had an altered response to insulin. In control animals, insulin rapidly inhibited glucagon-stimulated hepatic glucose output (HGO). In contrast, in the low-protein offspring, insulin caused an initial increase in HGO before inhibition occurred (Ozanne *et al.* 1996). These experiments reveal that poor nutrition during fetal and early postnatal life can have long-

term effects on the control of hepatic glucose output. A striking parallel exists between these findings and studies of HGO following the administration of oral glucose in Australian Aborigines, many of whom were destined to develop NIDDM (O'Dea, 1991).

We have also investigated the influence of maternal low-protein diet on the longevity of the offspring. The combined effect of maternal low-protein diet during pregnancy and lactation did not alter longevity in the offspring. However, a beneficial effect in terms of increasing the lifespan, particularly of the male offspring, was seen when the exposure to maternal protein restriction was limited to the nursing period only. Conversely, when the exposure to maternal protein restriction occurred during gestation alone, it had a detrimental effect and the lifespan of the male offspring was significantly shorter. A similar trend was observed in the female offspring, although it was not statistically significant. Thus, longevity can be permanently altered by events in early life in the rat, with reduced longevity being associated with 'catch-up' growth (Hales *et al.* 1996). It is possible that similar patterns may arise in the human male. Men who were light at birth but are of above median adult height have been found to have particularly high blood pressure (Leon *et al.* 1996).

#### *Early post-weaning*

We extended the studies described previously to determine the effect of lengthening the period of protein restriction to 10 weeks of age. Offspring of female rats fed on the 80 g protein/kg diet during pregnancy and lactation, were weaned onto the same reduced protein diet and compared with rats fed on the 200 g protein/kg diet. By 70 d of age female rats given the low-protein diet had reduced indices of growth: body weights (reduced 39 % compared with that of similar rats given the 200 g protein/kg diet), body lengths (15 % reduced) and BMI (15 % reduced; Fig. 1). This was mirrored by reductions in weights of individual organs. Pancreas, liver, spleen, heart, lungs and ovaries were reduced in proportion to total body weight. The tibialis anterior muscles and kidneys were so affected as to be reduced in weight relatively more than the reduction in total body weight. In contrast, the brain appeared to be somewhat preserved, in that although the protein reduction appeared to reduce its overall weight, this was proportionally less than the reduction in total body weight (only being 15 % reduced compared with the 39 % reduction in body weight).

#### *Adulthood*

Recently we have investigated the effect of a nutritionally-rich diet on adult rats who had been growth retarded by early protein restriction. For this study rats given the 80 g protein/kg diet until 10 weeks of age and rats who had been fed on a control (200 g protein/kg) diet during an equivalent period were given one of two different adult diets: some of them being fed on a standard laboratory chow in pellet form ('pellet' diet) and other rats being fed on a cafeteria-style diet containing a mixture of the laboratory chow with condensed milk and sugar ('cafeteria' diet; adapted from Wilding *et al.* 1992). These diets were continued until the rats reached 20 weeks of age. Whilst the postweaning rats were fed on the 80 or 200 g protein/kg diets, the energy intakes of female rats given the low-protein diet were significantly lower than those of the control rats (Fig. 2(a)). When the rats were transferred onto their adult diets, this difference in energy intake was preserved between the low-protein and control animals given the pelleted diet and between those

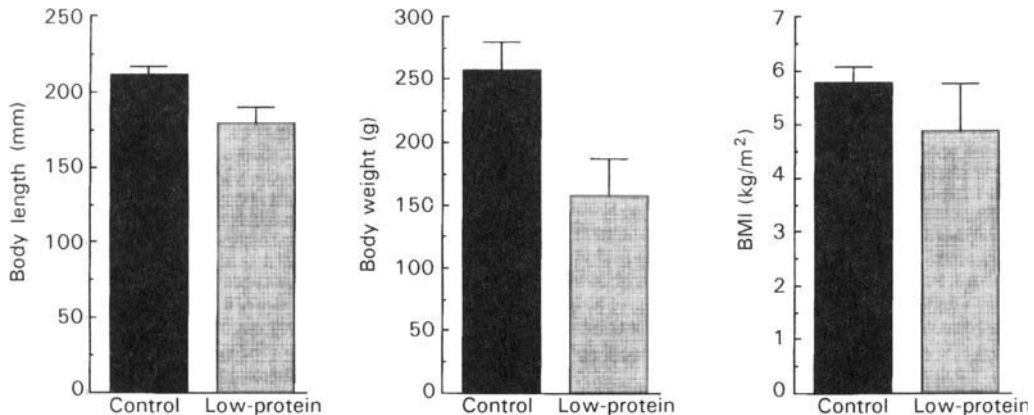


Fig. 1. The body lengths, body weights and BMI of 70-d-old female rats. Their mothers had received either a 200 g protein/kg (control) or an 80 g protein/kg (low-protein) diet throughout pregnancy and lactation, and then these rats were weaned onto the same diets. Values are means and standard deviations represented by vertical bars for sixteen rats per group (from at least eight different litters). The low-protein diet was associated with significant reductions in body lengths, body weights and BMI (all  $P < 0.001$ ; Student's  $t$  test).

animals given the cafeteria diet. However, those rats given the cafeteria diet had considerably higher energy intakes than equivalent rats given the pelleted diet (Fig. 2(a)). This meant that between 10 and 20 weeks of age low-protein rats subsequently given the cafeteria diet had higher energy intakes than control rats given the pelleted diet.

The body weights of the female rats given these diets largely reflect their energy intakes (Fig. 2(b)). At 10 weeks of age, when the diets were changed the low-protein rats weighed considerably less than the controls. This difference in body weight between control and low-protein rats was still evident at 20 weeks of age in both pelleted-diet and cafeteria-diet groups. However, those rats given the cafeteria diet put on considerably more weight than those rats given the pelleted diet. Overall, this had the effect that the rats growth retarded by early protein restriction, who were subsequently fed on the cafeteria diet, caught up in terms of body weight with control rats subsequently given the pelleted diet. Indeed, by 17 weeks of age there was no statistically significant difference in the body weights of these two groups ( $P > 0.05$ ; Student's  $t$  test). However, whilst their weights were not significantly different, the rats who were initially protein deprived were shorter than the control rats at 140d of age (mean length 225 (SD 6) mm v. 228 (SD 7) mm;  $P < 0.01$ ). A trend for shorter, fatter rats was also seen with BMI, although statistical significance was not reached (mean BMI 6.23 (SD 0.39) kg/m<sup>2</sup> v. 5.97 (SD 0.48) kg/m<sup>2</sup>;  $P = 0.11$ ; Fig. 3). It is thus apparent that a low-protein diet during fetal and early life can have permanent effects on body mass, body length and BMI irrespective of the adult diet.

Weight gain as a measure of growth of organs and tissues could be misleading in these animals since the body weight may also increase as a result of fat storage. For instance, unlike the body weight, most of the organs did not exhibit a corresponding increase in weight (brain, spleen, lungs and tibialis anterior muscles). Some organs showed a relative decrease in weight (pancreas, kidneys and heart), which could be interpreted as those organs showing no change in weight as a result of the cafeteria diet. The organs that increased proportionally with body weight were the liver and ovaries.

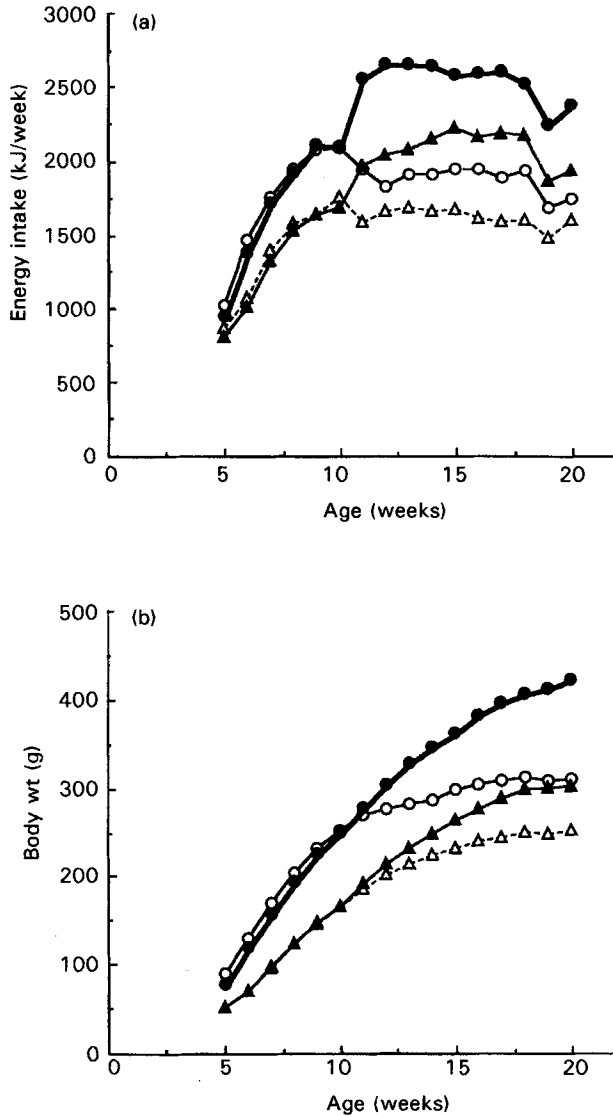


Fig. 2. (a) Energy intakes and (b) body weights of female rats. The mothers of these rats had been given either a 200 g protein/kg (control) or an 80 g protein/kg (low protein) diet throughout pregnancy and lactation. These rats were then weaned, at 4 weeks of age, onto the same diet as their mothers. They continued on these diets until they were 10 weeks old and then were transferred either onto a Porton combined (pelleted) diet or a highly-palatable (cafeteria) diet consisting of a mixture of the Porton combined diet, condensed milk, sugar and water. Values are means for sixteen rats (from at least eight different litters). Two-way ANOVA of energy intakes: association with early diet (control or low-protein)  $P < 0.05$  (at all ages), association with adult diet (pelleted or cafeteria)  $P > 0.05$  (before week 11) and  $P < 0.001$  (after week 10), statistical interactions between these two variables  $P < 0.05$  (weeks 12–14 and 20) and  $P > 0.05$  (all other weeks). Two-way ANOVA of body weights: association with early diet (control or low-protein)  $P < 0.001$  (at all ages), association with adult diet (pelleted or cafeteria)  $P > 0.05$  (before week 12) and  $P < 0.002$  (after week 11), statistical interaction between these two variables  $P > 0.05$  (before week 13) and  $P < 0.001$  (after week 12). (○), Control, pelleted; (●), control, cafeteria; (△), low-protein, pelleted; (▲), low-protein, cafeteria.

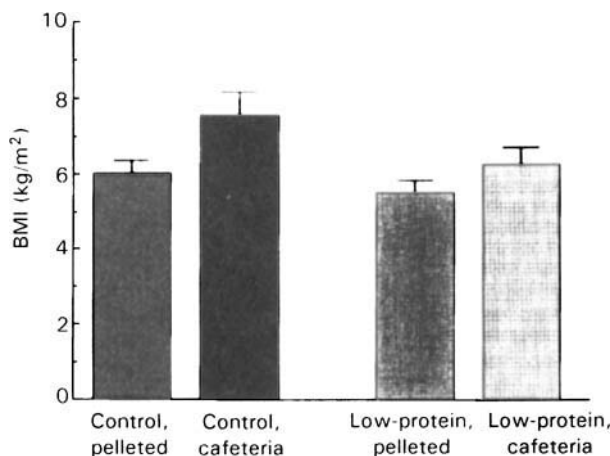


Fig. 3. BMI of 140-d-old female rats. The mothers of these rats were given either a 200 g protein/kg (control) or an 80 g protein/kg (low-protein) diet throughout pregnancy and lactation. These rats were then weaned, at 4 weeks of age, onto the same diet as their mothers. They continued on these diets until they were 10 weeks of age and were then transferred onto a Porton combined (pelleted) diet or a highly-palatable (cafeteria) diet consisting of a mixture of the Porton combined diet, condensed milk, sugar and water. Values are means and standard deviations represented by vertical bars for sixteen rats per group (from at least eight different litters). Two-way ANOVA: association with early diet (control or low-protein)  $P < 0.001$ , association with adult diet (pelleted or cafeteria)  $P < 0.001$ , statistical interaction between these two variables  $P = 0.002$ . The rats given the early low-protein diet followed by the cafeteria diet caught up in weight with those rats given the early control diet followed by the pelleted diet (see p. 237). The former group were significantly shorter than the latter group ( $P < 0.01$ ; Student's  $t$  test) but the differences in BMI did not reach statistical significance ( $P = 0.11$ ; Student's  $t$  test; see p. 237).

#### COMBINATION OF EARLY AND LATE DIETARY MANIPULATION

The test of early nutritional programming is whether the effects of early diets, i.e. protein deprivation in this case, hold true even after a period of nutritional recuperation, i.e. the use of the cafeteria diet in this case. In this respect a number of organ weights were shown to be permanently altered by the protein deprivation in early life. The kidneys were still reduced in weight in the rats which were protein restricted early in life and the reduction in organ weight was still greater than the reduction in total body weight attributable to protein deprivation. The liver, lungs, spleen and heart were still reduced in weight in proportion to the reduction in body weight. The brain, although reduced in weight, was still increased in relation to the reduction in body weight. These results demonstrate that early protein deprivation causes selective preservation of some organs, such as the brain, at the expense of others such as the kidneys. Observations such as these are the basis of the Thrifty Phenotype hypothesis (Hales & Barker, 1992) in which it is proposed that during times of nutritional thrift, the developing organism diverts essential nutrients to selected organs.

Not all organ weights were permanently altered by the early protein deprivation. Ovary weights after the cafeteria or pelleted diets were unaffected by early protein restriction. Thus, they were able to catch up their early growth restriction. Of interest is the partial recovery of organ weights seen in the pancreas and anterior tibialis muscles. Both organs were still reduced in weight by the early protein restriction, but the pancreas was now reduced relatively less than the reduction in total body weight and the muscles only in proportion to that of body weight. This opens the possibility that different organs have different critical 'windows' at which period of time nutritional deprivation is able to

permanently alter their future weights. In this case the period of protein deprivation may only have partially covered the critical period for the long-term alteration of pancreas and muscle weights. A more extended period of protein deprivation may well have permanently altered the weights of these organs. A change in organ weights is of course a very crude and incomplete index of the programming of the structure and function of an organ. Organs such as the liver may exhibit no change in adult organ weight after an earlier period of protein restriction (during pregnancy alone) and yet exhibit clear changes in enzyme composition (Desai *et al.* 1995).

### CONCLUSIONS

We have reviewed the evidence that there are a number of critical periods ('windows') in which the growth of the whole organism or individual organs can be determined ('programmed'). The effect of poor nutrition on organ growth is also gender specific. Epidemiological evidence strongly suggests that changes in organ growth consequent upon poor nutrition can have a major impact on disease susceptibility, particularly in the so-called 'degenerative' diseases which include NIDDM and the insulin-resistance syndrome. The precise outcome of poor nutrition is likely to depend on the critical window(s) and the particular nutrient involved. A summary of some of the windows is given in Table 1. Nutritional effects can be transmitted over more than one generation. Thus, it is likely that reversal of adverse consequences of poor nutrition will require improved nutrition over several generations. Furthermore, 'improved' nutrition in terms of increased energy intake leading to obesity can, at least within one generation, have detrimental effects. Obesity during pregnancy may lead to gestational diabetes which has been shown to predispose to diabetes in the offspring of Pima Indians (McCance *et al.* 1993). There is no doubt, therefore, that in attempting to disentangle the effects of nutrition to increase susceptibility

Table 1. *Some critical windows of nutrition*

Window	Consequence
Pregnant grandmother Grandmother starved during first two trimesters of pregnancy	Decreased birth weight of grandchild (Lumey, 1992)
Pre-pregnancy Mother's pre-pregnancy nutrition	Outcome of pregnancy (e.g. poor nutrition leading to a low birth weight, prematurity; Keen <i>et al.</i> 1993)
Pregnant mother High carbohydrate and/or energy intake in early pregnancy	Small placenta, adult hypertension (Campbell <i>et al.</i> 1996)
Low protein intake in late pregnancy	?Reduced islet $\beta$ -cell growth (Godfrey <i>et al.</i> 1996)
Lactation Offspring of adequate-protein-fed dams nursed by protein-restricted dams	Permanent growth retardation; increased lifespan (in males; Hales <i>et al.</i> 1996)
Offspring of low-protein-fed pregnant dams nursed by adequate-protein-fed dams	Catch-up growth; reduced lifespan (in males; Hales <i>et al.</i> 1996)
Adult Overnutrition	Obesity, IGT or NIDDM depending on early-life events

NIDDM, non-insulin-dependent diabetes mellitus; IGT, impaired glucose tolerance.



to diseases such as NIDDM a great deal of painstaking research both in different populations and different animal models is required. However, in view of the potential rewards in terms of long-term health benefits to be gained in understanding optimal nutrition before, during and after pregnancy, a high priority should be given to this research.

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