Non-insulin-dependent diabetes mellitus

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Non-insulin-dependent diabetes mellitus (NIDDM) occurs predominantly after the age of 50 years but is not easy to distinguish from late onset insulin-dependent diabetes. It is likely that misclassification is rare in a Caucasian population. Whilst NIDDM is widely believed to be genetically determined, recent epidemiological observations have consistently revealed statistical associations between indices of poor fetal and infant growth with susceptibility to loss of glucose tolerance in adult life. A possible explanation of these observations is that environmental constraints on fetal growth lead to permanent changes in organogenesis such that a poor capacity for insulin secretion and insulin resistance result. It is postulated that these adaptive responses serve to preserve the growth of certain organs, such as the brain, at the expense of others, such as the viscera. In addition, alterations in the function of organs, such as the liver, serve to aid survival of the offspring under conditions of poor postnatal nutrition. The results of studies of an animal model in which pregnant rats were fed a reduced protein diet are consistent with these concepts.

Diabetes mellitus

Definitions of types of diabetes

The diagnosis of diabetes mellitus continues to be based upon the measurement of the glucose concentration in the blood stream, usually after the consumption of 75 g of glucose by mouth (the oral glucose tolerance test). It is widely recognised that this procedure suffers from a number of defects, not the least being the relatively poor reproducibility on repeat testing of the same individual. However, until there is better understanding of the pathogenic processes which lead to the common forms of diabetes, it is unlikely that other tests will be accepted in replacement, particularly because of the loss of cross reference to the very large literature based on the outcome of the standard oral glucose tolerance test.
Most diabetes can be attributed to one of two types, insulin-dependent (IDDM or Type I) and non-insulin-dependent (NIDDM or Type II). Whilst the majority of sufferers from the former are first diagnosed under the age of 30 years and the latter after the age of 50 years, there is a significant overlap of the age distribution leading to potential problems of categorisation. The less difficult situation concerns the rare instances of non-insulin dependent diabetes of early onset. The recognition that such a patient can survive for several years without insulin treatment and of a clear pattern of inheritance over several generations reveals maturity onset diabetes of the young (MODY). Causative mutations of the glucose-sensing enzyme glucokinase have been described in a number of families, but there remain mutations of other molecules still to be discovered\(^1\) (see ‘Note added in proof below).

The more difficult distinction to make is between typical NIDDM and late onset IDDM. It is well recognised that many of the former eventually require insulin treatment and also that the rate of progression from first diagnosis to insulin requirement of the latter can be quite slow (over several years). The prevalence of late onset IDDM is not clear and may well vary quite widely amongst different populations. A major cause of this uncertainty is the lack of definitive markers of IDDM and the fact those markers which do exist (largely the demonstration of various circulating autoantibodies) are not consistent between themselves. In studies carried out in largely Caucasian groups of men and women in England, the frequency of detection of islet cell antibodies has been found to be very low (CHD Fall and GF Bottazzo, personal communication) encouraging us to believe that any error introduced by misclassification in this population is likely to be very small. It cannot be assumed that this finding is necessarily widely applicable and, therefore, tests for the presence of the common IDDM autoantibodies should probably be used in all present day studies of the epidemiology of NIDDM.

The pathogenic mechanisms leading to NIDDM are poorly understood and controversial. It is not clear whether the primary event is insulin resistance leading to a secondary failure of insulin secretion or whether insulin deficiency is the major abnormality or in turn there is a mixture of both processes.

**Genetics of NIDDM**

It is generally observed that NIDDM has a familial distribution. However, this distribution does not follow any clearly discernible pattern of inheritance, unlike that of MODY. There is a widespread
belief, nevertheless, that NIDDM is predominantly genetically determined and that the difficulty of defining a clear genetic pattern can be explained by a combination of the late onset of the disease plus its polygenic nature. Several years ago, Neel proposed that the widespread occurrence and high prevalence of NIDDM in certain populations was due to the causative genes conferring survival advantages during human evolution. His 'thrifty genotype' hypothesis envisaged that much of human evolution had occurred against a background of borderline nutrition. Thus it was suggested that genes which were beneficial to survival under these conditions ('thrifty') have been preserved in the population only to prove disadvantageous, in terms of the propensity to develop NIDDM, when nutrition became plentiful and obesity common.

Support for the genetic origin of NIDDM is derived mainly from studies of identical twins and of populations with different racial admixture. Differences in the prevalence of NIDDM have been shown to correlate with the degree of racial admixture. As has been argued elsewhere, such evidence can be readily challenged and does not in my opinion provide a secure basis for this aetiological hypothesis. Early data describing high concordance rates for NIDDM in monozygotic twins may have been due to an ascertainment bias from the sampling of diabetes clinics. More recent studies have found much lower concordance rates. Twin data may also be related to the similarities of the environment—particularly intrauterine—rather than to genetic factors. Studies of different racial groups may be confounded by differences in their socio-economic environment, an important determinant of NIDDM incidence (see below).

Epidemiology of NIDDM

Wide variation between populations

It has been estimated that between 100–200 million people worldwide have diabetes. Nevertheless, the prevalence of the disease varies very widely from one population to another. In certain groups of North American Indians and in some Pacific Island populations 30–40% of the adult population can be affected. At the other extreme, prevalences of diabetes of 1% or less have been described in rural Melanesian and Bantu populations. Clearly these large variations in prevalence should be providing clues as to the underlying processes leading to diabetes. It seems unlikely that they can be explained on a simple genetic basis because, even within a relatively homogeneous population with a high
prevalence of NIDDM, it is not possible to discern simple patterns of inheritance.

A rapid increase in the prevalence of NIDDM in a population is associated with a typical change in life style. It is the change from a rural environment with poor nutritional resources and high physical activity to an urban environment where these conditions are reversed. Well recognised examples of this process include populations of Australian Aborigines, rural to urban migration in India and Africa, emigration of Asians to the West and the movement of Ethiopian Jews to Israel.

Role of low socio-economic status

In contrast to the precipitating role of affluence and physical inactivity described above, which relates to populations coming from a very poor nutritional and economic background, is the finding that in developed countries it is people living in the worst socio-economic environment who have the highest prevalence of NIDDM. This has been demonstrated in England and is apparent amongst the black population of the US. It may be that in developed countries people who have been relatively well nourished over more than one generation are protected against the otherwise detrimental effects of exposure to abundant nutrition. Certainly in the Whitehall II study in the UK, there was a strong relationship between diseases such as ischaemic heart disease and diabetes and lower job status. There was also a strong relationship between the subject’s job status and the percentage of the fathers who were in the manual social class such that subjects with a low job status had the highest percentage of fathers who were in the manual social class. However, reports of parents having had a heart attack were more frequent in those with higher status jobs perhaps suggesting that the adverse effects of good nutrition on one generation may translate into beneficial effects on subsequent generations. A possible example of this phenomenon may be taking place on the island of Nauru in the Pacific. Earlier poor nutrition was abruptly terminated after the Second World War consequent upon prosperity acquired from phosphate mining. NIDDM became extremely common. However, recent studies have shown a striking reduction in the amount of impaired glucose tolerance in the generation conceived under better nutritional circumstances.
Early life anthropometry and subsequent risk of NIDDM and the insulin resistance syndrome

Epidemiology

Epidemiological studies showing statistical associations between indices of poor human fetal and infant growth and subsequent glucose intolerance have suggested novel mechanisms which may predispose to the development of NIDDM. The availability of archival data collected by Barker and his colleagues at the MRC Unit of Environmental Epidemiology at Southampton (see this issue) has provided the basis for determining whether poor growth in early life may lead to NIDDM in the middle-aged and elderly. A number of strong relationships have emerged—many of which have been confirmed by others in a variety of different populations. A summary of the findings is as follows:

1. Low birth weight or low weight at age 1 year was strongly related to glucose intolerance in men of average age 64 years whether glucose tolerance be defined by the 2 h plasma glucose concentration after a standard oral glucose tolerance test or whether subjects be defined as having newly diagnosed NIDDM or impaired glucose tolerance (IGT) by WHO criteria.\(^{10}\)

2. The relationship with weight at 1 year was not entirely explained by the relationship between birth weight and weight at 1 year.\(^{10}\)

3. In the same population similar but less strong relationships were observed in women.\(^{11}\)

4. Features of the insulin resistance syndrome (sometimes described as ‘syndrome X’), such as glucose intolerance, hypertension and hypertriglyceridaemia, were even more strongly related to birth weight. A male infant of birth weight less than 2.5 kg was 18 times more likely to show these features than one of birthweight greater than 4.31 kg. Data from a separate English community in which both men and women were studied showed relationships of a very similar nature and strength.\(^ {12}\)

5. A direct test of insulin resistance showed that it was most strongly predicted by thinness at birth.\(^ {13}\)

6. Both the relationship between birth weight and subsequent glucose intolerance and thinness at birth and subsequent insulin resistance interact with adult obesity so that the combined effect of poor early growth and subsequent adult obesity leads to the greatest changes.\(^ {13}\)

7. Whilst changes in adult life style leading for example to obesity added to the effects of poor early growth, the effects of poor early growth on glucose tolerance could be detected in much younger populations of
men (mean age 21 years) and of children (mean age 7 years). It is, therefore, clear that the relationships observed with poor early growth were not simply due to confounding by the possible relationship between poor early growth and subsequent adult life style. It appears that like the well known 'tracking' phenomenon of blood pressure throughout life such that children with higher blood pressure go on to become adults with higher blood pressure the same is likely to be true of glucose intolerance.

Although social class clearly affects early growth none of the above relationships are simply due to social class. Low social class is associated with low birth weight. Low social class in England (see above) is associated with an increased incidence of NIDDM. Nevertheless an offspring of low birth weight born to a family of higher social class is at increased risk of subsequent glucose intolerance.

**Thrifty phenotype hypothesis**

The above findings linking poor early growth to glucose intolerance and the insulin resistance syndrome could have a variety of explanations. They could be due to some unrecognised factor of adult life style which is linked to poor early growth and confounds the relationship. Without a specific suggestion as to what this might be this speculation is untestable. Poor early growth could be of genetic, infective or toxic origin. The evidence is that the genetic determination of growth, which is of undoubted importance, operates predominantly postnatally. There is no evidence that birth weight in general is determined by antenatal infection. Toxic factors cannot be excluded but there are no data to support this mechanism. Smoking is certainly an issue at the present time but is unlikely to have been of serious significance in these populations in the period 1920–1940.

In a study to investigate the relative role of environmental and genetic factors in the determination of birth weight, 62 cases of ovum donation were investigated. Donor weight, her own birth weight and the birth weight of the donor's own children were not significantly correlated with the birth weight of the child following ovum donation. However, the recipient's weight was, whereas her height was not. It was concluded that the environment provided by the human mother was more important than her genetic contribution to birth weight.

Thus the major factor determining fetal growth, whether it be human or mammalian, in general is undoubtedly environmental and usually nutritional. There exists a large volume of work which shows this
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clearly. Equally important, however, is the outcome of studies of experimental animals in which the irreversibility of deficits of early growth is documented. In broad outline the pattern emerges that restriction of growth via poor nutrition which leads to a deficit of tissue/organ DNA as a measure of cell number is irrecoverable. However, a restriction of growth which only reduces cell size or cell protein content may be recovered. Many tissues and organs are largely or completely formed with regard to cell numbers at or shortly after birth. Most of postnatal growth is a consequence of the enlargement of pre-existing cells rather than the accretion of additional cells. Such a generalisation is of course dependent on the particular tissue or organ concerned and certainly not absolute for all tissues\textsuperscript{16}.

We have, therefore, hypothesised that poor fetal nutrition imposes a strategy of ‘thrift’ upon this growing organism\textsuperscript{17}. The commonest cause of poor fetal nutrition world wide would undoubtedly be poor maternal nutrition. One must also put alongside the direct transmitted effects of poor maternal nutrition lending to poor fetal nutrition the indirect consequences of the maternal hormonal milieu to which the fetus is directly or indirectly exposed and which will be different if the mother is poorly nourished. One can, therefore, envisage that changes in maternal hormones may be responsible for some of the fetal changes which are the consequences of poor maternal nutrition. It is important also to take into account more remote nutritional influences on the outcome of pregnancy. Females who were \textit{in utero} in the first and second trimester of pregnancy during the Dutch famine (a war induced famine, November 1944 to May 1945) as adults gave birth to children of lower birth weight than matched controls\textsuperscript{18}. In this context, it is possible to envisage that environmental effects can be transmitted across the generations leading to the erroneous supposition that they are genetically transmitted. By the same token, one has a possible explanation of the observation that NIDDM is apparently more frequently transmitted from the mother than the father\textsuperscript{19}. It is also important to recognise that in attempting to observe or induce reductions in NIDDM due to the sustained improvement of nutrition of populations, with consequent increases in birth weight, that improvements will take more than one generation to reach maximum effect. Furthermore, improved nutrition of populations seems to be linked to the ever increasing prevalence of obesity. Increasing obesity will counteract the beneficial effects of improved fetal and infant growth.

It would be wrong, however, to conclude that the fetus that is undergoing a process of nutritional thrift is necessarily pathologically affected. We believe that these are normal adaptive strategies likely to be part of the general mechanisms by which at least mammalian organisms respond to the environment and hence enhance prospects for survival.
The outcome of these strategies only gives rise to pathological processes when the adaptations incurred conflict with the subsequent environment. The organism adapted to poor nutrition during fetal life responds poorly in the long term to normal or supranormal nutrition in postnatal life. Even these pathological consequences may have little significance for the survival of the species, since they occur mainly in late life when reproduction and the rearing of offspring is complete. Thus natural selection would have little or no effect on them.

The main adaptive strategy during poor fetal nutrition that has been identified thus far is the selective redistribution of nutrients to diminish the consequences for the growth of certain organs, such as the brain at the expense of greater loss of growth of other organs. In confirming this general pattern in the rat, we have also found that the prioritisation of organ growth differs between the male and the female (see below). Our recent results in an experimental rat model also now lead us to propose another important strategy that is adapted, that of a permanent shift of the set point in certain metabolic regulatory processes. This shift is in a direction beneficial to survival under the conditions the fetus 'expects' to encounter. Thus we propose that the fetus which is relatively starved in utero emerges with the metabolism of certain key organs, such as the liver, permanently biased towards a metabolic setting which would be adopted if it were starved as an adult. It is an interesting subject for future research to determine whether this phenomenon can be expanded into a general principle. The general question is whether environmental changes, which invoke a particular adaptive response in the adult, if applied to a pregnant rat or very young offspring permanently change the structure/function of that offspring in the direction adopted temporarily when the adult so reacts. Thus one could envisage fetal exposure and plasticity as leading to a certain permanent 'hard wiring' of the adult response.

The thrifty phenotype hypothesis also proposed that the combination of loss of glucose tolerance with other changes such as hypertension and hyperlipidaemia (sometimes referred to as syndrome X or the insulin resistance syndrome) could reflect other consequences of poor fetal growth and development. Because of the known detrimental effects of protein malnutrition both pre- and postnatally in rat and humans, we drew particular attention to this dietary constituent as being of importance. It is very unlikely that fetal growth retardation is entirely due to poor protein intake. Indeed recent studies in humans have drawn attention to the importance of a high energy/carbohydrate intake early in pregnancy as well as that of low protein intake late in pregnancy in reducing placental weight\(^{20,21}\). Thus the spectrum of adult diseases set in train by poor fetal and infant growth will be determined by the type and timing of the growth restraint.
Animal models

Testing the thrifty phenotype hypothesis

It is impossible within the life time of one investigator to directly test the effect of nutrition during human pregnancy on the emergence of diseases some 50–70 years later. Whilst in the relatively near future some trials of intervention will be necessary and it is clear that it is possible to improve human birth weight by nutritional interventions\(^2\), it is essential to proceed with considerable caution. High protein intakes during pregnancy have been found to reduce birth weight\(^3\). Furthermore, poorly nourished human communities exist in a relatively stable, if in the long term undesirable, equilibrium. An abrupt change in fetal size might well compromise delivery. The process of lactation might not improve in parallel with fetal growth, and so on. We also need to know much more about the range and outcome of growth restraints which operate during human pregnancy.

Therefore, in addition to the need for more epidemiological studies of a range of human populations and diets in relation to the outcome of pregnancy, much of the immediate research need is for studies of appropriate animal models. It is doubtful if any single model will be adequate. Rats have the advantage of relative cheapness, large size of litters and speed of maturation together with a diet and physiology which is reasonably close to the human equivalents. However, their small size restricts the scope of research which can be carried out antenatally. Sheep have provided an alternative which overcomes the latter problem but their diet and metabolism is very different from the human. In this respect pigs may prove a more attractive alternative with the additional benefit of a large litter size.

The low protein rat model

For many years it has been apparent both from human\(^24,25\) and animal\(^26,27\) studies that protein or protein/calorie malnutrition in early life is detrimental to the regulation of carbohydrate metabolism and the production of insulin. More recently experiments in which pregnant and postnatal rats were exposed to an isocaloric moderate (about 50\%) protein restriction have shown highly detrimental effects on \(\beta\) cell structure and function (particularly vascularisation)\(^28,29\). We have chosen to adopt this model in the first instance to examine two particular questions: (i) does short term early exposure (up to weaning) lead to long-term permanent changes in offspring?; and (ii) is it possible to
produce insulin resistance, as well as the insulin deficiency referred to above by this dietary manipulation?

In addressing these questions we have in the first instance concentrated on the liver and in particular two of the key liver enzymes regulated by insulin one involved in glycolysis (glucokinase, GK) and the other in gluconeogenesis (phosphoenolpyruvate carboxykinase, PEPCK). The results may be summarised as follows:

1. Offspring of dams fed on low protein diet during pregnancy and lactation at weaning showed an approximately 50% reduction in GK and 100% increase in PEPCK.
2. These changes in enzyme activity persisted 10 months later despite weaning onto a normal diet.
3. The results in two cross-over groups of offspring whose dams had either experienced the low protein regime during pregnancy or lactation showed that exposure during pregnancy alone was all that was required to produce the effect. This observation is particularly interesting since neither of these enzymes is expressed until after birth.
4. GK is expressed predominantly in the perivenous zone of the hepatic lobule whereas PEPCK is predominantly in the periportal zone. It was hypothesised that one explanation of the findings could be a permanent structural change in the lobule such that the periportal zone was expanded and the perivenous zone contracted.
5. Consistent with this hypothesis was the finding that two other enzymes expressed, respectively in the periportal or perivenous zones, namely carbamylphosphate synthetase and glutamine synthetase were changed in parallel with PEPCK and GK.
6. When the glucose output of isolated perfused livers was stimulated by glucagon the effect was found to be reduced in the livers of offspring of dams fed low protein. The addition of insulin to the glucagon-containing perfusate instead of suppressing the glucagon effect as in the control livers actually led to an immediate increase in output. The reduction in the glucagon effect could be due to an observed reduction in the content of glucagon receptors but the altered insulin effect coincided with a rise in the number of insulin receptors. Nevertheless, these changes are consistent with the suggestion of a change in the zonal distribution of metabolism since glucagon receptors have been observed to be higher in the perivenous and insulin receptors in the periportal zone of the lobule.
7. The small age-related loss of glucose tolerance seen in both control male and female rats was exaggerated in the offspring of dams fed low protein diets. At 15 months of age although the latter had worse glucose tolerance than the controls, the differences were small and the animals were
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certainly not diabetic. Loss of glucose tolerance in the males appeared
more related to insulin resistance and females to insulin deficiency.32.

Longevity

In the process of carrying out experiments on this model we decided that
it was also important to ask the more general question as to whether life
span itself might be affected by very early and short-lived dietarily-
induced growth retardation. Uniformly, and independently of early
growth patterns, male rats were found to have significantly shorter life
spans than females.31. Growth retardation in early life was found to exert
different and opposite effects depending on whether the retardation
occurred pre- or postnatally. Whilst the pattern of affects was the same
in males and females, the effects were larger and statistically significant
in the males. Postnatal growth retardation due to the suckling by pups of
protein-restricted dams proved to give a permanent reduction in weight
gain in adult life and to increased longevity. Antenatal growth restriction
as a consequence of a reduced maternal protein diet if followed by ‘catch
up’ growth postnatally during suckling by a normally fed dam led to a
reduction in longevity. The combination of the affects of pre and
postnatal growth retardation working in opposite directions cancelled
each other and led to unchanged longevity.32.

It has been known for many years that undernutrition increases
longevity in a variety of animals. As far as we are aware it has not been
previously shown that even a very short period of undernutrition
confined to the first 3 weeks of postnatal life not only permanently
reduces weight gain but also increases longevity. Perhaps of more
significance however is the possibility that ‘catch-up’ growth following
intra-uterine growth retardation is detrimental. We do not know
whether this finding is relevant to humans and if so which are the
critical time periods. The recent observation that men who were born
small but who grew to become above average height and obese as adults
became hypertensive suggests that this question merits careful study.

Implications and further research

The epidemiological evidence that indices of poor early growth in
humans are strongly linked to loss of glucose tolerance and the insulin
resistance syndrome is consistent and has been replicated in populations
as diverse as the Pima Indians34, Mexican Americans35 and in
Sweden.33,36. It is clearly very important to determine what underlies
these relationships. It is particularly important to discover whether they reflect environmental or genetic factors because of the consequences for intervention. How is a definitive answer to be obtained? One possibility is to wait upon the time and expenditure required to sequence the human genome. If no single gene defects emerge will that prevent a further wave of research into endless multiple gene combinations to satisfy those who favour the polygenic theory?

Investigations of potential infective or toxic factors as an underlying cause await the suggestion of possible candidates. None are apparent thus far. The nutritional theory suggests much further research although it has to be acknowledged that providing definitive support is difficult for the reasons discussed above. Despite this reservation, the strategy for further research seems clear. It requires an iterative sequence of studies from the human epidemiological to animal models and back. In this way we shall increasingly refine the understanding of what changes dietary factors during pregnancy and postnatally are capable of inducing in experimental animals. We may then test, inevitably relatively indirectly, whether similar changes occur in humans. Sooner or later intervention studies will be warranted and will ultimately prove the definitive test of these proposals. However it will be essential to generate shorter term end points than the emergence of NIDDM itself if understanding is to advance at a reasonable pace. Thus an important early requirement is to establish and validate indices of human organ growth and function which can be applied during fetal and early infant life. Answers to these questions are urgently required since the 'epidemic' of NIDDM is upon us.

**Note added in proof**

Gene mutations associated with MODY3 and MODY1 have recently been described.

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