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Review

Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis*

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In this contribution we put forward a novel hypothesis concerning the aetiology of Type 2 (non-insulin-dependent) diabetes mellitus. The concept underlying our hypothesis is that poor fetal and early post-natal nutrition imposes mechanisms of nutritional thrift upon the growing individual. We propose that one of the major long-term consequences of inadequate early nutrition is impaired development of the endocrine pancreas and a greatly increased susceptibility to the development of Type 2 diabetes. In the first section we outline our research which has led to this hypothesis. We will then review the relevant literature. Finally we show that the hypothesis suggests a reinterpretation of some findings and an explanation of others which are at present not easy to understand.

Insulin deficiency in Type 2 diabetes

The controversy concerning the relative roles of insulin deficiency and insulin resistance in Type 2 diabetes continues unresolved. Despite the early demonstration that obese people have elevated plasma insulin concentrations [1] many studies over the years have failed to control adequately for the influence of obesity. Another difficulty with the interpretation of plasma insulin concentrations is that sustained hyperglycaemia could have detrimental effects on insulin secretion.

In the 1960s one of us (CNH) attempted to determine whether subjects with a normal fasting glucose concentration but a delayed return of glucose to the fasting concentration after oral glucose (a condition similar to but not identical with that now defined as "impaired glucose tolerance") had poor insulin secretion early in a glucose tolerance test [2]. Subjects thus identified were studied again 5 years later to determine their tendency to deteriorate to diabetes [3]. Obese subjects in this group showed the greatest deterioration of glucose tolerance [3]. It was concluded that obese subjects with defective initial rises in plasma insulin concentration were those most likely to develop diabetes. Unfortunately the relatively small numbers of subjects who could be studied in those days meant that this finding could only be taken as suggestive rather than definitive.

Whilst this work was in progress the discovery of proinsulin [4], the later demonstration of its presence in plasma [5, 6] and of its elevation in the plasma of Type 2 diabetic subjects [7–9] raised a question concerning the specificity of insulin measurements in plasma. It was apparent from early days that proinsulin cross-reacted strongly in many insulin radioimmunoassays. A potential solution to the assay problem lay in the exploitation of immunoassay techniques involving the use of labelled antibodies termed "immunoradiometric" assays. These were developed in a variety of configurations in one of our laboratories over the years [10–14] leading to what was termed an "indirect two site immunoradiometric assay" of human proinsulin [15]. It was something of a surprise to discover subsequently, with the advent of bioengineered human proinsulin [16], that this assay did not detect intact human proinsulin but rather the sum of the partially proteolysed derivates on the pathway of conversion to insulin [17]. This finding led to the inevitable conclusion that a significant amount of the proinsulin-like material in plasma was partially split rather than intact. Further work, this time exploiting the monoclonal antibody technique [18], was required to devise assays with adequate specificity to resolve the complex mixture of insulin-like molecules present in plasma [19].

The new assays were applied to the re-investigation of plasma insulin concentrations in subjects with established Type 2 diabetes [20, 21]. The main conclusions to emerge from these studies were: (i) the major proinsulin-like molecule in the plasma of many Type 2 diabetic subjects was the 32–33 split form. (The assays produced do not discriminate between des 31, 32, des 32 and 32–33 split proinsulin or between des 64, 65 des 65 or 65–66 split proinsulin respectively. As pointed out [19] it is probable that des 31, 32 or des 64, 65 are the main products in plasma but for simplicity the term 32–33 split is used here.), (ii) the total concentration of proinsulin-like molecules in plasma from Type 2 diabetic subjects was one to two-thirds of the total

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concentration of insulin-like molecules in plasma, (iii) measuring the relatively biologically inactive proinsulin-like molecules as "insulin" could lead to the erroneous conclusion that a diabetic patient was insulin resistant rather than insulin deficient, (iv) specific measurement of insulin showed that there was a clear separation between the 30-min insulin responses of the control compared with the lower response of the Type 2 diabetic subjects, (v) insulin radioimmunoassays often measured the sum of all the insulin and proinsulin-like molecules present in plasma.

Another possibility that arose from this work was that 32–33 split proinsulin might have a pathogenic significance [22]. Risk factors for ischaemic heart disease such as plasma cholesterol, triglyceride, HDL-cholesterol, plasminogen activator inhibitor and blood pressure were more strongly correlated with 32–33 split proinsulin than was insulin itself. We return to the interpretation of this finding below.

Although the finding of a uniformly reduced early insulin response to oral glucose in Type 2 diabetic subjects seemed to be received as something of a shock a couple of years ago, review of the literature of obesity-controlled studies over the years shows this to be an almost universal finding (e.g. [23–32]). Disagreement over the insulin status of Type 2 diabetic subjects has often resulted from the use of different aspects of the plasma insulin response to oral glucose to assess status. Emphasis has been variously placed on the early insulin response, the 2 h insulin concentration or the area under the insulin curve or a combination of these. It is now clear that the early insulin response is of critical importance in determining glucose tolerance [33]. Thus in the absence of a normal early insulin response, elevated 2-h insulin concentrations or an elevated area of insulin concentration under the 2-h curve cannot be accepted as evidence of insulin resistance. Studies of subjects with impaired glucose tolerance [IGT] give less clear cut findings than in Type 2 diabetes, but provide little evidence of universally raised early insulin responses as might be expected of a condition which has been suggested to be largely determined by insulin resistance (e.g. [25–28, 32, 34–37]).

Studies such as those listed above however cannot determine whether insulin deficiency, insulin resistance or a combination of the two leads to Type 2 diabetes. A large prospective study of adult men and women living in Ely, Cambridgeshire, UK has been initiated to address this issue. An early and surprising finding to emerge from this work is that there is in this population a continuous relationship between height and glucose tolerance and that both men and women subjects with impaired glucose tolerance are significantly shorter than matched control subjects [38].

Fetal and infant growth and Type 2 diabetes

Previous work by one of us (DJPB) has led to the conclusion that cardiovascular disease in adult life results from restraint of growth during fetal life and infancy [39]. Cardiovascular disease is viewed as a 'programmed' effect of interference with early growth and development. (Programming may be defined as a permanent or long-term change in the structure or function of an organism resulting from a stimulus or insult acting at a critical period of early life [40]). The first evidence for this came from geographical studies which showed that differences in death rates from cardiovascular disease in different areas of England and Wales were closely related to differences in neonatal mortality (deaths before one month of age) 70 and more years ago [41]. Since most neonatal deaths were associated with low birthweight these findings suggest that cardiovascular disease is linked to impaired fetal growth.

This link was subsequently demonstrated in studies of individual men and women whose fetal and infant growth was recorded at the time. The first study was carried out in the county of Hertfordshire, England, where since 1911 all babies born have been weighed at birth and at one year. Among 5654 men those who had the lowest weight at birth and at one year had the highest death rates from ischaemic heart disease as adults [42]. The differences in death rates were large, around three-fold. This posed the question of what processes link lower rates of fetal and infant growth with cardiovascular disease. Subsequent studies in Hertfordshire and in the city of Preston showed that lower birthweight, especially if associated with disproportionately high placental weight, is linked to raised blood pressure in adult life and to elevated plasma levels of fibrinogen [43, 44]. It was concluded that these long-term associations reflect restraint of growth of certain tissues, including blood vessels and the liver, by an adverse environment during a critical period of fetal or infant development. Poor maternal nutrition was suggested as an important environmental influence [39].

The known associations of Type 2 diabetes and IGT with ischaemic heart disease and hypertension [45, 46] plus awareness of the rapid growth of Beta cells during fetal life [47] suggested to us that reduced glucose tolerance may be another outcome of early growth restraint.

Of the Hertfordshire men who still live in the county 468 attended for venous blood sampling in the fasting state. Of these men 370 agreed to have a full 75 g oral glucose tolerance test. From this study some strong relationships have emerged [47]. The percentage of men with impaired glucose tolerance or Type 2 diabetes fell progressively with increasing birth weight and weight at one year [48]. Forty percent of men with birth weights of 2.5 kg (5.5 pounds) or less had a 2–4 h plasma glucose of 7.8 mmol/l or over compared with 14% of men with birthweights over 4.3 kg (9.5 pounds). Forty three percent of men with weights at one year of 8.2 kg (18 pounds) or less had a 2-h plasma glucose of 7.8 mmol/l or over compared with 13 % of men with weights at one year of 12.3 kg (27 pounds) or more. It is possible that some infants with heavier birth weights were the outcome of pregnancies complicated by gestational diabetes. However, the number of such babies would have been small and their survival 60 or more years ago would probably have been poor. Though there is evidence that gestational diabetes predisposes to diabetes in the offspring [49], this could not explain our finding that the largest babies are those least likely to develop diabetes.

Analysis of the effects of obesity, measured as body-mass index, showed that its diabetogenic effect adds to

that of poor early growth. The mean 2-h glucose concentration ranged from 5.8 mmol/l in men who were in the highest tertile of weight at one year but the lowest tertile of current BMI (≤ 25.4), to 7.7 mmol/l in men in the lowest tertile of weight at one and the highest tertile of current BMI (> 28). Interestingly there was a similar addition of the effects of obesity and low weight at one year on current fasting 32–33 split proinsulin concentration. The extremes of the range defined as above were 2.1 and 4.8 pmol/l respectively. When the subjects were divided into quintiles according to the fasting 32–33 split proinsulin concentration this measurement was highly correlated with systolic blood pressure (Table 1). This association is consistent with earlier findings linking 32–33 split proinsulin and risk factors for ischaemic heart disease [22] and requires an explanation.

The concentrations of 32–33 split proinsulin measured in the Hertfordshire study are in the low pmolar range. Any biological activity of this derivative at these low concentrations has yet to be described. It seems to us that a more likely explanation of its association with blood pressure is that the pathogenic mechanisms leading to changes in both measurements are linked. This is reminiscent of the proposal by Reaven [50] in relation to what he termed "Syndrome X" which includes glucose intolerance, hypertension and some types of hyperlipidaemia. He has hypothesised that insulin resistance is the underlying factor linking these phenomena.

Our data suggests a different interpretation. Consistent with previous findings [43] blood pressure in the Hertfordshire men was inversely related to birth weight though unlike 2-h plasma glucose it was not related to weight at one year. Factors affecting early growth may therefore lead to either high blood pressure or impaired glucose tolerance/Type 2 diabetes, or a mixture of hypertension and glucose intolerance, depending on the exact timing of the growth impairment during fetal or infant life. Our working hypothesis is that the varying components and combinations of Syndrome X, possibly including insulin resistance, are late outcomes of abnormal growth and development processes occurring in fetal and early infant life.

At first sight it may seem improbable that events occurring in the first 2 years of existence could produce changes 50–70 years later. However looked at in another way it is perhaps less surprising. It has been calculated that the fertilised ovum in developing into a full-term infant goes through some 42 rounds of cell division [51]. After birth there need be only a further 5 cycles of division. The number of these divisions and their timing in development varies widely between different tissues. For example at birth a virtually full complement of brain neurons and of renal glomeruli are present and, available data suggest, at the age of 1 year at least half the adult complement of Beta cells is present [52]. Adverse influences, in particular poor nutrition, acting at this early time could permanently impair the size and structure of organs and tissues. Poor intrauterine nutrition may lead either to generalised growth retardation, or growth of the brain may be protected at the expense of the viscera. Evidence for selective growth retardation comes from the studies of blood pressure in Preston, UK where one group of people with high blood pressure as adults was characteristed at birth by their shortness in relation to their head circumference [43]. There is good reason to believe that development of Beta cells, which proceeds rapidly during fetal life and early infancy [47], would be vulnerable to poor nutrition. Poor fetal nutrition may be caused by poor maternal nutrition. A link with poor maternal nutrition would explain the high rates of impaired glucose tolerance and diabetes in parts of the third world and is also consistent with the occurrence of Type 2 diabetes in more affluent countries. A recent survey in Oxford, UK, for example, found evidence of iron deficiency in 47 % of all pregnant women [53].

Thus we propose that poor nutrition of the fetus and infant leads to permanent changes of the structure and function of certain organs and tissues. The timing and precise nature of the deficiencies determine the pattern of metabolic and functional abnormalities seen in later life, including diabetes and hypertension and possibly also including some hyperlipidaemias and even insulin resistance. We suggest that poor early development of islets of Langerhans and Beta cells is a major factor in the aetiology of Type 2 diabetes.

In referring to poor early development we do not at this stage consider this necessarily to be a solely quantitative deficiency of Beta cells but include the possibility that the cells themselves may be altered, or that the more complex aspects of islet structure and function, such as vasculature [54] and innervation may be abnormally developed. There is a disproportionately large flow of blood to the islets (10–20 %) compared to that of the pancreas as a whole. Therefore major changes in islet vasculature such as have been described [54] could make a large contribution to changes in islet and particularly Beta cell function.

Table 1. Relationship of 32–33 split proinsulin to systolic blood pressure in men aged 59–70 years. 32–33 split proinsulin was measured in plasma from a sample of blood taken after an overnight fast

32–33 split proinsulin (pmol/l)	Mean systolic[a] pressure (mm Hg)	Number of men
−1.5	161	96
−2.5	164	90
−3.6	163	93
−5.8	165	96
>5.8	170	93
Total	164 (SD 23)	468

p-value for trend = 0.003

[a] (adjusted for BMI, age, room temperature)

Brief review of evidence

We briefly review six key questions central to the hypothesis.

1.) Is there a deficiency of Beta cells in Type 2 diabetes?

Many of the histological studies which have been carried out thus far have failed to control for the effects of obesity on Beta cells. However, as reviewed by Klöppel and col-

leagues [55], there is now a general consensus that the number and total area of islets are reduced, mainly due to a decrease in the volume of Beta cells. However, one may wonder whether a 50% reduction is really enough to cause diabetes? Dogma has it that an 80–90% loss is needed to produce diabetes.

2.) What degree of deficiency of Beta cells is required to reduce glucose tolerance?

A recent paper from the University of Minnesota pancreas transplant programme showed that even hemipancreatectomy in humans leads to a considerable deterioration of insulin secretion and glucose tolerance. The early insulin response was virtually arithmetically halved in these subjects and 7 out of 28 developed severely abnormal glucose tolerance [56].

In parallel with this data, work from Weir's laboratory has shown that careful quantitation of the degree of deficiency produced by pancreatic ablation in animals is needed. Both after neonatal streptozotocin and pancreatectomy considerable regeneration of Beta cells occurs in the rat. This group has been able to produce good models of Type 2 diabetes in rats which retain 46 and 42% of normal Beta cell mass after neonatal streptozotocin or pancreatectomy respectively [57].

Our assertion that poor fetal growth is associated with Type 2 diabetes in later life begs the question:

3.) What are the major nutritional determinants of fetal growth?

Many studies have shown the key role of amino acids in fetal growth. Not only are they essential for laying down the protein required by the growing fetus but interestingly they are also a major source of substrate for energy production [58]. Looking at it teleologically this is not too surprising since the fetus clearly has to gear its growth to the availability of amino acids.

The availability of amino acids may be monitored by the Beta cell, just as the Beta cell senses the availability of nutrients in the adult. Thus, it is important to understand what effect amino acids have on the development and growth of Beta cells in the fetus and also whether they control fetal insulin secretion. Evidence available to date strongly suggests that amino acids are the major factors controlling Beta-cell growth and development and insulin secretion until late fetal life. Glucose has little effect until late gestation [59, 60]. Insulin in turn appears to be a key regulator of fetal growth [61, 62].

If the key sequence of events is the supply of amino acids leading to insulin secretion leading to fetal growth then we should ask:

4.) Is the amino acid supply abnormal in growth retarded babies?

A recent collaborative study between Milan and Denver has shown that this is indeed the case and that the deficiency is large [63]. Furthermore, whether as a cause or effect, there is deficient amino acid transport in placentae of small babies [64].

If a major cause of defective intrauterine and early post natal growth is linked to insulin deficiency and this in turn leads to adult diabetes, then we should be able to show that there is defective production and performance of Beta cells in this situation and that such defects are irreversible.

5.) Does defective Beta-cell growth and function result from malnutrition? If so is it irreversible?

There is in fact quite a considerable body of evidence both in man and experimental animals that the answer to both these questions is "Yes".

James and Coore studied treated malnourished children and suggested that they showed a permanent reduction of insulin response to glucose [65]. Milner studied malnourished children before and after treatment and found the same. He even questioned whether this might predispose to adult diabetes [66]. These two studies were of postnatally malnourished children, although it is possible of course that the children might also have been malnourished in the uterus. Certainly there is evidence of a major effect of intrauterine malnutrition. Growth retarded new born infants have reduced numbers of Beta cells and reduced insulin secretion [67]. Studies in experimental animals show clearly that these changes can be reproduced by subjecting either fetal or early post natal animals to general protein/calorie malnutrition [68] or interestingly to protein deficiency alone [54]. It is significant that the degree of loss of insulin secretion in protein/calorie malnutrition is much more severe than would have been expected from the degree of reduction of islet volume [68]. This of course is reminiscent of the situation in human Type 2 diabetes. An explanation of the discrepancy between the deficit of Beta cells and the severe loss of insulin secretion may lie in the finding that protein deficiency not only reduced Beta cells mass but produced an even larger effect on islet vascularisation [54]. Thus, poor insulin secretion may be due not only to less Beta cells but also to abnormal islet structure and vascularisation. Indeed one cannot help wondering whether poor vascularisation might lead to poor clearance of insoluble peptides. Or in other words could amyloid deposition be secondary to vascular changes? This would of course have an accelerating effect on the underlying pathology.

In addition underfeeding young rats lowers adult plasma insulin. This is not restored by refeeding normally [69]. Indeed the finding of irreversible loss from an early growth failure applies generally to tissue growth. Work in the 1960s and early 1970s showed clearly that a failure of early cell multiplication leads to an irrecoverable deficit in cell numbers [70, 71].

In reviewing the effect of poor fetal and early postnatal nutrition on Beta-cell growth and function we have placed great emphasis on the role of protein and amino acids. We have done this because there is considerable evidence that, as far as insulin production is concerned, protein and amino acid supply are critically important. However, optimum nutrition in pregnancy and early life depends on a complex interaction of many nutrients con-

cerning which we are still largely ignorant. It is probable that other nutrients play a role in Type 2 diabetes and other components of "Syndrome X".

Relation of hypothesis to current concepts of the aetiology of Type 2 diabetes

How do we reconcile the view which we are putting forward with the widely accepted theory that Type 2 diabetes is totally genetically determined? In the first place the mechanisms we propose by no means exclude genetically based changes. We do suggest however that in thinking of candidate genes in Type 2 diabetes we should widen our horizons considerably and consider genes involved in fetal growth and development.

The evidence that we have presented raises a question about the interpretation of concordance in identical twin data. A genetic interpretation of concordance rates of Type 2 diabetes in identical twins may not be justifiable since identical twins share a common early nutritional environment. The familial pattern of Type 2 diabetes may have a similar explanation. Family members share a similar socio-economic environment, which is known to be linked to the incidence and prevalence of Type 2 diabetes [72]. Poor maternal nutrition may be the key influence associated with low socio-economic status. The stronger maternal than paternal influence on the development of Type 2 diabetes [73] is consistent with our hypothesis. So too are the results of a large genetic study of Type 2 diabetes [74]. This study of families with Type 2 diabetes looked for evidence of genetic inheritance of poor insulin secretion. Instead it was discovered that the strongest influence was the common environment shared by the siblings.

We should also reconsider the Neal "thrifty genotype" hypothesis – that the diabetogenic gene or genes persist at a high level in the population because they somehow confer a survival advantage in times of nutritional deprivation, though detrimental at times of adequate or over nutrition [75, 76]. For reasons outlined above we are suggesting a thrifty phenotype hypothesis. We propose that Type 2 diabetes is the outcome of the fetus and early infant having to be nutritionally thrifty. This thrift results in impaired growth of the Beta cells and the islets of Langerhans. As long as the individual persists in the undernourished state there is no need to produce much insulin. However, a sudden move to good or over-nutrition exposes the reduced state of Beta-cell function and diabetes results. This situation was demonstrated recently in the Ethiopian Jews transported to Isreal among whom a high prevalence of diabetes was observed [77]. The effect of a rapid transition from subsistence to good or overnutrition was also seen in the Nauruan islanders who suffered severe nutritional deficiency before and during the last World War. After the war, they became affluent from phosphate mining. Diabetes on the island became epidemic. An interesting consequence of what we are suggesting is that the advent of good nutrition should start to result in better infant and fetal growth which in turn will reduce the incidence of diabetes, provided always of course that the population does not become fatter and less active. It was therefore interesting to see the outcome of the most recent survey of the islanders [78]. Though obesity, exercise and other risk factors had not decreased since 1975/1976, when the first survey was carried out, there had nevertheless been a dramatic reduction in impaired glucose tolerance and Type 2 diabetes. The authors attributed this to a eugenic affect of lower reproduction of diabetic subjects. However, the size and speed of the improvement makes this explanation unlikely. We suggest it was due to a great improvement of fetal and infant nutrition consequent upon post-war affluence. Thus, infants born after 1945 are now up to 46 years old. It was among them that the reduction in diabetes was seen.

Conclusions

We propose a "thrifty phenotype" hypothesis of the aetiology of Type 2 (non-insulin-dependent) diabetes. The essence of the hypothesis is that poor nutrition in fetal and early infant life are detrimental to the development and

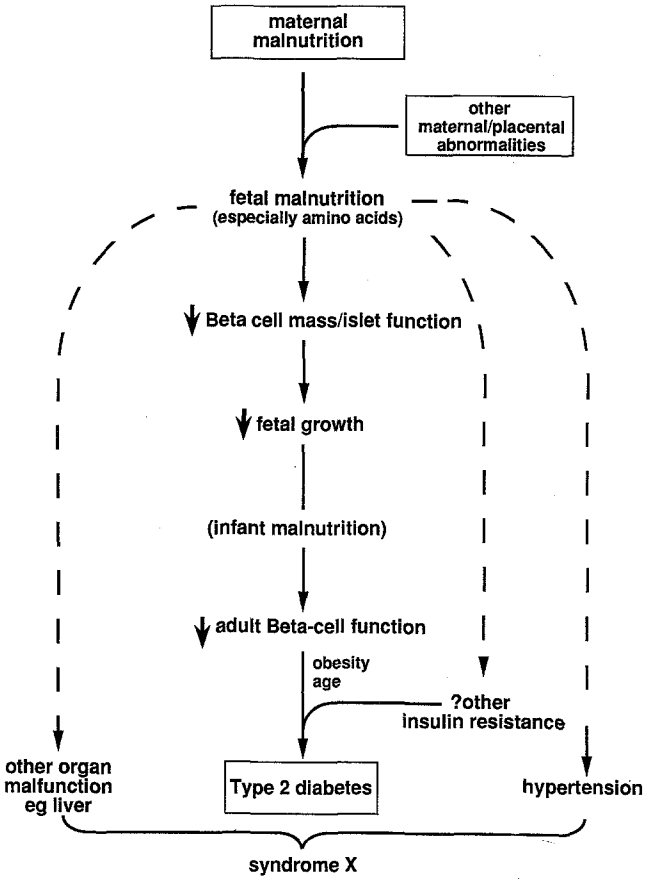

Fig. 1. Diagramatic representation of key features of the "thrifty phenotype" hypothesis of the aetiology of Type 2 (non-insulin-dependent) diabetes. Also outlined is the suggestion that the features of Syndrome X [49] may have closely related origins in failures of early growth and development. Not shown for the sake of simplicity and clarity are the additional possibilities that (i) an early reduction of insulin production could have secondary consequences for the growth and development of other organs involved in Syndrome X; (ii) infant malnutrition may be involved in processes contributing to components of Syndrome X

function of the Beta cells of the islets of Langerhans. Such defects of structure and function, which may include more complex features of islet anatomy such as the vasculature and innervation, predispose to the later development of Type 2 diabetes. Existing evidence points to a key role for protein and amino acids in this process but other nutritional defects are not excluded. Indeed the complex interactions of the type and timing of nutritional defects in early life are suggested as underlying the pathogenesis of the variable abnormalities sometimes described as "Syndrome X". Whilst these early changes powerfully determine susceptibility, additional factors such as obesity, ageing, physical inactivity, and possibly other processes leading to insulin resistance must also play a role in deciding the time of onset and severity of Type 2 diabetes (Fig. 1).

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References

1. Karam JH, Grodsky GM, Forsham PH (1963) Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. Diabetes 12: 197–204
2. Hales CN, Greenwood FC, Mitchell FL, Strauss WT (1968) Blood-glucose plasma-insulin and growth hormone concentrations of individuals with minor abnormalities of glucose tolerance. Diabetologia 4: 73–82
3. Strauss WT, Hales CN (1974) Plasma insulin in minor abnormalities of glucose tolerance: a 5-year follow-up. Diabetologia 10: 237-243
4. Steiner DF, Oyer PE (1967) The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc Natl Acad Sci USA 57: 473–480
5. Roth J, Gorden P, Pastan I (1968) "Big insulin": a new component of plasma insulin detected by immunoassay. Proc Natl Acad Sci USA 61: 138–145
6. Rubenstein AH, Cho S, Steiner DF (1968) Evidence for proinsulin in human urine and serum. Lancet I: 1353–1355
7. Duckworth WC, Kitabchi AE, Heinemann M (1972) Direct measurement of plasma proinsulin in normal and diabetic subjects. Am J Med 53: 418–427
8. Gorden P, Hendricks CM, Roth J (1974) Circulating proinsulin-like component in man: increased proportion in hypoinsulinaemic states. Diabetologia 10: 469–474
9. Mako ME, Starr JI, Rubenstein AH (1977) Circulating proinsulin in patients with maturity onset diabetes. Am J Med 63: 865–869
10. Miles LEM, Hales CN (1968) Labelled antibodies and immunological assay systems. Nature 219: 186–189
11. Miles LEM, Hales CN (1968) The preparation and properties of purified ^{125}I-labelled antibodies to insulin. Biochem J 108: 611–618
12. Addison GM, Hales CN (1971) The immunoradiometric assay. In: Kirkham KE, Hunter WM (ed) Radioimmunoassay methods. Churchill Livingstone, Edinburgh, Scotland, pp 481–487
13. Addison GM, Hales CN (1971) Two site assay of human growth hormone. Horm Metab Res 3: 59–60
14. Beck P, Hales CN (1975) Immunoassay of serum polypeptide hormones by using ^{125}I-labelled anti-(immunoglobulin G) antibodies. Biochem J 145: 607–616
15. Rainbow SJ, Woodhead JS, Yue DK, Luzio SD, Hales CN (1979) Measurement of human proinsulin by an indirect two site immunoradiometric assay. Diabetologia 17: 229–234
16. Frank BH, Pettee JH, Zimmerman RE, Burck PH (1981) The production of human proinsulin and its transformation to human insulin and C-peptide. In: Rich DH, Gross E (ed) Peptides: synthesis-structure-function. Proceedings of the Seventh American Peptide Symposium. Pierce Chemical Company, pp 729–738
17. Gray IP, Siddle K, Docherty K, Frank BH, Hales CN (1984) Proinsulin in human serum: problems in measurement and interpretation. Clin Endocrin 21: 43–47
18. Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256: 495–497
19. Sobey WJ, Beer SF, Carrington CA et al. (1989) Sensitive and specific two-site immunoradiometric assays for human insulin proinsulin 65–66 split and 32–33 split proinsulins. Biochem J 260: 535–541
20. Temple RC, Carrington CA, Luzio SD et al. (1989) Insulin deficiency in non-insulin dependent diabetes. Lancet I: 293–295
21. Temple RC, Clark PMS, Nagi DK, Schneider AE, Yudkin JS, Hales CN (1989) Radioimmunoassay may overestimate insulin in non-insulin dependent diabetics. Clin Endocrinol 32: 689–693
22. Nagi DK, Hendra TJ, Ryle AJ et al. (1990) The relationship of concentrations of insulin, intact proinsulin and 32–33 split proinsulin with cardiovascular risk factors in Type 2 (non-insulin-dependent) diabetic subjects. Diabetologia 33: 532–537
23. Perley MJ, Kipnis DM (1967) Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J Clin Invest 46: 1954–1962
24. Chiles R, Tzagounis M (1970) Excessive serum insulin response to oral glucose in obesity and mild diabetes. Diabetes 19: 458–464
25. Jackson WPU, Van Mieghem W, Keller P (1972) Insulin excess as the initial lesion in diabetes. Lancet I: 1040–1044
26. Savage PJ, Dippe SE, Bennett PH et al. (1975) Hyperinsulinemia and hypoinsulinemia. Insulin responses to oral carbohydrate over a wide spectrum of glucose tolerance. Diabetes 24: 362–368
27. Reaven GM, Bernstein R, Davis B, Olefsky JM (1976) Nonketotic diabetes mellitus: insulin deficiency or insulin resistance? Am J Med 60: 80–88
28. Kosaka K, Hagura R, Kuzuya T (1977) Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. Diabetes 26: 944–952
29. Savage PJ, Bennion LJ, Flock EV et al. (1979) Diet-induced improvement of abnormalities in insulin and glucagon secretion and in insulin receptor binding in diabetes mellitus. J Clin Endocrinol Metab 48: 999–1007
30. Mohan V, Sharp PS, Cloke HR, Burrin JM, Schumer B, Kohner EM (1986) Serum immunoreactive insulin responses to a glucose load in Asian Indian and European Type 2 (non-insulin-dependent) diabetic patients and control subjects. Diabetologia 29: 235–237
31. Deacon CF, Schleser-Mohr S, Ballmann M, Willims B, Conlon JM, Creutzfeldt W (1988) Preferential release of proinsulin relative to insulin in non-insulin-dependent diabetes mellitus. Acta Endocrinol (Copenhagen) 119: 549–554
32. Yoshioka N, Kuzuya T, Matsuda A, Taniguchi M, Iwamoto Y (1988) Serum proinsulin levels at fasting and after oral glucose load in patients with Type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 31: 355–360
33. Bruce DG, Chisholm DJ, Storlein LH, Kraegen EW (1988) Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. Diabetes 317: 736–744
34. Johansen K (1972) Normal initial plasma insulin response in mild diabetes. Metabolism 21: 1177–1180
35. Danowski TS, Khurana RC, Nolan S et al. (1973) Insulin patterns in equivocal glucose tolerance tests (chemical diabetes). Diabetes 22: 808–812
36. Reaven GM, Olefsky JM (1977) Relationship between heterogeneity of insulin responses and insulin resistance in normal

subjects and patients with chemical diabetes. Diabetologia 13: 201–206
37. Berntorp K, Eriksson KF, Lindgärde F (1986) The importance of diabetes heredity in lean subjects on insulin secretion, blood lipids and oxygen uptake in the pathogenesis of glucose intolerance. Diab Res 3: 231–236
38. Brown DC, Byrne CD, Clark PMS et al. (1991) Height and glucose tolerance in adults. Diabetologia 34: 531–533
39. Barker DJP (1991) The intrauterine origins of cardiovascular and obstructive lung disease in adult life. The Marc Daniels Lecture 1990. J Roy Coll Phys Lond 25: 129–132
40. Lucas A (1991) Programming by early nutrition in Man. In: Bock GR, Whelan J (ed) The childhood environment and adult disease. Ciba Found. Symp. John Wiley & Sons, Chichester, England, pp 38–50
41. Barker DJP, Osmond C (1986) Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. Lancet I: 1077–1081
42. Barker DJP, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic disease. Lancet II: 577–580
43. Barker DJP, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. Br Med J 30: 259–262
44. Barker DJP, Meade TV, Fall CHD et al. (in press) The relation of fetal and infant growth to plasma fibrinogen and factor VII levels in adult life. Br Med J 304: 148–152
45. Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H (1980) Coronary heart disease risk and impaired glucose tolerance. Lancet I: 1373–1376
46. Modan M, Halkin H, Almog S et al. (1985) Hyperinsulinaemia: a link between hypertension, obesity and glucose tolerance. J Clin Invest 75: 809–817
47. Hellerström C, Swenne I, Andersson A (1988) Islet cell replication and diabetes. In: Lefebvre PJ, Pipeleers DG (ed) The pathology of the endocrine pancreas in diabetes. Springer, Heidelberg, pp 141–170
48. Hales CN, Barker DJP, Clark PMS et al. (1991) Fetal and infant growth and impaired glucose tolerance at age 64 years. Br Med J 303: 1019–1022
49. Pettitt DJ, Aleck KA, Baird HR, Carraker MJ, Bennett PH, Knowler WC (1988) Congenital susceptibility to NIDDM. Role of intrauterine environment. Diabetes 37: 622–628
50. Reaven GM (1988) Role of insulin resistance in human disease. Diabetes 37: 1595–1607
51. Milner RDG (1989) Mechanisms of overgrowth. In: Sharp F, Fraser RB, Milner RDG (ed) Fetal growth. Proceedings of the twentieth study group of the Royal College of Obstetricans and Gynaecologists. Royal College of Obstetricians and Gynaecologists. London, pp 139–148
52. Rahier J, Wallon J, Henquin J-C (1981) Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia 20: 540–546
53. Godfrey KM, Redman CWG, Barker DJP, Osmond C (1991) The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. Br J Obstet Gynaecol 98: 886–891
54. Snoeck A, Remacle C, Reusens B, Hoet JJ (1990) Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. Biol Neonate 5: 107–118
55. Klöppel G, Löhr M, Habich K, Oberholzer M, Heitz PU (1985) Islet pathology and the pathogenesis of Type 1 and Type 2 diabetes mellitus revisited. Surv Synth Path Res 4: 110–125
56. Kendall DM, Sutherland DER, Najarian JS, Goetz FC, Robertson RP (1990) Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. N Engl J Med 322: 898–903
57. Weir GC, Leahy JL, Bonner-Weir S (1986) Experimental reduction of B-cell mass: implications for the pathogenesis of diabetes. Diab Metab Res 2: 125–161
58. Battaglia FC, Meschia G (1978) Principle substrates of fetal metabolism. Physiol Rev 58: 499–527
59. De Gasparo M, Milner GR, Norris PD, Milner RDG (1978) Effect of glucose and amino acids on foetal rat pancreatic growth and insulin secretion in vitro. J Endocrinol 77: 241–248
60. Swenne I (1992) Pancreatic Beta-cell growth and diabetes mellitus. Diabetologia 35: 193–201
61. Fowden AL (1989) The role of insulin in prenatal growth. J Develop Physiol 12: 173–182
62. Philipps AF, Rosenkrantz TS, Clark RM, Knox I, Chaffin DG, Raye JR (1991) Effects of fetal insulin deficiency on growth in fetal lambs. Diabetes 40: 20–27
63. Cetin I, Marconi AM, Bozzetti P et al. (1988) Umbilical amino acid concentrations in appropriate and small for gestational age infants: a biochemical difference present in utero. Am J Obstet Gynecol 158: 120–126
64. Dicke JM, Henderson GI (1988) Placental amino acid uptake in normal and complicated pregnancies. Am J Med Sci 295: 223–227
65. James WPT, Coore HG (1970) Presistant impairment of insulin secretion and glucose tolerance after malnutrition. Am J Clin Nutr 23: 386–389
66. Milner RDG (1971) Metabolic and hormonal responses to glucose and glucagon in patients with infantile malnutrition. Pediat Res 5: 33–39
67. Van Assche FA, Aerts L (1979) The fetal endocrine pancreas. Contr Gynec Obstet 5: 44–57
68. Weinkove C, Weinkove EA, Pimstone BL (1974) Insulin release and pancreatic islet volume in malnourished rats. SA Med J 48: 1888
69. Swenne I, Crace CJ, Milner RDG (1987) Persistant impairment of insulin secretory response to glucose in adult rats after limited period of protein-calorie malnutrition early in life. Diabetes 36: 454–458
70. Winick M, Noble A (1966) Cellular response in rats during malnutrition at various ages. J Nutr 89: 300–306
71. Widdowson EM, Crabb DE, Milner RDG (1972) Cellular development of some human organs before birth. Arch Dis in Childhood 47: 652–655
72. Barker DJP, Gardner MJ, Power C (1982) Incidence of diabetes amongst people aged 18–50 years in nine British towns: a collaborative study. Diabetologia 22: 421–425
73. Alcolado JC, Alcolado R (1991) Importance of maternal history of non-insulin dependent diabetic patients. Br Med J 302: 1178–1180
74. Iselius L, Lindsten J, Morton NE et al. (1982) Evidence for an autosomal recessive gene regulating the persistance of the insulin response to glucose in man. Clin Genet 22: 180–194
75. Neal JV (1962) Diabetes mellitus: a thrifty genotype rendered detrimental by "progress"? Am J Human Genet 14: 353–362
76. Editorial (1989) Thrifty genotype rendered detrimental by progress? Lancet II: 839–840
77. Cohen MP, Stern E, Rusecki Y, Zeidler A (1988) High prevalence of diabetes in young adult Ethiopian immigrants to Israel. Diabetes 37: 824–828
78. Dowse GK, Zimmet PZ, Finch CF, Collins VR (1991) Decline in incidence of epidemic glucose intolerance in Naruans: implications for the "thrifty geontype". Am J Epidemiol 133: 1093–1104

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