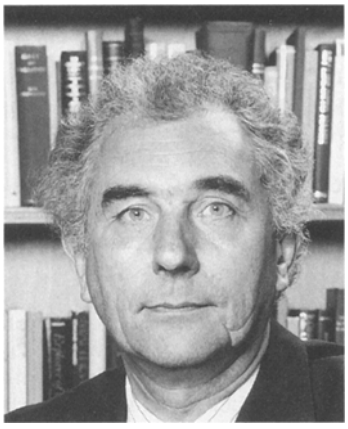
Diabetologia (1994) 37 [Suppl 2]: S162–S168

Diabetologia

Charles Nicholas Hales Minkowski Award, 1971, Southampton



Dr. Hales was born in 1935 in Stafford, UK. He obtained a Ph. D. in 1964 and an M. D. in 1971 from Cambridge University, UK. Dr. Hales was a House Officer at University College Hospital, London, UK from 1959 to 1960. Dr. Hales was successively: Research Fellow at the Department of Biochemistry (1960–1963); Stothert Research Fellow of the Royal Sociey (1963–1964) and Lecturer in the Department of Biochemistry, at the University of Cambridge. From 1970–1977 Dr. Hales was Professor of Chemical Pathology at the Welsh National School of Medicine. Dr. Hales has been Professor of Clinical Biochemistry at the University of Cambridge since 1977.

The pathogenesis of NIDDM

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Summary Improvements in the specificity and sensitivity of assays for insulin-related molecules in the circulation have proved to be necessary and informative in studies of the pathogenesis of non-insulin-dependent diabetes (NIDDM). Of particular interest has been the close relationship between increases in des 31,32 split proinsulin and susceptibility to loss of glucose tolerance and the insulin resistance syndrome. It is suggested that the analogy can be drawn between this measurement and the measurement of HbA_{1c}. The amount of this partially processed precursor of insulin in the circulation indicates the degree of glucose stimulus applied to the beta cell combined with the inherent capacity of the insulin secretory system to respond. Further improvements of the sensitivity and specificity of the assay of proinsulin related molecules are desirable. Deterioration of the early insulin response to oral glucose is a major feature of the loss of glucose tolerance associated with the transition from normal to impaired glucose tolerance and to NIDDM. The extent to which this loss of insulin secretion reflects a major predisposing factor in the aetiology of this type of diabetes or is secondary to glucose toxicity or amyloid accumulation remains to be determined. A relationship between birth weight and impaired glucose tolerance, NIDDM and the insulin resistance syndrome has now been observed in two populations in the UK, in Mexican Americans and in Pima Indians. It is therefore reproducible and applicable to widely differing populations. Much further research is indicated to determine, amongst many questions, how much diabetes is associated with this link and what factors explain it. [Diabetologia (1994) 37 [Suppl 2]: S162–S168]

Key words Non-insulin-dependent diabetes mellitus, impaired glucose tolerance, immunoassay, pro-insulin-related molecules, birth weight.

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Abbreviations: NIDDM, Non-insulin dependent diabetes mellitus; IGT, impaired glucose tolerance; MODY, maturity onset diabetes of the young.

The underlying causes of NIDDM remain controversial. It is not agreed whether insulin deficiency, insulin resistance or a combination of the two represent the primary pathogenic process. However, as we understand in greater detail the basic mechanisms which subserve the physiology of insulin secretion and insulin action it does at least become clearer why it is proving so difficult to unravel the pathogenic sequence of events. The main reason for the difficulty is the way in which insulin secretion and insulin action interact one upon the other. Thus, a major consequence of insulin deficiency is to generate insulin resistance through a variety of secondary events. Conversely insulin resistance can lead through chronic hyperglycaemia to secondary damage to the insulin secretory process.

A further problem relates to the fact that insulin resistance leads to a compensatory increase in insulin secretion. Thus, in studying the maximum secretory response of an individual it is not at present possible to distinguish between the inherent genetically or environmentally endowed capacity of the system and any increment or decrement induced by changes in demand. To these problems of the interactions of the basic pathophysiological processes involved in the metabolic abnormalities of NIDDM has been added the difficulty of measuring insulin specifically in the plasma of patients with this condition when the plasma concentrations of intact and partially processed proinsulin are also elevated [1].

Despite these fundamental problems there are important areas of agreement resulting from many studies of NIDDM. Over the past 25 years it has been consistently observed that the early insulin response (30 min after an oral glucose tolerance test) is reduced in established diabetes [1] There is also increasing evidence that the same defect, albeit on a reduced scale, is present in subjects with IGT [2–4]. There is also general agreement from prospective studies that both insulin resistance and poor insulin secretion are predictors of the subsequent development of NIDDM [5–10]. It is probable that the condition is heterogeneous with some subjects being predominantly characterised by insulin deficiency and others by a mixture of resistance and deficiency [11, 12]. A further example of heterogeniety is provided by the variable conjunction of glucose intolerance, hypertension and hyperlipidaemia (variously known as syndrome X [13] or the insulin resistance syndrome). This "syndrome" is really a statistically increased frequency of association between these and other metabolic abnormalities. The fact that these abnormalities are by no means invariably associated must mean that they are the outcome of a variety of pathological processes.

The research which we have been pursuing for more than 30 years into the basis of NIDDM has followed three main themes:

(i) the development of assay methods for the specific and sensitive assay of insulin and insulin-related peptides for use in clinical and epidemiological studies [14–17].
(ii) Studies of plasma insulin and insulin related peptides in subjects with diabetes or IGT.
(iii) Epidemiological studies into the relationship of fetal and infant growth and development with the later development of diabetes, IGT and/or syndrome X.
The following is a summary of the outcome of this research together with a discussion of the interpretation of the findings.

Assay development

The main thrust of this research in recent years has been to apply the monoclonal antibody technique [18] and bioengineered human proinsulin and insulin molecules [19] to the development of specific and sensitive two-site immunometric assays [20] of insulin and proinsulin-like molecules [17, 21], (Fig. 1). As a result of this work it has been found that, other than intact proinsulin, the major insulin-like molecule present in plasma is "32–33" split proinsulin which in the plasma of normal subjects is approximately equimolar with intact proinsulin. A recent comparison of the results of the "32–33" split proinsulin assay with those obtained in Polonsky's laboratory using a combination of HPLC separation and immunoassay has shown that most, if not all, of the material measured as 32–33 split proinsulin is in fact lacking the C-terminal basic amino acids of the B-C chain junction region and is des 31, 32 split proinsulin (Ostrega et al., unpublished data). Thus far it has not been possible to detect with confidence the alternative partially processed proinsulin, namely that split at the 65–66 site. Both for this reason and because in normal plasma the concentration of intact and des 31,32 split proinsulin may be below the limit of detection of assays currently available, some further improvement of assay sensitivity is desirable and continues to be a subject of our research.

Studies of NIDDM and IGT

It has been known for many years that proinsulin is present in human plasma [22, 23] and that the concentration of proinsulin is raised in the plasma of subjects with NIDDM [24–26]. The availability of biosynthesised human proinsulin and its partially processed derivatives [19] led to the discovery that partially processed proinsulin is also present in the circulation [27]. It soon became apparent that many "insulin" radioimmunoassays cross-react strongly with both intact and partially processed proinsulin.

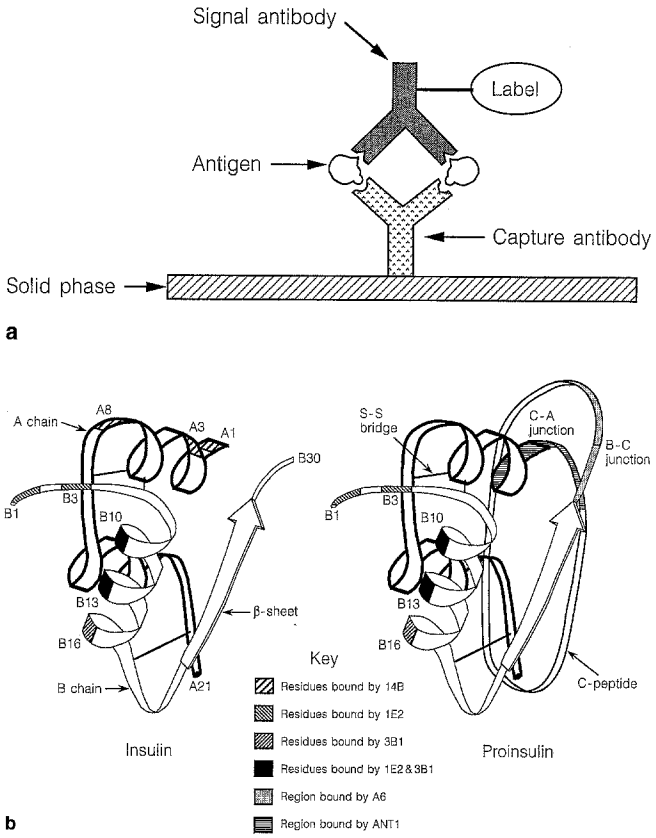

Fig. 1 a, b. Diagram illustrating (**a**) principle of two-site immunometric assays and (**b**) approximate location of epitopes for the monoclonal antibodies used in different combinations to measure insulin, intact and partially processed proinsulins (In press. Reproduced from Protein Engineering, with permission)

It therefore became imperative to re-examine the insulin status of diabetic patients using assays specific for insulin and to define the amount of intact and partially processed proinsulin in their plasma. A specific insulin assay [17] re-emphasised the important role of a poor early insulin response to oral glucose even in diabetics with a relatively modest elevation of fasting glucose [28, 29]. Both intact and partially processed (des 31,32 split) proinsulin concentrations were considerably raised. A similar, although less severe, pattern of changes was discovered in people with IGT [4, 30]. When this data is put together the critical relationship of the early, as opposed to 120 min, insulin response becomes apparent as has been previously shown in studies of IGT [2] and those using injected insulin [31]. There was an almost linear inverse relationship between the height of the 30 min insulin response and the height of the 120 min glucose when data from normal, IGT and diabetic responses were pooled and plotted (Fig. 2). The relationship of the 120 min glucose to the 120 min insulin gives the misleading impression that individuals with IGT or mild diabetes are primarily insulin resistant and only become insulin deficient when there is a severe loss of glucose tolerance (Fig. 2).

In studies of glucose tolerance in three separate populations in East Anglia, UK, an interesting incidental finding has been that individuals with IGT are significantly shorter than subjects with normal glucose tolerance [30, 32]. One such study of 346 subjects from the population of Ely, Cambridgeshire, UK, showed that there was a significant negative correlation between height and 120 min glucose after an oral glucose tolerance test in both men and women [32]. This link between height and glucose tolerance would suggest that changes linked to loss of glucose tolerance are already operating before the cessation of growth in height.

The relationships between fetal and infant growth and development with the later development of NIDDM, IGT and syndrome X

Studies linking fetal and infant growth with death from ischaemic heart disease and the prevalence of hypertension [33, 34] raised the possibility that IGT and NIDDM might show a similar linkage. Such a linkage has now been found in two populations in the UK, in Hertfordshire and Preston, and in men and women [35, 36]. This linkage is independent of social class, either currently or of the parents and of gestational age. Men and women in Preston who had IGT or newly-diagnosed NIDDM were 5.0 cm shorter, 5.1 kg heavier and had a higher waist to hip ratio. Their mean birth weight was 0.3 kg less; the ratio of placental to birth weight was greater; their head circumference was less at birth and they were thinner at birth as judged by the ponderal index (weight/length^3).

These results showing strong associations between impaired fetal and infant growth and the subsequent development of IGT or NIDDM imply that events very early in life are major factors leading to loss of glucose tolerance. Since loss of glucose tolerance is statistically associated with hypertension and hyperlipidaemia, a further analysis was performed to determine whether this collection of abnormalities might itself be linked to early growth and development. Strong associations were found between birth weight and syndrome X in two populations. In 64-year-old men in Hertfordshire the relative risk for an individual of birth weight less than 2.50 kg having syndrome X in comparison with one of birth weight more than 4.31 kg was increased 18 fold. In men and women in Preston at a mean age of 50 years, the increase in risk between birth weight of less than 2.50 kg and more than 3.41 kg was 13.5 fold [37]. A similar finding has now been reported in ca. 44-year-old Mexican-Americans [38]. It has also been observed that in Pima Indians low birth weight is associated with increased risk of developing NIDDM [39]. Interestingly in the latter population there is also an

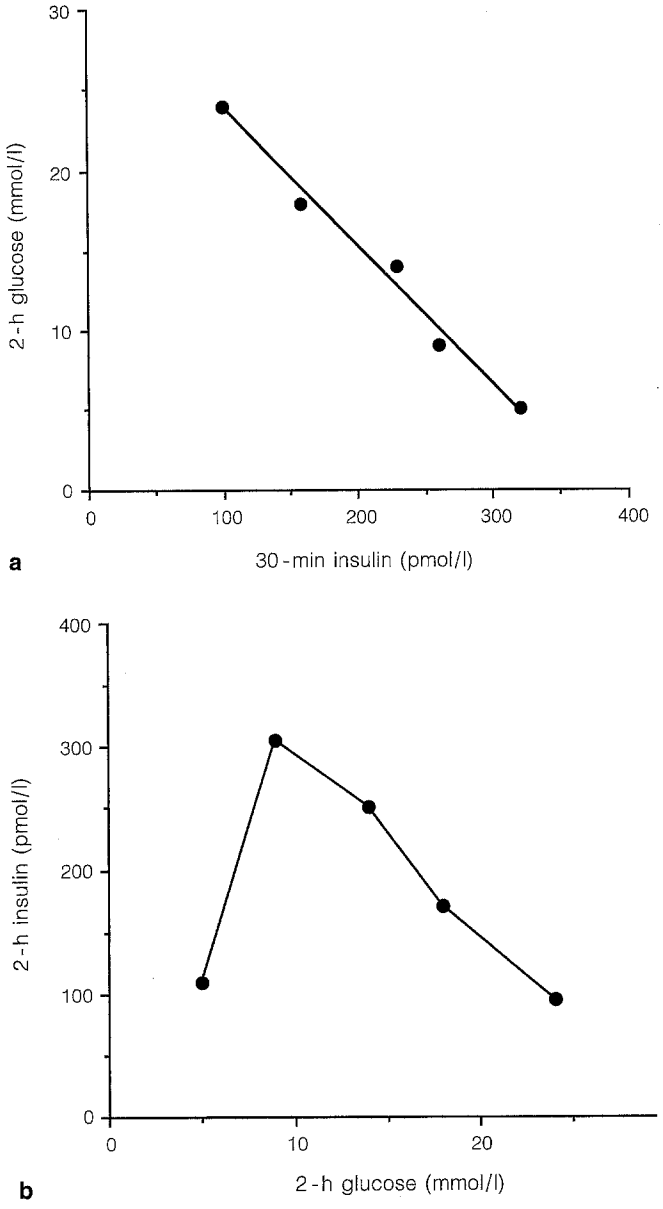

Fig. 2a, b. Relationships between 2-h glucose concentration and (**a**) 30-min; (**b**) 2-h insulin concentrations in subjects with normal, impaired and three grades of NIDDM oral glucose tolerance tests

increased risk associated with the higher birth weight presumably because of the large proportion of pregnancies associated with gestational diabetes which is also a factor predisposing to subsequent NIDDM in the offspring [40].

The association of NIDDM, IGT and syndrome X with poor early growth and development raises the question as to whether insulin deficiency, insulin resistance or both can be linked to changes in fetal and infant growth. There is a considerable amount of evidence both in experimental animals and man that poor nutrition and growth are associated with long-term defects in insulin secretion [41]. We have recently studied insulin resistance in 103 men and women in Preston, UK. Of the subjects 81 were known to have normal glucose tolerance and 22 had IGT [42]. Insulin resistance was measured by the fall in blood glucose concentration after the intravenous injection of insulin, a test which we have found to be simple and reproducible for population studies [43] and others have been shown to give results which correlate closely with the euglycaemic clamp [44, 45]. We found, as expected that subjects with IGT were more resistant to insulin than normoglycaemic individuals. Men and women who were thin at birth as measured by their ponderal index were more insulin resistant. This finding was independent of the duration of gestation, adult body mass index and waist to hip ratio and of the potentially confounding variables social class currently or at birth and current cigarette smoking. These findings suggest that insulin resistance may have its origin in defects of growth and development in early life.

Thus, these studies of adult populations for whom we have varied anthropometry at birth or the age of one show that a number of indices of poor early growth and development are linked with the risk of IGT, NIDDM and syndrome X in adult life. These include reduced weight at birth and age one year; less teeth at one year; a raised placental to birth weight ratio; reduced head circumference and a lower ponderal index. These links are not explained by prematurity or the social class of the parent or person concerned.

Discussion

Improvements in immunoassay specificity and sensitivity have refined our ability to probe the changes in insulin production and release which accompany NIDDM. The close structural relationships between insulin and its precursor molecules and the very low concentration of the latter in the circulation make difficult technical demands of assay systems. The increased concentrations of intact and des 31,32 split proinsulin in the plasma of individuals with IGT suggest that changes in the release of these molecules may give an early indication of the pathogenic events leading to NIDDM. Improving the assays of these molecules would therefore seem to be a worthwhile investment of time. Improvement is also desirable since a number of normal subjects have undetectable concentrations of intact and des 31,32 split proinsulin in the circulation (< 1 pmol/l) and it is not yet possible to measure des 64, 65 split proinsulin reliably.

In many of our studies of conditions related to NIDDM and in epidemiological studies relating fetal growth to the subsequent development of diabetes, IGT or syndrome X it has been apparent that the measurement of des 31,32 split proinsulin often pro-

vides the strongest association with these changes. Recent investigations of families with genetically determined maturity onset diabetes of the young (MODY) have also shown differences between them and subjects with NIDDM with regard to the proinsulin-like molecules in the circulation [46]. Intact proinsulin concentrations were not raised in MODY and des 31,32 split proinsulin was undetectable. Whilst the genetic defects leading to MODY in these individuals have not all been characterised, many of them are known to have glucose sensing defects due to mutations in the beta-cell glucokinase enzyme. Thus, it would appear probable that increased secretion of des 31,32 split proinsulin is the result of an increased, but sensed, glucose stimulus to the beta cell. Recent work on the pathway of proinsulin processing and the control of the synthesis of the processing enzymes provides an explanation of why the release of this molecule provides a particularly good signal of the glucose "pressure" on insulin secretion. Proinsulin is converted to insulin via the action of three enzymes known as PC1(3), PC2 and carboxypeptidase H. Kinetic studies suggest that the preferred route of processing is via cleavage at the 32–33 site rather than at 65–66 [47]. It is not clear what are the relative activities of PC1(3) and PC2 in the beta cell, but it is possible that PC2 is present in much smaller amounts (see article by Halban this issue). Irrespective of this, glucose strongly stimulates the synthesis of proinsulin and of PC1(3), but not PC2 [48]. Thus, as plasma glucose concentrations rise processing of des 31,32 split proinsulin to insulin is, or becomes, rate-limiting. Thus, it is suggested that the concentration of des 31,32 split proinsulin in the circulation provides a unique signal related (a) to the intensity of glucose stimulation of the beta cell, and (b) to the ability of the beta cell to adapt its performance to increased demands for insulin. In obesity not complicated by deterioration of glucose tolerance, there is an increased release of all these insulin-related molecules with maintenance of their relative proportions. However, increased demands which cannot be met by the normal compensatory mechanisms lead to a relative increase in the proportion of proinsulin-like molecules released and in particular that of des 31,32 split proinsulin. An analogy can be drawn with the relationship of glycaemia to the concentration of HbA_{1c}. The concentration of des 31,32 split proinsulin in the circulation could be regarded as the "HbA_{1c} of the beta cells" since it measures the degree of exposure of the beta cells to glucose.

The sequence of events accompanying the loss of glucose tolerance from normal to IGT and then to NIDDM is becoming clearer. In the normal subject insulin secretion is closely linked to insulin sensitivity such that any insulin resistance is balanced by an increased production and release of insulin. The ability to undertake this compensatory adjustment is governed, we believe, by environmental factors operating in fetal and possibly early infant life which determine the structure and function of the beta cells with which the adult is eventually endowed. What genetic factors are involved remains unclear except in rare conditions such as glucokinase deficient MODY subjects. Because of the close interplay between insulin resistance and production it is difficult to obtain measures of the inherent performance of the beta cell independent of resistance. Whilst we have found relationships between insulin secretion and birth weight in young men [49] it has not yet been possible to establish such linkages in older adults [50].

However, the transition from normal to IGT and IGT to NIDDM is clearly associated with loss of early insulin secretion during an oral glucose tolerance test. Our bias is to believe that this is the stage at which the inherent limitation of the beta cell's capacity to compensate for resistance is reached. Nevertheless it must be acknowledged that damage to the beta cell secondary to "glucose toxicity" or amyloid accumulation cannot be ruled out at present as causing the beta cell defect.

The association between low birth weight and IGT, NIDDM and syndrome X have now been observed in two populations in the UK [35–37], in Mexican Americans [38] and in Pima Indians [39]. Thus, it must be accepted as reproducible and as applying to widely differing populations. These findings open up a new field of enquiry in studies of the pathogenesis of NIDDM. Much further research is indicated. It is important to discover how much of the prevalence of diabetes in different populations can be attributed to this linkage. This calculation is fraught with difficulty since whatever factors are involved they are clearly not totally limited to the lowest birth weight infant (even though the effect is strongest in these infants) since the relationship is graded and continuous through the birth weight range. We also need to discover what it is which determines the observed epidemiological link. Again our bias is to believe that it is predominantly environmental and dietary, but infections or genetic factors cannot be ruled out at this stage. A reason for exploring the environmental and dietary hypothesis in the first instance is the existence of good experimental data in rats that such factors have major effects [41]. Furthermore, if maternal dietary factors play a major role in this aspect of fetal development then it is a matter of considerable urgency to unravel and prevent this process in human populations.

Acknowledgements. The work reviewed here has been supported by the British Diabetic Association, the Medical Research Council of the UK, the British Heart Foundation, Bayer Diagnostics and Lilly Research Laboratories.

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