A glimpse of the ‘natural history’ of established Type 2 (non-insulin dependent) diabetes mellitus from the spectrum of metabolic and hormonal responses to a mixed meal at the time of diagnosis

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Received 18 November 1993; revision received 15 August 1994; accepted 5 September 1994

Abstract

The reported glucose and immunoreactive insulin (IRI) responses to oral and intravenous glucose in subjects with Type 2 diabetes have not always been consistent. This may have resulted from variations in the method of glucose administration, the ethnic backgrounds of subjects, the diagnostic criteria applied, the duration of the disease or IRI assay methods. The use of a mixed meal rather than glucose has been shown to provide a more physiological stimulus to the pancreatic \(\beta\)-cell due to both glucose and non-glucose secretagogues. We have analysed the metabolic and hormonal responses of 188 newly diagnosed Caucasian subjects with Type 2 diabetes and 38 non-diabetic subjects to a 500 kcal mixed meal. The diabetic subjects were stratified according to fasting plasma glucose (FPG) (< 9, 9–12, 12–15 and ≥ 15 mmol/l) and body mass index (BMI) (< 26.5, 26.5–30 and ≥ 30 kg/m\(^2\)). Increasing FPG was associated with higher peak glucose concentrations and increasing failure to achieve basal glucose levels by 4 h. Median fasting IRI concentrations were similar to those of normal subjects, but all diabetic subjects had reduced early-phase insulin secretion. Diabetic subjects with FPG < 9 mmol/l showed augmented IRI area under the curve (AUC) at 2 and 4 h, whereas those with FPG > 9 mmol/l had progressive falls in IRI AUC to below that of the normal subjects \((P < 0.0001\) for the trend). Peak IRI concentrations declined progressively with increasing FPG. Despite equivalent glucose exposure simple trends of increasing AUC, IRI with increasing BMI were statistically significant \((P < 0.001, P < 0.02, P < 0.01\) and \(P < 0.01\), respectively for each FPG group). Both fasting and AUC non-esterified fatty acid concentrations increased significantly with FPG regardless of BMI \((P < 0.001\) for the trends). These results using a more physiological mixed meal challenge in a large number of recently diagnosed Type 2 diabetic subjects demonstrate a marked and increasing loss of \(\beta\)-cell secretory function with increasing fasting hyperglycaemia aggravated by insulin resistance with increasing obesity.

Keywords: Mixed meal test; Metabolic/hormonal responses; Type 2 diabetes mellitus

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1. Introduction

When established, Type 2 diabetes mellitus represents a heterogeneous disorder resulting from the interaction between insulin insensitivity and β-cell dysfunction. Evidence for the co-existence of these two pathological processes has been exhaustively presented [1]; however, controversy persists as to which represents the primary event. In addition, these abnormalities are already detectable to some extent in glucose tolerant individuals considered ‘at risk’ of developing the syndrome [2–5]. Patients presenting with fasting hyperglycaemia commensurate with the diagnosis of Type 2 diabetes have evidence of both β-cell dysfunction and insulin resistance of varying degrees. The pathophysiological picture is also often complicated by the co-existence of obesity, itself associated with insulin resistance, in up to 60% of patients with this form of diabetes [6]. An attempt to ‘metabolically stage’ these patients near to the time of diagnosis prior to any therapeutic intervention in terms of their fasting hyperglycaemia, glucose tolerance and pancreatic β-cell response to a mixed meal challenge may help to indicate the possible sequence of pathophysiological events in the ‘natural history’ of the disease which could have significant therapeutic and prognostic implications [7]. Previous attempts to define the metabolic and hormonal responses of such patients possessing a wide range of fasting plasma glucose (FPG) levels and using a variety of challenges have been further complicated by the heterogeneity of the results obtained. Studies in the last 30 years utilising both oral and intravenous glucose tolerance tests have observed either low, normal or high fasting immunoreactive insulin (IRI) concentrations in Type 2 diabetic subjects coupled with a reduced or absent ‘early-phase’ IRI secretory response in over 70% of the reports [8–13]. Similarly the ‘late-phase’ and total IRI responses to an oral glucose challenge have also been shown to be either reduced, normal or increased when compared to healthy non-diabetic subjects [12,13]. The lack of homogeneity in these findings derives from a variety of test protocols, using subjects from different ethnic backgrounds and without controlling for degrees of obesity and treatment, has limited the opportunities for metabolic and hormonal stratification of subjects with Type 2 diabetes.

Our aim has been to ‘metabolically stage’ a large number of individuals with Type 2 diabetes at or near the time of diagnosis prior to any dietary or therapeutic intervention, based on their fasting plasma glucose concentration. The diabetic subjects were allocated to one of four sub-groups defined arbitrarily based on the fasting glucose being < 9 mmol/l, 9–12 mmol/l, 12–15 mmol/l and ≥ 15 mmol/l. The cohort of diabetic subjects were also stratified according to the level of obesity as defined by the body mass index (BMI) i.e., BMI < 26.5, 26.5–30 and ≥ 30 kg/m². A group of healthy normal subjects were also recruited for the purpose of comparison. To accomplish our aim we used a standardised mixed meal tolerance test (MTT). The mixed meal was chosen so as to provide a more physiological challenge for the metabolic assessment of such patients. Similar post-prandial glucose and insulin responses have been observed with mixed meals and an oral glucose load in both non-diabetic subjects [14–17] and subjects with Type 2 diabetes [15–17]. Additionally, a mixed meal provides stimulus to the β-cell via both glucose and non-glucose insulin secretagogues representing a more physiological challenge for the metabolic assessment of such patients. The metabolic and hormonal responses of subjects in this study were analysed according to the stratification of subjects by FPG and degree of obesity as measured by body mass index.

2. Materials and methods

2.1. Subjects

With local ethical committee approval, newly diagnosed subjects with Type 2 diabetes were recruited at their first visit to the diabetic clinic at the University Hospital of Wales as part of an ongoing prospective study of NIDDM. A total of 188 islet cell antibody negative subjects gave informed consent to take part in the study. None had received dietary advice or oral hypoglycaemic agents prior to study and no patients had any evidence of clinically significant cardiac, hepatic, renal or other endocrine disease at the time of investigation, although diabetic retinopathy was present to varying degrees in a proportion of the subjects at presentation (see Fig. 1). Tables 1a and 1b contain the characteristics of the diabetic subjects stratified ac-
2.2. Study protocol

For each of the diabetic subjects a meal tolerance test (MTT) took place within 2 weeks of first presentation at the diabetic outpatient clinic, after full clinical, biochemical and haematological assessments and a pre study day 10 h overnight fast. All subjects remained resting in a supine position throughout the test period with no smoking allowed. An intravenous cannula was placed in an antecubital vein for sampling purposes and maintained patent by a slow running infusion of 0.9% saline. Baseline blood samples for plasma glucose (PG), IRI and glycosylated haemoglobin (HbA1) were taken after 15 min rest at times designated −30 min, −15 min and 0 min. Immediately following blood sampling at time 0 min, the subjects consumed over a 10-min period a 500-kcal mixed meal (cereal/fruit juice/chicken/bread) the calorie contribution being 55% carbohydrate, 25% fat and 20% protein. Sampling for PG and IRI continued according to FPG concentrations and body mass index, respectively. Additionally, a group of 38 non-diabetic subjects underwent identical investigation. All non-diabetic subjects were healthy volunteers with no family history of diabetes and were taking no medication at the time of study.

![Figure 1](image.png)

Fig. 1. Prevalence and severity of diabetic retinopathy at presentation. Trend for increasing prevalence of retinopathy with increasing FPG highly significant (P < 0.001).

Table 1

(a) Subject characteristics (median (interquartile range)) by FPG (mmol/l)

<table>
<thead>
<tr>
<th></th>
<th>Normals (n = 38)</th>
<th>FPG group</th>
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<tbody>
<tr>
<td></td>
<td>&lt;9 (n = 39)</td>
<td>9–12 (n = 66)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.5 (16.0)</td>
<td>52 (14.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (3.9)</td>
<td>27.9 (5.5)</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>5.5 (0.4)</td>
<td>8.0 (1.0)</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>6.6 (1.2)</td>
<td>8.5 (1.1)</td>
</tr>
<tr>
<td>SEX (%m,%f)</td>
<td>42.58</td>
<td>87.13</td>
</tr>
</tbody>
</table>

(b) Subject characteristics (median (interquartile range)) by BMI (kg/m²)

<table>
<thead>
<tr>
<th></th>
<th>BMI group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;26.5 (n = 65)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 (16.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 (2.6)</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>12.3 (4.9)</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>11.4 (3.5)</td>
</tr>
<tr>
<td>SEX (%m,%f)</td>
<td>88.12</td>
</tr>
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ated centrifuge, and immediately stored at \(-20^\circ\text{C}\) until assay. Non-diabetic subjects underwent an identical meal tolerance test also following a 10-h overnight fast.

2.3. Analytical methods

Plasma glucose was assayed on an autoanalyser (Chemlab Instruments Ltd, Hornchurch, Essex, UK) using an enzymatic colorimetric method with intra-assay and inter-assay coefficients of variation (CV) of \(<2\%\). IRI was measured by a modification of the technique of Heding [18] using a second antibody to separate free and antibody bound \(^{125}\text{I}\) insulin. The intra-assay and inter-assay CVs were 4.6\% and 7.3\%, respectively. HbA\(_1\) (normal range 5.5–7.8\%) was measured by column chromatography (Test-combination HbA\(_1\), BCL Ltd, Lewes, Sussex, UK). NEFAs were determined by an enzymatic colorimetric assay using standard reagents (DAKO NEFA-C, Alpha Labs, Eastleigh, Hants, UK).

2.4. Statistical Analysis

Fasting values (PG and NEFAs) were calculated by averaging pre-meal values (–30, –15 and 0 min). Subjects were classified according to a stratification based on (approximate) quartiles of the FPG data and tertiles of the BMI data. FPG categories were \(<9, 9-12, 12-15\) and \(\geq 15\) mmol/l, and BMI categories were \(<26.5, 26.5-30\) and \(\geq 30\) kg/m\(^2\). Whilst these categories were somewhat arbitrary, they maintain a balance between sensitivity and group size.

Normality assumptions were significantly departed from in a number of the intended groupings of the data. FPG categories were analysed for homogeneity of BMI, HbA\(_1\) and gender by Kruskal-
Wallis and proportion tests with $\chi^2$ approximations using non-specific alternative hypotheses. BMI categories were similarly analysed for homogeneity of FPG and gender. AUC provides a useful summary of individual time series data and is closely related to the mean value of the time series measurements [19]. In the present context AUC gives an indication of the amount of substance (PG, IRI, NEFA) to which a subject was exposed during the MTT and, unless otherwise indicated, are 0–240 min areas. The nonparametric method of Meddis [20,21] for the analysis of variance by ranks

![Figure 3](image-url)

**Fig. 3.** (a) Plasma glucose profiles (±S.E.M.) for diabetic subjects (grouped by FPG) and normal subjects during a meal tolerance test. Boxplots show the median (white mark inside box), interquartile range (height of box), a robust estimate of the range (the 'whiskers') and possible outliers (lines outside the whiskers). (b) Boxplots illustrating immunoreactive insulin profiles for diabetic (categorised by FPG) and normal subjects.
of unbalanced, multiple sample, multiple block designs was thus used to examine the statistical support for hypothesised trends in sample medians. Unless otherwise indicated, a priori specific alternative hypotheses were used. Analyses were blocked by BMI or FPG as appropriate and were undertaken using methods developed with S-Plus [22] software.

3. Results

Fig. 1 shows the prevalence of diabetic retinopathy at presentation according to fasting plasma glucose. A total of 18% of the NIDDM subjects had retinopathy at presentation. The trend of increasing prevalence of retinopathy with increasing FPG was highly significant ($P < 0.001$). Fig. 2 presents the mean (±S.E.M.) PG and IRI responses to the MTT for both normal and the four diabetic subgroups and Figs. 3a and 3b use boxplots to show the same data in a form which gives a more thorough summary of the data than mean curves.

The null hypothesis of equality of median BMI values for FPG sub-groups could not be rejected ($X^2 = 3.5$, d.f. = 3, $P = 0.3$) although Table 1a suggests that a fall in BMI as FPG increased might be
revealed by a study specifically directed towards this question. When stratified by BMI (Table 1b) no difference could be shown for median FPG values ($\chi^2 = 5.46$, d.f. = 2, $P = 0.24$). Additionally, both tables suggest unequal proportions of men and women in the lower FPG ($\chi^2 = 5.46$, d.f. = 2, $P = 0.06$) and lowest BMI categories ($\chi^2 = 12.49$, d.f. = 2, $P = 0.002$).

Fig. 3a and Table 2 indicate that as fasting plasma glucose rose, the median maximum glucose excursion in response to the mixed meal increased from 2.6 mmol/l in normal subjects to between 4.4 and 5.7 mmol/l in the diabetic subjects. Additionally the fasting plasma glucose concentration in 82% of subjects with FPG < 9 mmol/l had returned to basal (fasting) levels at 4 h compared to 42%, 57% and
27% of those diabetic subjects with FPG 9–12, 12–15 and ≥ 15 mmol/l, respectively. These changes occurred in conjunction with a generally reduced early-phase (0–60 min) IRI secretion, with particularly depressed insulin concentrations in the first 30–60 min of the test in all diabetic subjects (Fig. 3b), and an apparent augmentation of IRI response during the late-phase (60–240 min) in those subjects with FPG < 9 mmol/l. Thereafter a progressive decrease in the overall IRI response was observed with increasing FPG concentrations above this level. Median fasting IRI concentrations were similar for normal and diabetic subjects (Table 2). Median peak IRI concentrations fell progressively from 0.59 nmol/l in normals to 0.19 nmol/l in subjects with FPG ≥ 15 mmol/l. Median IRI AUC values in the first hour of the test were subnormal for all NIDDM groups (Fig. 4, panel 1), whereas over the two (Fig. 4, panel 6) and 4 post-prandial h (Fig. 4, panel 3) the median total IRI AUC was supranormal in subjects with FPG < 9 mmol/l, similar to normal in subjects with FPG 9–12 mmol/l and becoming subnormal in the remaining diabetic groups with FPG in excess of 12 mmol/l. Trends in the fall of median IRI AUC with increasing FPG over 1, 2 and 4 h post-prandially were statistically significant (P < 0.0001, whether adjusted for BMI or not). In comparing the three arbitrarily defined BMI groups, glucose exposure (AUC) was relatively constant within each FPG sub-groups (Fig. 5a, non-specific hypotheses, P > 0.95, P > 0.1, P > 0.9 and P > 0.3, respectively). Within each of the four FPG subgroups, increasing obesity was associated with increasing concentrations of IRI (P < 0.001, P < 0.02, P < 0.001 and P < 0.01, respectively for trends). However, as the FPG rose the difference between the median IRI AUC for each BMI group fell (Fig. 5b). Fig. 6 presents both fasting and cumulative post-prandial NEFA profiles (AUC 0–240 min). Fig. 7 (panel 1) illustrates the presence of increasing fasting NEFA with increasing FPG (P < 0.0001 for the trend). The degree of NEFA suppres-

![Fig. 6 Boxplots showing NEFA profiles for FPG categories of diabetic subjects](image-url)
Fig. 7. Boxplots of fasting and AUC NEFA versus FPG category in diabetic subjects. Simple trends of increasing fasting or AUC value with increasing FPG were statistically significant ($P < 0.001$ whether corrected for BMI or not).

4. Discussion

The current study attempts to describe the spectrum of metabolic and hormonal responses to a standard test meal of a large group of normal subjects and subjects with Type 2 diabetes at time of presentation stratified according to the fasting plasma glucose and degree of obesity. The normal subjects were included for comparative purposes with respect to insulin secretion but were not specifically matched for BMI or sex.

Previous attempts to describe the metabolic and hormonal responses of with Type 2 diabetes to glucose stimulation have produced varied results which may be related to the use of oral or intravenous glucose, differing assay procedures, the inclusion of various ethnic groups with variable duration of diabetes and treatment and also the possibility of inclusion of subjects who would now be classified as having impaired glucose tolerance [13]. Using data from oral glucose tolerance tests only, fasting IRI concentrations have been shown to increase with increasing FPG up to the level of 7.8 mmol/l and subsequently decline [1]. Additionally, integrated insulin concentrations over a 2-h glucose tolerance test are said to be augmented compared to normal subjects, in those subjects with only mildly elevated FPG (6.4 mmol/l) but then progressively decline with increasing fasting hyperglycaemia — the so-called ‘Starling curve’ of the pancreas [1,10,23–26]. The data from our newly diagnosed subjects with Type 2 diabetes, using a mixed meal stimulus, broadly supports this view, although the fasting median IRI concentrations in our mildly hyperglycaemic group with FPG < 9 mmol/l (median 8.0 mmol/l) were similar to normals in whom the median FPG was lower at 5.5 mmol/l. In our diabetic subjects, including those subjects with FPG < 9 mmol/l there was a gross reduction in IRI AUC during the first hour of the test which deteriorated exponentially with increasing fasting plasma glucose concentrations. Augmentation of the insulin response compared to the normal subjects only occurred in the second hour of the test and only in subjects in the lowest FPG group. Total insulin response then fell progressively with increasing fasting hyperglycaemia in the remaining diabetic groups. The adverse impact of obesity on insulin effectiveness is also suggested by our data. Despite equivalent glucose exposure within each FPG group, the most obese subjects had higher IRI AUC concentrations. This trend was greatest for the ‘mildest’ diabetic subjects (FPG < 9 mmol/l) and least in the most hyperglycaemic group (FPG ≥ 15 mmol/l).
Although the aetiology of severe hyperglycaemia in obese subjects with Type 2 diabetes is often believed to be mainly insulin resistance [27], similar subjects in our study also had marked relative insulinopenia, not too dissimilar to their less obese equally hyperglycaemic counterparts and the role of hyperglycaemia itself as a desensitiser of the β-cell cannot be discounted. Additionally, our findings and those of others aiming to describe the pathophysiology of Type 2 diabetes must be viewed with a degree of caution as they are based on the radioimmunoassay (RIA) of insulin. It is well known that proinsulin cross-reacts with IRI in standard insulin RIA assays and that subjects with Type 2 diabetes secrete large quantities of proinsulin both in the fasting state and after glucose stimulation compared to normal controls [28]. The current insulin RIA may therefore have resulted in an overestimation of circulating plasma insulin concentrations [29]. The future availability of highly specific insulin and proinsulin assays using the ELISA technique [30] or immunoradiometric assay [31] will allow better characterisation of the β-cell secretory dysfunction in Type 2 diabetes.

This study has attempted to describe the spectrum of metabolic and hormonal responses observed in Type 2 diabetes as near to the time of diagnosis as possible and prior to the institution of any treatment. The metabolic and hormonal responses were observed utilising a physiological challenge in the form of a standard meal without the complicating factor of any therapeutic intervention with the patients arbitrarily divided into sub-groups according to the fasting glucose concentrations and degree of obesity. We have been able to clearly demonstrate a marked and increasing loss of the early-phase β-cell secretory response as depicted by the 1-h IRI secretion in response to the mixed meal in all subgroups of diabetic subjects. Compensatory hypersecretion over 2 and 4 h after the mixed meal compared to healthy subjects occurred only in those subjects with the mildest fasting hyperglycaemia (<9 mmol/l). Thereafter with FPG ≥ 9 mmol/l the insulin response was subnormal despite increasing ambient plasma glucose concentrations. Increasing obesity was accompanied by increasing insulin secretion counterbalanced by insulin resistance which was most evident in the lowest FPG group (<9 mmol/l).

These results offer an opportunity to observe part of the natural history of Type 2 diabetes in subjects who fulfil the diagnostic requirements of a fasting plasma glucose of ≥ 7.8 mmol/l. The data indicates the heterogeneity of the syndrome and the variability in the metabolic and hormonal responses even attempting to control for the level of FPG and degree of obesity. In conclusion subjects with established Type 2 diabetes have an inadequate early-phase response to a mixed meal, accompanied by an apparent and transient compensatory late-phase response only in the mildest subjects with FPG < 9 mmol/l. Obesity at all levels of hyperglycaemia was associated with relative hypersecretion of insulin indicating a state of insulin resistance.

The pathophysiological sequence resulting in Type 2 diabetes requires the more difficult and long-term study of subjects at risk of developing the syndrome.

Acknowledgements

We would like to thank Dr. T.M. Hayes and Dr J.R. Peters for kindly allowing us to study their patients and Sister Hilary Smith and Nurse Miriam Khan for help in carrying out the meal tolerance tests.

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