The Metabolic Associations of Intravenous Glucose Tolerance in the 10 Years from Diagnosis of Type 2 Diabetes

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Intravenous glucose tolerance was measured at diagnosis and during the subsequent 10 years in 103 Type 2 diabetic patients not treated with insulin. K_G (the rate constant for clearance of intravenous glucose) was inversely related to fasting plasma glucose at all review times (at diagnosis being $R_s = -0.77$, $p < 0.001$), and at times to the circulating concentrations of ketone bodies (at diagnosis being $R_s = -0.52$, $p < 0.001$) and glycerol ($R_s = -0.29$, $p < 0.01$). In the first year of treatment, most metabolic abnormalities improved. One to 10 years after diagnosis, fasting glucose concentration and intravenous glucose tolerance deteriorated (median glucose from 6.4 to 7.4 mmol l^{-1}, $p < 0.001$; median K_G from 0.81 to 0.69 % min^{-1}, $p < 0.01$). Likewise, the 'homeostatic model assessment' of insulin insensitivity deteriorated (median from 2.3 to 3.7 arbitrary units, $p < 0.001$) over the same period but first-phase insulin secretion remained steady or improved. This suggests that increases in insulin insensitivity have a predominant effect on slowly deteriorating glucose tolerance from 1 to 10 years after diagnosis in Type 2 diabetes.

KEY WORDS **Intravenous glucose tolerance Type 2 diabetes Intermediary metabolites K_G rate constant**

Introduction

The rate constant for clearance of an intravenous bolus of glucose[1] (K_G) is a measure of glucose tolerance that is unaffected by gastrointestinal absorption of glucose[2] and is more reproducible than oral glucose tolerance.[3,4] It is reduced in subjects with ischaemic heart disease[5] and hypertension[6] and is inversely associated with mortality rate in Type 2 diabetic patients.[7,8] We have examined the changes in intravenous glucose tolerance over 10 years from diagnosis in a group of Type 2 diabetic patients. Those patients who came to treatment with insulin were excluded both to be sure of their Type 2 status and because of difficulty in assessing their islet B-cell function.

In non-diabetic or only mildly diabetic subjects there are inverse correlations between fasting plasma glucose concentration and K_G,[3,9] and between K_G and fasting plasma insulin levels,[10] while K_G correlates closely with the ratio of early to late insulin secretory areas.[9,11] This probably reflects two principles, firstly, that a large first-phase insulin response is associated with a high K_G,[12] and secondly, that the greater the sensitivity to the hypoglycaemic action of the insulin, the faster the blood glucose levels will fall, so reducing the hyperglycaemic stimulus[13,14] to later insulin secretion.

We have now examined these relationships in a group of Type 2 diabetic patients over a period of 10 years from diagnosis. Glucose tolerance changed over time in this study. Glucose tolerance reflects a balance between insulin sensitivity and islet B-cell function, although little is known about how these progress for so long after diagnosis. The aim was to determine whether changes in K_G predominantly reflect insulin secretion. In order to avoid the problem of ambient glucose concentration affecting insulin secretion,[13] we used first-phase insulin area and homeostatic model analysis[15] ('HOMA-B') as indices of islet B-cell function over the 10-year follow-up. In the management of Type 2 diabetes, insulin sensitivity is increased by diet, exercise, and weight loss.[16] Associations between K_G and sensitivity/resistance, as estimated by homeostatic model assessment[15] ('HOMA-R'), were therefore also examined.

Hyperglycaemia is by no means the only metabolic abnormality in diabetes.[8,17] Some of the other metabolic derangements may influence glucose tolerance.[17,18] Therefore we have tested whether circulating concentrations of intermediary metabolites other than glucose are independently related to K_G, as would be expected, for instance, from the glucose/non-esterified fatty acid cycle.[18] According to this theory high circulating concentrations of 'alternative fuels' such as fatty acids, ketone

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bodies or glycerol would impair net glucose clearance, at least by some tissues.[17]

Patients and Methods

Patients

Newly diagnosed diabetic patients aged 65 years or less, attending the Radcliffe Infirmary Diabetic Clinic, Oxford, between April 1973 and March 1976, were admitted to the study, provided that they were willing to co-operate, were not suffering from any co-existent major illness, and were not insulin-requiring at that time. Other details of this prospective study have been reported previously.[7,8,19]

Some patients were not studied to the end of the 10-year follow-up period because of death (34 patients), conversion to insulin therapy (27 patients) or other reasons, such as having moved away, declining follow-up, or incomplete data collection. This left 103 patients available for analysis throughout the follow-up period. In this group of 103, not all patients had complete data at all time-points for all the measurements listed in Tables 2 and 3 but for each variable at least 74 patients had complete data. At diagnosis and at 10 years 103 of this group attended, 101 at 1 year, 95 at 3 years, and 84 at 5 years. The results were qualitatively similar whether statistical analysis was performed on the 74 with complete data (paired statistics) or the 103 with incomplete data (unpaired statistics).

The patient data at entry are shown in Table 1 for all the 249 patients and the 103 tested again at 10 years. The values for both the patients attending for follow-up and those who did not attend at 10 years ($n = 146$) were similar, except for a lower fasting glucose ($p < 0.05$) in the subgroup followed. As those with poorer glucose tolerance were more likely to die within 10 years [7,8] this difference was not unexpected.

To isolate the effect of oral hypoglycaemic agents, we have analysed separately a subgroup of 27 patients who were managed on diet alone for the whole 10 years. In this group, although median fasting glucose was lower and K_G higher at diagnosis than for the 76 patients on oral hypoglycaemic agents when reviewed at 10 years, these differences were not significant, nor was the trend to higher body mass index (BMI). However, there were significant differences in fasting immunoreactive insulin and HOMA-B (both $p < 0.05$), with higher values in the diet-only patients. When first enrolled into the study at clinic, only three subjects had a fasting glucose below that diagnostic of diabetes mellitus by World Health Organisation criteria.[20] They had been diagnosed by clinical criteria and had K_G below 0.90.[4,19,21,22]

Protocol

An intravenous glucose tolerance test (IVGTT, 20 g m^{-2} body surface) was performed at admission into the study ('at diagnosis'), after advice to eat at least 250 g carbohydrate for at least 2 days before the test and before definitive treatment was commenced. Blood and plasma samples were collected for measurement of glucose, immunoreactive insulin, lactate, pyruvate, ketone bodies (the sum of 3-hydroxybutyrate and acetoacetate), glycerol, and non-esterified fatty acid (NEFA) concentrations twice in the fasting state and serially after injection of IV glucose. Triacylglycerol and total cholesterol were measured in the fasting state. Weight and height were measured at each review, and BMI calculated. The IVGTTs and metabolic tests were repeated 1,3,5, and 10 years thereafter (fasting and omitting morning medication) but

Table 1. Clinical characteristics including fasting metabolites at diagnosis of Type 2 diabetes for whole group and subgroup followed throughout 10 years

	Whole group	Sub-group followed for 10 yr
n	249	103
Age (yr)	53.3 (44.8–59.9)	53.5 (46.2–60.7)
Gender (percent male)	57	55
BMI (kg m^{-2})	27.3 (24.2–30.9)	27.3 (24.7–30.6)
Glucose (mmol l^{-1})	11.4 (8.0–15.1)	10.3 (7.6–14.0)
Insulin (mU l^{-1})	9.2 (5.6–14.5)	9.5 (6.1–12.7)
K_G (% min^{-1})	0.53 (0.43–0.71)	0.54 (0.43–0.75)
HOMA-R (arbitrary units)	4.18 (2.52–7.07)	3.73 (2.50–6.45)
HOMA-B (arbitrary units)	25.7 (12.7–51.5)	27.7 (14.6–44.4)
$Insulin_{AUC}$ (1st phase) (mU l^{-1} min)	56.1 (29.4–99.8)	54.3 (29.6–80.4)
Lactate (mmol l^{-1})	1.01 (0.80–1.26)	1.05 (0.82–1.27)
Ketone bodies (mmol l^{-1})	0.12 (0.07–0.29)	0.10 (0.06–0.25)
Glycerol (mmol l^{-1})	0.11 (0.09–0.14)	0.10 (0.08–0.13)
NEFA (mmol l^{-1})	0.71 (0.55–0.85)	0.66 (0.51–0.83)
Cholesterol (mmol l^{-1})	5.19 (4.42–6.02)	5.43 (4.79–6.08)
Triacylglycerol (mmol l^{-1})	1.45 (1.05–2.00)	1.49 (1.09–1.87)

Median (interquartile range).

without change in the usual therapeutic regimen, which was decided on clinical grounds in a separate clinic.

Assays

Plasma glucose was measured from samples preserved in fluoride, without freezing, on an auto-analyser by the glucose oxidase method of Technicon Instruments (Tarrytown, NY, USA), and other metabolites were measured as previously described.[7,8,19] Plasma immunoreactive insulin concentration was measured by radioimmunoassay.[23]

Quality control techniques were used to ensure consistency of all assays, and avoid drift of assay sensitivity. The glucose assay was part of an external quality control scheme throughout. Other metabolite assays were controlled throughout by the use of 'in-house' standards made up from Analar grade chemicals, external quality control procedures being used during the later part of the study follow-up. Hormone radioimmunoassays used antibodies, standards, and other reagents from a constant source throughout (Wellcome Reagents, Beckenham, UK). 'In-house' pooled control plasma was used until an external quality control scheme for immunoreactive insulin was joined part-way through the 5-year review. Retrospective comparisons demonstrated that no drift of the immunoreactive insulin assay had occurred between 5-year and 10-year measurements. The normal range of the immunoreactive insulin assay was unchanged throughout (median fasting 6.0–6.7 mU l^{-1}).

Calculations and Statistical Analysis

The rate constant, K_G, for decline in blood glucose during IVGTT was calculated from plasma glucose values from 10 to 60 min after IV injection of glucose.[1] Homeostatic model assessment (HOMA) analysis was as described by Matthews *et al.*[15] The HOMA value for estimates of insulin insensitivity is termed HOMA-R, and that for islet B-cell function is HOMA-B. HOMA values are calculated from the fasting insulin and glucose concentrations, units are arbitrary, and the magnitudes of the HOMA values are a function of the insulin and glucose assays used. First-phase insulin area was calculated as the area under the time vs absolute-immunoreactive-insulin curve for 5 min from the end of the glucose injection.

Statistical analyses were performed using the SPSS[24] statistical package on the Oxford University Digital Vax cluster. As some of the data is not normally distributed, medians and interquartile ranges are given throughout. The large number of statistical tests performed means that only those with fairly extreme probability values should be considered reliable, unless forming part of a consistent pattern.[25] Wilcoxon's paired tests were used to examine the changes in variables from diagnosis to 1 year (Tables 2 and 3). Trend tests[26] were used to examine the changes from 1 to 10 years after diagnosis.

We carried out univariate analysis by a Spearman's correlation coefficient to study the interactions of metabolic factors with K_G. Then multiple linear regression analyses were performed on those factors associated with K_G ($p < 0.1$ by Spearman), to determine which of them were independently related. Factors not significantly associated at any time-point with K_G by univariate analysis were not submitted to the multiple regression analysis. For the multiple regression analyses, variables were logarithmically transformed (as necessary) to ensure that no value of skewness exceeded 2.0 before submission to the regression analysis. Variables were entered stepwise into the regression equation until the *F*-value probability for the inclusion of the next variable rose above 0.05. At each stage in the multiple logistic regression analysis the best regression line was calculated from the regression equation. However, as all points do not lie on the regression line, the fraction of the total variance of the K_G accounted for by the regression equation is recorded at each stage of the regression analysis.

Because HOMA-B and HOMA-R were calculated using the fasting glucose and immunoreactive insulin concentrations, multiple linear regression analyses were performed with and without these derived variables.

Results

Changes with Time of Metabolic Defects in Diabetes

There were significant and substantial improvements in most measured variables between diagnosis and the 1-year review (Table 2). Thereafter, there were significant increases in fasting glucose and insulin concentrations, and in HOMA-R and insulin_{AUC} (1st phase), without a significant change in HOMA-B between year 1 and year 10.

At 1 year, the fasting glucose concentration, BMI and K_G in the diet only group (Table 3) were less abnormal ($p < 0.05$) than for those who subsequently required oral hypoglycaemic agents. In the diet only group, although HOMA-R, fasting insulin, and glucose concentrations rose significantly ($p < 0.05$) between 1 and 10 years, K_G, BMI, and insulin_{AUC} (1st phase) did not change significantly. The insulin_{AUC} (1st phase) at 10 years was significantly higher than in those treated with oral hypoglycaemic agents ($p < 0.05$). In those prescribed oral hypoglycaemic agents, insulin_{AUC} (1st phase) did not change significantly between 1 and 10 years after diagnosis.

Univariate Associations Between K_G and Metabolic Factors

Older patients and females tended to have a lower K_G, but these relationships rarely reached statistical significance (Table 4).

Table 2. Changes with time in measures related to glucose tolerance in members of a group of 103 Type 2 diabetic patients

	At diagnosis	Time from diagnosis (years) 1	3	5	10
Fasting glucose (mmol l^{-1})	10.3 (7.6–14.0)	6.4 (5.3–7.8)[b]	6.8 (5.6–8.3)	7.2 (5.9–9.0)	7.4 (6.0–10.3)[d]
Fasting insulin (mU l^{-1})	9.5 (6.1–12.7)	7.5 (4.2–11.2)[a]	7.3 (4.1–10.7)	7.5 (5.0–11.5)	10.0 (7.0–13.8)[d]
K_G (% min^{-1})	0.54 (0.43–0.75)	0.81 (0.66–0.99)[b]	0.84 (0.61–1.12)	0.63 (0.55–0.84)	0.69 (0.61–0.86)[c]
HOMA-R (arbitrary units)	3.7 (2.5–6.5)	2.3 (1.2–3.7)[b]	2.0 (1.2–3.7)	2.2 (1.4–4.4)	3.7 (2.2–5.6)[d]
HOMA-B (arbitrary units)	28 (15–44)	55 (29–78)[b]	40 (27–59)	43 (25–68)	55 (33–85)
$Insulin_{AUC}$ (1st phase) (mU l^{-1} min)	54 (30–80)	57 (37–87)	54 (30–81)	57 (30–88)	66 (45–108)[c]
BMI (kg m^{-2})	27 (25–31)	25 (23–28)[b]	25 (24–29)	26 (24–29)	26 (23–29)[d]
Oral hypoglycaemic agent treated (%)	0	18[b]	22	35	74[d]
On other medication (%)	21	25	28	33	43[d]

Median (interquartile range), or percent.
Significant change at 1 year from values at diagnosis; [a] $p < 0.05$; [b] $p < 0.001$, by Wilcoxon's paired test. Significant change in values from 1 to 3 to 5 to 10 years; [c] $p < 0.01$; [d] $p < 0.001$, by trend test.

Table 3. Changes with time of measures related to glucose tolerance in 27 Type 2 diabetic patients treated with diet alone for 10 years

	At diagnosis	Time from diagnosis (years) 1	10
Fasting glucose (mmol l^{-1})	8.8 (7.0–13.6)	5.4 (4.9–6.2)[b]	6.3 (5.3–8.1)[d]
Fasting insulin (mU l^{-1})	11.9 (6.7–14.1)	6.7 (5.7–9.4)[a]	9.7 (7.6–12.2)[c]
K_G (% min^{-1})	0.60 (0.36–1.05)	1.03 (0.76–1.16)[b]	0.97 (0.67–1.10)
HOMA-R (arbitrary units)	3.9 (2.4–6.9)	1.7 (1.1–2.5)[b]	2.6 (1.9–3.6)[d]
HOMA-B (arbitrary units)	38 (21–80)	61 (52–86)	61 (42–123)
$Insulin_{AUC}$ (1st phase) (mU l^{-1} min)	63 (37–96)	61 (44–96)	85 (56–200)
BMI (kg m^{-2})	28 (25–30)	24 (22–27)[b]	25 (23–28)
On medication (%)	30	22	33

Median (interquartile range), or percent.
Significant change at 1 year from values at diagnosis; [a] $p < 0.05$; [b] $p < 0.001$, by Wilcoxon's paired test. Significant change between 1 and 10 years; [c] $p < 0.05$; [d] $p < 0.01$, by trend test.

Table 4. Factors related to K_G by zero order analysis (Spearman's R_s) in members of a group of 103 Type 2 diabetic patients

	At diagnosis	Time from diagnosis (years) 1	3	5	10
Age	−0.31[b]	−0.19	−0.14	−0.15	−0.16
Glucose	−0.77[c]	−0.46[c]	−0.72[c]	−0.56[c]	−0.53[c]
HOMA-R	−0.46[c]	−0.17	−0.27[a]	−0.41[c]	−0.31[b]
HOMA-B	0.47[c]	0.25[a]	0.58[c]	0.29[a]	0.39[c]
$Insulin_{AUC}$ (1st phase)	0.32[b]	0.27[a]	0.34[b]	0.30[b]	0.26[a]
Lactate	−0.13	−0.07	−0.08	−0.04	−0.28[a]
Ketone bodies	−0.52[c]	−0.32[b]	−0.18	−0.40[c]	−0.23[a]
NEFA	−0.22	−0.02	−0.17	−0.31[b]	−0.21
Glycerol	−0.29[b]	−0.15	−0.09	−0.21	−0.20

Numbers are Spearman correlation coefficients between K_G and fasting metabolite concentrations and other measures related to glucose tolerance. Levels of significance of correlation (not corrected[25]); [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. In view of the large numbers of tests undertaken, only highly significant correlations should be considered reliable unless forming part of a consistent pattern.

Fasting glucose was the strongest and most consistent correlate of K_G (inversely). The strength of this correlation was greatest at diagnosis (Table 4). Fasting concentrations of ketone bodies were also negatively related to K_G (consistently but with variable strengths of correlations and levels of significance). Glycerol, NEFA, and lactate were also inversely related to K_G, although such correlations were often weak and not statistically significant. BMI, fasting insulin, cholesterol, and triacylglycerol were never significantly related to K_G.

$Insulin_{AUC}$ (1st phase) was consistently and directly related to K_G. HOMA-R was inversely associated with K_G (usually significantly so) while HOMA-B was directly related.

Multiple Linear Regression Analyses

In the initial multiple linear regression analyses (Table 5), fasting glucose concentration was confirmed as the predominant numerical associate of K_G, and accounted for the largest fraction of the variance of K_G. $Insulin_{AUC}$ (1st phase) was also significantly related to K_G, with a consistency and strength of association exceeded only by that of fasting glucose. However, its contribution to the variance was quite small, 4–11 %. Other significantly related factors were age and the fasting blood ketone bodies and lactate concentrations. However, the contribution of such factors to the variance never exceeded 9 %, and their presence in the regression equation was inconsistent.

When HOMA values were submitted along with the other factors from Table 4, the HOMA-R and HOMA-B values usually displaced fasting glucose from the regression equation, doubtless because the HOMA values are calculated from the fasting glucose and insulin concentrations, and hence are strongly co-linear with them. HOMA-R entered regression equations at three of the five time-points, improving the total variance accounted for by up to 8 %. Even when HOMA-R entered the regression equation first-phase insulin area remained an independent associate of K_G. HOMA-B entered the regression equation only at diagnosis.

Analysis by multiple linear regression analyses of data from either the 27 diet-alone patients or only the 76 treated with oral hypoglycaemic agents did not change the overall findings.

Discussion

Data are presented from a group of 103 Type 2 diabetic patients followed for 10 years, to show the sequential changes in intravenous glucose tolerance, and the statistical associations of the K_G with concentrations of intermediary metabolites, hormones, and indices of islet B-cell function and insulin insensitivity. Few prospective studies on diabetes mellitus have a large enough cohort of patients studied long enough from diagnosis to observe the metabolic evolution of diabetes mellitus under usual clinic management, whose therapeutic effects are seen soon after diagnosis.[19,27,28]

Our patients were enrolled in the study before the World Health Organisation defined their current criteria for diabetes mellitus.[20] Nonetheless, our patients at diagnosis had biochemical abnormalities very similar to[29] (or perhaps slightly worse than[30]) patients in subsequent studies.

The present observations confirm the expected relationships between the K_G rate constant and (a) the fasting blood glucose concentration,[1,3] (b) the area under the insulin curve,[9–11,31,32] and (c) increasing age.[31,32] The disposal of an IV glucose load involves non-insulin-mediated[34] as well as insulin-mediated pathways, and tests first-phase insulin secretory response.[35,36] K_G reflects the glucose tolerance during this dynamic, hyperglycaemic stress. The correlation between the K_G and fasting glucose concentration illustrates their strong relationship

Table 5. Results of multiple linear regression analysis in members of a group of 103 Type 2 diabetic patients

	At diagnosis	Time after diagnosis (years) 1	3	5	10
Glucose	−0.73 (61)[c]	−0.44 (20)[c]	−0.69 (41)[c]	−0.52 (28)[c]	−0.37 (21)[c]
$Insulin_{AUC}$ (1st phase)	0.21 (4)[b]	0.27 (7)[b]	0.34 (11)[b]	0.31 (11)[c]	0.30 (8)[c]
Age	NS	NS	−0.27 (8)[a]	−0.21 (4)[a]	NS
Ketone bodies	NS	−0.33 (9)[b]	−0.32 (4)[b]	NS	NS
Lactate	NS	NS	−0.21 (4)[a]	NS	NS
Total variance (%)	65	37	68	43	29

At each year K_G is the dependent variable and those factors which enter the regression equation are indicated together with the standardized regression slope, the significance of entry into the regression equation ([a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$), and the percentage (in brackets) of the total variance of K_G accounted for by that factor. Only highly significant correlation factors should be considered reliable unless forming part of a consistent pattern. NS indicates the factor did not make a significant contribution to the regression equation. Metabolite concentrations were measured in the fasting state and ketone body concentration and $insulin_{AUC}$ were logarithmically transformed before submission to the regression analysis. HOMA values were not submitted to these regression analyses.

despite the very different circumstances of their measurement.

Previously unreported associations of K_G include correlations with the circulating concentrations of various lipid metabolites. As shown in Table 4, the correlations of ketone bodies, NEFA, lactate, and glycerol with K_G were relatively weak and frequently did not attain statistical significance. This suggests that the biological impact of these metabolite concentrations on the observed glucose tolerance is minor compared with those of fasting glucose and islet B-cell function. The inverse relationships observed accord with the underlying theory of the glucose/non-esterified fatty acid cycle,[18] sometimes generalized as the 'alternative fuel hypothesis', whose main premise is that glucose disposal by cells is likely to be reduced when other metabolic fuels are present in competing amounts. From both the univariate and multivariate analyses it appears that, among the 'alternative fuels', the circulating concentration of ketone bodies has the strongest relationship to K_G, although the reason for this primacy of ketone bodies is unclear, as NEFA are usually considered the principal 'alternative fuel'.[17,18]

Previous workers showed associations between K_G and first-phase insulin secretion in normal and mildly diabetic subjects.[9–11,31,32] Our similar observation in diabetic patients supports the role of early insulin secretion in the disposal of an IV glucose load. There is evidence from other studies that the onset of Type 2 diabetes occurs as the islet B-cells fail, and the patient can no longer sustain the hyperinsulinaemic state which often precedes frank diabetes.[37]

Improvements in most indices of metabolic severity of diabetes began between diagnosis and 1 month,[27] and continued to the 1-year review (Table 3), as previously reported.[19] Thereafter, these indices deteriorated.[30] Fasting glucose continued to be the strongest associate of K_G, although this relationship weakened with time. Lipid metabolites made inconsistent and small contributions to the regression equations. The association between K_G and 1st phase insulin area (Table 4) was consistent and independent (Table 5). Our results for serial changes in HOMA values, fasting glucose, and insulin are similar to those of Rudenski *et al.*,[30] although the patients in their 6-year study had lower initial glucose concentrations. The validity of the HOMA values in prospective studies of diabetic patients has been discussed by others.[30,38] After an initial rise, we saw a non-significant fall in HOMA-B between 1 and 3 years in line with Rudenski *et al.*'s observations (Table 2), but this trend was reversed 5–10 years after diagnosis as more of our patients were commenced on oral hypoglycaemic agents.

No previous study has reported the sequential changes in insulin deficiency, insulin insensitivity, and intravenous glucose disposal in Type 2 diabetic patients for so long after diagnosis. Although fasting insulin increased after its nadir 1–3 years from diagnosis, the time-trend of first-phase insulin area is more variable. Thus, the 27 patients treated by diet only showed an increase in first-phase secretion while those prescribed oral hypoglycaemic agents showed a possible decrease (data not shown, not statistically significant). Our findings of preserved or increasing fasting insulin and HOMA-R were unexpected, and although similar effects of aging in non-diabetic subjects have been reported,[30,31,38,39] they are usually of a lesser magnitude. Thus in our diabetic group HOMA-R increased by 50 % in 9 years while a group of 56- to 65-year-old non-diabetic subjects had a HOMA-R 14 % higher than a group 46 to 55 years old.[38]

A drift of the insulin assay could have produced some of the observed changes. However, there was no evidence of such a change in the sensitivity of our immunoreactive insulin assay. The increase with time of first-phase insulin secretion occurred only in the diet-only patients, not in those requiring oral hypoglycaemic therapy, a finding which cannot be explained by a drift in the immunoreactive insulin assay. There is current uncertainty as to the exact interpretation of conventional radioimmunoassays for insulin, following Hales and colleagues' emphasis on cross-reactivity with split and intact proinsulin molecules.[40] Because a different ratio of true insulin to its precursor molecules is secreted in the basal state compared with the glucose stimulated state,[40] the relative constancy in the present study of the ratio between fasting insulin concentration and the first-phase insulin secretion suggests that the specificity of the assay to insulin precursors did not change substantially.

In our 103 patients followed for 10 years, the increasing use of oral hypoglycaemic agents may have maintained or increased basal insulin secretion (while having a lesser effect on first-phase insulin secretion), although data on the long-term efficacy of such drugs is sparse.[41,42] The cause of the apparent increase with time in insulin insensitivity is also uncertain. As stated above, aging may play a role,[33,38,43] as may increasing tissue damage,[44] or increasing resort to non-hypoglycaemic drug therapy (Tables 2 and 3).[45]

Causality cannot be attributed to any relationships in a study of the present kind. Nevertheless it is possible to test hypotheses by determining whether predicted relationships are indeed present. Thus it has been possible to find relationships consistent with the 'alternative fuel' hypothesis, and evidence that insulin secretion is important in determining the glucose tolerance of people with Type 2 diabetes. However, in these Type 2 diabetic patients the deteriorating glucose tolerance 1–10 years after diagnosis was associated with increasing insulin insensitivity while insulin secretion was maintained. The results from the small number of these patients who have now been reviewed at the 16-year anniversary of diagnosis continue the same trend.[46]

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