Metabolic Heterogeneity in Impaired Glucose Tolerance

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Type II (non-insulin-dependent) diabetes mellitus is a metabolically heterogeneous condition, and is invariably preceded by impaired glucose tolerance (IGT). We examined whether metabolic heterogeneity is a feature of IGT. Three subject groups were studied: IGT subjects with two or more living non-insulin-dependent diabetic relatives (IGT_{WF}, n = 17), and IGT subjects (IGT_{WOF}, n = 17) and subjects with normal glucose tolerance (NGT, n = 25) without a family history of diabetes. Glucose tolerance, glucose (K_{ITTG}) and nonesterified fatty acid (K_{ITTNEF}) insulin sensitivity, and first-phase insulin secretion (FPIS) were assessed by oral glucose tolerance (OGTT), insulin tolerance (ITT), and intravenous glucose tolerance (IVGTT) tests, respectively. Comparison of groups was made by ANOVA and *t* test. The three groups were matched for age, gender, body mass index (BMI), and waist to hip ratio (WHR). IGT_{WOF} and IGT_{WF} subjects had comparable 2-hour plasma glucose levels on OGTT, and insulin secretion and K_{ITTG} were decreased to comparable degrees. However, in comparison to IGT_{WF} subjects, IGT_{WOF} subjects had increased fasting serum triglyceride (geometric mean, 1.8 [range, 0.8 to 4.5] *v* 1.1 [0.4 to 2.5] mmol · L^{-1}, $P = .02$) and 2-hour plasma nonesterified fatty acid ([NEFA] mean ± SD, 0.12 ± 0.07 *v* 0.08 ± 0.03 mmol · L^{-1}, $P < .02$) levels and decreased K_{ITTNEF} values (4.0 [1.7 to 8.9] *v* 6.2 [2.8 to 12.1] % · min^{-1}, $P < .02$). Thus, the two IGT groups had comparable changes in glucose metabolism, but IGT_{WOF} subjects had additional abnormalities of lipid metabolism. In conclusion, metabolic heterogeneity is a feature of IGT, and this may reflect underlying etiological heterogeneity.

TYPE II (non-insulin-dependent) diabetes mellitus is a complex metabolic and clinically heterogeneous condition. It is characterized by impaired insulin secretion and decreased insulin sensitivity, although the relative importance of these features varies between patients. Thus, there is a distinct difference between the type II diabetic patient with the metabolic syndrome in which insulin resistance and absolute hyperinsulinemia are predominant features and the lean insulin-treated patient in whom impaired insulin secretion represents the major deficit.

Impaired glucose tolerance (IGT) almost invariably precedes the development of type II diabetes mellitus, and has been studied to try to define early predisposing metabolic abnormalities. However, distinct metabolic changes would be difficult to define if metabolic heterogeneity was a feature of IGT and clearly established before the development of diabetes. Evidence for such a situation comes from a recent study in which two subgroups of IGT subjects were identified on the basis of metabolic parameters.[1]

Twin and family studies provide support for the role of genetic factors in the development of type II diabetes mellitus.[2,3] The aim of this study was therefore to examine the metabolic features of IGT subjects with a strong family history of type II diabetes compared with IGT subjects with no family history of diabetes, in whom environmental factors are likely to be important.

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SUBJECTS AND METHODS

Subjects

Three subject groups were recruited: IGT subjects with two or more living type II diabetic relatives (one of whom was a first-degree relative) and no family history of type I diabetes (IGT_{WF}, n = 17), IGT subjects with no family history of diabetes mellitus (IGT_{WOF}, n = 17), and control subjects with normal glucose tolerance (NGT) and no family history of diabetes (n = 25). The subjects were recruited from two larger well-characterized samples of IGT (n = 68) and NGT (n = 154) subjects. Subject groups were matched for age, sex, and body mass index (BMI), and IGT groups were matched for degree of glucose intolerance. None of the subjects were taking medication known to influence carbohydrate or lipid metabolism. Informed written consent was obtained from the subjects, and the study was approved by the Newcastle Health Authority and University of Newcastle upon Tyne Joint Ethics Committee.

Protocol

All subjects were eating a standard weight-maintaining diet, and were asked to avoid alcohol and severe exercise for 48 hours before each study.

Each subject underwent a 75-g (388 mL degassed Lucazade; Smithkline Beecham, Brentford, UK) oral glucose tolerance test (OGTT) following an overnight fast, and glucose tolerance was classified using World Health Organization criteria.[4] The diagnosis of IGT was confirmed by a second OGTT at least 3 weeks after the initial test. The waist to hip ratio (WHR)[5] and supine blood pressure ([BP] mean of three measurements taken over 5 minutes) were also recorded.

An intravenous glucose tolerance test (IVGTT) was conducted to examine first-phase insulin secretion (FPIS). Following an overnight fast, two cannulae were introduced under local anesthetic. The first was placed retrogradely in a dorsal hand vein for arterialized venous blood sampling.[6] The hand was warmed in a heated hand box set at a temperature of 50°C. The second cannula was placed in an antecubital vein, and at time 0 minutes, glucose 0.3 g · kg^{-1} was administered over 1 minute.[7]

A short insulin tolerance test (ITT) was performed[8] on a separate occasion to assess whole-body insulin sensitivity for glucose and nonesterified fatty acid (NEFA) metabolism. Following an overnight fast, two cannulae were inserted and prepared as described for the IVGTT. A bolus (0.05 U/kg) of soluble insulin (Novo Nordisk,

Copenhagen, Denmark) was administered at time 0 minutes, and the test was terminated at 15 minutes by administration of oral glucose.

Sampling and Analytical Procedures

During the OGTT, fasting and 2-hour samples were taken to determine serum insulin and plasma glucose and NEFA levels; and fasting samples were taken for serum lipid levels. Arterialized venous blood was sampled for plasma glucose and serum insulin concentrations at intervals throughout the IVGTT as previously described.[7] During the ITT, arterialized venous blood was sampled for plasma glucose and NEFA levels at 1-minute intervals from 3 to 15 minutes.

Glucose concentrations were measured by the glucose oxidase method using a glucose analyzer (interstudy coefficient of variation [CV], 3.5%; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin concentrations were determined using a two-site monoclonal enzyme-linked immunosorbent assay[9] that is highly specific for insulin (cross-reactivity, intact proinsulin 0.5% at 1,000 pmol · L^{-1} and 32,33-split proinsulin 1.2% at 4,200 pmol · L^{-1}). Plasma NEFA levels were measured by centrifugal enzymatic analysis with an interassay CV of 3% at 0.53 mmol · L^{-1} (Wako NEFA kit; Wako Chemicals, Neuss, Germany) based on the method of Knox and Jones.[10] Serum triglyceride and high-density lipoprotein (HDL) cholesterol concentrations were measured using commercial kits (Roche, Welwyn Garden City, UK).

Calculations and Statistical Analysis

FPIS was defined as δ 0- to 10-minute insulin area divided by δ 0- to 10-minute glucose area, which has been shown to be the most reproducible assessment compared with other standard methods.[7]

During the ITT, plasma glucose and NEFA levels showed an exponential decline with time. A plot of the natural logarithmic substrate concentration against time produced a linear response,[11] with the slope (K_{ITT} value) of the line representing insulin sensitivity. Thus, whole-body insulin sensitivity for glucose and NEFA metabolism was defined by K_{ITTG} and K_{ITTNEF} values, respectively.

The distributions of serum insulin and triglyceride, K_{ITTNEF}, and FPIS measurements were all skewed and were normalized by $\log_{10}$ transformation. Comparison between the three subject groups was made by ANOVA. If the F value was significant at the 5% level, subsequent comparisons were performed between groups using the *t* test. Qualitative data were compared by chi-square analysis. Data are presented as the mean ± SD or geometric mean [range].

RESULTS

There were no significant differences between the three groups for age, BMI, WHR, composition by sex, and weekly alcohol intake (Table 1). Systolic BP was comparable across the groups, although diastolic BP was lower in IGT_{WOF} compared with NGT subjects (Table 1). However, more IGT_{WOF} subjects (n = 7) were taking antihypertensive medication (angiotensin-converting enzyme [ACE]) inhibitors and/or calcium-channel blockers) compared with IGT_{WF} (n = 1) and NGT (n = 3) groups, although this failed to reach statistical significance (chi square = 5.2, 2 *df*, .1 > *P* > .05).

Table 1. Subject Characteristics

Characteristic	NGT	IGT_{WF}	IGT_{WOF}
Sex (M:F)	16:9	11:6	11:6
Age (yr)†	52 ± 9	48 ± 9	51 ± 9
BMI (kg · m^{-2})†	28.2 ± 4.3	30.7 ± 6.9	29.2 ± 5.7
WHR†	0.86 ± 0.10	0.87 ± 0.11	0.87 ± 0.05
Systolic BP (mm HG)†	134 ± 14	137 ± 16	135 ± 13
Diastolic BP (mm Hg)†	85 ± 8	83 ± 9	78 ± 7*
Alcohol intake (U/wk)			
Geometric mean	7	5	5
Range	0-24	0-27	0-18

*$P < .01$ *v* NGT.
†Mean ± SD.

Table 2. OGTT Data: Fasting Values

Parameter	NGT	IGT_{WF}	IGT_{WOF}
Plasma glucose			
(mmol · L^{-1})‡	4.6 ± 0.3	5.3 ± 0.8*	5.3 ± 0.5*
Serum insulin (mU · L^{-1})			
Geometric mean	10	13	15
Range	3-25	5-40	4-33
Serum C-peptide			
(nmol · L^{-1})‡	0.60 ± 0.25	0.66 ± 0.31	0.68 ± 0.21
Plasma NEFA			
(mmol · L^{-1})‡	0.58 ± 0.20	0.68 ± 0.22	0.70 ± 0.24
Triglyceride (mmol · L^{-1})			
Geometric mean	1.4	1.1	1.8†
Range	0.5-4.3	0.4-2.5	0.8-4.5
HDL cholesterol			
(mmol · L^{-1})‡	1.4 ± 0.5	1.2 ± 0.2	1.2 ± 0.3

*$P < .001$ *v* NGT.
†$P = .02$, *v* IGT_{WF}.
‡Mean ± SD.

OGTT

Fasting OGTT measurements are summarized in Table 2. Fasting plasma glucose concentrations were comparable in the two IGT groups, and were significantly increased compared with levels in the NGT subjects (both $P < .001$). Fasting serum insulin, C-peptide, and plasma NEFA levels were not significantly different between the three groups. However, fasting triglyceride levels were increased in IGT_{WOF} compared with IGT_{WF} subjects ($P = .02$), whereas the levels were similar in IGT_{WF} and NGT groups. There was no significant difference between the three groups for HDL cholesterol levels.

The 2-hour measurements are summarized in Table 3. Plasma glucose levels were increased to a comparable degree in IGT groups. There was no significant difference in serum insulin levels between the two IGT groups, whereas both groups showed an increase compared with NGT subjects (both $p < .01$). Serum C-peptide levels showed a similar pattern, although differences between the groups were not significant. However, plasma NEFA levels were higher in IGT_{WOF} compared with IGT_{WF} subjects ($P < .02$), with no significant difference between IGT_{WF} and NGT subjects.

IVGTT and ITT

There was no significant difference between the two IGT groups for FPIS (geometric mean, 1.8 [range, 0.6 to 11.9] and 2.5 [0.5 to 15.2] mU · mmol), but this was significantly decreased in both groups relative to the NGT subjects (5.6 [2.0 to 16.4] mU · mmol; comparison with IGT groups, both $P < .02$). Similarly, K_{ITTG} was decreased to a comparable degree in the IGT groups (2.2 ± 0.6 and 2.4 ± 0.9 % · min^{-1}, IGT_{WOF} and IGT_{WF}, respectively) compared with NGT subjects (3.4 ± 1.4

Table 3. OGTT Data: 2-Hour Values

Parameter	NGT	IGT_{WF}	IGT_{WOF}
Plasma glucose (mmol · L^{-1})§	5.3 ± 0.8	8.2 ± 1.5*	8.7 ± 1.2*
Serum insulin (mU · L^{-1})			
Geometric mean	52	106†	120†
Range	18-295	31-285	43-235
Serum C-peptide (nmol · L^{-1})§	2.20 ± 1.01	2.56 ± 1.16	2.70 ± 1.06
Plasma NEFA (mmol · L^{-1})§	0.07 ± 0.03	0.08 ± 0.03	0.12 ± 0.07‡

*$P < .001$, †$P < .01$: *v* NGT.
‡$P < .02$ *v* IGT_{WF}.
§Mean ± SD.

% · min^{-1}; comparison with IGT groups, both $P < .01$). Conversely, K_{ITTNEF} was normal in the IGT_{WF} group (6.2 [2.8 to 12.1] and 6.4 [2.6 to 16.9] % · min^{-1}, IGT_{WF} and NGT), but was significantly decreased in IGT_{WOF} subjects (4.0 [1.7 to 8.9] % · min^{-1}; comparison with IGT_{WF}, $P < .02$).

DISCUSSION

The purpose of this study was to determine whether important metabolic differences exist between IGT_{WF} and IGT_{WOF} subjects after matching for key anthropometric indices and for glucose intolerance. All IGT subjects had persistent IGT based on repeat OGTTs.

FPIS was decreased to a comparable degree in the two IGT groups, and a decrease in early insulin secretion has been previously noted in persistent IGT, although the family history of diabetes was not specified.[12,13] Decreased insulin sensitivity for glucose metabolism has been reported in previous studies of IGT,[14-16] and was found to be comparable in the two IGT groups based on K_{ITTG} and 2-hour insulin values. Thus, the combination of decreased FPIS and insulin insensitivity is important in the development of abnormal glucose tolerance in both IGT groups irrespective of differences in the family history of diabetes.

However, there were important differences between the IGT subject groups in terms of lipid metabolism. Thus, fasting serum triglyceride and 2-hour plasma NEFA levels were significantly increased in IGT_{WOF} subjects, but were comparable to normal values in IGT_{WF} subjects. Similarly, K_{ITTNEF} was decreased in IGT_{WOF} subjects but normal in the IGT_{WF} group. The decreased K_{ITTNEF} measurement is consistent with decreased insulin sensitivity for NEFA metabolism in IGT_{WOF} subjects, and is supported by the observation that 2-hour plasma NEFA levels were inadequately suppressed despite the increased 2-hour insulin concentrations.

Further study is required to define whether the decreased insulin sensitivity is due to changes in lipolysis and/or reesterification. Nonetheless, it is probable that the abnormality of NEFA metabolism represents a fundamental abnormality that secondarily contributes to the changes in triglyceride metabolism by increased substrate supply. We have previously shown decreased suppression of lipid oxidation in response to insulin in IGT_{WOF} subjects,[14] and postulated that this might contribute to the peripheral insulin resistance for glucose metabolism via operation of the glucose/fatty acid cycle. In addition, there is recent evidence to suggest that elevated NEFA levels can also impair insulin secretion.[17] More study is needed to determine the contribution of these changes in lipid metabolism to the abnormal glucose tolerance in IGT_{WOF} subjects.

Previous studies have examined the metabolic features of IGT_{WF} and IGT_{WOF} subjects.[18-20] However, there has been no previous attempt to match IGT groups for key anthropometric indices and degree of glucose intolerance. Indeed, circulating NEFA levels have only been examined in the most recent study,[20] but the high insulin levels achieved during hyperinsulinemic clamps resulted in complete suppression of circulating NEFA levels in all subject groups. The acute sensitivity of NEFA metabolism to insulin[21] therefore means that any differences between IGT groups will have been overlooked.

Although there was no difference in BP measurements between the two IGT groups, the use of antihypertensive medication tended to be greater in IGT_{WOF} subjects. This suggests that IGT_{WOF} subjects had a greater propensity to have hypertension, which together with the lipid abnormalities points to an increased cardiovascular risk along the lines of syndrome X.[22] Importantly, all subjects in the study who were using antihypertensive medication were treated with either an ACE inhibitor and/or a calcium-channel blocker (amlodipine or nifedipine), which do not influence circulating triglyceride levels.[23-25]

In conclusion, we have identified important differences in lipid metabolism in carefully matched IGT_{WF} and IGT_{WOF} subjects. This metabolic heterogeneity almost certainly reflects underlying etiological heterogeneity in IGT. However, it is clear that our study represents a "snapshot" of metabolism and that longitudinal assessment is required to examine how the identified metabolic changes progress with time.

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