Multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families

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Summary Non-diabetic first degree relatives of non-insulin-dependent diabetic (NIDDM) families are at increased risk of developing diabetes mellitus, and have been studied to identify early metabolic abnormalities. Hormone concentrations measured by specific enzyme immunoassays were assessed in non-diabetic relatives of North European extraction, and control subjects with no family history of diabetes were matched for age, sex and ethnicity. A 75-g oral glucose tolerance test was conducted and those with newly diagnosed NIDDM were excluded. Basal insulin resistance was determined by homeostasis model assessment (HOMA), and hepatic insulin clearance by C-peptide:insulin molar ratio. Relatives ($n = 150$) were heavier (BMI: $p < 0.0001$) than the control subjects ($n = 152$), and had an increased prevalence of impaired glucose tolerance (15 vs 3 %, $p < 0.01$). The relatives had increased fasting proinsulin levels and decreased C-peptide levels following the glucose load, while insulin levels were increased at all time

points. To examine whether the differences in hormone levels were secondary to the differences in glucose tolerance and adiposity, we studied 100 normal glucose tolerant relatives and control subjects pair-matched for age, sex, waist-hip ratio and BMI. The differences in proinsulin levels were no longer apparent. However, the relatives remained more insulin resistant, and had decreased C-peptide levels and C-peptide:insulin ratios at all time points. In conclusion, we have identified several metabolic abnormalities in the normal glucose tolerant relatives, and propose that the decreased hepatic insulin clearance helps to maintain normoglycaemia in the face of combined insulin resistance and decreased insulin secretion. [Diabetologia (1997) 40: 1185–1190]

Keywords C-peptide, proinsulin, insulin, insulin secretion, insulin resistance, insulin clearance, families, adiposity, glucose intolerance.

Non-insulin-dependent diabetes mellitus (NIDDM) is a complex metabolic condition characterised by both impaired insulin secretion and insulin resistance [1]. It is also recognised that elevated circulating

proinsulin levels are an important and consistent feature of NIDDM [2–5], and may represent a specific defect of pancreatic beta-cell function. There is continuing debate as to whether insulin deficiency or insulin resistance represents the primary abnormality which predisposes to the development of NIDDM [6], although it is clear that comparative insulin deficiency is a *sine qua non* for NIDDM to develop [7].

In an attempt to identify the early fundamental abnormalities, non-diabetic first degree relatives of NIDDM patients have been studied, as they have a 40 % lifetime risk of progressing to diabetes [8]. However, such an approach has so far failed to clarify matters, as some studies have found a principal defect of insulin secretion [9–13], several have suggested that

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; IGT, impaired glucose tolerance; WHR, waist:hip ratio; CV, coefficient of variation; NEFA, non-esterified fatty acid; HOMA, homeostasis model assessment; IVGTT, intravenous glucose tolerance test.

insulin resistance is the primary abnormality [14–19], while three studies have reported evidence for both impaired insulin secretion and action in normal glucose tolerant relatives [20–22]. A number of factors might account for these divergent observations, and include differences in study populations, for example in ethnicity, and methodological differences. Certainly, insulin resistance appears to be of fundamental importance in the Mexican-American [18] and Pima Indian populations [23], while evidence of decreased insulin secretion has tended to come from studies of Caucasians [9–13, 20–22].

It is now well recognised that proinsulin-like molecules can cross-react in conventional insulin radioimmunoassays [24], and this could have been important in earlier studies [14–17, 20] in which “hyperinsulinaemia” was detected in non-diabetic relatives and presumed to represent insulin resistance. This has been explored further with the advent of specific hormone assays. Proinsulin levels have been reported to be normal [5, 25] and slightly increased [26] in normal glucose tolerant relatives of North European extraction, and increased in non-diabetic Mexican Americans with a parental history of diabetes [18].

The aim of the present study, therefore, was to explore changes in beta-cell function, insulin metabolism and insulin action using specific hormone assays in non-diabetic relatives of NIDDM families of North European extraction and control subjects with no family history of diabetes.

Subjects and methods

Subjects. We recruited 154 non-diabetic first degree relatives (age 20–65 years) of NIDDM patients from 62 families of North European extraction with two or more living NIDDM patients. The relatives were related to the NIDDM family members in the following ways; 108 were offspring, 2 were siblings, and 44 were both offspring and siblings. For comparison, 154 non-diabetic control subjects with no family history of diabetes and matched for age, sex and ethnic background were recruited from a randomly selected subgroup of the local background population. This subgroup comprised over 1000 residents of the Newcastle and North Tyneside District that had been selected from the Family Health Services Authority Register for a health survey conducted by the Department of Epidemiology, University of Newcastle upon Tyne. Ethnicity was based upon both parents being of North European origin. None of the subjects was taking agents known to influence lipid or glucose metabolism. Informed written consent was obtained from all subjects and the study was approved by the Newcastle and North Tyneside District Ethics Committee.

Protocol. Subjects were studied following an overnight fast and a 24-h period of abstinence from alcohol and vigorous exercise. An indwelling cannula was placed in an antecubital vein for blood sampling. At 0 min, 75 g of glucose (388 ml degassed Lucozade, Smithkline Beecham, Brentford, UK) was given orally; blood was sampled fasting (0 min), and 30 and 120 min following the glucose load. Glucose tolerance was classified

according to 1985 World Health Organization criteria [27], and those subjects classified as having impaired glucose tolerance (IGT) had a repeat oral glucose tolerance test within 2 months to identify subjects with persistent IGT. Waist: hip ratio (WHR) was assessed by standard methods [28], and blood pressure was measured after 10 min resting supine.

Laboratory methods. Blood glucose was measured by the glucose oxidase method using a Yellow Springs glucose analyser (inter-assay coefficient of variation [CV] 3.5%: Yellow Springs Instrument Company, Yellow Springs, Ohio, USA). Serum creatinine (Boehringer Mannheim, Lewes, UK) and serum triglyceride (Roche, Welwyn Garden City, UK) concentrations were measured using commercial kits. Plasma non-esterified fatty acid (NEFA) levels were measured by centrifugal enzymatic analysis (inter-assay CV 3.0%, Wako, Neuss, Germany). Serum for hormone determinations was separated at 4°C and stored at –40°C, and relative and control subject samples were subsequently analysed together in batches. Total proinsulin, intact proinsulin, insulin and C-peptide concentrations were all measured by specific enzyme immunoassays (DAKO Diagnostics Ltd, Ely, UK). For the insulin assay (inter-assay CV 4.8% at 34 pmol/l), the detection limit was 6 pmol/l with 0.3% cross-reactivity with both intact and 32–33 split proinsulin, and 0% cross-reactivity with C-peptide. For the C-peptide assay (inter-assay CV 3.5% at 160 pmol/l), the detection limit was 50 pmol/l with 63% and 73% cross reactivities with intact and 32–33 split proinsulins, respectively, and 0% cross reactivity with insulin. Both total (inter-assay CV 4.7% at 16 pmol/l) and intact (inter-assay CV 6.6% at 10 pmol/l) proinsulin assays had detection limits of 0.15 pmol/l, and 0% cross-reactivity with insulin and C-peptide.

Statistical analysis. Basal insulin resistance was determined using the homeostasis model assessment (HOMA) modified from Matthews et al. [29]. Proinsulin:insulin and proinsulin:C-peptide molar ratios are presented as percentages, and C-peptide:insulin molar ratios are shown as absolute values. Initial comparison of the two subject groups was by unpaired *t*-test. HOMA values and hormone concentrations and ratios were all $\log_{10}$ transformed to normalise distributions, and these data are presented as the geometric mean [range]. For the second analysis of normal glucose tolerant subjects, relatives and control subjects were individually pair-matched and analysed by paired *t*-test. For every variable, the differences were approximately normally distributed and so the data are presented as means for relatives and control subjects, and the SD of the differences. All analyses were conducted using the Minitab Statistical Package.

Results

Four relatives and two control subjects were diagnosed as having diabetes on oral glucose tolerance test and were excluded from further study. The remaining 150 relatives and 152 control subjects were matched for age and sex (Table 1) and all subjects had serum creatinine concentrations within the normal population range. BMI was significantly increased in the relatives, while WHR showed a similar trend ($p = 0.07$). The relatives also had increased basal insulin resistance ($p < 0.0001$) and a higher prevalence of IGT ($p < 0.01$). Blood glucose and serum

Table 1. Anthropometric and metabolic characteristics of the non-diabetic relatives and control subjects

		Relatives (<i>n</i> = 150)	Control subjects (<i>n</i> = 152)	<i>p</i> value
Age (years)		39 ± 10	41 ± 12	NS
Sex (male, female)		66 : 84	72 : 80	–
BMI (kg/m ²)		27.4 ± 5.1	25.3 ± 4.1	0.0001
WHR		0.84 ± 0.09	0.82 ± 0.11	NS
HOMA (mmol · mU ⁻¹ · l ⁻²)		2.3 [0.5, 10.9]	1.6 [0.2, 8.3]	0.0001
Impaired glucose tolerance (%)		15	3	0.01
Blood glucose (mmol/l)	Fasting	4.8 ± 0.9	4.6 ± 0.6	0.01
	30'	7.9 ± 1.5	7.6 ± 1.4	0.05
	120'	5.6 ± 2.3	5.1 ± 1.5	0.01
C-peptide (pmol/l)	Fasting	465 [70, 1970]	486 [130, 1520]	NS
	30'	1588 [480, 4480]	1790 [420, 5100]	0.04
	120'	1524 [90, 4660]	1918 [600, 5280]	0.0014
Insulin (pmol/l)	Fasting	65 [14, 317]	48 [6, 202]	0.00001
	30'	474 [93, 2761]	387 [56, 1989]	0.003
	120'	261 [28, 2191]	211 [27, 1826]	0.02
Total proinsulin (pmol/l)	Fasting	12 [1, 144]	10 [3, 78]	0.03
	30'	66 [6, 240]	40 [11, 245]	NS
	120'	66 [5, 333]	61 [17, 317]	NS
Intact proinsulin (pmol/l)	Fasting	4 [1, 41]	3 [1, 34]	0.05
	30'	9 [3, 49]	9 [1, 44]	NS
	120'	18 [5, 94]	18 [5, 112]	NS

Data presented as mean ± SD or geometric mean [range]

insulin concentrations were significantly raised in the relatives at all time points (Table 1). Fasting total and intact proinsulin levels were also increased in the relatives, but not after the glucose load. Conversely, C-peptide levels were significantly lower in the relatives following the glucose load.

To investigate whether the differences in the hormone levels between relatives and control subjects were secondary to the differences in glucose tolerance and adiposity, normal glucose tolerant (120 min glucose less than 6.7 mmol/l) relatives and control subjects were pair-matched for age, sex, WHR and BMI. This gave 45 male pairs matched for age (means for relatives, control subjects [SD of differences]; 37, 38 [4] years), BMI (26.3, 26.0 [1.5] kg/m²), and WHR (0.89, 0.89 [0.06]), and 55 female pairs similarly matched for age (39, 39 [5] years), BMI (25.7, 25.6 [1.7] kg/m²), and WHR (0.77, 0.76 [0.05]). As differences between the relatives and control subjects for all variables were comparable in both sexes (i.e. no gender effect), the data for the male and female pairs are presented together. The prevalence of hypertension (blood pressure ≥ 160/95 mmHg) was comparable in the relatives (1%) and control subjects (3%) with one subject from each group using an antihypertensive agent (both amlodopine). The prevalence of hypertriglyceridaemia (fasting level > 2.5 mmol/l) was also comparable in the relatives (4%) and control subjects (5%), and no subject had a value greater than 3.7 mmol/l.

Table 2 summarises the metabolic data for the paired analyses. Basal insulin resistance remained significantly increased in the relatives. Fasting plasma non-esterified fatty acid (NEFA) levels were marginally lower in the relatives, although there were no differences between the groups following the glucose load. Total and intact proinsulin, and insulin concentrations were comparable between the two subject groups, while the C-peptide levels remained significantly decreased in the fasting and glucose-stimulated states. The C-peptide:insulin molar ratios were markedly decreased and the total proinsulin:C-peptide (%) ratios were increased in the relatives at all time points. Conversely, the total proinsulin:insulin (%) ratios were similar in the two groups.

Discussion

Proinsulin, insulin and C-peptide levels were measured by specific immunoassays in non-diabetic relatives of NIDDM families and control subjects with no family history of diabetes. Considering all of the non-diabetic relatives (*n* = 150) and control subjects (*n* = 152), the key findings were that the relatives were more insulin resistant, and had increased insulin and decreased C-peptide concentrations. Total and intact proinsulin levels were also higher in the relatives in the fasting state. However, the relatives also had a higher prevalence of persistent IGT, and were also significantly heavier and tended to have a higher

Table 2. Metabolic measurements for the 100 pair-matched normal glucose tolerant relatives and control subjects

		Relatives	Control subjects	SD of differences	<i>p</i> value
HOMA (mmol · mU ⁻¹ · l ⁻²)		2.1	1.8	1.3	0.02
Blood glucose (mmol/l)	Fasting	4.5	4.6	0.8	NS
	30'	7.6	7.4	1.8	NS
	120'	4.7	4.6	1.4	NS
NEFA (mmol/l)	Fasting	0.51	0.58	0.30	0.03
	30'	0.31	0.33	0.24	NS
	120'	0.07	0.08	0.05	NS
C-peptide (pmol/l)	Fasting	442	516	262	0.01
	30'	1712	2082	1071	0.0003
	120'	4060	5280	1200	0.005
Insulin (pmol/l)	Fasting	64	56	37	NS
	30'	529	496	387	NS
	120'	265	246	289	NS
Total proinsulin (pmol/l)	Fasting	11	11	9	NS
	30'	48	48	43	NS
	120'	67	69	71	NS
Intact proinsulin (pmol/l)	Fasting	4	4	4	NS
	30'	10	11	9	NS
	120'	18	21	19	NS
C-peptide/insulin molar ratio	Fasting	7.8	11.0	7.8	0.0002
	30'	3.5	5.0	2.1	0.0001
	120'	7.7	10.8	6.6	0.0001
Total proinsulin/C-peptide ratio (%)	Fasting	2.8	2.1	1.9	0.001
	30'	3.3	2.3	2.4	0.0003
	120'	5.4	3.4	8.3	0.01
Total proinsulin/Insulin ratio (%)	Fasting	20	22	17	NS
	30'	10	11	6	NS
	120'	31	33	23	NS

All data are presented as the respective means and the SD of the differences

WHR. A key question, therefore, was whether the differences in hormone levels and insulin resistance between the two groups simply due to these differences in adiposity and glucose tolerance.

To address this issue, we pair-matched 100 normal glucose tolerant relatives and control subjects on the basis of age, sex, WHR and BMI. The striking observation was that important metabolic differences remained between the relatives and control subjects (Table 2). First, basal insulin resistance remained increased in the relatives. Although the HOMA index provides an indirect assessment of insulin sensitivity, it has been shown to be closely correlated ($r = 0.88$, $p < 0.0001$) with the index of insulin sensitivity (M value) derived from the hyperinsulinaemic euglycaemic clamp [29]. Other studies have reported insulin resistance in normal glucose tolerant relatives of NIDDM families [15–22], although the biochemical mechanisms remain to be fully defined. Decreased insulin-stimulated glycogen deposition in skeletal muscle appears to be a key abnormality [16, 19], while physical fitness, an important determinant of insulin sensitivity in normal man [30], has recently been reported to be decreased in a comparable group of normal glucose tolerant relatives [22]. Second, C-peptide levels in the fasting and glucose stimulated states were decreased in the relatives indicating impaired insulin

secretion. Thus, we have found evidence for both decreased insulin action and decreased insulin secretion in the normal glucose tolerant relatives that supports the observations of other recent studies [20–22].

This does, however, beg the question as to how the relatives maintain normoglycaemia in the presence of these two metabolic defects. One previously suggested mechanism involves a compensatory increase in glucose-mediated glucose disposal as determined using the frequent sampled intravenous glucose tolerance test (IVGTT) [21]. Our observations lead us to propose a second complimentary mechanism whereby peripheral insulin levels are maintained through a decrease in hepatic insulin clearance. The C-peptide:insulin ratio is an index of hepatic insulin clearance [31–33], and was found to be markedly decreased in the relatives (Table 2). Haffner and colleagues reported decreased hepatic insulin clearance in non-diabetic Mexican Americans when compared with non-Hispanic white control subjects [33], and proposed that it represented an adaptive response to maintain adequate circulating insulin levels in the presence of insulin resistance. An alternative explanation is that the decreased insulin clearance is simply secondary to some other metabolic abnormality in the relatives. Increased NEFA levels have been shown to decrease hepatic insulin clearance [34], and is thought

to be the mechanism by which hyperinsulinaemia develops in subjects with abdominal obesity [35]. However, there was no evidence that this mechanism was important in the relatives in the present study. First, although visceral fat stores were not directly assessed, previous studies have reported a significant positive correlation between WHR and visceral fat stores as determined by computed tomography [36, 37]. Importantly, WHR was one of the parameters used to pair-match the normal glucose relatives and control subjects. Second, plasma NEFA levels were not increased in the relatives following the glucose load, and, indeed, were lower in the fasting state. Thus, the available evidence strongly suggests that the decreased hepatic insulin clearance in the relatives was not secondary to abdominal obesity, and this therefore provides support for an adaptive response the nature of which requires further investigation.

Hepatic insulin clearance is receptor-mediated and linked in part to intracellular insulin action [38]. Inhibition of rat hepatocyte insulin clearance and degradation has been shown to impair the action of insulin on amino acid but not glycogen metabolism [39]. The biological significance of decreased hepatic insulin clearance for hepatic metabolism in the normal glucose tolerant relatives requires further study, although it is interesting to note that hepatic glucose output was found to be normal in a group of comparable relatives of NIDDM families [16].

The elevated fasting total and intact proinsulin levels observed in the whole group of relatives (Table 1) were no longer apparent when the pair-matched normal glucose tolerant subjects were considered (Table 2). This suggests that the increased fasting proinsulin levels were secondary to the differences in glucose tolerance and adiposity, and is in keeping with other reports of normal proinsulin levels in normal glucose tolerant relatives [5, 25]. However, the reports that split proinsulin levels were increased during an IVGTT in comparable normal glucose tolerant relatives [26] and that total proinsulin levels were raised independently of metabolic factors in non-diabetic Mexican-Americans [18], suggests that subtle primary abnormalities of circulating proinsulin levels may be present in at-risk relatives under certain conditions.

As anticipated from the changes in the absolute hormone concentrations, the proinsulin:C-peptide ratio was increased in the normal glucose tolerant relatives, with no difference between the groups for the proinsulin:insulin ratios. This underlines the important point that the proinsulin:insulin ratio is not a reliable index of beta-cell function under conditions of altered hepatic insulin clearance, and under such circumstances, the proinsulin:C-peptide ratio should be used. Certainly a close and inverse relationship has been demonstrated between the proinsulin:C-peptide ratio and first phase insulin secretion as an index of beta-cell function [40].

In conclusion, we have identified multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families. We propose that the potential impact of decreased insulin secretion and decreased insulin action to raise blood glucose levels is limited by compensatory mechanisms, one of which includes decreased hepatic insulin clearance that will increase peripheral insulin levels and help to maintain normoglycaemia. Longitudinal studies are clearly needed to delineate how these metabolic changes progress with time and contribute to the ultimate development of diabetes.

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