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Insulin resistance in relatives of NIDDM patients: the role of physical fitness and muscle metabolism

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Summary First degree relatives of patients with non-insulin-dependent diabetes mellitus (NIDDM) are often reported to be insulin resistant. To examine the possible role of reduced physical fitness in this condition 21 first degree relatives of NIDDM patients and 22 control subjects without any history of diabetes were examined employing a 150-min hyperinsulinaemic (0.6 mU insulin · kg^{-1} · min^{-1}) euglycaemic clamp combined with the isotope dilution technique (3-^{3}H-glucose, Hot GINF), the forearm technique and indirect calorimetry. During hyperinsulinaemia glucose disposal (Rd) and forearm glucose extraction were significantly diminished in the relatives ($p < 0.01$ and $p < 0.05$), but glucose oxidation and the suppressive effect on hepatic glucose production were normal. Arteriovenous differences across the forearm of the gluconeogenic precursors lactate, alanine and glycerol as well as the increments in forearm blood flow during hyperinsulinaemia were similar in the two groups. Maximal oxygen uptake (VO_2 max) was lower in the relatives than in the control subjects (36.8 ± 1.9 vs 42.1 ± 2.0 ml · kg^{-1} · min^{-1}; $p = 0.03$). There was a highly significant correlation between Rd and VO_2 max in both relatives and control subjects ($r = 0.68$ and 0.66, respectively; both $p < 0.001$). Comparison of the linear regression analyses of insulin-stimulated Rd on VO_2 max in the two groups showed no significant differences between the slopes (0.10 ± 0.03 vs 0.09 ± 0.02) or the intercepts. In stepwise multiple linear regression analyses with insulin-stimulated Rd as the dependent variable VO_2 max significantly determined the level of Rd ($p < 0.01$), whereas forearm blood flow and anthropometric data did not. In conclusion, the insulin resistance in healthy first degree relatives of patients with NIDDM is associated with a diminished physical work capacity. Whether, this finding is ascribable to environmental or genetic factors (e.g. differences in muscle fibre types, capillary density etc) remains to be determined. [Diabetologia (1996) 39: 813–822]

Keywords Insulin resistance, relatives, non-insulin-dependent diabetes mellitus, oral glucose tolerance test, physical fitness, forearm blood flow, muscle metabolism.

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Abbreviations: BMI, Body mass index; W/H ratio, waist to hip ratio; FFM, fat free mass; NEFA, non-esterified fatty acids; A-V, arteriovenous; VO_2 max, maximal oxygen uptake; GIR, glucose infusion rate; Rd, isotopically determined glucose disposal; HGP, hepatic glucose production; Rd(ox), glucose oxidation rate; Rd(nonox), non-oxidative glucose disposal; lipid(ox), lipid oxidation rate; Protein(ox), protein oxidation rate; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test.

Insulin resistance is considered to be one cardinal feature in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) [1–3]. Employing various techniques for assessment of insulin sensitivity both cross-sectional [4–13] and prospective studies [14–16] in subjects with an increased risk for developing NIDDM such as offspring of patients with NIDDM and Pima Indians, support this hypothesis. Normal glucose tolerant healthy first degree relatives of patients with NIDDM are characterized by an impaired insulin-stimulated glucose uptake [non-oxidative) compared to subjects without any family history of diabetes [5, 6, 11, 12].

Physical work capacity is a major determinant of insulin sensitivity. Using the hyperinsulinaemic euglycaemic clamp technique many investigators have demonstrated a strong correlation between whole-body insulin-stimulated glucose uptake and maximal oxygen uptake in healthy humans [e.g. 17, 18]. Furthermore, a single bout of exercise improves insulin action on glucose metabolism for several hours to days [19, 20], whereas detraining conversely causes a deterioration in insulin action [18]. An association between a low level of physical activity and an increased occurrence of NIDDM has recently been demonstrated in two large prospective studies [21, 22]. Nevertheless, a possible direct relationship between the magnitude of insulin resistance in relatives of NIDDM patients and their physical fitness has not been tested.

In addition, an impaired ability of insulin to stimulate skeletal muscle blood flow has been suggested to be an important mechanism contributing to the impaired insulin-stimulated glucose uptake in full-blown insulin resistance conditions, such as NIDDM and obesity [23–26]. The abnormality could provide an important link between hypertension and insulin resistance [27]. Whether the impact of hyperinsulinaemia on muscle blood flow is normal in subjects with an enhanced risk of developing NIDDM has so far not been examined.

Thus, the present investigation was undertaken to gain further insight into the possible roles of: (i) a diminished physical capacity; and (ii) abnormalities in insulin-stimulated skeletal muscle blood flow as pathogenic factors behind the insulin resistance of first degree relatives of NIDDM patients.

Subjects and methods

Subjects. Twenty-one healthy offspring of NIDDM patients and 22 healthy control subjects without any family history of diabetes participated in the study. The relatives were recruited via their NIDDM parents attending the outpatient clinic, Medical Department M, Aarhus Kommunehospital. Ten of the relatives had one known family member (first degree) with NIDDM (one parent), ten had two or more (one first degree and one or more second degree relatives) and one had two first degree relatives with NIDDM. Ten had a maternal and 11 had a paternal history. All were healthy and were taking no medication. No family history of any other endocrine disorder was present. Additional exclusion criteria were age over 54 years, BMI over 30 kg/m^2 and non-Caucasian origin. If two or more offspring were available in a family, only one was randomly (by chance) selected to participate. The control group was recruited from volunteer blood donors, medical students, physicians and nurses. The pertinent clinical data of the two groups are depicted in Table 1.

Table 1. Clinical data of the two groups

	Relatives ($n = 21$)	Control subjects ($n = 22$)
Gender (male/female)	11/10	13/9
Age (years)	37.6 (27–53)	35.4 (21–50)
BMI (kg/m^2)	24.8 (16.8–30.0)	24.0 (18.8–29.2)
Waist/hip ratio	0.90 (0.77–1.03)	0.89 (0.73–1.06)
Fat free mass (kg)	56.9 (39.3–75.7)	56.9 (40.9–73.1)
VO_2 max (ml · kg^{-1} · min^{-1})	36.8 (19.5–55.5)	42.1 (24.0–65.6)[a]
Number of smokers	9	8
Systolic blood pressure (mm Hg)		
Day-time	127 (110–135)	128 (96–134)
Night-time	110 (95–120)	112 (90–130)
24-h	122 (106–132)	123 (94–135)
Diastolic blood pressure (mm Hg)		
Day-time	79 (61–85)	79 (63–84)
Night-time	63 (51–75)	63 (50–80)
24-h	74 (59–84)	74 (60–84)
Urinary albumin/creatinine ratio (mg/mmol)	0.51 (0.10–1.33)	0.46 (0.07–0.87)
Serum lipids (mmol/l)		
Total cholesterol	5.23 (3.3–6.5)	4.94 (3.2–6.4)
HDL-cholesterol	1.41 (0.80–2.52)	1.27 (0.57–1.83)
Triglycerides	1.04 (0.41–2.28)	1.07 (0.53–2.09)

Data are mean (range)
[a] $p = 0.03$. All other comparisons $p > 0.25$

All were instructed to consume a weight-maintaining diet containing at least 300 g of carbohydrate for 3 days prior to both examinations (see below) and none was engaged in heavy physical exercise in the same periods. None had a history of infectious disease within the 4 weeks prior to the study. All females were examined in the follicular phase of the menstrual cycle, one in each group was postmenopausal.

The protocol was approved by the ethical committee of the County of Aarhus.

Design. All subjects were tested twice on two separate occasions (Test I and II) with an interval of 2 to 4 weeks.

Test I

After an overnight fast the subjects underwent a physical examination including determination of waist to hip (W/H) ratio and fat free mass (FFM) employing bioelectric impedance (Animeter; HTS-Engineering APS, Odense, Denmark) [28]. At 08.00 hours an oral glucose tolerance test (OGTT) (75 g glucose) was performed. Blood for determination of plasma glucose, serum insulin, C-peptide, and non-esterified fatty

acids (NEFA) was taken at time 0, 30, 60, 90 and 120 min. Blood for determination of serum triglycerides, total cholesterol, and HDL-cholesterol was collected at time 0.

After termination of the OGTT a 6-min submaximal exercise test with continuous monitoring of the heart rate, was performed on a bicycle ergometer (Monark Ergometric 829 E; Monark Exercise AB, Varberg, Sweden) using a workload of 300–1500 kpm/min, depending on age, gender and reported physical activity by the subject. The mean heart rate during the last 2 min of work (> 120–130 beats/min) was used for calculation of the maximal aerobic capacity (VO_2 max) as described by Åstrand [29]. This indirect measure of VO_2 max has been shown to correlate well to VO_2 max as determined by direct measurements with a coefficient of variance of less than 10 % [30, 31]. The level of habitual physical activity, both during work and leisure, was quantified using a questionnaire according to Saltin and Grimby [32].

Finally, after a light meal measurement of 24-h ambulatory blood pressure was started by a portable lightweight monitor (Spacelabs 90202, Redmond, Wash., USA). The monitor was programmed to measure blood pressure every 20 min during daytime (06.00 to 24.00 hours) and every 60 min during the night. The procedure has been described elsewhere [33]. On the following morning subjects were instructed to collect their first morning urine sample for assessment of the albumin/creatinine ratio.

Test II

All studies started at 08.00 hours in the clinical research unit after a 10-h overnight fast. One catheter was inserted retrogradely into a deep antecubital vein of one arm for sampling of blood derived from the forearm muscles. The criteria for satisfactory positioning was that the oxygen saturation in blood drawn from the catheter was under 70 %. In the contralateral arm, one catheter was placed in a heated dorsal hand vein for sampling of arterialized blood (oxygen saturation > 90 %), and another was positioned in the antecubital vein for all infusions.

At 08.30 hours (time 0 min) a 20 μCi bolus dose of 3-^{3}H glucose (DuPont-New England Nuclear, Boston, Mass., USA) was given followed by infusion at a constant rate of 0.20 μCi/min throughout the study. As assessed by HPLC the tracer contained no significant contamination [34]. After 150 min, insulin (Actrapid; Novo-Nordisk, Copenhagen, Denmark) was infused intravenously at a constant rate of 0.6 mU · kg^{-1} · min^{-1} for 150 min (time, 150–300 min). Plasma glucose was clamped at 5 mmol/l as described by DeFronzo et al. [35]. To minimize rapid dilution of the labelled glucose pool with unlabelled glucose, 3-^{3}H glucose was added to the glucose infused during the clamp (100 μCi/500 ml 20 % glucose) [36]. Blood for determination of glucose specific activity and serum insulin were drawn at times 0, 90, 120, 135, 150, 180, 210, 240, 270, 285 and 300 min. The intervals between 120 and 150 min and 270 and 300 min were defined as the basal state and the hyperinsulinaemic 'steady-state' period, respectively. Indirect calorimetry [37] (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland), measurements of forearm blood flow [38] (Venous occlusion plethysmography; Digimatic 2000; Medimatic A/S, Copenhagen, Denmark) and examination of arteriovenous (A-V) substrate balances [39] were performed in these two periods.

Plasma glucose was determined every 5–10 min during the clamp. Serum NEFA and blood alanine, lactate, glycerol and 3-hydroxy-butyrate were measured every 15 min in the basal state and the steady-state period.

Analytical methods. Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, Calif., USA). Serum insulin was determined by RIA as described by Ørskov et al. [40] with modifications. Circulating insulin during the OGTT was also assayed by ELISA employing a two-site immunoassay [41], which does not detect proinsulin, split(32–33)-, and des(31, 32)-proinsulin, whereas split(65–66)- and des(64–65)-proinsulin crossreact 30 % and 63 %, respectively. The intraassay coefficient of variation (C.V.) was 2.0 % (n = 75) at a serum level of 200 pmol/l. Serum C-peptide was determined according to Heding [42]. Serum NEFA was determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany). Blood lactate, glycerol, 3-hydroxy-butyrate and alanine were assayed using a Cobas Bio centrifugal analyzer with a fluorometric attachment [43].

Calculations. After counting plasma glucose specific activity the non-steady-state equation as described by Finegood et al. [36] was used for calculation of glucose appearance/disposal rates. A pool fraction of 0.65 and a distribution volume of 220 ml/kg were employed. Respiratory exchange ratios were assessed employing indirect calorimetry. Protein oxidation rates were estimated from urinary excretion of urea. Net lipid oxidation and glucose oxidation rates were computed from the above measurements, and non-oxidative glucose disposal was calculated by subtracting the glucose oxidation rate from total isotopically determined glucose disposal.

Calculation of forearm blood flow was performed blind and was based upon the average of measurements made in triplicate in each of the two periods.

Statistical analyses

Data in the text and figures are given as means ± SEM. Student's two-tailed t-tests for unpaired and paired data were used for comparison of data between and within groups, respectively. Student's one-tailed t-test for unpaired data was used to test the hypothesis that VO_2 max was decreased in relatives of NIDDM patients. When data were not parametrically distributed (urinary albumin/creatine ratio) Mann-Whitney's Rank sum test for unpaired data was used. In addition two-way analysis of variance (ANOVA) for repeated measures, Pearson Product moment correlation test, linear- and stepwise multiple linear regression analyses were used. Finally, chi square analysis was employed to test the results of the questionnaire.

Results

Oral glucose tolerance test. Neither fasting plasma glucose nor serum insulin and C-peptide were different ($p > 0.10$) in the basal state prior to the OGTT between the two groups (Table 2). HbA_{1c} tended, however, to be higher in the relatives (5.15 ± 0.11 %) than in the control subjects (4.85 ± 0.13 %, $p = 0.09$). During the OGTT plasma glucose was more elevated in the relatives after 60, 90 and 120 min compared to the control subjects ($p = 0.05$, $p < 0.05$ and $p = 0.08$, respectively) (Fig. 1). However, all subjects exhibited a normal OGTT according to the criteria of the National Diabetes Data Group [44]. Following the glucose challenge average serum insulin values as determined by a

Table 2. Basal plasma glucose, serum NEFA and circulating insulin and C-peptide prior to OGTT

	Relatives	Control subjects
Plasma glucose (mmol/l)	5.3 ± 0.1	5.1 ± 0.1
Serum NEFA (mmol/l)	0.62 ± 0.06	0.57 ± 0.07
Serum insulin (RIA) (pmol/l)	38 ± 8	43 ± 5
Serum insulin (ELISA) (pmol/l)	44 ± 8	39 ± 3
Serum C-peptide (nmol/l)	0.47 ± 0.04	0.41 ± 0.04

Data are mean ± SEM

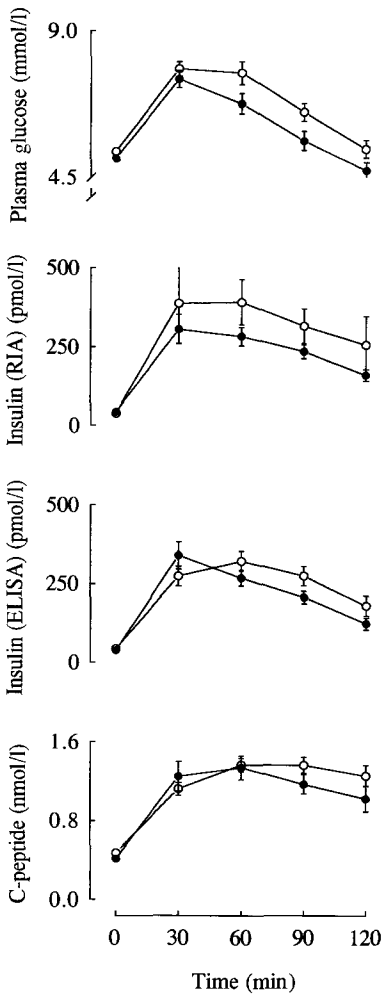

Fig. 1. Plasma glucose (mean ± SEM), serum insulin (measured by RIA and by ELISA using a two-site immunoassay) and C-peptide during a 75-g OGTT in 21 first degree relatives of patients with NIDDM (○) and 22 control subjects with no family history of diabetes mellitus (●)

traditional RIA tended to be higher in the relatives although these differences were not statistically significant owing to large variations in the group. By contrast, the profiles of circulating insulin as measured by ELISA using a highly specific two-site immunoassay and circulating C-peptide following OGTT were different in the relatives and the control subjects (ANOVA; $p < 0.01$ and $p = 0.05$). Both curves showed a sluggish initial response in the relatives, but the areas under the curves using the trapezoidal rule were comparable in the two groups ($p > 0.10$). Basal NEFA was comparable in the two groups.

Physical capacity/activity. There was a large overlap in maximal oxygen consumption as assessed by bicycle ergometer in the two groups. However, on average VO_2 max was approximately 15 % higher in the control subjects than in the relatives ($p = 0.03$, Table 1). Assessment of habitual physical activity by questionnaire [32] did not disclose any differences in the level of activity between the relatives and the control subjects, during work or during leisure.

Glucose metabolism. There was no difference in basal metabolism between the two groups (Table 3). During hyperinsulinaemia (steady-state serum insulin: 379 ± 20 vs 371 ± 13 pmol/l; steady-state plasma glucose: 5.1 ± 0.1 vs 5.1 ± 0.0 mmol/l; relatives vs control subjects) rates of glucose infusion (GIR), isotopically determined total glucose disposal (Rd) and non-oxidative glucose disposal (Rd(nonox)) were all decreased in the relatives as compared to the control subjects ($p = 0.05$ or less, Table 3). Similar discrepancies were found when expressing the data as mg · kg · FFM^{-1} · min^{-1} (data not shown). By contrast, oxidative glucose disposal (Rd(ox)), lipid oxidation (lipid(ox)) and hepatic glucose production (HGP) were all similar during hyperinsulinaemia in the two groups (Table 3). As indicated in Figure 2, GIR and Rd were diminished in the relatives after 60 min of hyperinsulinaemia, while HGP was uniformly suppressed during the period of hyperinsulinaemia. Insulin-stimulated Rd was similar in the subjects with a paternal (4.59 ± 0.35 mg · kg^{-1} · min^{-1}) and a maternal history of NIDDM (4.81 ± 0.49 mg · kg^{-1} · min^{-1}). Likewise, Rd was comparable in the relatives with only one known and two or more known relatives with NIDDM (4.52 ± 0.38 vs 4.85 ± 0.45 mg · kg^{-1} · min^{-1}).

Relationship between glucose metabolism and physical capacity. There was a highly significant correlation between Rd and VO_2 max in both relatives and control subjects ($r = 0.68$ and 0.66, respectively; p(both) < 0.001). A linear model was found to give the best description of this relationship in the two groups separately and together (Fig. 3). When comparing the linear regression analyses of insulin-stimulated Rd on VO_2 max in the two groups no significant differences between the slopes (0.10 ± 0.03 vs 0.09 ± 0.02) or the intercepts (0.92 ± 0.95 vs 1.71 ± 1.05 mg · kg^{-1} · min^{-1}) were found. A linear relationship was also found between GIR and Rd(nonox) and VO_2 max ($p < 0.01$).

In stepwise multiple linear regression analyses with insulin-stimulated Rd as the dependent variable

Table 3. Basal and insulin-stimulated glucose and lipid metabolism (mg · kg^{-1} · min^{-1})

	Relatives	Control subjects	*p* value
Basal			
Rd	1.75 ± 0.08	1.75 ± 0.06	NS
Rd (ox)	0.99 ± 0.11	1.03 ± 0.07	NS
Rd (nonox)	0.76 ± 0.10	0.72 ± 0.08	NS
Lipid (ox)	1.04 ± 0.04	1.07 ± 0.05	NS
HGP	1.79 ± 0.09	1.78 ± 0.07	NS
Protein (ox)	0.58 ± 0.03	0.57 ± 0.04	NS
Hyperinsulinaemia			
GIR	4.82 ± 0.32	6.03 ± 0.33	$p = 0.01$
Rd	4.69 ± 0.29	5.67 ± 0.29	$p < 0.05$
Rd (ox)	2.26 ± 0.15	2.41 ± 0.11	NS
Rd (nonox)	2.56 ± 0.22	3.73 ± 0.25	$p < 0.01$
Lipid (ox)	0.48 ± 0.06	0.47 ± 0.04	NS
HGP	− 0.24 ± 0.13	− 0.36 ± 0.12	NS

Data are mean ± SEM
Rd, Rd (ox) and Rd (nonox): Isotopically determined total glucose disposal, glucose oxidation and nonoxidative glucose disposal, respectively. Lipid (ox): Lipid oxidation. Protein (ox): Protein oxidation

VO_2 max statistically significantly determined the level of Rd in both relatives and control subjects ($p < 0.01$), whereas basal and insulin-stimulated blood flow, anthropometric data, age, gender, basal insulin and NEFA levels, 24-h ambulatory blood pressure, lipid profile and urinary albumin/creatinine ratio (entered separately and together with VO_2 max as independent variables) did not further significantly contribute to the level of Rd.

Forearm blood flow. Forearm blood flow did not differ significantly in the basal state between the relatives (1.62 ± 0.15 ml · 100 ml tissue^{-1} · min^{-1}) and the control subjects (2.06 ± 0.22 ml · 100 ml tissue^{-1} · min^{-1}; $p = 0.12$). During the clamp (and hyperinsulinaemia) forearm blood flow rose in both groups (relatives and control subjects: $p < 0.05$ and $p < 0.01$; respectively) (Fig. 4). Forearm blood flow was higher in the control subjects (2.53 ± 0.27 ml · 100 ml tissue^{-1} · min^{-1}) than in the relatives (1.86 ± 0.12 ml · 100 ml tissue^{-1} · min^{-1}; $p < 0.05$). The increments in blood flow did not, however, differ between the two groups (relatives vs control subjects: 0.24 ± 0.10 vs 0.47 ± 0.14 ml · 100 ml tissue^{-1} · min^{-1}; $p = 0.18$). There was no correlation between increments in blood flow and Rd in the two groups ($r = 0.16$ and $r = 0.07$; p (both) > 0.50).

NEFA, metabolites and A-V balances. Basal arterialized levels of NEFA, glycerol, 3-hydroxy-butyrate, lactate and alanine were similar in the relatives and the control subjects (Table 4). Following hyperinsulinaemia, the variables were affected equally in the two groups. Serum NEFA, blood 3-hydroxy-butyrate and blood glycerol were greatly reduced ($p < 0.001$), while blood lactate increased significantly ($p < 0.001$).

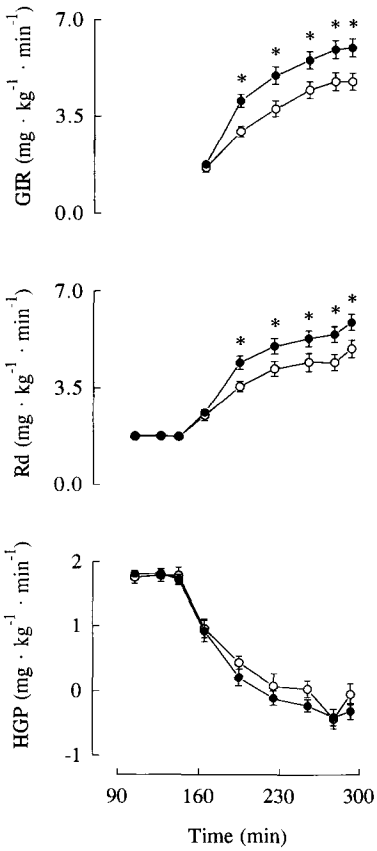

Fig. 2. Rates of glucose infusion (GIR) (mean ± SEM), isotopically determined glucose disposal (Rd) and hepatic glucose production (HGP) in the basal state and during the hyperinsulinaemic clamp (time, 150–300 min) in relatives (○) and control subjects (●). *$p < 0.05$

Blood alanine was not influenced by the insulin infusion (Table 4). A-V differences of the metabolites during insulin exposure did not differ between the two groups. A-V differences of the gluconeogenic precursors, lactate and alanine were –0.040 ± 0.015 and –0.01 ± 0.003 mmol/l, respectively in the relatives and –0.036 ± 0.013 and –0.028 ± 0.003 mmol/l in the control subjects. A-V differences in blood glycerol and 3-hydroxy-butyrate were close to zero in both groups during hyperinsulinaemia.

A-V differences of plasma glucose across the forearm during insulin stimulation were comparable in the relatives (0.91 ± 0.11 mmol/l) and the control subjects (1.05 ± 0.15 mmol/l, $p = 0.39$). However, there was a significant correlation between A-V differences in plasma glucose and isotopically determined Rd among all subjects ($r = 0.76$, $p < 0.001$). In contrast insulin-stimulated forearm glucose disposal, determined by multiplying the forearm blood flow and A-V differences in plasma glucose [39, 45], was significantly reduced in the relatives (1.37 ± 0.20 μmol · 100 ml tissue^{-1} · min^{-1}) as compared to the control subjects (1.94 ± 0.26 μmol · 100 ml tissue^{-1} · min^{-1}; p(ANOVA) < 0.05; Fig. 5). As expected, there

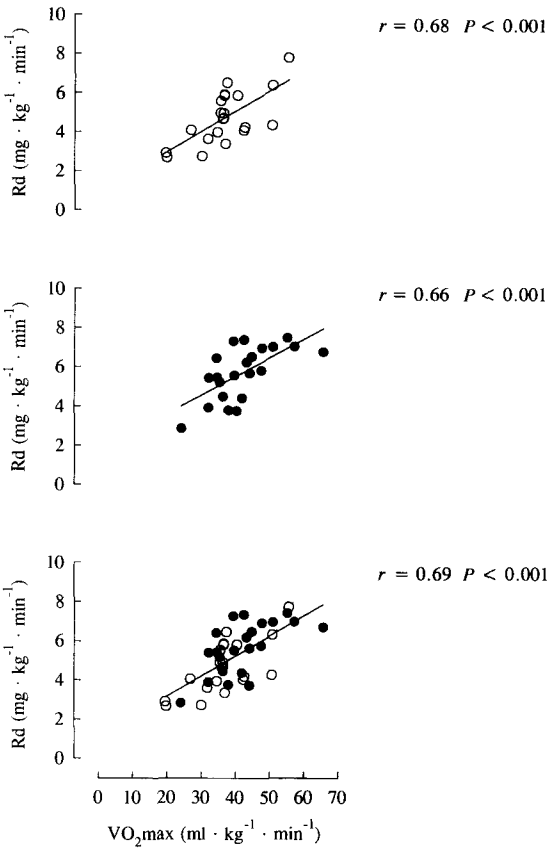

Fig. 3. Linear regression line between insulin-stimulated Rd and VO_2 max in relatives of patients with NIDDM (○) and control subjects (●), and in the two groups together

Table 4. Arterialized serum NEFA, blood glycerol, 3-hydroxy-butyrate, lactate and alanine in the basal state and during hyperinsulinaemic clamp (mmol/l)

	Relatives	Control subjects
Basal		
NEFA	0.655 ± 0.033	0.604 ± 0.032
Glycerol	0.056 ± 0.002	0.051 ± 0.003
3-hydroxy-butyrate	0.130 ± 0.029	0.090 ± 0.018
Lactate	0.521 ± 0.015	0.509 ± 0.025
Alanine	0.207 ± 0.027	0.205 ± 0.019
Hyperinsulinaemia		
NEFA	0.036 ± 0.005	0.036 ± 0.004
Glycerol	0.021 ± 0.001	0.021 ± 0.001
3-hydroxy-butyrate	0.003 ± 0.001	0.005 ± 0.001
Lactate	0.641 ± 0.020	0.644 ± 0.012
Alanine	0.195 ± 0.007	0.191 ± 0.006

Data are mean ± SEM

was a highly significant relationship between forearm glucose disposal and whole-body Rd ($r = 0.75$, $p < 0.001$).

Blood pressure, lipid parameters and urinary albumin /creatinine ratio. 24-h ambulatory blood pressure, daytime and night-time blood pressure were almost identical in the two groups. There was no correlation

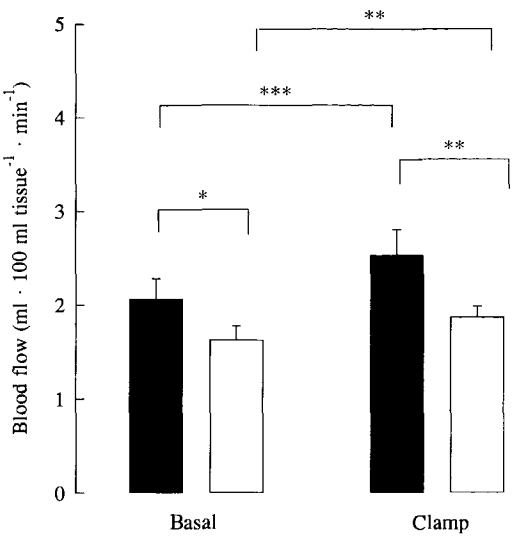

Fig. 4. Forearm blood flow (mean ± SEM) in the basal state and during the hyperinsulinaemic clamp in relatives (□) and control subjects (■). $^{*}p$ = NS; $^{**}p < 0.05$; $^{***}p < 0.01$

between either systolic blood pressure or diastolic blood pressure and GIR or Rd (p (all) > 0.5). Similarly, serum levels of total cholesterol, HDL-cholesterol and triglycerides were also comparable, and no difference in urinary albumin/creatinine ratio was observed (Table 1).

Discussion

One of the novel observations in the present study was that the insulin resistance of the relatives of patients with NIDDM was related to their maximal aerobic oxygen uptake. The relationship between VO_2 max and insulin-stimulated glucose disposal was close and almost identical in the relatives and control subjects, but VO_2 max was on average 15 % lower in the relatives. The data does not allow conclusions about causality, but it is tempting to hypothesyse the decreased physical work capacity as being one cardinal determinant behind the insulin resistance of the relatives of NIDDM patients.

In some of the previous studies evaluating insulin sensitivity in subjects with increased risk of developing NIDDM [5–16, 46–52] groups were well matched in terms of age, gender and BMI. Although there is abundant evidence in the literature [for review, 18 and 53] that maximal aerobic power is significantly correlated to insulin-stimulated glucose disposal (in the current study we found an r^2 at 0.46 in the relatives and 0.44 in the control subjects), maximal oxygen uptake has only been measured in one of the former reports [6]. Laws et al. [6], however, found relatives of NIDDM subjects to be insulin resistant as assessed by the insulin suppression test despite having comparable VO_2 max values to those found in control

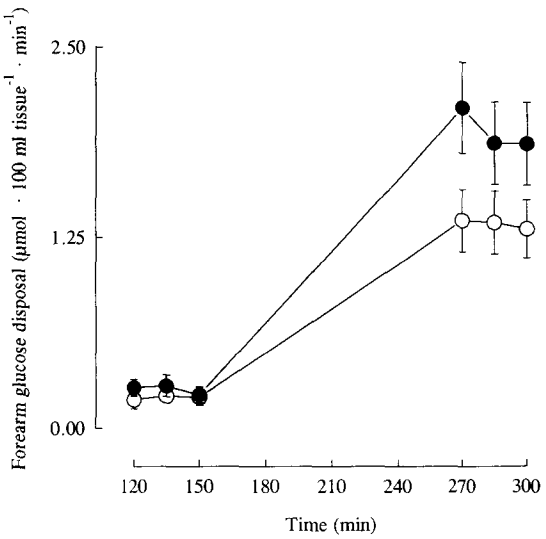

Fig. 5. Forearm glucose disposal (mean ± SEM) in relatives of patients with NIDDM (○) and control subjects (●) during the basal period (time, 120–150 min) and the hyperinsulinaemic clamp (time, 270–300 min). $p < 0.05$ (ANOVA)

subjects, suggesting that factors other than impaired physical fitness appear to contribute to the insulin resistance of the relatives. The discrepancy between their study and ours might be due to notable differences in body composition and physical activity. VO_2 max was considerably lower and all were overweight (120–160 % of ideal body weight) implying that the impact of physical activity on insulin action could be partly concealed by an obesity-related insulin resistance.

Whilst the questionnaire quantifying physical activity both during work and leisure times showed similar levels in the two groups, the maximal aerobic power was significantly higher in the control subjects. Though a questionnaire may be fraught by uncertainty, this finding together with the reduced maximal aerobic capacity of the relatives might be grounded in differences/abnormalities in skeletal muscle fibre composition, capillary density and also perhaps in abnormalities in the skeletal muscle phospholipids [54]. An association between fibre composition and density of capillaries vs insulin sensitivity has been recognized for more than a decade [55], and has been confirmed by Lillioja et al. [56] who using the clamp technique demonstrated correlations between insulin-stimulated glucose disposal rates and capillary density, percentage of type I fibres and percentage of type II B fibres (an inverse relationship), and analogous correlations were seen between VO_2 max and the muscle morphology. Recently, it has been shown that subjects with abdominal obesity (with and without NIDDM) exhibit a low percentage of type I fibres and a low capillary density [57], but whether such variations are ascribable to genetic factors or environmental factors (including secondary hyperinsulinaemia, [58]) is not known. The W/H ratios were similar in our two groups, but it should be noted that the ability of this classic measurement to estimate the amount of the visceral fat deposits is limited [59]. The content of intraabdominal fat has been suggested to be an important determinator of the insulin sensitivity in men with NIDDM [60].

The coupling of insulin resistance and lack of physical fitness in relatives of NIDDM subjects is parallel to findings in another facet of the metabolic syndrome, namely essential hypertension [61, 62]. Offspring of hypertensive subjects exhibit impaired insulin-stimulated glucose metabolism [63], which is apparently related to maximal oxygen uptake [64].

Examining forearm A-V balances our study demonstrates for the first time direct evidence of reduced insulin-stimulated extraction of glucose in skeletal muscle in relatives of NIDDM patients. This finding is, however, not unexpected bearing in mind the work of DeFronzo et al. [65] indicating that 80–90 % of plasma glucose removal during hyperinsulinaemia is confined to skeletal muscle in healthy humans and studies in relatives of NIDDM patients showing abnormalities in pivotal intracellular processings of muscle cells, e.g. the activities of glycogen synthase [11] and tyrosine kinase [66]. Based upon indirect calorimetry our study is in line with Eriksson et al. [5], Vaag et al. [11] and Gulli et al. [12] all finding that mainly/only non-oxidative insulin-stimulated glucose disposal is affected in relatives. The diminished non-oxidative glucose disposal has been assumed to be due to impaired storage of glycogen which is the major non-oxidative event during short-term moderate insulin exposure, but lactate and alanine formation and release are other important non-oxidative events. However, the arterialized concentrations and the A-V differences of the two metabolites were not different between the relatives and the control subjects. In addition, we support previous data indicating that the restraining effect of insulin on HGP is normal in relatives of NIDDM patients. Employing the hot glucose infusion protocol approach our design, however, allows assessment of the time-course of effects of insulin on the liver.

As mentioned before a reduced insulin-stimulated blood flow has been suggested to be one cardinal factor behind insulin resistance in obesity and NIDDM [23–26]. In the present study neither basal forearm blood flow, insulin-stimulated blood flow nor the incremental blood flow significantly influenced insulin-stimulated glucose disposal, thus not supporting differences in the blood flow as being major determinants for the insulin resistance of our relatives of NIDDM patients. The findings on forearm blood flow warrant two comments. First, the muscle mass of the forearm constitutes less than 10 % of the total body muscle mass and there may be some heterogeneity in the ability of insulin to increase blood flow

in other regions. Second, our observation of an increment in forearm blood flow during the hyperinsulinaemic euglycaemic clamp (both in relatives and in control subjects) seems difficult to reconcile with previous reports where even pharmacologic doses of insulin failed to modulate forearm blood flow significantly [67].

All subjects had a normal glucose tolerance; however, the average plasma glucose concentrations were higher 60, 90 and 120 min after the glucose ingestion in the relatives suggesting a relative glucose intolerance. Circulating insulin concentrations before and after the oral glucose were assayed not only by employing a traditional RIA but also an ELISA using a two-site immunoassay, which do not detect proinsulin, split (32–33)- and des(31, 32)-proinsulin, the latter being the only proinsulin products present in significant amounts in circulation. Basal levels of circulating insulin were comparable in the two groups, irrespective of the assay, whilst the patterns of the post-glucose insulin curves differed substantially in the two assays. Using the traditional (non-specific) assay serum insulin peaked after 30 min in both groups, and was consistently elevated in the relatives as compared to the control subjects, while the rise in serum insulin in the relatives using the two-site assay was clearly delayed (and exhibited a pattern comparable to that of circulating C-peptide). This strongly indicates that the initial insulin release of the relatives includes a large fraction of proinsulin and split-products, thus substantiating the composite pathogenesis of NIDDM. A nonsignificant difference in fasting insulin in the two groups is not contradictory to the presence of insulin resistance in the relatives, because the majority of basal glucose disposal takes place in non-insulin-dependent tissues [68]. Moreover, the insulin resistance may be compensated for by a slight elevation of blood glucose (HbA_{1c} tended to be higher in the relatives, $p = 0.09$), and could also be through an exaggerated glucose effectiveness [13]. Our findings support the notion of an early beta-cell dysfunction in prediabetic subjects and are thus in line with the data of Pimenta et al. [52] demonstrating a diminished first- and second-phase insulin response in relatives during a hyperglycaemic clamp.

An increasing amount of evidence from both epidemiological and from case-control studies indicate a clustering of insulin resistance, hypertension, dyslipidaemia, NIDDM and obesity, with insulin resistance playing a pivotal role [63, 69, 70]. In addition, microalbuminuria has lately been suggested to be associated with insulin resistance in NIDDM [71] and to be a predictor of NIDDM [72]. The present study did not show any significant differences in the 24-h ambulatory blood pressure and the lipid variables between the relatives and the control subjects. Furthermore, urinary albumin/creatinine ratios were comparable in the two groups. Obviously, this may partly be due to the limited number of participants, but probably also relates to the heterogeneity of this syndrome.

In conclusion, relatives of patients with NIDDM are characterized by impaired insulin-stimulated glucose uptake in skeletal muscle when compared to an age and anthropometrically matched control group without any family history of diabetes mellitus. This abnormality seems, however, to be associated with a diminished maximal aerobic uptake. Whether the reduced physical fitness of the relatives is due to environmental or genetic factors remains to be elucidated.

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