Defects in Insulin Secretion and Insulin Action in Non–insulin-dependent Diabetes Mellitus Are Inherited

Metabolic Studies on Offspring of Diabetic Probands

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Abstract

No studies are available that have compared early defects in glucose metabolism in the offspring of insulin-deficient and insulin-resistant probands with non–insulin-dependent diabetes mellitus (NIDDM). To investigate this issue, we evaluated insulin secretion capacity with oral and intravenous glucose tolerance tests and with the hyperglycemic clamp, and insulin action with the euglycemic insulin clamp in 20 offspring of NIDDM patients with low fasting C-peptide (±450 pmol/liter), reflecting deficient insulin secretion (IS-group), 18 offspring of NIDDM patients with high fasting C-peptide (≥ 880 pmol/liter), reflecting insulin resistance (IR-group), and 14 healthy control subjects without a family history of NIDDM. The frequency of impaired glucose tolerance was 45.0% in the IS-group and 50% in the IR-group. The IS-group had lower insulin–glucose response at 30 min in the oral glucose tolerance test (85.2±10.0 pmol insulin per mmol glucose) than the control group (136.4±23.1 pmol insulin per mmol glucose; $P < 0.05$) and the IR-group (115.6±11.8 pmol insulin per mmol glucose; $P = 0.05$). Furthermore, the acute insulin response during the first 10 min of an intravenous glucose tolerance test was lower in the IS-group than in the IR-group. Maximal insulin secretion capacity evaluated by C-peptide levels during the hyperglycemic clamp did not differ between the groups. The IR-group had lower rates of whole body glucose uptake (60.1±4.6 μmol per lean body mass per minute) than did the control group (84.2±5.0 μmol per lean body mass per minute; $P < 0.001$) or the IS-group (82.6±5.9 μmol per lean body mass per minute; $P < 0.01$) and this was due to reduced glucose nonoxidation. To conclude, both impaired insulin secretion and insulin action seem to be inherited and could represent the primary defects in glucose metabolism in the offspring of NIDDM probands. (*J. Clin. Invest.* 1997. 100:86–96.) Key words: glucose tolerance • insulin secretion • insulin sensitivity • fat distribution • maximal oxygen uptake

Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is a common disorder affecting 3–5% of individuals among westernized populations. The key mechanisms causing hyperglycemia are disturbed insulin secretory capacity of the pancreatic β cells and decreased sensitivity to insulin in target tissues, e.g., in skeletal muscle and liver (1).

There is a strong evidence that NIDDM is genetically determined (2, 3). The inheritance pattern, however, is unknown and in only a small number of cases the genetic defect resulting in NIDDM is well documented (4, 5). Furthermore, it is likely that the genetic background predisposing to NIDDM can be different in obese and nonobese NIDDM patients (6).

Since hyperglycemia per se impairs both insulin secretion and insulin action (7), this can hamper studies of glucose metabolism in patients with overt NIDDM. This problem can be overcome by studying individuals at high risk of developing NIDDM, i.e., their first-degree relatives (8–16). However, even these studies have given controversial results regarding the primary defect in NIDDM. Some studies have suggested that insulin resistance is the primary cause for NIDDM (8–14) while others have emphasized the role of insulin secretion defect (15, 16). As NIDDM is a heterogenous disorder, the drawback of previous studies is that they have not classified study subjects according to the phenotype of NIDDM.

Since there are no studies that have compared early defects in glucose metabolism in the offspring of NIDDM patients with different phenotypes, we performed metabolic studies on offspring of NIDDM patients with deficient insulin secretion phenotype and on offspring of NIDDM patients with insulin resistant phenotype. We compared these two groups with respect to the occurrence of glucose intolerance, insulin secretory capacity and insulin resistance.

Methods

Subjects

The subjects for this study were offspring of patients with newly diagnosed NIDDM who were originally studied in 1979–1981. The formation and representativeness of the study population have been described earlier in detail (17–20). We have followed these patients for over 10 yr and performed repeated oral glucose tolerance tests (baseline, 5 and 10 yr). The index patients were subdivided into two groups

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1. *Abbreviations used in this paper:* BMI, body mass index; GAD, glutamic acid decarboxylase antibody; IR-group, offspring of the probands with insulin resistant phenotype; IS-group, offspring of the probands with deficient insulin secretion phenotype; IVGTT, intravenous glucose tolerance test; LBM, lean body mass; NIDDM, non–insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test.

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on the basis of fasting C-peptide value at the 10 yr follow-up study: (*a*) NIDDM patients with low fasting C-peptide level (< 450 pmol/liter) reflecting deficient insulin secretion capacity (12.1% of the whole diabetic study population), and (*b*) NIDDM patients with high fasting C-peptide level (> 880 pmol/liter) reflecting insulin resistance (9.1% of the whole diabetic study population). Probands with glutamic acid decarboxylase (GAD) (21) and/or islet cell antibody positivity (22) (11 patients altogether) were excluded. Additional exclusion criteria for the selection of the offspring were: (*a*) diabetes mellitus in both parents; (*b*) diabetes mellitus; (*c*) dyslipidemia (serum total triglycerides > 2.5 mmol/liter); (*d*) drug treatment or any disease that could potentially disturb carbohydrate metabolism; (*e*) pregnancy; (*f*) overt psychiatric disease; and (*g*) age under 30 or over 55 yr.

Offspring of the probands with low fasting C-peptide (< 450 pmol/liter) (IS-group). Altogether 35 randomly selected subjects from 40 eligible subjects were invited in the study. Seven subjects did not reply or refused to participate (participation rate 80%). Eight subjects had at least one of the exclusion criteria (2 diabetic subjects, 4 subjects with dyslipidemia, 1 subject with epilepsy, and 1 subject with pregnancy). Thus, the IS-group consisted of 20 subjects (15 women and 5 men) who were offspring of 11 probands. One to three subjects from each family were included. The mean age of the probands was 68.4 yr, their mean body mass index (BMI) was 28.1 kg/m^2 and their mean fasting C-peptide was 350 pmol/liter at the 10 yr follow-up.

Offspring of the probands with high fasting C-peptide (> 880 pmol/liter) (IR-group). Altogether 40 subjects randomly selected from 56 eligible subjects were invited to participate in the study. Six subjects did not reply or refused to participate (participation rate: 85%). 16 subjects had at least one of the exclusion criteria (10 subjects whose both parents had diabetes, one subject with diabetes, three subjects with dyslipidemia, one subject with schizophrenia, and one subject with pregnancy). Thus, the IR-group consisted of 18 subjects (11 women and 7 men) who were offspring of nine probands. One to three offspring from each family were included. The mean age of the probands was 65.7 yr, their mean BMI was 32.0 kg/m^2 and their mean fasting plasma C-peptide was 980 pmol/liter.

Control group. The control group was also recruited from the same follow-up study (17–20). Originally the control group consisted of 144 subjects from whom 53 subjects had repeatedly normal glucose tolerance according to the World Health Organization criteria (23) determined by an oral glucose tolerance test (OGTT) (baseline, 5 and 10 yr). From these 53 subjects, 2 subjects had GAD antibody positivity, 9 subjects had no offspring, and 10 subjects had a diabetic spouse. From the remaining 32 available subjects 10 subjects were randomly selected to serve as probands. These 10 probands had 28 children to whom a questionnaire was sent including questions on weight, height, drug treatment, chronic diseases, and the family history of diabetes and hypertension. The control subjects had to fulfill the following inclusion criteria: (*a*) age from 30 to 55 yr; (*b*) no diabetes; (*c*) first degree relatives without a history of diabetes; (*d*) no drug treatment nor any disease that could potentially disturb carbohydrate metabolism; (*e*) BMI within the range of mean±2 SD of the BMI of the IS- and IR-groups; and (*f*) no history of hypertension. Altogether, 17 subjects met the inclusion criteria. 3 of the 17 subjects had dyslipidemia in the examination and were therefore excluded. Thus, the control group consisted of 14 offspring (5 men and 9 women) of eight probands. One to three subjects from each family were examined. The mean age of the probands was 64.7 yr, their mean BMI was 26.0 kg/m^2 and their mean fasting plasma C-peptide was 480 pmol/liter.

Study protocol

The subjects were admitted to the metabolic ward of the Department of Medicine of the Kuopio University Hospital for 2 d. On the first day after 12 h fasting the bioelectric impedance measurement was performed, followed by an OGTT and the hyperglycemic clamp, respectively. On the second day an intravenous glucose tolerance test (IVGTT) followed by the hyperinsulinemic euglycemic glucose clamp test were performed. Within 1 mo after these examinations, a computed tomography of the abdominal fat and cardiopulmonary exercise test was performed.

The protocol was approved by the Ethics Committee of the University of Kuopio. Informed consent was given by all the subjects studied.

Oral glucose tolerance test

In a 2-h OGTT (75 g of glucose) samples for blood glucose, plasma insulin, and plasma C-peptide were drawn at 0, 30, 60, 90, and 120 min to evaluate the degree of glucose tolerance and the β cell response to the oral glucose load.

Hyperglycemic clamp

At 120 min immediately after the 2-h oral glucose tolerance test blood glucose was acutely increased to 20 mmol/liter by a constant glucose infusion and clamped at 20 mmol/liter until 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5 min intervals applying the hyperglycemic clamp technique. At 150, 165, and 180 min, samples were taken for the measurement of plasma insulin and C-peptide.

Intravenous glucose tolerance test

IVGTT was performed to determine the first phase insulin secretion capacity. At 8 A.M. after a 12-h overnight fast, an intravenous catheter was placed in the antecubital vein for the infusion of glucose. Another cannula for blood sampling was inserted into a wrist vein surrounded by a heated box (40°C). After baseline blood collection and measurement of gas exchange (see Indirect calorimetry, below), a bolus of glucose (300 mg/kg in a 50% solution) was given (within 30 s) into the antecubital vein to acutely increase the blood glucose level. Samples for the measurement of blood glucose and plasma insulin were drawn at −5, 0, 2, 4, 6, 8, and 10 min.

Euglycemic clamp

The degree of insulin resistance was evaluated with the euglycemic hyperinsulinemic clamp technique (24). After IVGTT, a priming dose of insulin infusion (Actrapid 100 IU/ml; Novo Nordisk, Gentofte, Denmark) was administrated during the initial 10 min to acutely raise plasma insulin to the desired level, where it was maintained by a continuous insulin infusion of a rate of 80 mU/m^2 body surface area per minute. Blood glucose was clamped at 5.0 mmol/liter for the next 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals (mean coefficient of variation of blood glucose was < 4% in both study groups and control group). The data were calculated for each 20-min interval; the mean value for the period from 120 to 180 min was used to calculate the rates of whole body glucose uptake. In fasting and at 120, 140, 160, and 180 min, samples for the measurement of plasma lactate, insulin, and serum free fatty acid were drawn.

Indirect calorimetry

Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (DELTATRAC®; TM Datex, Helsinki, Finland) as previously described (25) in the fasting state and in connection with the euglycemic clamp. This device has a precision of 2.5% for O_2 consumption and 1.0% for CO_2 production. On the day of the experiment, gas exchange (O_2 consumption and CO_2 production) was measured for 30 min after a 12-h fast before the euglycemic clamp and during the last 30 min of the euglycemic clamp. The values obtained during the first 10 min of both data set were discarded, and the mean value of the remaining 20-min data was used for calculation. Protein, glucose, and lipid oxidation were calculated according to Ferrannini (26). Protein oxidation was calculated on the basis of the urinary nonprotein nitrogen excretion rate before and during the clamp studies by multiplying this value by 6.25. The fraction of carbohydrate nonoxidation during the euglycemic clamp was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycemic clamp).

Body fat composition and fat distribution

Body composition was determined by bioelectrical impedance (RJL Systems, Detroit, MI) in the supine position after 12 h fast (27).

Abdominal fat distribution was evaluated by computed tomography (Somatom Plus S; Siemens AG, Munich, Germany) according to the method of Sjöström et al. (28). Briefly, the scanning was performed with 120 kV and the slide thickness was 10 mm. The subjects were examined in the supine position with their arms stretched above their heads. The fourth lumbal vertebra (L4) was mapped with a radiograph of the vertebral spine and one scan from that level was obtained. Total and intra-abdominal fat areas were calculated by delineating the area by graph pen and then computing the adipose tissue surfaces with an attenuation range of −30 to −190 HU (28, 29). Intra-abdominal fat area was calculated by drawing a line within the muscle wall delineating the abdominal cavity. Subcutaneous fat area was measured by subtracting the amount of intra-abdominal fat from the total fat area. The radiologist (S. Kainulainen) evaluated the amount of intra-abdominal and abdominal fat blindly without the knowledge of the group which the subjects belonged to.

Cardiopulmonary exercise test

Cardiopulmonary exercise test was performed with a bicycle ergometer (Siemens-Elema AB, Solna, Sweden) in the upright position until exhaustion (30). The initial workload was 20 W with subsequent increments of 20 W per minute. During the test, a 12-lead ECG, blood pressure, heart rate, subjective symptoms, and perceived exertion were recorded. Respiratory gas exchange was analyzed continuously during the exercise test with a computer based system (Metabolic Measurement Cart/System; Sensor Medix 2900, Yorba Linda, CA). The average values of oxygen uptake measured during the last 30 s of exercise were used for maximum attainable oxygen uptake (VO_2 max).

Assays and calculations

Blood glucose and plasma lactate in the fasting state, during clamp studies, and blood glucose during the oral glucose tolerance test were measured by glucose and lactate oxidase methods, respectively (Glucose & Lactate Analyzer 2300 Stat Plus; Yellow Springs Instrument Co., Inc., Yellow Springs, OH). For the determination of plasma insulin and C-peptide, blood was collected into EDTA tubes. After centrifugation, the plasma for the determinations of insulin and C-peptide was stored at −20°C until the analysis. Plasma insulin and C-peptide were determined by RIA (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden) and C-peptide of insulin by 125J RIA kit (INCSTAR Corp., Stillwater, MN). Insulin assay also detects proinsulin and proinsulin conversion products with sensitivity of 47%. Serum lipid and lipoprotein concentrations were determined from fresh serum samples drawn after a 12-h overnight fast. Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation (31) as described previously (32). Cholesterol and triglyceride levels from whole serum and from lipoprotein fractions were assayed by automated enzymatic methods (Boehringer Mannheim, Mannheim, Germany). Serum free fatty acids were determined by an enzymatic method from Wako Chemicals GmbH (Neuss, Germany). Nonprotein urinary nitrogen was measured by an automated Kjeldahl method (33).

The incremental insulin and glucose areas under the curve were calculated by the trapezoidal method. The maximal insulin and C-peptide secretion during the hyperglycemic clamp study were calculated as mean insulin and C-peptide values measured at 150, 165, and 180 min. The acute insulin response was calculated as a ratio of the increment of plasma insulin (pmol per liter) to that of blood glucose (mmol per liter) 30 min after the oral glucose load or during the 10 min of IVGTT. Plasma C-peptide/plasma insulin ratio was calculated as an index of the hepatic insulin extraction in the fasting state and during the hyperglycemic clamp (mean value of three measurements during the last 30 min of the clamp).

Statistical analysis

All calculations were performed with the SPSS for Windows program (SPSS Inc., Chicago, IL). Data are shown as mean±SEM. The differences among the three groups were tested by the one way ANOVA for continuous variables and by Mantel-Henzel's test for dichotomized variables. Only in case where P value was < 0.05 the two groups were compared. The differences between the two groups were analyzed by the Student's t test for unpaired samples or by the χ^2 test when appropriate. The analysis of covariance (ANCOVA) was used to adjust for confounding variables. Plasma insulin, C-peptide, and serum triglyceride levels were analyzed after logarithmic transformation.

Table I. Clinical and Biochemical Characteristics of the Study Subjects

	Controls	IS-group	IR-group	P value ANOVA
	n = 14	*n = 20*	*n = 18*	
Age (yr)	40.1±1.5	41.3±1.4	40.5±0.9	NS
Gender (M/F)	5/9	5/15	7/11	NS
BMI (kg/m^2)	25.0±1.0	24.6±0.5	28.8±0.9*‡	< 0.001
Total fat mass (kg)	19.0±2.6	20.5±1.6	26.4±2.1§‖	< 0.05
LBM (kg)	52.0±2.7	49.1±2.6	58.1±3.0	NS
Antihypertensive drug				
Treatment (percent)	0	15.0	22.2	NS
Total cholesterol (mmol/liter)	5.04±0.20	5.58±0.22	5.64±0.14	NS
HDL-cholesterol (mmol/liter)	1.41±0.09	1.56±0.07	1.34±0.08	NS
Total triglycerides (mmol/liter)	1.10±0.12	0.98±0.09	1.04±0.08	NS
Fasting blood glucose (mmol/liter)	4.5±0.1	4.5±0.1	4.7±0.1	NS
Fasting insulin (pmol/liter)	48.6±4.8	49.2±3.6	72.6±7.2§¶	< 0.01
Fasting C-peptide (pmol/liter)	470±30	500±40	640±60§	< 0.05
VO_2max (ml/LBM/min)	47.1±2.6	47.9±2.9	41.9±1.6	NS

The results are given as means±SEM or n (percent). *$P < 0.01$ versus controls. ‡$P < 0.001$ versus IS-group. §$P < 0.05$ versus controls. ‖$P < 0.05$ versus IS-group. ¶$P < 0.01$ versus IS-group. IS-group = offspring of diabetic probands with deficient insulin secretion phenotype; IR-group = offspring of diabetic probands with insulin resistant phenotype.

Results

Clinical and biochemical characteristics of the study groups

Table I shows the clinical and biochemical characteristics of the study groups. The groups were comparable with respect to age and gender. The offspring with the insulin resistant phenotype (IR-group) had higher BMI and total fat mass (TFM) than the control group ($P < 0.01$ and $P < 0.05$, respectively) or the offspring with the deficient insulin secretion phenotype (IS-group) ($P < 0.001$ and $P < 0.05$, respectively). Lean body mass (LBM), antihypertensive drug treatment, serum total cholesterol, HDL-cholesterol, total triglycerides, fasting blood glucose, and VO_2 max did not differ among the study groups. The IR-group had higher fasting plasma insulin as compared with that of the control group ($P < 0.05$) or the IS-group ($P < 0.01$). In addition, the IR-group had higher plasma fasting C-peptide than the control group ($P < 0.05$).

Glucose tolerance

Impaired glucose tolerance was frequent in both study groups (IS-group: 45.0% and IR-group: 50.0%; P = NS), whereas there were no subjects with impaired glucose tolerance in the control group.

Fig. 1 depicts the glucose response in an OGTT. Fasting blood glucose levels were comparable among the groups (control group: 4.5±0.1 mmol/liter; IS-group: 4.5±.1 mmol/liter; IR-group: 4.7±0.1 mmol/liter). The blood glucose levels were higher in the IR-group compared with those in the control group at all time points measured after the oral glucose load. In addition, the IS-group had higher blood glucose levels than the control group at 60, 90, and 120 min. Oral glucose response, when expressed as an incremental glucose area under the curve differed among the three groups ($P < 0.01$) and was significantly higher in IS- and IR-groups compared with that in the control group (IS-group: 4.50±0.50 mmol/liter·hours, IR-group: 5.06±0.50 mmol/liter·hour and control group: 2.68± 0.41 mmol/liter·hour; $P < 0.05$ and $P < 0.001$, respectively).

Fig. 2 shows the plasma insulin response in the OGTT. The IR-group had higher plasma insulin levels at all time points compared with those in the control group and the IS-group. In addition, the IS-group had higher plasma insulin level than the control group at 120 min ($P < 0.05$). The plasma insulin response expressed as an incremental insulin area under the curve (IIAUC) was higher in the IR-group (624.6±57.0 pmol/liter·hour) than in the control group (318.6±41.4 pmol/liter·hour; $P < 0.001$). The difference, however, lost its statistical significance after adjustment for BMI. The IIAUC of the IS-group was 446.2±90.0 pmol/liter·hour. The IS-group had lower insulin/glucose response (AIR) at 30 min during the OGTT (85.2±10.0 pmol insulin per mmol glucose) than the control group (136.4±23.1 pmol insulin per mmol glucose, $P < 0.05$) and the IR-group (115.6±11.8 pmol insulin per mmol glucose, $P = 0.05$). The AIR of the IR-IGT subgroup was significantly lower than that of the IR-NGT subgroup (93.0±11.4 pmol insulin per mmol glucose and 138.0±18.6 pmol insulin per mmol glucose, respectively; $P < 0.05$) but did not significantly differ from that of the control group. The IR-group had higher plasma C-peptide in the OGTT at 90 and 120 min

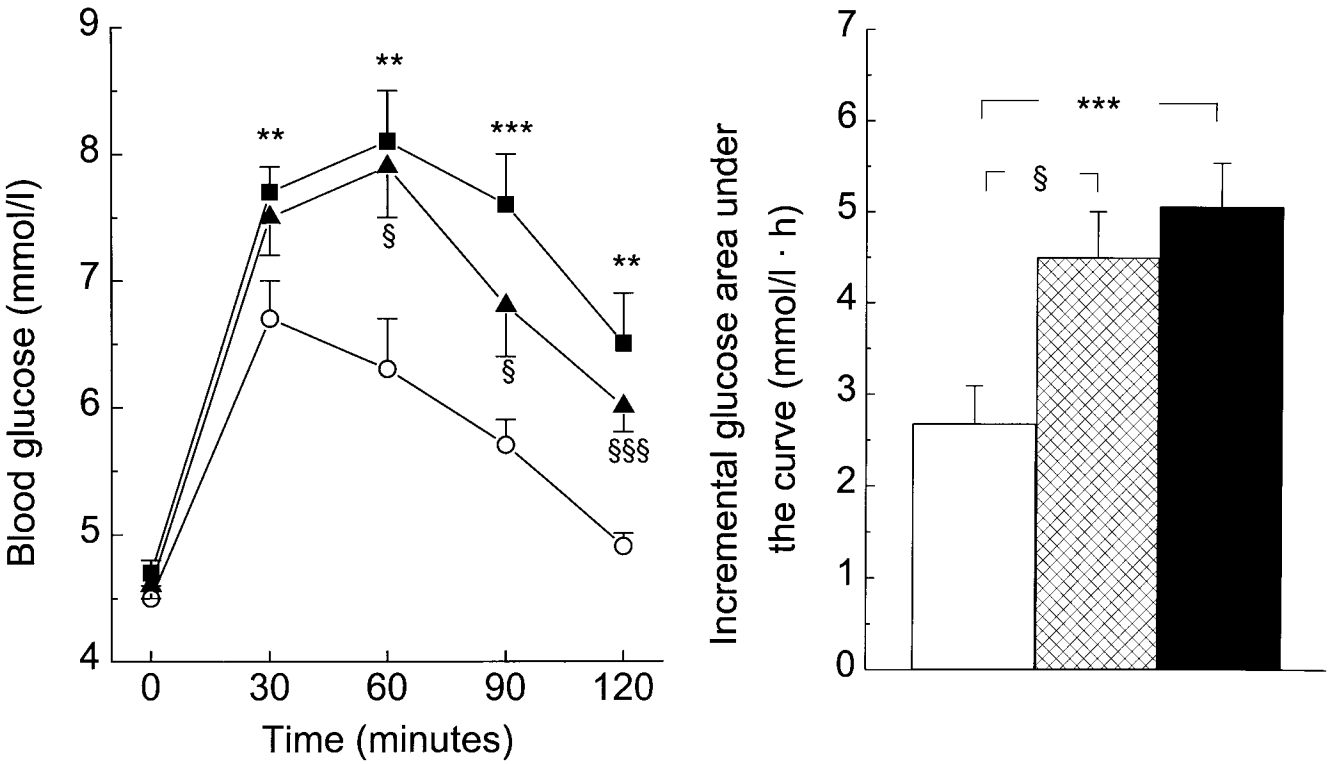

Figure 1. Line plots show the blood glucose concentrations during the OGTT: the control group (*open circles*); the IS-group (*black triangles*); the IR-group (*black squares*). Bar graphs present the incremental blood glucose area under the curve: the control group (*white bar*); the IS-group (*hatched bar*); the IR-group (*black bar*). $^{**}P < 0.01$; $^{***}P < 0.001$ (IR-group versus control group); $^{\S}P < 0.05$; $^{\S\S\S}P < 0.001$ (IS-group versus control group).

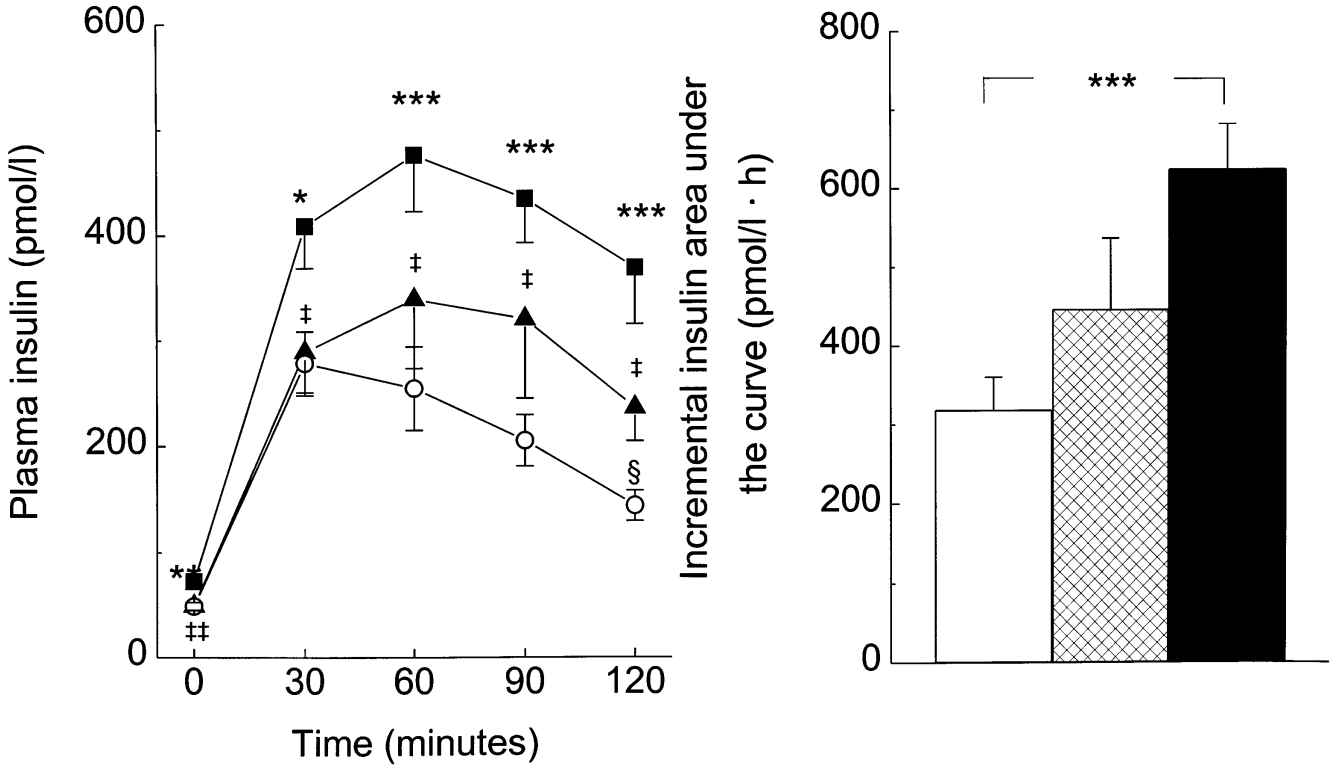

Figure 2. Line plots show the plasma insulin concentrations during the OGTT: the control group (*open circles*); the IS-group (*black triangles*); the IR-group (*black squares*). Bar graphs present the incremental plasma insulin area under the curve: the control group (*white bar*); the IS-group (*hatched bar*); the IR-group (*black bar)*.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (IR-group versus control group); ‡$P < 0.05$ (IR-group versus IS-group); §$P < 0.05$ (IS-group versus control group).

(2547.2±158.0 pmol/liter and 2255.0±178.0 pmol/liter , respectively) than the control group (1800±130 pmol/liter and 1560±90 pmol/liter; $P < 0.01$ and $P < 0.01$, respectively). Moreover, the IS-group had higher plasma C-peptide at 120 min (1997.0 ±178.0 pmol/liter) than the control group ($P < 0.05$). The incremental C-peptide area under the curve was higher in the IR-group (2835.8±158.0 pmol/liter·hour) than in the control group (2237.1±183.0 pmol/liter·hour, $P < 0.05$). The respective area under the curve for the IS-group was 2638.4±270.0 pmol/liter·hour. The hepatic insulin extraction (pmol C-peptide/pmol insulin) in the fasting state tended to be lower in the IR-group (9.02±0.31) than in the control group (10.30±0.83, $P = 0.13$) or in the IS-group (10.32±0.50, $P < 0.05$).

Insulin secretion

First phase insulin secretion. Fig. 3 shows the acute plasma insulin response in an IVGTT. The IR-group had higher fasting plasma insulin level (78.6±11.4 pmol/liter) than the control group (48.0±4.2 pmol/liter; $P < 0.01$) and the IS-group (51.6±4.2 pmol/liter; $P < 0.05$). Plasma insulin levels at 2 min were comparable among the groups but the IR-group had higher plasma insulin levels at 4, 6, 8, and 10 min than the IS-group. The IS-group had the lowest insulin levels at each time point. The incremental insulin area under the curve was higher in the IR-group (4023.0±586.2 pmol/liter·minute) than in the IS-group (2530.8±347.4 pmol/liter·minute; $P < 0.05$), but did not differ from that of the control group was (3445.2±637.8 pmol/liter·minute). The difference between the IR and IS-group disappeared after adjustment for BMI. The incremental glucose area under the curve was higher in the IR-group (84.6±2.3 mmol/liter·minute) compared with that of the control group (73.1±2.8 mmol/liter·minute, $P < 0.01$) and the IS-group (76.1±1.6 mmol/liter·minute, $P < 0.01$). The acute insulin response divided by the acute glucose response tended to be lower in the IS-group (33.0±4.3 pmol insulin per mmol glucose) than in the control group (46.5±7.6 pmol insulin per mmol glucose, $P = 0.11$) and in the IR-group (46.6±6.3 pmol insulin per mmol glucose, $P = 0.08$).

Maximal insulin secretion. Fig. 4 illustrates the maximal plasma insulin and C-peptide secretion response in the hyperglycemic clamp. The mean blood glucose level during the hyperglycemic clamp for the period 160–180 min were similar among the groups: 21.2±0.1 mmol/liter for the control group, 21.3± 0.2 mmol/liter for the IS-group and 21.4±0.2 mmol/liter for the IR-group. The IR-group had higher maximal plasma insulin secretion (1541.4±297.0 pmol/liter) than the control group (838.8±143.4 pmol/liter; $P < 0.05$) and the IS-group (832.8±130.2 pmol/liter; $P < 0.01$). These differences, however, disappeared after adjustment for BMI. The maximal plasma C-peptide secretion was similar among the groups (control group: 3829.9±270.0 pmol/liter; IS-group: 4006.2± 315.0 pmol/liter; IR-group: 4625.2±392.0 pmol/liter). The hepatic insulin extraction was lower in the IR-group (3.90±0.39) than in the control group (5.80 ±0.88, $P < 0.05$) and in the IS-group (5.57±0.35, $P < 0.01$).

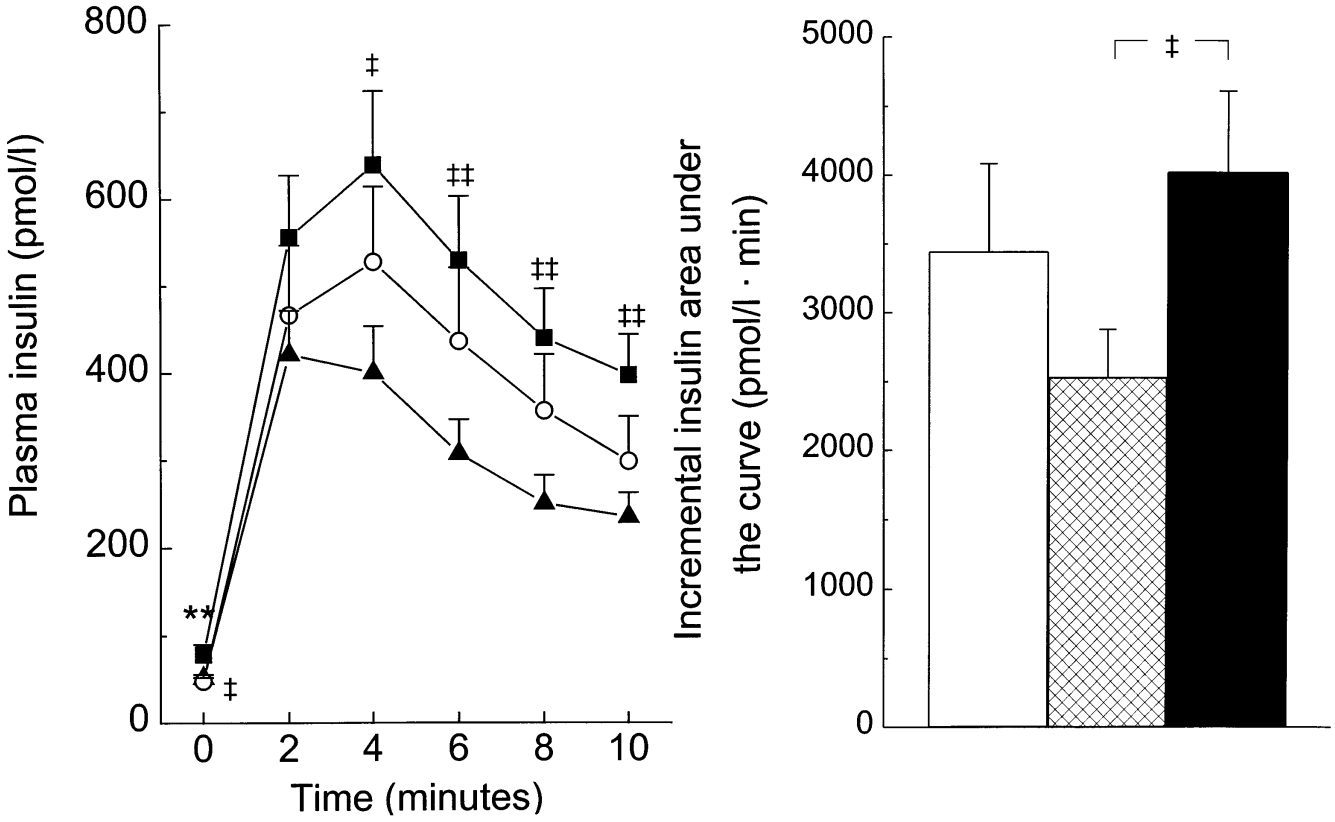

Figure 3. Line plots show the plasma insulin concentration during the IVGTT: the control group (*open circles*); the IS-group (*black triangles*); the IR-group (*black squares*). Bar graphs present the incremental plasma insulin area under the curve: the control group (*white bar*); the IS-group (*hatched bar*); the IR-group (*black bar*). $^{**}P < 0.01$ (IR-group versus control group). $^{‡}P < 0.05$; $^{‡‡}P < 0.01$ (IR-group versus IS-group).

Insulin sensitivity

The steady-state insulin levels during the euglycemic clamp were higher in the IR-group (1093.2±73.8 pmol/liter) than in the control group (889.8±45.0 pmol/liter; $P < 0.05$) or in the IS-group (878.4±34.8 pmol/liter; $P < 0.01$). In spite of that the IR-group had lower rates of whole body glucose uptake (60.1±4.6 μmol/LBM/min) than did the control group (84.2±5.0 μmol/LBM/min; $P < 0.001$) or the IS-group (82.6±5.9 μmol/LBM/min; $P < 0.01$) (Fig. 5). The lower rates of whole body glucose uptake in the IR-group were mainly accounted for by lower glucose nonoxidation (34.2±4.1 μmol/LBM/min for the IR-group; 55.5±3.7 μmol/LBM/min for the control group; and 48.3±4.3 μmol/LBM/min for the IS-group; $P < 0.001$ and $P < 0.05$, respectively). Moreover, glucose oxidation was lower in the IR-group (25.9±1.5 μmol/LBM/min) than in the IS-group (34.4±2.6 μmol/LBM/min; $P < 0.01$). The respective glucose oxidation for the control group was 28.7±1.6 μmol/LBM/min. The differences in the rates of whole body glucose uptake persisted after the adjustment for age, gender and total fat mass (ANCOVA: $F = 6.16$; $P < 0.01$) or for age, gender and abdominal subcutaneous fat (ANCOVA: $F = 2.77$; $P < 0.01$) or for age, gender and intra-abdominal fat (ANCOVA:

Table II. Glucose Oxidation, Lipid Oxidation, and Energy Expenditure in the Fasting State and Fasting Serum Free Fatty Acid and Plasma Lactate Levels of the Study Subjects

	Controls	IS-group	IR-group	*P* value (ANOVA)
Glucose oxidation (μmol/LBM/min)	10.7±1.4	8.9±0.8	9.7±1.1	NS
Lipid oxidation (mg/LBM/min)	1.02±0.11	1.50±0.10‡§	1.17±0.12	< 0.05
Energy expenditure (kcal/LBM)	29.3±0.8	33.6±1.5*‡	29.3±0.9	< 0.05
Fasting serum free fatty acids (mmol/liter)	0.50±0.06	0.56±0.06	0.55±0.06	NS
Fasting plasma lactate (mmol/liter)	0.91±0.09	0.90±0.07	1.08±0.07	NS

The results are given as means±SEM or *n* (percent). $^{*}P < 0.05$ versus control group. $^{‡}P < 0.05$ versus IR-group. $^{§}P < 0.01$ versus control group. IS-group = offspring of diabetic probands with deficient insulin secretion phenotype; IR-group = offspring of diabetic probands with insulin resistant phenotype.

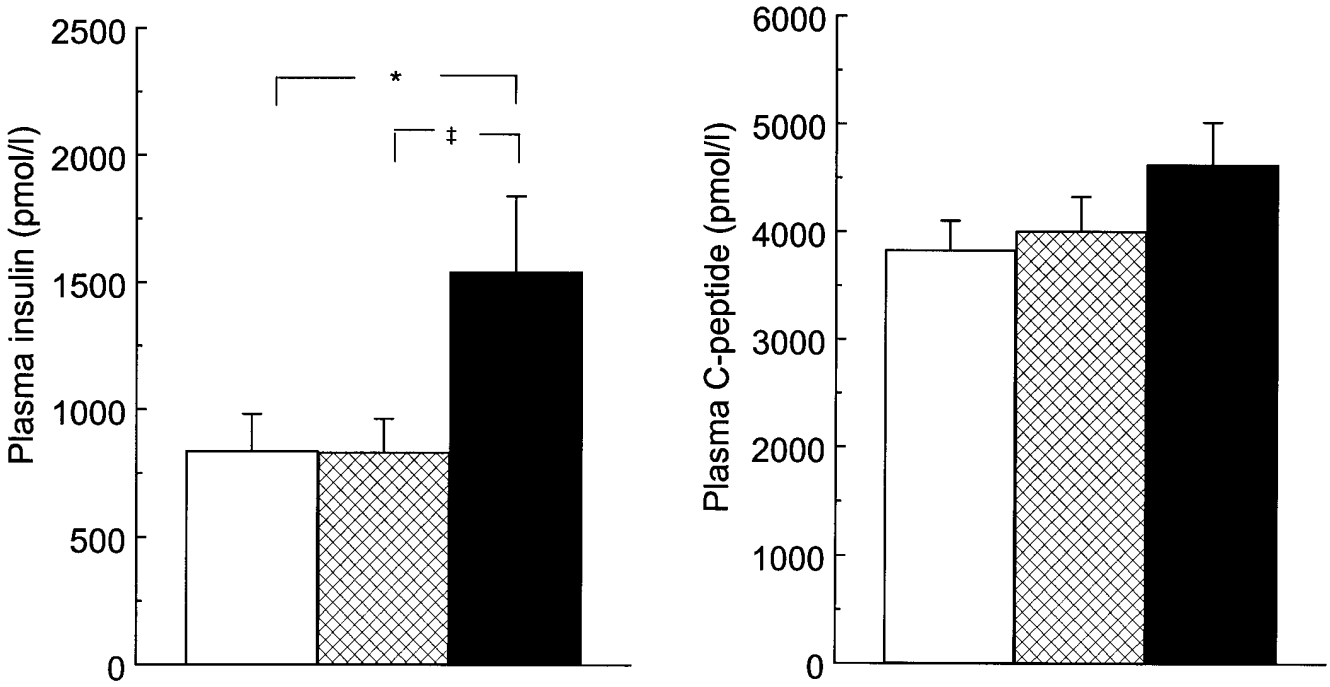

Figure 4. Bar graphs present the mean plasma insulin and C-peptide levels during the hyperglycemic clamp study: the control group (*open bar*); the IS-group (*hatched bar*); the IR-group (*black bar*). *$P < 0.05$ (IR-group versus control group); ‡$P < 0.05$ (IR-group versus IS-group).

F = 3.41; $P < 0.05$). In the fasting state glucose oxidation was similar in the study groups (Table II).

Energy expenditure, lipid oxidation, serum free fatty acid, and plasma lactate levels

The IS-group had higher energy expenditure expressed as kilocalories per LBM in the fasting state than the control group ($P < 0.05$) or the IR-group ($P < 0.05$). Similarly, during the euglycemic clamp, the IS-group had higher energy expenditure (38.2±2.1 kcal/LBM) than the IR-group (33.1±1.0 kcal/LBM; $P < 0.05$) but energy expenditure was not significantly different from that in the control group (33.5±1.2 kcal/LBM). The IS-group had higher lipid oxidation expressed as milligrams of LBM per minute in the fasting state than the control group ($P < 0.01$) or the IR-group ($P < 0.05$) (Table II). During the euglycemic clamp, lipid oxidation was less fully suppressed in the IR-group (0.36 ±0.07 mg/LBM/min) than in the control group (0.16±0.06 mg/LBM/min; $P < 0.05$) or in the IS-group (0.10±0.07 mg/LBM/min; $P < 0.05$) (Fig. 6). Serum free fatty acid levels and plasma lactate levels were similar among the groups in the fasting state (Table II) and during the euglycemic clamp (control group: 0.06±0.01 mmol/liter; IS-group: 0.03±0.01 mmol/liter and IR-group: 0.06±0.01 mmol/liter) (Fig. 6). Plasma lactate concentrations did not differ between the groups in the fasting state (Table II) and during the eugly-

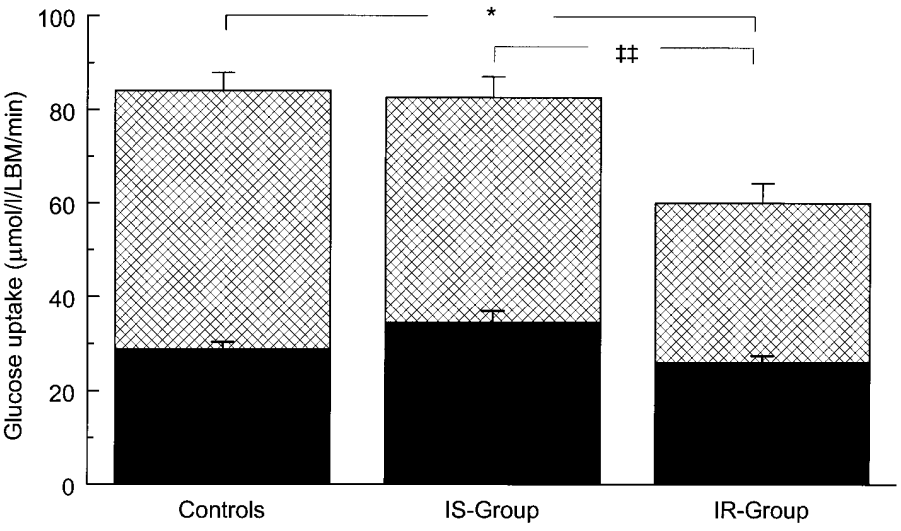

Figure 5. Bar graphs show the mean glucose uptake, glucose oxidation (*hatched sections*) and nonoxidation (*black sections*) expressed as mmol per kg LBM per minute during the euglycemic clamp study. * $P <$ 0.001 (IR-group versus control group); ‡‡$P < 0.01$ (IR-group versus IS-group).

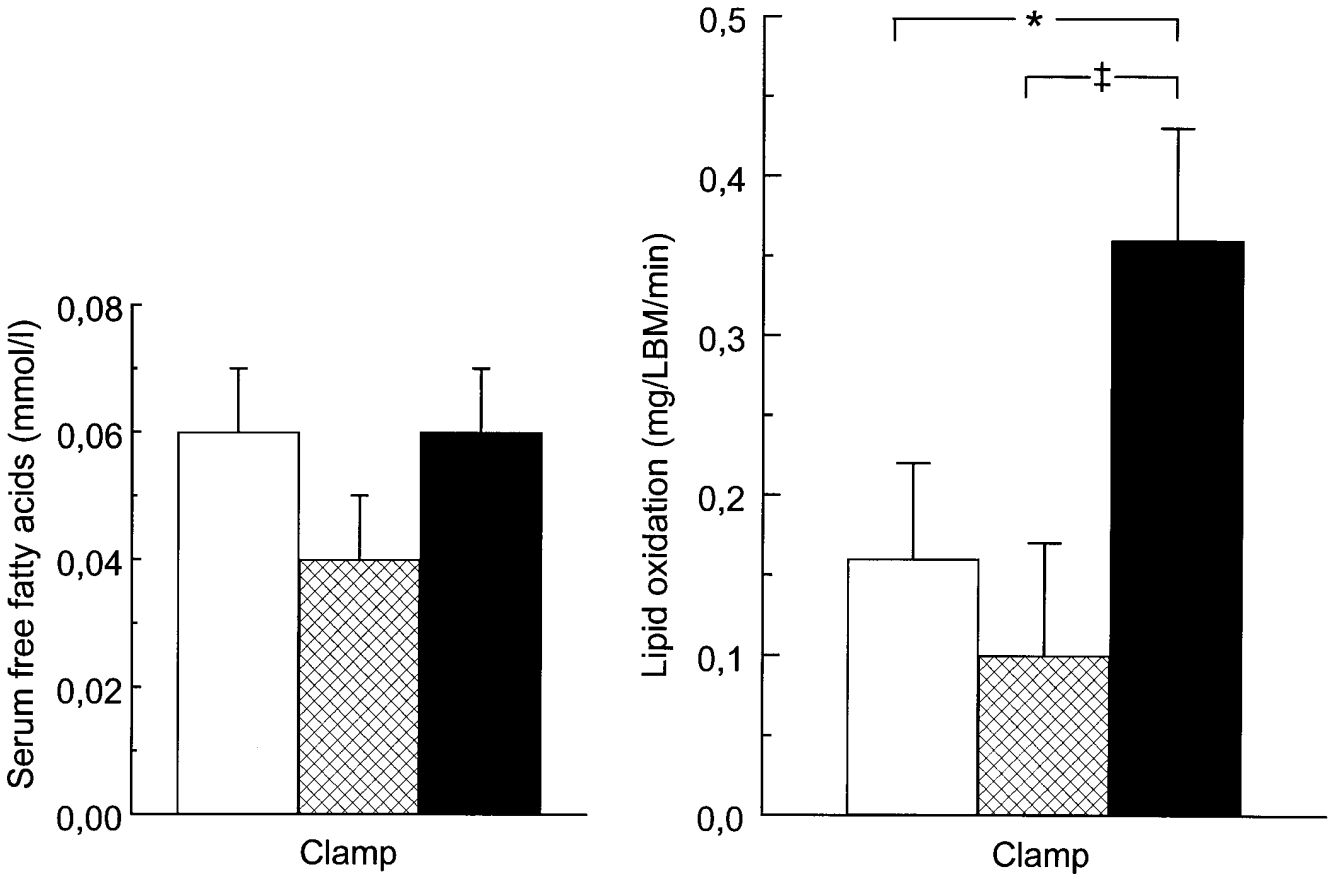

Figure 6. Bar graphs show the serum free fatty acid levels and the lipid oxidation expressed as mg per kg LBM per minute during the euglycemic clamp. The control group (*white bar*); the IS-group (*hatched bar*); the IR-group (*black bar*). $^{*}P < 0.05$ (IR-group versus control group); $^{‡}P < 0.01$ (IR-group versus IS-group).

cemic clamp (control group: 1.36±0.07 mmol/liter; IS-group: 1.27±0.07 mmol/liter; IR-group: 1.16±0.07 mmol/liter; $P <$ NS between the groups).

Intra-abdominal and subcutaneous abdominal fat

The IR-group had higher intra-abdominal fat area (120 ±16 cm^2) than did the control group (74±12 cm^2; $P < 0.05$) or the IS-group (84±12 cm^2; $P = 0.058$) (Fig. 7). This difference between the IR-group and the control group disappeared after adjustment for total fat mass. Furthermore, the IR-group had higher abdominal subcutaneous fat area (303±25 cm^2) than the control group (214±37 cm^2; $P < 0.05$) and the IS-group (215±15 cm^2; $P < 0.01$). These differences, however, disappeared after adjustment for total fat mass.

Discussion

The novel finding of our study was that defects in insulin secretion and insulin action in NIDDM seem to be inherited since the phenotype of probands with NIDDM was related to the defects in glucose metabolism in their nondiabetic offspring. The offspring of probands with deficient insulin secretion phenotype of NIDDM had impaired insulin secretion capacity, but normal insulin action, whereas the offspring of probands with insulin resistant phenotype of NIDDM had impaired insulin action but quite normal insulin secretion capacity. This verifies the genetic heterogeneity of NIDDM and emphasizes the importance of clinical characterization of the phenotype of NIDDM when trying to search metabolic and genetic defects predisposing to NIDDM.

Several studies suggest that defects in insulin secretion capacity could be the primary defect in glucose metabolism in NIDDM (8–14). Indeed, in our study the nondiabetic offspring of probands with deficient insulin secretion phenotype of NIDDM had impairment in their insulin secretion capacity in OGTT and IVGTT. This defect was most clearly seen in insulin response measured by the ratio of the increment of plasma insulin to that of plasma glucose at 30 min after an oral glucose load. In addition, the offspring of the diabetic probands with deficient insulin secretion phenotype had significantly lower first phase insulin response to intravenous glucose than the offspring of diabetic probands with insulin resistant phenotype but no statistically significant difference was observed compared with that in control subjects. Furthermore, the glucose-intolerant offspring of NIDDM probands with insulin resistant phenotype had an impaired acute insulin response to oral glucose, whereas the glucose tolerant subjects in the same group had normal response. This suggests that the functional β cell defect appears very early also in this subgroup as shown in previous studies (8, 9, 34–36) and could be due to a mild hyperglycemia. Impaired first phase insulin secretion could lead to more severe defect in insulin secretion because of deleterious

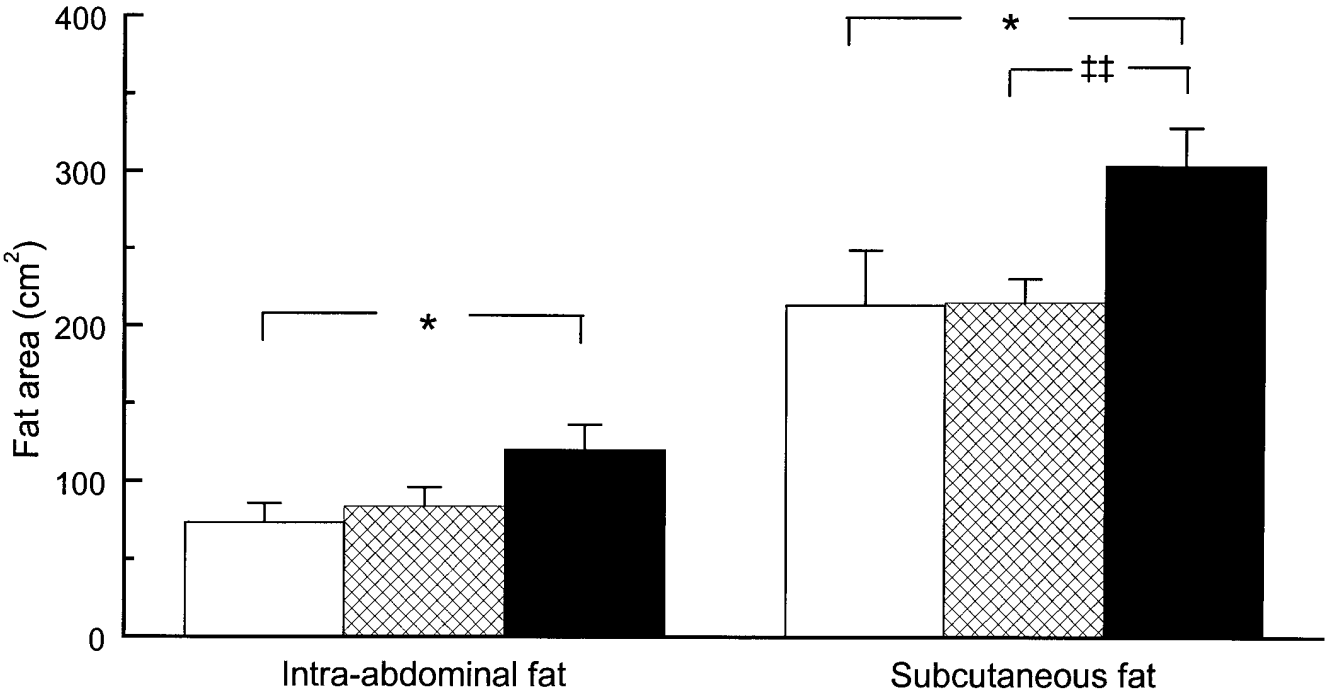

Figure 7. Bar graphs present the fat distribution pattern: the control group (*white bar*); the IS-group (*hatched bar);* the IR-group (*black bar*). $^*P < 0.05$ (IR-group versus control group); $^{‡‡}P < 0.01$ (IR-group versus IS-group).

effects of hyperglycemia per se. Indeed, hyperglycemia inhibits the first phase insulin secretion in humans (37) and in animal models (38).

A reduction of the pancreatic β cell mass has been suggested previously to be of etiological significance for the development of NIDDM (39, 40). Indeed, NIDDM has been associated with reduced maximal insulin secretion capacity (41, 42). In this study the offspring of probands with deficient insulin secretion phenotype as well as the offspring of probands with insulin resistant phenotype had no impairment in maximal insulin secretion capacity compared with that in the control group evaluated by C-peptide levels in hyperglycemic clamp (Fig. 4). This finding does not support the hypothesis that reduced β cell mass is an early defect in the development of NIDDM.

Impaired insulin action has been suggested to be the primary defect in glucose metabolism in NIDDM in several studies (8–14). In this study we found that the nondiabetic offspring of diabetic probands with insulin resistant phenotype had impaired insulin action (Fig. 5). Because skeletal muscle is mainly responsible for glucose uptake during hyperinsulinemia the primary defect in insulin action is likely to be located in skeletal muscle. This impairment was accounted mainly for by decreased rates of glucose nonoxidation, a phenomenon that has been documented in previous studies (8, 9). The defect in glucose nonoxidation is likely to be due to glycogen storage because no difference in anaerobic glycolysis (lactate levels) were found between the groups. The IR-group exhibited also an impairment in insulin's effect to suppress lipid oxidation during hyperinsulinemia (Fig. 6), which is probably one of the causes for reduced rates of whole body glucose uptake.

Obesity and particularly visceral obesity are strongly associated with insulin resistance (43, 44). The causal relationship between these two in NIDDM has, however, remained controversial (45–47). In this study the offspring of diabetic parents with insulin resistant phenotype were more obese than the control subjects. This suggests that insulin resistance in these subjects could be at least in part due to overall (43) or visceral obesity (44). However, fatness is unlikely to be the sole explanation for insulin resistance in the offspring of probands with insulin resistant phenotype since the adjustment for the amount of total or abdominal (either subcutaneous or intra-abdominal) fat mass did not abolish the differences in the rates of whole body glucose uptake between the insulin resistant group and the control group. Moreover, although the offspring of probands with insulin resistant phenotype had more visceral fat than the control group or the offspring of probands with insulin deficient phenotype, this could be accounted for by a higher amount of total fat mass. This is in accordance with recent studies by Abate et al. (48, 49). This study suggests that visceral fat and associated metabolic alterations can not completely explain the impairment in insulin action in the offspring of NIDDM probands with insulin resistant phenotype.

Recently, the physical work capacity has been shown to be associated to insulin resistance in healthy first degree relatives of NIDDM patients (50), although also contradictory results have been reported (51). Furthermore, Lillioja et al. (52) showed an association between insulin action and muscle fiber composition, capillary density, and VO2 max. Whether these associations are primarily due to genetic factors affecting muscle fiber composition or capillary density, or alternately due to secondary environmental factors, such as hyperinsulinemia or diminished physical activity is, however, still unknown. In this study, however, the physical work capacity was not clearly associated with insulin resistance. Therefore, it could not explain

the differences in the rates of whole body glucose uptake between the groups.

We have shown in this study that the phenotype of NIDDM is inherited. Since the frequency of IGT in offspring of diabetic parents was remarkably high ($\sim$ 50%) this strongly suggests that defects either in insulin secretion or in insulin action separately could lead to NIDDM. These findings have important implications for genetic studies. Low insulin response to oral glucose has been suggested to have a genetic basis (53, 54). Although the reduced β cell mass in our study did not explain low insulin response in the offspring of probands with insulin deficient phenotype, other mechanisms, however, could be involved. The β cell could have a defect in glucose-sensing (55) meaning that the β cell is incapable to respond adequately to prevailing glucose level. The specific defect could lie for example in the β cell ATP-sensitive K-channel or in its subunit, sulfonylurea receptor (56), or in hepatocyte nuclear factor-1α (57), or in hepatocyte nuclear factor-4α (58).

We demonstrated impaired insulin action in the offspring of probands with insulin resistant phenotype. More accurately, since in this group only glucose nonoxidation was impaired, it is likely that the defect lies in metabolic pathway of glucose storage. Previously, this defect has been suggested to be related to impaired activation of glycogen synthase (59) and this impairment could in turn to be due to some defect in signaling pathway, which eventually leads to the activation of glycogen synthase, e.g., in glycogen synthase phosphatase (PP-1) (60) or in ras-related protein (Rad) (61). Whether or not a defect in insulin action alone can lead to NIDDM can not be solved by this study. However, it is important to note that glucose intolerant offspring of probands with insulin resistant phenotype tended to have a simultaneous defect in insulin secretion which might indicate that in this phenotype the defect in both insulin secretion and insulin action are needed for the development of NIDDM.

In conclusion, both insulin secretion and impaired insulin action seem to be inherited and represent primary defects in glucose metabolism in the offspring of NIDDM probands. When studying metabolic and genetic features of this common disease, the phenotype characterization of NIDDM has to be done in future studies more carefully than it has until this time.

Acknowledgments

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