

# Glucose Turnover and Adipose Tissue Lipolysis Are Insulin-Resistant in Healthy Relatives of Type 2 Diabetes Patients

## Is Cellular Insulin Resistance a Secondary Phenomenon?

Jan W. Eriksson, Ulf Smith, Finn Waagstein, Marian Wysocki, and Per-Anders Jansson

To elucidate potential mechanisms for insulin resistance occurring early in the development of type 2 diabetes, we studied 10 young healthy individuals, each with two first-degree relatives with type 2 diabetes, and 10 control subjects without known type 2 diabetic relatives. They were pairwise matched for age ( $35 \pm 1$  vs.  $35 \pm 1$  years), BMI ( $23.6 \pm 0.6$  vs.  $23.1 \pm 0.4$  kg/m<sup>2</sup>), and sex (four men, six women). Glucose turnover was assessed during a euglycemic clamp at two insulin levels (low ~20 mU/l; high ~90 mU/l), and abdominal subcutaneous adipose tissue (SAT) lipolysis and blood flow were concomitantly studied with microdialysis and <sup>133</sup>Xe clearance. HbA<sub>1c</sub> was higher in patients with type 2 diabetic relatives than in control subjects ( $4.8 \pm 0.1$  vs.  $4.5 \pm 0.1\%$ ,  $P < 0.02$ ), but fasting glucose, insulin, and C-peptide levels were similar. During the clamp, the insulin sensitivity index for glucose disposal was lower ( $P < 0.03$ ) in relatives than in control subjects (low  $12.0 \pm 1.6$  vs.  $18.1 \pm 1.4$ ; high  $9.4 \pm 0.8$  vs.  $12.9 \pm 0.6$  [ $100 \cdot \text{mg} \cdot \text{l} \cdot \text{kg}^{-1} \cdot \text{mU}^{-1} \cdot \text{min}^{-1}$ ]). This difference was partially attributed to slightly higher clamp insulin levels in the relatives ( $P < 0.03$ ), suggesting an impaired rate for insulin clearance. SAT lipolysis measured as *in situ* glycerol release did not differ under basal conditions ( $2.0 \pm 0.2$  vs.  $2.1 \pm 0.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), but the suppression during the insulin infusion was less marked in relatives than in control subjects (glycerol release: low  $0.92 \pm 0.09$  vs.  $0.68 \pm 0.16$ ; high  $0.71 \pm 0.10$  vs.  $0.34 \pm 0.10$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.03$ ). Plasma nonesterified fatty acids also tended to be higher in relatives than in control subjects during the insulin infusion (NS). In contrast, *in vitro* experiments with isolated

subcutaneous adipocytes displayed similar effects of insulin in relatives and control subjects with respect to both glucose uptake and antilipolysis. In conclusion, insulin action *in vivo* on both lipolysis and glucose uptake is impaired early in the development of type 2 diabetes. Since this impairment was not found in isolated adipocytes, it may be suggested that neural or hormonal perturbations precede cellular insulin resistance in type 2 diabetes. *Diabetes* 48:1572–1578, 1999

The mechanisms causing type 2 diabetes are to a great extent unknown. It is established that resistance to insulin's effects on glucose turnover occurs early in the development of the disease and can predict the risk of future type 2 diabetes (1,2). To elucidate the early perturbations involved in the pathogenesis of type 2 diabetes, several studies have focused on young, healthy first-degree relatives of type 2 diabetic patients, who have a marked risk of developing type 2 diabetes later in life. With this approach, it has been demonstrated that insulin-resistant glucose uptake and intracellular glucose metabolism (such as glucose phosphorylation and glycogen synthesis) as well as elevated nonesterified fatty acid (NEFA) levels are early hallmarks of the insulin resistance that precedes frank type 2 diabetes (2–5). However, the primary mechanisms leading to these metabolic alterations are still unknown, although some authors have suggested the involvement of candidate genes governing cellular insulin action, such as glycogen synthase (2,3,6,7). Neuroendocrine alterations could be involved, and an enhanced activity in the hypothalamus-pituitary-adrenal (HPA) system, for example, as well as in the sympathetic nervous system can produce insulin resistance (8–11). Type 2 diabetes is often associated with hypertension and lipid abnormalities such as elevated triglyceride and NEFA levels, and these conditions often cluster in the so-called insulin resistance syndrome (12,13). In this study, to assess insulin action on adipose tissue lipid mobilization, we used subcutaneous microdialysis to measure lipolysis as reflected by glycerol release into the interstitial fluid (14). To study possible cellular defects in insulin action, we also obtained isolated subcutaneous adipocytes through needle biopsies and investigated the regulation of glucose transport and lipolysis *in vitro*. In addition, we studied physical work

From the Lundberg Laboratory for Diabetes Research (J.W.E., U.S., P.-A.J.); the Departments of Cardiopulmonary Medicine (F.W.) and Internal Medicine (M.W.), Sahlgrenska University Hospital, Göteborg; and the Department of Medicine (J.W.E.), Umeå University Hospital, Umeå, Sweden.

Address correspondence and reprint requests to Dr. Jan Eriksson, Department of Medicine, Umeå University Hospital, S-90185 Umeå, Sweden. E-mail: jan.eriksson@medicin.umu.se.

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ADA, adenosine deaminase; ATBF, adipose tissue blood flow; BSA, bovine serum albumin; CV, coefficient of variation; GIR, glucose infusion rate; HPA, hypothalamus-pituitary-adrenal; IA, interstitial-arterial; Ins 1, first insulin infusion step during clamp ( $10 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ); Ins 2, second insulin infusion step during clamp ( $60 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ); ISI, insulin sensitivity index; MBF, muscle blood flow; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; PIA, *N*<sup>6</sup>-(*R*-phenylisopropyl) adenosine;  $R_{\text{e}}$ , endogenous glucose production;  $R_{\text{d}}$ , endogenous glucose disposal; SA, specific activity; SAT, subcutaneous adipose tissue.

capacity assessed as maximal oxygen uptake, since insulin resistance in subjects with type 2 diabetic relatives has previously been reported to be associated with a reduction in maximal oxygen uptake (15).

## RESEARCH DESIGN AND METHODS

**Subjects.** Ten relatives of type 2 diabetic patients were studied. They all had two first-degree relatives—parents and/or siblings—with type 2 diabetes and, in several instances, additional relatives with the disease. It is established that such individuals are at a very high risk (~80%) of developing type 2 diabetes during their lifetime (16). As a control group, we recruited 10 subjects with no known type 2 diabetes among their relatives. All participating subjects gave their informed consent, and the study was approved by the Ethical Committee of the Göteborg University. The subjects were matched pairwise with respect to sex, age, BMI, smoking, and physical activity (time per week spent on leisure physical activity). All subjects were nonobese and healthy, and none took regular medication. Body composition (lean body mass and fat mass) was estimated with the bioelectrical impedance method (BLA-103; RJL Systems, Detroit, MI) (17). Anthropometric data are given in Table 1. A 75-g oral glucose tolerance test (OGTT) was performed with blood sampling for plasma insulin and glucose at 0, 15, 30, 60, 90, and 120 min.

**Euglycemic insulin clamp.** Whole body glucose turnover, subcutaneous adipose tissue (SAT) lipolysis, indirect calorimetry, and skeletal muscle and adipose tissue blood flow (ATBF) were studied (Fig. 1). After an overnight fast, the subjects arrived at the laboratory at 8:00 A.M. During the study, they reclined in a comfortable bed in a room kept at 26–28°C. Two polytetrafluoroethylene cannulas (Venflon; Viggo, Helsingborg, Sweden) were positioned intravenously, one in an antecubital vein for all infusions and a second one, inserted distally, in a vein of the contralateral arm for taking blood samples. The forearm used to draw blood samples was heated with electric pads to arterialize venous blood (18).

First, a 120-min equilibration period was instituted with a primed infusion of [ $^3\text{H}$ ]glucose (10  $\mu\text{Ci}$ ) followed by a constant infusion at 6  $\mu\text{Ci/h}$  during 120 min. Semisynthetic regular human insulin (Actrapid; Novo Nordisk, Copenhagen) was infused as a priming dose for the first 10 min followed by a continuous infusion at 10  $\text{mU} \cdot \text{m}^2 \cdot \text{body surface} \cdot \text{min}^{-1}$ , and the insulin infusion continued for a further 110 min to maintain a constant, moderately elevated insulin level (Ins 1). In parallel, a glucose infusion (200 mg/ml) with [ $^3\text{H}$ ]glucose added was started, and the infusion rate was adjusted to maintain a blood glucose level of 5.0 mmol/l measured at 5-min intervals with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). After this insulin infusion period, another priming dose of insulin was administered during 10 min and then a continuous infusion at 60  $\text{mU} \cdot \text{m}^2 \cdot \text{body surface} \cdot \text{min}^{-1}$  was administered for another 110 min to achieve steady-state hyperinsulinemia (Ins 2). The variable glucose infusion (now without [ $^3\text{H}$ ]glucose) continued throughout the study to maintain blood glucose at 5.0 mmol/l. During the Ins 1 period, steady-state blood glucose during the final 40 min was  $5.08 \pm 0.03$  and  $5.03 \pm 0.02$  mmol/l in relatives and control subjects, respectively; during the Ins 2 period, it was  $5.13 \pm 0.04$  and  $5.05 \pm 0.07$  mmol/l, respectively. The coefficient of variation (CV) for steady-state blood glucose was 6 and 8% for relatives and control subjects, respectively, indicating a stable glucose level.

Steady-state plasma insulin concentrations were calculated as the mean of the two values obtained at 20-min intervals during the final 40 min of the clamp study at each insulin level. The glucose infusion rate (GIR) during the glucose clamp, taken as a measure of insulin sensitivity (19), was calculated as the M value, GIR divided by body weight (milligrams per kilogram per minute). The M value is obtained from the average GIR of the final 40 min at each step of the clamp, when steady state was clearly reached. Insulin sensitivity index (ISI) is calculated by dividing M by the mean insulin concentration during the same period of the clamp (20).

**Calculation of glucose kinetics.** At steady state during clamps, whole body insulin-stimulated glucose uptake equals the amount of glucose infused if endogenous glucose production ( $R_g$ ) is entirely suppressed; this should be true during the Ins 2 infusion period with a high insulin level, ~90  $\text{mU/l}$  (21). During the baseline and low-dose insulin infusion (Ins 1) periods,  $R_g$  and endogenous glucose disposal ( $R_d$ ) were calculated using the Steele equation (22). This was done by assessing [ $^3\text{H}$ ]glucose specific activity (SA) in plasma as previously reported (23). These measurements were made for the final 40 min of the baseline and Ins 1 periods. For the determination of [ $^3\text{H}$ ]glucose SA, plasma was deproteinized with  $\text{Ba}(\text{OH})_2$  and  $\text{ZnSO}_4$  and evaporated (22). The dried glucose residue was resuspended, counted in a liquid scintillation counter after adding scintillation liquid, and corrected for quenching. The [ $^3\text{H}$ ]glucose SA was calculated by dividing disintegrations per minute in plasma by the plasma glucose concentration.

**Blood flow measurements.** The blood flow assessments were performed under steady-state conditions during the final 30 min at each step in the glucose clamp procedure (Fig. 1). ATBF measurements and estimation of apparent glycerol release were essentially done as previously reported (14,24). In brief, 3–6 MBq  $^{133}\text{Xe}$  (Mallinckrodt, Petten, the Netherlands) was injected at a depth of ~5 mm in the periumbilical subcutaneous tissue with >3 cm distance to the nearest microdial-

TABLE 1

Anthropometric data on relatives of type 2 diabetes patients ( $n = 10$ ) and control subjects ( $n = 10$ )

	Relatives	Control subjects	P value
Sex (M/F)	4/6	4/6	NS
Smokers/nonsmokers	2/8	2/8	NS
Age (years)	$35 \pm 1$	$35 \pm 1$	NS
Body weight (kg)	$71.8 \pm 2.5$	$70.5 \pm 3.4$	NS
BMI ( $\text{kg/m}^2$ )	$23.6 \pm 0.6$	$23.1 \pm 0.4$	NS
Lean body mass (kg)	$52.2 \pm 2.8$	$54.5 \pm 3.4$	NS
Body fat (kg)	$19.4 \pm 1.0$	$16.2 \pm 1.2$	0.021

Data are  $n$  or means  $\pm$  SE.

ysis catheter, and 30-min registrations were made throughout the day, as indicated in the study protocol. A mean value of 10.0 ml/g was used for the tissue/blood partition coefficient for  $^{133}\text{Xe}$ , and ATBF was calculated as described previously (24,25). Skeletal muscle blood flow (MBF) at rest was measured in the right leg by plethysmography (Pletysmograph; Elektromedicin AB, Kullavik, Sweden) using the calf volume gain detected by the mercury-in-sialistic strain gauge during venous occlusion (26). A cuff placed just above the knee was used for the blood pressure measurements and was then inflated to a venous occlusion pressure of 50 mmHg. MBF was expressed as milliliters per 100 ml muscle tissue per minute.

**Subcutaneous microdialysis.** The microdialysis procedure has been described in detail (14,27) and is only briefly presented here. Two 30-mm Cuprophane membranes (Gambro, Lund, Sweden) were placed in the abdominal subcutaneous tissue 5 cm lateral to the umbilicus, and the catheters were always more than 5 cm apart. The perfusion fluid contained 2.5 mmol/l glucose and 30  $\mu\text{mol/l}$  glycerol to prevent drainage (27), and the perfusion rate was 3  $\mu\text{l/min}$  using a high-precision syringe pump (CMA 100; CMA Microdialysis, Solna, Sweden). Dialysates were collected every 15 min throughout the study; sampling started 30 min after the insertion of the catheters. Steady-state sampling was performed during the final 30 min at each step of the clamp. Each catheter was calibrated in situ for little less than 3 h according to the no net flux method (27). The relative recovery was estimated for glycerol by adding four different concentrations of glycerol (0–300  $\mu\text{mol/l}$ ) to the perfusion liquid, and the net change of glycerol after dialysis was determined. Regression analysis showed a good linear correlation during the calibration period ( $r > 0.9$ ), and the recovery of glycerol was assessed.

**Calculations of glycerol release.** Apparent glycerol release (in micromoles per 100 grams per minute) was estimated according to Fick's equation, substrate release =  $(V - A) \times Q \times (1 - \text{hematocrit} \times 10^{-3})$ , where  $V$  is capillary venous plasma substrate concentration,  $A$  is arterialized venous plasma substrate concentration, and  $Q$  is blood flow. Conversion of interstitial to capillary venous plasma substrate concentration was done according to the equation  $V = (I - A) \times (1 - e^{-PS/Q}) + A$ , where  $I$  is the interstitial substrate concentration,  $PS$  is the permeability surface product area, and  $Q$  is the plasma flow rate (28,29). A  $PS$  of 5  $\text{ml} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$  for glycerol was adopted (14). Mean values from the final 30 min during each step were used for the calculations.

**Indirect calorimetry.** Respiratory exchange measurements were performed with a flow-through canopy gas analyzer system (Deltatrac, Helsinki, Finland). Samples of expired air, which were suctioned at 40 l/min, were analyzed for  $\text{O}_2$  and  $\text{CO}_2$  concentration differences using paramagnetic  $\text{O}_2$  and infrared  $\text{CO}_2$  analyzers. The hood was placed on the subject's head during the final 30 min at each clamp step, and mean values were calculated for the final 20 min. The following

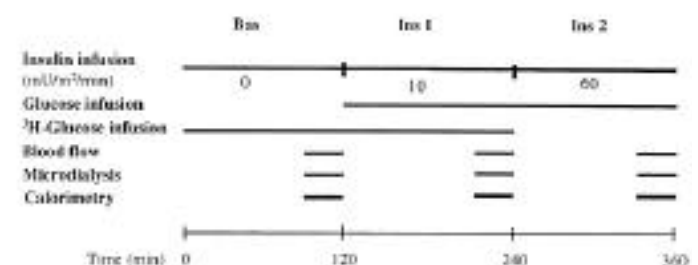


FIG. 1. Schematic presentation of the study procedure for the euglycemic clamp experiment and concomitant adipose tissue microdialysis, calorimetry, and blood flow measurements.

constants were used for calculation of substrate oxidation rates (30): 1 g of glucose requires 746 ml O<sub>2</sub> and produces 746 ml CO<sub>2</sub>, oxidation of 1 g of lipid requires 2,029 ml O<sub>2</sub> and produces 1,430 ml CO<sub>2</sub>.

**Analytical methods.** Glycerol levels were analyzed by using a radiometric method (31). Glucose was determined by a YSI 2300 glucose analyzer (Yellow Springs Instruments). NEFA concentrations were determined using a commercial enzymatic kit (Wako Chemicals GmbH, Neuss, Germany). Insulin was analyzed with a radioimmunoassay (Pharmacia Upjohn, Uppsala, Sweden).

**Ergometer bicycle test.** Vo<sub>2max</sub> was measured by means of a submaximal bicycle exercise test with concomitant analysis of expiratory gases as described by Åstrand and Ryhning (32). Vo<sub>2max</sub> declines with increasing age (33), but this was adjusted for since the subjects were matched for age. The equipment used was the CS-100 Ergo-Spirometry-Module (Schiller AG, Baar, Switzerland).

**Adipocyte studies**

**Materials.** [<sup>14</sup>C]U-D-glucose (SA 246 µCi/µmol) was purchased from Amersham (Buckinghamshire, U.K.). [<sup>32</sup>P]ATP (SA 3 Ci/µmol) was from Du Pont (Geneva, Switzerland). 8-br-cAMP, bovine serum albumin (BSA) (fraction V), and collagenase were from Sigma Chemical (St. Louis, MO). Adenosine deaminase (ADA), N<sup>5</sup>-(R-phenylisopropyl) adenosine (PIA), and glycerokinase were from Boehringer Mannheim (Mannheim, Germany). Medium 199 was obtained from Gibco BRL, Life Technologies (Paisley, U.K.). Human monocomponent insulin was purchased from Novo Nordisk (Copenhagen, Denmark).

**Needle biopsies and isolation of adipocytes.** Needle biopsies of abdominal SAT were performed at 8:00–9:00 A.M. after an overnight fast. The skin was anesthetized with Lidocaine, and ~2 g fat from the paraumbilical region was aspirated into a plastic syringe. The fat tissue was washed with preformed medium 199, and adipocytes were isolated in medium containing 0.6 mg/ml collagenase and 4% BSA at 37°C for 1 h in a shaking water bath (~120 rpm), filtered through a nylon mesh, and washed with fresh medium four times. Cell size and number were determined as previously reported (34,35).

**Lipolysis.** The lipolysis assay was performed essentially as previously reported (36). Briefly, isolated cells were incubated at a lipocrit of 1–3% in medium 199 containing 5.6 mmol/l glucose, 4% BSA, ADA (1 U/ml), PIA (1 µmol/l), and the indicated agents at 37°C for 1 h. The incubation was stopped by cooling on ice, and adipocytes were rapidly separated from medium by centrifugation through silicone oil. Lipolysis was assessed by determining the glycerol content in the medium (31). Glycerol was phosphorylated in the presence of glycerokinase and [<sup>32</sup>P]ATP at 37°C for 30 min. Residual [<sup>32</sup>P]ATP was then hydrolyzed in perchloric acid at 95°C for 1 h. Free [<sup>32</sup>P]phosphate was precipitated on ice in the presence of ammonium molybdate and triethylamine. After centrifugation, radioactivity of the supernatant (reflecting phosphorylated glycerol) was measured.

**Glucose uptake.** For glucose uptake assessment, adipocytes (lipocrit 3–5%) were incubated as described above but in medium 199 with a tracer amount of [<sup>14</sup>C]U-D-glucose (0.86 µmol/l) but with no more glucose at 37°C in the presence or absence of insulin. After 1 h, the glucose uptake was terminated by transferring cells and medium to prechilled tubes on ice. Adipocytes were then immediately separated by centrifugation through silicone oil. Cell-associated radioactivity was determined, and remaining extracellular [<sup>14</sup>C]U-D-glucose, assessed by adding the same amount of the isotope at the end of incubation, was subtracted. The cellular clearance of glucose from the medium was calculated according to the formula

$$\text{Cellular clearance of medium glucose} = \frac{\text{cell radioactivity}}{(\text{medium radioactivity} \times \text{cell number} \times \text{seconds})}$$

and taken as an index of the rate of glucose uptake (36).

**Statistical analysis.** Unless otherwise indicated, data are means ± SE and represent observations on 10 relatives of type 2 diabetes patients and 10 control sub-

TABLE 2  
Fasting blood chemistry

	Relatives	Control subjects	P value
Glucose (mmol/l)	5.1 ± 0.1	4.9 ± 0.1	NS
Insulin (mU/l)	8.2 ± 1.0	6.1 ± 0.7	NS
C-peptide (nmol/l)	0.49 ± 0.06	0.47 ± 0.08	NS
HbA <sub>1c</sub> (%)	4.8 ± 0.1	4.5 ± 0.1	0.019
NEFA (mmol/l)	0.50 ± 0.03	0.48 ± 0.04	NS

Data are means ± SE.

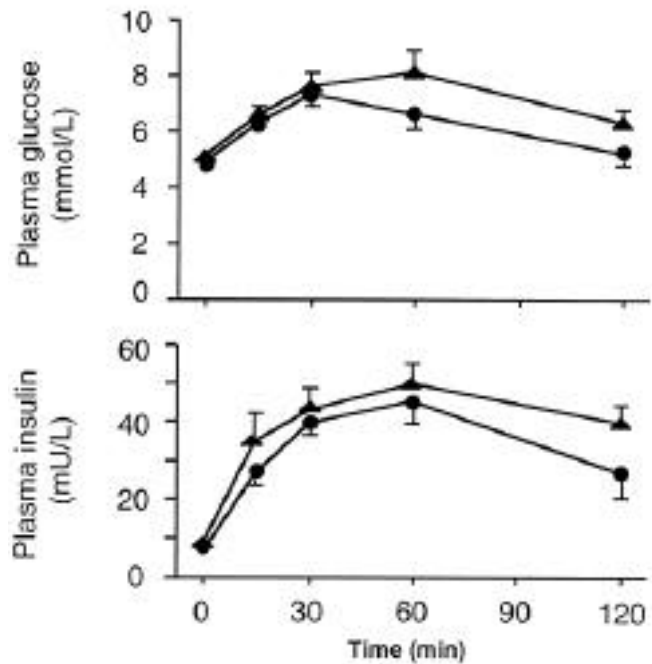


FIG. 2. Plasma glucose and insulin during OGTT in relatives of type 2 diabetes patients (▲, n = 10) and control subjects (●, n = 10).

jects, respectively. Statistical significance of differences was analyzed with Student's two-tailed t test for paired data or as specified. A P value <0.05 was considered significant.

**RESULTS**

**Blood chemistry and OGTT.** Blood chemistry reflecting lipid and glucose metabolism is shown in Table 2. The investigated variables did not differ between the groups, except for HbA<sub>1c</sub> which, surprisingly, was slightly but significantly higher in the relatives than in the control subjects. All values were within the normal range (3.3–5.3%), and the findings would be compatible with a latent elevation in glycemia in the relatives of type 2 diabetes patients (relatives). During the OGTT, there was a tendency toward higher insulin and glucose levels during the second hour among relatives, but no significant differences were found (Fig. 2). Fasting insulin also

TABLE 3  
Data from ergometer cycle test

	Relatives	Control subjects	P value
Maximum work load (W)	191 ± 11	193 ± 17	NS
Vo <sub>2max</sub> (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	33.8 ± 2.4	34.9 ± 2.8	NS
Anaerobic threshold (Vo <sub>2</sub> , l/min)	1.77 ± 0.13	1.80 ± 0.15	NS
Heart rate (beats/min)			
Resting	86 ± 3	83 ± 7	NS
Maximum	187 ± 4	184 ± 4	NS
Systolic blood pressure (mmHg)			
Resting	121 ± 5	118 ± 6	NS
Maximum	186 ± 4	182 ± 6	NS
Diastolic blood pressure (mmHg)			
Resting	87 ± 3	81 ± 3	NS
Maximum	88 ± 3	83 ± 4	NS

Data are means ± SE. Resting, sitting at rest on ergometer cycle; maximum, at maximum exercise.

TABLE 4  
Insulin levels and glucose turnover during clamp

	Relatives	Control subjects	P value
Insulin (mU/l)			
Basal	7.7 ± 1.0	5.7 ± 0.4	NS
Ins 1	23.4 ± 1.7	17.9 ± 0.7	0.024
Ins 2	97.2 ± 5.5	83.1 ± 2.9	0.025
M value (mg · kg <sup>-1</sup> · min <sup>-1</sup> )			
Ins 1	2.6 ± 0.3	3.2 ± 0.3	NS
Ins 2	9.0 ± 0.7	10.6 ± 0.5	NS
ISI (100 · mg · l · kg <sup>-1</sup> · min <sup>-1</sup> · mU <sup>-1</sup> )			
Ins 1	12.0 ± 1.6	18.1 ± 1.4	0.028
Ins 2	9.4 ± 0.8	12.9 ± 0.6	0.017

Data are means ± SE. M value, glucose infusion rate.

tended to be higher in the relatives. Normal glucose tolerance was found in all subjects except one female relative who displayed a slightly impaired glucose tolerance, with a 120-min glucose of 9.1 mmol/l.

**Ergometer cycle test.** As shown in Table 3, relatives and control subjects were very similar in maximal working capacity, oxygen uptake, anaerobic threshold, and pulse and blood pressure reaction during a maximal exercise test. This indicates that there are no major differences between the groups with respect to cardiopulmonary and muscular working capacity and is evidence that the groups were well matched for physical activity.

**Euglycemic clamp.** The data from the euglycemic clamp experiments are shown in Tables 4–6. The insulin levels attained during both steps of the insulin infusion were somewhat higher in relatives compared with control subjects, suggesting an impairment in insulin clearance. The glucose infusion rate reflecting net whole body glucose utilization was slightly lower in the relatives than in control subjects during both steps of insulin infusion, but the difference did not reach statistical significance. However, when assessed as the so-called ISI—i.e., adjusted for the prevailing insulin concentration—there was a clear impairment in insulin-mediated glucose utilization in the relatives (Fig. 3A). Using labeling with [<sup>3</sup>H]glucose, the  $R_a$  and  $R_d$  could be estimated.

TABLE 5  
Indirect calorimetry during clamp

	Relatives	Control subjects	P value
Glucose oxidation (μmol · kg <sup>-1</sup> LBM · min <sup>-1</sup> )			
Basal	14.4 ± 1.4	11.8 ± 3.5	NS
Ins 1	19.6 ± 1.7	18.4 ± 1.8	NS
Ins 2	30.0 ± 2.3	30.6 ± 1.9	NS
Lipid oxidation (μmol · kg <sup>-1</sup> LBM · min <sup>-1</sup> )			
Basal	4.5 ± 0.5	4.9 ± 0.3	NS
Ins 1	2.7 ± 0.4	3.0 ± 0.5	NS
Ins 2	1.1 ± 0.3	0.7 ± 0.3	NS

Data are means ± SE. LBM, lean body mass.

TABLE 6  
Blood flow in adipose tissue and skeletal muscle during clamp

	Relatives	Control subjects	P value
ATBF (ml · 100 g <sup>-1</sup> · min <sup>-1</sup> )			
Basal	2.4 ± 0.4	3.6 ± 0.5	NS
Ins 1	2.4 ± 0.5	3.5 ± 0.5	NS
Ins 2	2.8 ± 0.6	2.3 ± 0.3	NS
MBF (ml · 100 ml <sup>-1</sup> · min <sup>-1</sup> )			
Basal	2.6 ± 0.3	2.5 ± 0.4	NS
Ins 1	2.9 ± 0.3	2.6 ± 0.4	NS
Ins 2	3.1 ± 0.3*	3.1 ± 0.4	NS

Data are means ± SE. \* $P = 0.046$  compared with basal.

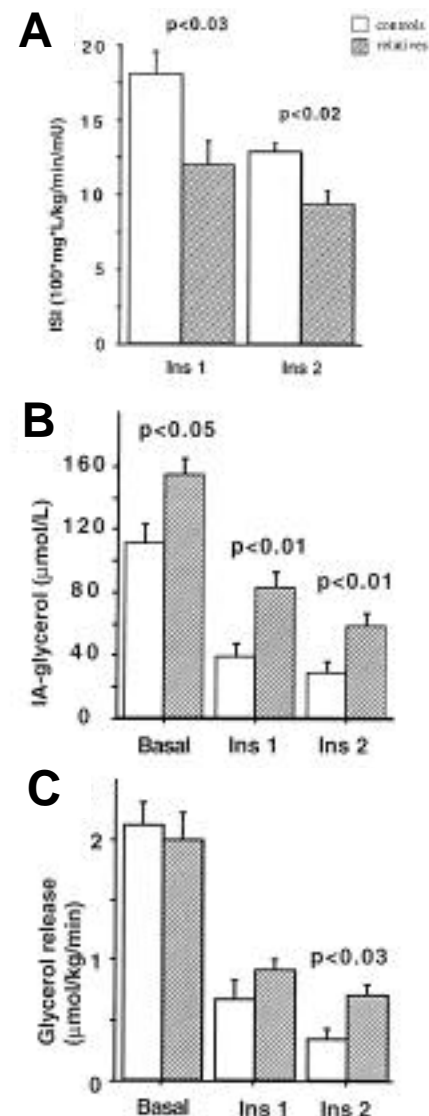


FIG. 3. Insulin action in vivo on glucose utilization (A) and adipose tissue lipolysis reflected by the glycerol gradient between interstitial fluid and arterial plasma (B) or net glycerol release (C). The assessments were performed during the euglycemic clamp with insulin infusions at 10 and 60 mU · m<sup>-2</sup> · min<sup>-1</sup> (Ins 1 and 2, respectively). Insulin-mediated glucose turnover is assessed as the glucose infusion rate divided by the ambient insulin level to obtain the ISI. Lipolysis was studied by using the subcutaneous microdialysis technique in parallel with the insulin-glucose clamp, and data from the initial equilibration period are included to represent basal lipolysis.

Neither differed significantly between the groups, but there was a tendency toward an impaired insulin-induced suppression of  $R_a$  in relatives (data not shown). Indirect calorimetry indicated that glucose and lipid oxidation (Table 5) as well as energy expenditure (not shown) were similar between the two groups during the baseline and insulin infusion periods.

Plasma adrenaline and noradrenaline were measured in samples during the baseline period and during both insulin steps of the clamp (at 0, 240, and 360 min). Baseline levels of adrenaline and noradrenaline did not differ significantly between relatives and control subjects; neither were there any consistent alterations during the insulin infusions (data not shown).

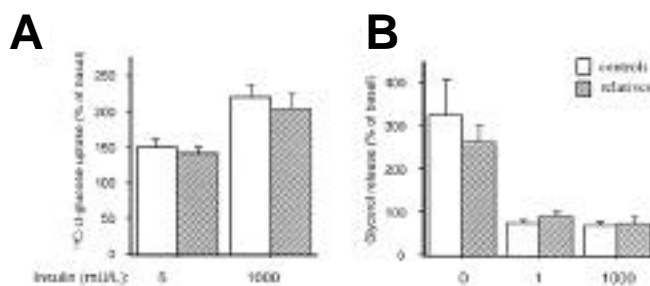
Microdialysis of the abdominal SAT showed a similar basal lipolysis rate assessed as the interstitial-arterial (IA) difference in glycerol concentration, whereas the decrease seen during insulin infusion was more pronounced in control subjects than in relatives (Fig. 3B). This result was also confirmed when net glycerol release from adipose tissue was estimated from IA difference and SAT blood (Fig. 3C). Blood flow per se was not significantly changed by the insulin infusions, and it did not differ between the groups (Table 6). Plasma NEFAs tended to be higher in relatives than in control subjects both before and during the insulin infusions (baseline:  $498 \pm 27$  vs.  $483 \pm 41$ ; Ins 1:  $145 \pm 37$  vs.  $94 \pm 19$ ; Ins 2:  $77 \pm 24$  vs.  $55 \pm 18$   $\mu\text{mol/L}$ ; NS).

MBF was assessed by venous occlusion plethysmography in the right calf; data are shown in Table 6. There was a tendency toward increased blood flow during the two insulin infusion periods. There was no difference between the groups.

**In vitro effects of insulin in isolated subcutaneous adipocytes.** The isolated fat cells obtained from needle biopsies were incubated for 60 min with different concentrations of insulin. Data are shown in Fig. 4. Basal [ $^{14}\text{C}$ ]glucose uptake was similar between relatives and control subjects ( $66 \pm 16$  vs.  $51 \pm 9$   $\text{fl} \cdot \text{cell}^{-1} \cdot \text{s}^{-1}$ , NS), and it was stimulated by two different concentrations of insulin to a similar degree in cells from both groups, thus suggesting similar insulin responsiveness and sensitivity (Fig. 4A). Due to limited amounts of biopsy material, lipolysis measurements had to be omitted in two control subjects and in one relative. Basal nonstimulated lipolysis of adipocytes varied greatly between individual subjects and was not significantly different between the groups (not shown). As shown in Fig. 4B, when lipolysis was stimulated by 8-bromo cAMP (5 mmol/l), there was no difference in glycerol release between adipocytes from control subjects and relatives. Also, concomitant addition of different concentrations of insulin counteracted lipolysis activation, and this antilipolytic effect did not differ between the groups. Cell size was slightly greater in subcutaneous fat obtained from relatives than from control subjects (cell diameter  $96 \pm 2$  vs.  $85 \pm 3$   $\mu\text{m}$ ,  $P = 0.0053$ ).

## DISCUSSION

As previously reported, we found that glucose uptake was resistant to insulin action in the subjects who had close relatives with type 2 diabetes. This finding was evident only when glucose utilization was adjusted to the prevailing insulin concentration during the clamp experiments, however. The higher insulin levels found in relatives of type 2 diabetes patients compared with control subjects thus contributed to the result. It is not obvious that the higher plasma insulin also means a higher concentration at the target cells, but accord-



**FIG. 4.** Insulin action in vitro in isolated subcutaneous adipocytes from relatives of type 2 diabetes patients and control subjects. **A:** [ $^{14}\text{C}$ ]glucose uptake,  $n = 10$  in each group. **B:** Lipolysis,  $n = 9$  relatives and 8 control subjects. Assessments were performed as described in METHODS. Lipolysis was studied by measuring cellular release of glycerol under basal conditions and after stimulation with 4 mmol/l 8-bromo cAMP and concomitant addition of the indicated insulin concentrations. Data are expressed as percent of basal nonstimulated glucose uptake and glycerol release, respectively, neither of which differed significantly between relatives and control subjects.

ing to previous results, interstitial insulin concentrations of skeletal muscle should be proportional to those of plasma (37). It was previously reported (38) that the pre-type 2 diabetic state is associated with an impaired clearance of insulin, which can explain the present finding with an elevated clamp insulin concentration in the relatives.

We found an impairment in insulin action on glucose turnover in the relatives despite the fact that they were matched to control subjects with respect to physical activity. (Similar physical capacity was also confirmed with an ergometer cycle test including measurement of oxygen uptake rate and anaerobic threshold.) One previous report suggested that insulin resistance in relatives of type 2 diabetes patients is caused by a reduced physical capacity as reflected by maximal oxygen uptake, and that this in turn is probably due to less physical exercise (15). Our study argues against that concept, since insulin resistance in the relatives was found independent of physical activity and working capacity. Moreover, on regression analysis, we found no association between glucose uptake during clamps and the maximal oxygen uptake (not shown). The results thus suggest similar cardiopulmonary function in the two groups. Our findings do not rule out an altered cardiac function in the prediabetic state, however, since the correlation between aerobic capacity and myocardial systolic function is rather poor (39).

In addition to insulin-resistant glucose turnover, we also found that the antilipolytic effect of insulin was impaired in the relatives of type 2 diabetes patients. This was demonstrated by using the microdialysis technique to assess glycerol release into the interstitial water in SAT. During the insulin-glucose clamps, the suppression of lipolysis produced by insulin was clearly attenuated in the relatives. Thus, we propose that along with insulin resistance occurring with respect to glucose metabolism, there is an impairment in the antilipolytic effect of insulin that occurs very early in the development of type 2 diabetes. The enhanced release of NEFAs can also be an accelerating factor for the metabolic perturbations, because this promotes insulin resistance (40), and elevated NEFA levels may predict future risk of type 2 diabetes (4). Some previous data also suggest an early impairment in insulin's antilipolytic action (41). We have also found an impaired suppression in NEFAs following a mixed meal in

patients with type 2 diabetic relatives (41a), giving further support to this concept. In fact, the alterations in lipid metabolism could precede those in glucose metabolism. Our present finding, that lipid oxidation during the insulin clamp did not differ between the groups despite less suppressed lipolysis in relatives of type 2 diabetes patients, is somewhat surprising. However, lipid oxidation does not always occur in parallel to lipolysis, and the enhanced mobilization of NEFAs and glycerol in relatives of type 2 diabetes patients might instead be used in the liver synthesis of triglyceride-rich lipoproteins.

Insulin can stimulate skeletal MBF, which may be impaired in different insulin-resistant conditions (42). In the present study, we found that insulin enhanced MBF during the clamps, but there were no differences between insulin-resistant relatives and control subjects. In addition, subcutaneous ATBF was slightly but not significantly lower in the relatives both in the basal state and during low-dose insulin infusion. We previously found an impaired ATBF in type 2 diabetic subjects (43).

To investigate whether the insulin resistance found in vivo in relatives of type 2 diabetes patients could be explained by a primary defect in cellular insulin action, we studied insulin effects in isolated subcutaneous adipocytes. In the two groups, we found similar in vitro activation of fat cell lipolysis by a cAMP analog. With respect to insulin action in vitro, we found no consistent alterations in the effects of insulin on either glucose uptake or lipolysis in adipocytes; this result was in contrast to the in vivo findings. Of course, this finding does not exclude the possibility that other cell types, such as muscle fibers, are insulin-resistant in vitro. In contrast, in adipocytes from patients with established type 2 diabetes, we and others have found cellular insulin resistance with respect to glucose uptake in vitro; also, the insulin signal transduction system may be perturbed (36,44–46). There is a well-known secondary component of insulin resistance in type 2 diabetes that is at least partly due to hyperglycemia, the glucotoxic effect, which is possibly mediated by the hexosamine pathway (47,48); this component may explain why impaired cellular insulin action is found in type 2 diabetes but not in the pre-type 2 diabetic state represented by healthy relatives of type 2 diabetes patients.

The discrepancy between whole body insulin resistance and normal cellular insulin action found in the relatives of type 2 diabetes patients in the present study is not fully explained. One possibility is that factors of the nervous system or in the circulation play a role to produce insulin resistance in the in vivo milieu, and that the cells can respond normally to insulin once they are removed from this environment. It should also be appreciated, however, that the normal insulin action found in fat cells of relatives of type 2 diabetic patients in vitro does not exclude an inherited cellular insulin resistance that becomes evident only in vivo. The mechanisms causing whole body insulin resistance are not clarified but are expected to be at least partly inherited. Of course, the critical genes are not known, but they may potentially govern neuroendocrine regulation. An enhanced activity of the sympathetic nervous system could theoretically contribute to both the insulin-resistant glucose turnover and lipolysis, even though the level of circulating catecholamines did not differ between the groups in our study. Moreover, other neural and hormonal factors (or other humoral factors) may contribute to insulin resistance in the prediabetic state. We are presently pursuing studies in relatives of type 2 diabetes patients

addressing the regulation of the sympathetic nervous system and the HPA system; preliminary observations suggest alterations in these neuroendocrine systems. Certainly, acquired factors, such as physical activity, smoking, nutritional state, and the socioeconomic environment, can also contribute to insulin resistance in relatives of type 2 diabetes patients, although we adjusted for some of these in the present study.

In conclusion, several metabolic defects occur early in the development of type 2 diabetes. Along with an impaired insulin action on glucose turnover, our data show resistance to the antilipolytic effect of insulin. The resulting NEFA mobilization from adipose tissue can promote insulin resistance with respect to glucose turnover. It is speculated that the primary perturbations involve neural or hormonal mechanisms rather than cellular defects in the adipose tissue.

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