

# Postabsorptive and Insulin-Stimulated Energy Homeostasis and Leucine Turnover in Offspring of Type 2 Diabetic Patients

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**OBJECTIVE** — This study was performed to ascertain whether insulin resistance with respect to protein metabolism is an additional primary metabolic abnormality affecting insulin-resistant offspring of type 2 diabetic parents, along with insulin resistance with respect to glucose and lipid metabolism.

**RESEARCH DESIGN AND METHODS** — We studied 18 young, nonobese offspring of type 2 diabetic parents and 27 healthy matched (by means of dual-energy X-ray absorption) individuals with the bolus plus continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose and [1-<sup>13</sup>C]leucine in combination with the insulin clamp (40 mU · m<sup>-2</sup> · min<sup>-1</sup>).

**RESULTS** — Fasting plasma leucine, phenylalanine, alanine, and glutamine concentrations, as well as the glucose and leucine turnover (reciprocal pool model: 155 ± 10 vs. 165 ± 5 μmol · kg lean body mass<sup>-1</sup> · h<sup>-1</sup> in offspring of type 2 diabetic patients and healthy matched individuals, respectively), were also not different. During the clamp, glucose turnover rates were significantly reduced in offspring of type 2 diabetic patients (7.1 ± 0.5) in comparison with healthy matched individuals (9.9 ± 0.6 mg · kg lean body mass<sup>-1</sup> · min<sup>-1</sup>; *P* < 0.01). Also, the suppression of leucine turnover was impaired in offspring of type 2 diabetic patients (12 ± 1%) in comparison with healthy matched individuals (17 ± 1%; *P* = 0.04) and correlated with the degree of the impairment of insulin-stimulated glucose metabolism (*R*<sup>2</sup> = 0.13; *P* = 0.02).

**CONCLUSIONS** — Nonobese, nondiabetic, insulin-resistant offspring of type 2 diabetic patients were characterized by an impairment of insulin-dependent suppression of protein breakdown, which was proportional to the impairment of glucose metabolism. These results demonstrate that in humans, a primary in vivo impairment of insulin action affects glucose and fatty acid metabolism as previously shown and also protein/amino acid metabolism.

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**Abbreviations:** ELF, endogenous leucine flux; FFA, free fatty acid; REE, resting energy expenditure; α-TNF-R2, α-tumor necrosis factor receptor-2.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Type 2 diabetic patients are characterized by an impairment of in vivo insulin action with respect to glucose metabolism involving glucose transport, glycogen synthesis, glycolysis, and glucose oxidation (1). Multiple abnormalities in insulin-stimulated lipid and fatty acid metabolism were also found in type 2 diabetes (2). In contrast, a clear-cut dissociation between the effects of insulin on glucose/fatty acid metabolism and amino acid metabolism in type 2 diabetes was reported (3,4). Leucine turnover assessed by means of radioactive or stable isotopes dilution techniques was found to be normal in the postabsorptive condition and in the insulin-stimulated state in normal-weight and obese patients with type 2 diabetes (3–5). The normal insulin sensitivity with respect to leucine metabolism in type 2 diabetic patients was challenged by the observation that in other nondiabetic human model of impaired insulin-stimulated glucose and fatty acid metabolism (obesity), insulin resistance with respect to amino acid metabolism was detected (6), and by the report of a defect of whole-body protein breakdown estimated by means of the assessment of the in vivo nitrogen flux in patients with poorly controlled type 2 diabetes, which was reversed by exogenous insulin administration (7). The early studies in type 2 diabetic patients are difficult to interpret because of differences in the subject populations and intravenous insulin infusion protocols, but most importantly because of the presence of hyperglycemia versus euglycemia or because of the use of the overnight low-dose insulin infusion to achieve euglycemia on the morning of the study. Approximately 25–75% (8) of the healthy, young, nondiabetic, nonobese first-degree relatives of type 2 diabetic patients show the same moderate-to-mild abnormalities of insulin-stimulated glucose (9) and fatty acid (10) metabolism affecting their parents with type 2 diabetes and are considered a model of primary

Table 1—Anthropometry, body composition, endocrine profile, and energy expenditure of study groups

	Offspring		Normal subjects	
	Basal	Insulin	Basal	Insulin
Patients (F/M)	10/8		17/10	
Age (years)	27 ± 2		26 ± 1	
Body weight (kg)	69.0 ± 5.9		63.8 ± 2.2	
Height (cm)	171 ± 2		169 ± 2	
BMI (kg/m <sup>2</sup> )	23.7 ± 1.7		22.2 ± 0.5	
Body fat mass (kg)	20.1 ± 3.5		16.8 ± 1.3	
Body fat (%)	28 ± 3		26 ± 2	
Arms fat content (%)	24.0 ± 2.8		20.6 ± 1.9	
Trunk fat content (%)	26.9 ± 3.3		21.7 ± 1.6	
Legs fat content (%)	31.3 ± 2.7		31.2 ± 2.2	
Lean body mass (kg)	47.5 ± 3.1		45.9 ± 1.9	
Total cholesterol (mg/dl)	188 ± 9		171 ± 5	
HDL cholesterol (mg/dl)	44 ± 6		46 ± 3	
LDL cholesterol (mg/dl)	123 ± 10		111 ± 5	
Triglycerides (mg/dl)	102 ± 14†		72 ± 5	
Physical activity index	8.6 ± 0.5		9.0 ± 0.5	
Insulin (pmol/l)	55 ± 18		39 ± 3	
Glucagon (ng/l)	76 ± 9		108 ± 10	
Cortisol (ng/ml)	68 ± 12		64 ± 5	
Growth hormone (ng/ml)	0.97 ± 0.15		1.09 ± 0.14	
IGF-1 (ng/ml)	186 ± 9		213 ± 13	
Leptin (ng/ml)	9.5 ± 2.1		7.2 ± 1.3	
Adiponectin (μg/ml)	9.7 ± 1.3†		13.8 ± 1.3	
α-TNF-R2 (ng/ml)	1.25 ± 0.09		1.40 ± 0.07	
REE (kcal/day)	1,584 ± 96	1,602 ± 119	1,515 ± 50	1,541 ± 64
REE/kg body weight (kcal · kg <sup>-1</sup> · day <sup>-1</sup> )	22.8 ± 0.8	22.9 ± 1.0	23.5 ± 0.5	23.7 ± 0.6
REE/kg lean body mass (kcal · kg <sup>-1</sup> · day <sup>-1</sup> )	33.7 ± 1.2	33.8 ± 1.4	33.2 ± 0.8	33.6 ± 0.9

Data are means ± SE. The range of possible scores for the physical activity index is 3–15; the lowest value corresponds to the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 MJ/h, e.g., bowling) and who participates in sedentary activities during leisure time. The highest value corresponds to the level of physical activity of a person who is very physically active at work (e.g., a construction worker), who plays heavy sports (energy expended is at least 1.76 MJ/h, e.g., boxing, basketball, football, or rugby), and who is very physically active during leisure time (e.g., walking >1 h/day or biking >45 min/day). †Indicates  $P < 0.05$  vs. normal subjects (Student's unpaired  $t$  test and Bonferroni correction).

insulin resistance. In this work, to circumvent the methodological problems of studying diabetic individuals and to ascertain whether insulin resistance with respect to amino acid metabolism also is part of the genetic cluster of type 2 diabetes, we assessed postabsorptive and insulin-stimulated leucine turnover in healthy, young, nondiabetic, nonobese offspring of type 2 diabetic parents.

## RESEARCH DESIGN AND METHODS

Eighteen offspring of type 2 diabetic patients were recruited at Istituto Scientifico H San Raffaele. The main criteria for their inclusion in the study were the following: 1) both parents with type 2 diabetes or one parent and a first- or second-degree relative with type 2 diabetes; 2) age 24–45 years; 3) white race; 4) sedentary lifestyle; and 5) no

history of hypertension, endocrine/metabolic disease, or cigarette smoking. Habitual physical activity was assessed using a questionnaire (11). Offspring of type 2 diabetic parents were compared with 27 healthy subjects matched by anthropomorphic features with the exception that they had no family history of diabetes and hypertension traced through their grandparents. The characteristics of the study groups are summarized in Table 1. Body weight was stable for at least 6–12 months, and women were not taking oral steroidal contraceptives for at least 12 months. All subjects were in good health as assessed by medical history and physical examination. Informed consent was obtained from all subjects after explanation of purposes, nature, and potential risks of the study. The protocol was ap-

proved by the ethical committee of the Istituto Scientifico H San Raffaele.

Subjects were instructed to consume an isocaloric diet (~250 g carbohydrate/day) and to abstain from exercise activity for 3 weeks before the studies. Women were studied between days 3 and 10 of the menstrual cycle. They were studied by means of the euglycemic-hyperinsulinemic clamp and indirect calorimetry to assess whole-body insulin sensitivity and resting energy expenditure (REE) and glucose and lipid oxidation after a 10-h overnight fast period and during the insulin clamp. Within 2–3 days they were also studied by means of dual-energy X-ray absorption to assess body composition. Dual-energy X-ray absorption was performed in the Department of Science, Nutrition, and Microbiology, Nutrition Section, Università degli Studi di Milano.

**Euglycemic-hyperinsulinemic clamp**

Subjects were admitted to the Metabolic Unit of the Division of Internal Medicine I of the Istituto Scientifico H San Raffaele at 7:00 A.M. after a 10-h overnight fast. A Teflon catheter was inserted into an ante-cubital vein for infusions and an additional one was inserted retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box throughout the experiment to allow sampling of arterialized venous blood. A bolus of [6,6-<sup>2</sup>H<sub>2</sub>]glucose (5 mg/kg body wt) and of [1-<sup>13</sup>C]leucine (0.5 mg/kg body wt), followed by a continuous infusion (0.05 mg · kg body wt<sup>-1</sup> · min<sup>-1</sup> and 0.007 mg/kg body wt, respectively) obtained from massTrace (Woburn, MA) was administered. Blood samples for postabsorptive plasma glucose, total cholesterol, HDL cholesterol, triglycerides, free fatty acids (FFAs), insulin, leptin, growth hormone, IGF-1 glucagon, cortisol,  $\alpha$ -tumor necrosis factor receptor-2 ( $\alpha$ -TNF-R2), and adiponectin were performed in duplicate in the postabsorptive condition. After a 150-min tracer equilibration period, a euglycemic-hyperinsulinemic clamp was performed as previously described (10). Insulin was infused at 40 mU · m<sup>-2</sup> · min<sup>-1</sup> and plasma glucose concentration was kept at ~5 mmol/l for an additional 150 min by means of a variable infusion of 20% dextrose infusion. Blood samples for plasma hormones, substrates, and tracer enrichments were drawn every 15 min.

**Indirect calorimetry**

Indirect calorimetry was performed continuously while the subjects were lying quietly for 30 min during the basal equilibration period and at the end of the clamp with a ventilated hood system (Sensor Medics 2900, Metabolic Measurement Cart). The mean coefficients of variation within the session for O<sub>2</sub> and CO<sub>2</sub> measurements were <2%. Urine samples were collected at the end of the tracer equilibration period and at the end of the 150-min clamp period. Blood samples for the assessment of blood urea nitrogen were also collected in the basal equilibration period and at the end of the insulin clamp.

**Body composition**

Dual-energy X-ray absorption was performed with a Lunar-DPX-IQ scanner (Lunar, Madison, WI) as previously described (10).

**Analytical procedures**

Plasma glucose was measured with a Beckman glucose analyzer (10). Plasma FFAs and plasma total cholesterol, HDL cholesterol, and triglycerides were measured as previously described (10). LDL cholesterol was calculated using the Friedwald formula. Plasma insulin was measured with a microparticle enzyme immunoassay technology (10) (IMx Insulin assay; Abbott Laboratories, Rome, Italy). Plasma growth hormone, glucagon, and cortisol concentrations were assessed as previously described (12); IGF-1 was measured by radioimmunoassay (RIA-coated; Medgenics Diagnostics, Fleurus, Belgium) as previously described (12).  $\alpha$ -TNF-R2 was measured with an enzyme immunoassay (Immunotech Beckman Coulter, Marseille, France) as previously described (12). Plasma leptin concentrations were determined as previously described (10) by radioimmunoassay with a human kit (Linco Research, St. Charles, MO). Plasma adiponectin was measured using a commercially available radioimmunoassay kit (Linco Research) as previously described (13). Serum urea nitrogen was measured in the postabsorptive and hyperinsulinemic conditions using an enzymatic method on an Hitachi 747(12). The [6,6-<sup>2</sup>H<sub>2</sub>]glucose and [1-<sup>13</sup>C]leucine enrichment were measured by gas chromatography–mass spectrometry as previously described (14).

**Calculations**

Glucose turnover was calculated in the basal state by dividing the [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion rate by the steady-state plateau of plasma [6,6-<sup>2</sup>H<sub>2</sub>]glucose enrichment achieved during the last 45 min of the basal period. Glucose kinetics during the insulin clamp were calculated using the Steele equations for the non-steady state (15). We did not attempt to describe the intracellular leucine kinetics with equations for the nonsteady state because this approach would imply many more assumptions than the simpler monocompartmental approach. To define the leucine release from proteolysis (endogenous leucine flux [ELF]), the intracellular leucine enrichments were estimated by plasma [1-<sup>13</sup>C]ketoisocaproate enrichments, which are derived from the intracellular leucine reciprocal pool approach with the standard steady-state equation as previously described (12,14). Plasma amino acid concentrations were

also assessed by means of gas chromatography–mass spectrometry as previously described (12,14). Endogenous glucose production was calculated by subtracting the glucose infusion rate from the rate of glucose appearance measured with the isotope tracer technique. Total body glucose uptake was determined during the clamp by adding the rate of residual endogenous glucose production to the exogenous glucose infusion rate. REE was calculated by the Weir standard equation from the O<sub>2</sub> consumption rate and the CO<sub>2</sub> production rates measured by means of indirect calorimetry (excluding the first 10 min of data acquisition) and from the urinary nitrogen excretion as previously described (12). Glucose, lipid, and protein oxidation were estimated as previously described (12) in the postabsorptive state. During the insulin clamp, protein oxidation rates were corrected for changes in urea pool. Nonoxidative glucose disposal was calculated subtracting the glucose oxidation rate from the tissue glucose disposal.

**Statistical analysis**

Data are means  $\pm$  SE. Analyses were performed using the SPSS (version 10.0; SPSS, Chicago, IL). The steady state for plasma tracers enrichment was defined as a nonsignificant correlation with time ( $P > 0.05$ ) using linear regression. Comparisons between groups in the fasting condition were performed using Student's unpaired *t* test and Bonferroni correction. Repeated-measures ANOVA was used to assess the main effects of group, insulin, and group-by-insulin interactions on the metabolic and endocrine parameters. If the repeated-measures ANOVA was significant, then pairwise comparisons were made by Tukey method. Linear regression analysis was performed to assess relationships between variables. First, a significant effect of group-by-ELF percentage suppression interaction using percentage stimulation of insulin-stimulated glucose disposal as the dependent variable was performed using the general linear model procedure. Regression analysis was then performed in the entire population and separately in the offspring of type 2 diabetic parents and healthy matched individuals.

Table 2—Glucose and protein metabolism in the postabsorptive (basal) and insulin-stimulated conditions (insulin)

	Offspring		Normal subjects		% change	
	Basal	Insulin	Basal	Insulin	Offspring	Normal Subjects
Glucose metabolism						
Plasma glucose (mmol/l)	5.00 ± 0.08	4.98 ± 0.16	5.06 ± 0.04	4.98 ± 0.17	—	—
Endogenous glucose production (mg · kg <sup>-1</sup> · min)	2.12 ± 0.06	0.40 ± 0.13	2.31 ± 0.07	0.48 ± 0.13	-82 ± 5	-80 ± 6
Glucose disposal (mg · kg LBM <sup>-1</sup> · min <sup>-1</sup> )*	3.14 ± 0.07	7.09 ± 0.53†	3.29 ± 0.11	9.89 ± 0.60	+73 ± 6‡	+200 ± 14
Glucose oxidation (mg · kg LBM <sup>-1</sup> · min <sup>-1</sup> )	1.99 ± 0.19	3.27 ± 0.32	2.04 ± 0.11	3.66 ± 0.25	+190 ± 28	+188 ± 15
Nonoxidative glucose disp (mg · kg LBM <sup>-1</sup> · min <sup>-1</sup> )	—	3.85 ± 0.86§	—	6.23 ± 0.23	—	—
Protein metabolism						
Leucine (μmol/l)	119 ± 6	73 ± 5	109 ± 4	68 ± 2	-38 ± 2	-37 ± 1
α-Ketoisocaproate (μmol/l)	30 ± 4	18 ± 2	47 ± 8	31 ± 5	-36 ± 9	-29 ± 6
Phenylalanine (μmol/l)	53 ± 3	41 ± 2	51 ± 1	39 ± 1	-23 ± 2	-24 ± 1
Glutamine (μmol/l)	468 ± 35	375 ± 25	471 ± 22	404 ± 22	-19 ± 2	-15 ± 1
Alanine (μmol/l)	276 ± 32	238 ± 14	306 ± 16	281 ± 10	-8 ± 5	-5 ± 3
Endogenous leucine flux (μmol · kg LBM <sup>-1</sup> · h <sup>-1</sup> )	155 ± 10	136 ± 9	165 ± 5	137 ± 4	-12 ± 1	-17 ± 1
Protein oxidation (mg · kg LBM <sup>-1</sup> · min <sup>-1</sup> )	0.97 ± 0.06	0.62 ± 0.08	0.98 ± 0.04	0.71 ± 0.05	-35 ± 8	-29 ± 4

Data are means ± SE. \*Significant main effect of group ( $P = 0.04$ ) and significant main effect of insulin ( $P < 0.01$ ) but no significant main effect of group-by-insulin interaction ( $P = 0.26$ ). † $P < 0.05$  vs. normal subjects (ANOVA for repeated-measures Tukey's post hoc test). ‡ $P < 0.01$  vs. normal subjects (Student's unpaired  $t$  test and Bonferroni correction). §Significant main effect of insulin ( $P < 0.05$ ) was found for all parameters with the exception of plasma glucose and alanine. § $P < 0.05$  vs. normal subjects (Student's unpaired  $t$  test and Bonferroni correction). LBM, lean body mass.

## RESULTS

### Anthropometric characteristics and energy homeostasis

Anthropometric parameters of study subjects are summarized in Table 1 and were not different between groups. The REE was not different in offspring of type 2 diabetic patients in comparison with healthy matched individuals in absolute values, when expressed per kilogram of body weight and when expressed per kilogram of lean body mass.

### Endocrine profile

The fasting plasma hormonal profile is summarized in Table 1. Plasma insulin, cortisol, growth hormone, IGF-1, leptin, and α-TNF-R2 concentrations were not different between groups. Plasma glucagon showed a trend to be lower in offspring of type 2 diabetic patients than in healthy matched individuals ( $P = 0.09$ ); meanwhile, plasma adiponectin was significantly lower ( $P = 0.03$ ). During the clamp, the plasma insulin concentration reached a plateau that was not different between groups ( $351 ± 42$  vs.  $357 ± 39$  pmol/l in offspring of type 2 diabetic patients and healthy matched individuals, respectively;  $P = 0.80$ ).

### Glucose metabolism

Plasma glucose and the endogenous glucose production rates were not different in offspring of type 2 diabetic patients and healthy matched individuals in the postabsorptive and insulin-stimulated states (Table 2). The insulin-stimulated total body glucose disposal was lower in the offspring of type 2 diabetic patients compared with the healthy matched individuals (Table 2 and Fig. 1A) because of an impairment of the nonoxidative glucose disposal; meanwhile, glucose oxidation was preserved (Table 2).

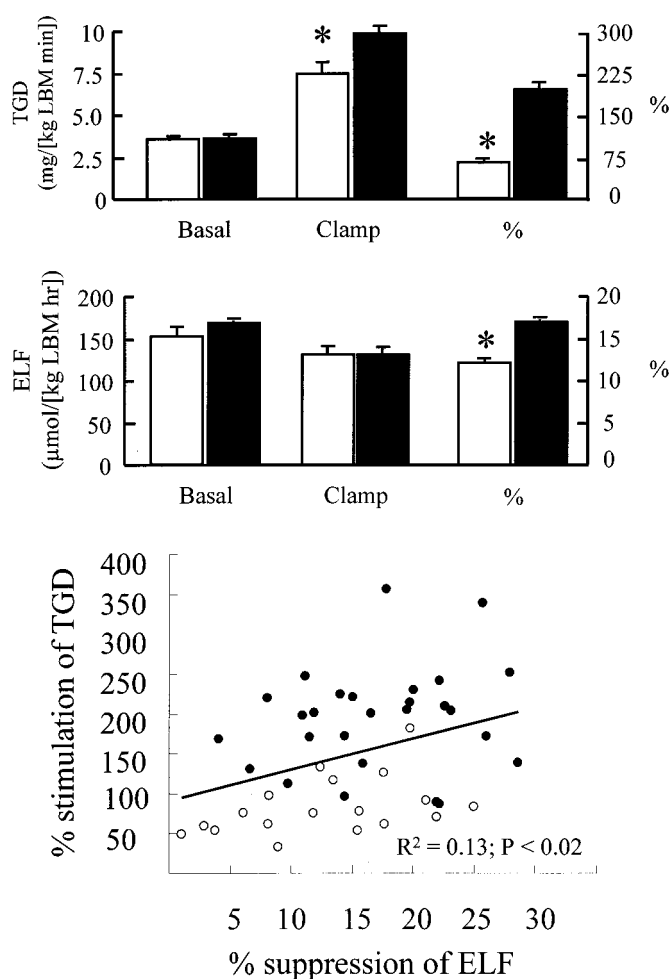
### Lipid metabolism

Fasting plasma total cholesterol ( $P = 0.06$ ) and triglycerides ( $P < 0.05$ ) were higher in the offspring of type 2 diabetic patients compared with healthy matched individuals (Table 1). Plasma FFAs were higher in offspring of type 2 diabetic patients in comparison with healthy matched individuals in both the postabsorptive ( $612 ± 33$  vs.  $447 ± 40$  μmol/l;  $P = 0.006$ ) and insulin-stimulated condition ( $89 ± 12$  vs.  $51 ± 6$  μmol/l;  $P = 0.002$ ), even if the percent suppression during the clamp was not different ( $84 ± 3$  vs.  $86 ± 2$ %;  $P = 0.64$ ). Lipid oxidation was not different in offspring of type 2 diabetic patients in comparison with

healthy matched individuals in both the postabsorptive ( $1.23 ± 0.10$  vs.  $1.17 ± 0.06$  mg · kg lean body mass<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.59$ ) and insulin-stimulated ( $0.51 ± 0.09$  vs.  $0.40 ± 0.06$  mg · kg lean body mass<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.47$ ) states.

### Protein metabolism

Postabsorptive plasma leucine, α-ketoisocaproic acid, phenylalanine, glutamine, and alanine concentrations were not different in offspring of type 2 diabetic patients in comparison with healthy matched individuals (Table 2). Postabsorptive ELF, which represents a parameter of whole-body proteolysis, was also not different ( $P = 0.14$ ; Table 2). During insulin stimulation, the suppression of plasma leucine, α-ketoisocaproic acid, phenylalanine, glutamine, and alanine in offspring of type 2 diabetic patients was not different from that in healthy matched individuals (Table 2); however, the ELF showed resistance to the insulin action with the percent suppression being significantly smaller than that in healthy matched individuals ( $P = 0.04$ ; Table 2 and Fig. 1B). Protein oxidation, calculated using the urea nitrogen excretion rates and changes in the urea pool size during the clamp, was not different be-



**Figure 1**—A: Total glucose disposal (TGD) in the postabsorptive (basal) and insulin-stimulated conditions (clamp) and percent stimulation in the study groups. B: ELF in the postabsorptive (basal) and insulin-stimulated conditions (clamp) and percent inhibition in the study groups. C: The relationship between the percent suppression of the ELF and the percent stimulation of the TGD is represented. □ and ○, offspring of type 2 diabetic parents; ■ and ●, normal subjects. Data are means  $\pm$  SE. \* $P < 0.05$  (Student's unpaired *t* test and Bonferroni correction).

tween groups in both the basal and insulin-stimulated states (Table 2).

### Regression analysis

A preliminary search for significant interactions revealed the effect of a group-by-ELF percentage suppression using percentage stimulation of insulin-stimulated glucose disposal as a dependent variable ( $P = 0.05$ ). Then, we performed the linear regression analysis in the entire population (Fig. 1C;  $R^2 = 0.13$ ,  $P < 0.02$ ) and separately in the offspring of type 2 diabetic patients ( $R^2 = 0.23$ ,  $P = 0.08$ ) and in the healthy matched individuals ( $R^2 = 0.03$ ,  $P = 0.37$ ).

**CONCLUSIONS**— This work demonstrates that young, nondiabetic, non-obese offspring of type 2 diabetic parents are characterized as expected by whole-body insulin resistance with respect to glucose and fatty acid metabolism and also, as a novel result, to the insulin's antiproteolytic action.

The 30% impairment of insulin-induced glucose metabolism (Table 2 and Fig. 1A) is a typical feature (8–10) in offspring of type 2 diabetic parents. Also, lipid metabolism was altered in the offspring of type 2 diabetic parents. They had higher plasma triglycerides and higher plasma FFAs in both the postabsorptive and insulin-stimulated conditions. In type 2 diabetes, insulin

resistance with respect to several pathways of glucose and fatty acid metabolism is well established. To date, insulin-dependent control of protein breakdown has been considered preserved in type 2 diabetic patients; a profound, but reversible, alteration of whole-body protein turnover has been found only in patients with poorly controlled type 1 diabetic patients (16). Based on these results, it might be assumed that abnormality of the insulin's signal system possibly involved in the pathogenesis of type 2 diabetes must reside downstream to the steps regulating the control on proteolysis. In contrast, this work, demonstrating that insulin resistance with respect to glucose and fatty acid metabolism is paralleled by insulin resistance also with respect to protein breakdown (Table 2 and Fig. 1B) would rather suggest that the abnormality of the signal system of insulin involved in the pathogenesis of type 2 diabetes must be upstream to the key steps regulating glucose, fatty acid, and amino acid metabolism. This scenario is also supported by the fact that in dose-response studies in the offspring of type 2 diabetic parents, when appropriate insulin infusion rates were used for comparison of insulin-mediated metabolic effects, impairment of insulin-stimulated glucose metabolism (assessed at an insulin infusion rate of 40  $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ) was associated with the impairment of whole-body lipolysis (assessed at insulin infusion rate of 10  $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ) (17). This study demonstrated that the severity of the impairment of the suppression of the ELF was proportional to the severity of insulin resistance with respect to glucose metabolism (Fig. 1C), supporting the hypothesis of an alteration of a common key regulatory step as the cause of the inherited trait of insulin resistance.

The finding of an impairment of the insulin-dependent inhibitory effect on protein breakdown in the offspring of type 2 diabetic parents but not in their parents (3,4) was surprising. It is possible that in previous works the type 2 diabetic patients represented a peculiar group of diabetic but normal-weight patients (3) not fully representative of the features of type 2 diabetes. It may be speculated that the overnight low-dose insulin infusion, often used to study type 2 diabetic patients in the condition of euglycemia (4), may blunt this abnormality. We previously observed that in type 2 diabetic pa-

tients, recipients of heart transplants who did not receive an overnight insulin infusion had an impairment of the antiproteolytic action of insulin that was detected despite the fact that nondiabetic heart recipients did not show the same defect (18). Finally, it cannot be excluded that in type 2 diabetic patients, chronic adaptations to hyperglycemia, hyperinsulinemia, or treatment with oral hypoglycemic agents may enable proteolysis to remain within the normal range, as suggested by Halvatsiotis et al. (4).

Protein homeostasis is regulated by multiple hormonal, nutritional, neural, and inflammatory factors, and among them insulin certainly plays a crucial role, regulating both protein degradation and synthesis (19). The cellular mechanisms underlying insulin antiproteolytic actions are poorly understood, and it is unknown where the regulatory action of insulin takes place within the insulin-signaling system, even if mounting evidence suggests that the ATP-dependent ubiquitin-proteasome proteolytic pathway plays a central role in insulin-regulated protein degradation (20). Much more is known about the control of protein synthesis by insulin; unfortunately, in this study we are limited because of the lack of assessment of leucine oxidation and consequently of the nonoxidative leucine disposal (marker of protein synthesis).

Because studies have also implicated several other hormones, cytokines, and adipocyte-derived peptides in causing insulin resistance, we measured those reported in Table 1, seeking a relationship with insulin resistance with respect to protein breakdown. The data suggested that although plasma cortisol, growth hormone, IGF-1, and leptin were not different between groups, a trend for higher postabsorptive plasma insulin concentration and lower glucagon and adiponectin levels were found in the offspring of type 2 diabetic parents. The possible effects of a moderate chronic hyperinsulinemia on leucine turnover are controversial. Hyperinsulinemia, artificially maintained in normal subjects, was able to induce within 24 h an insulin-resistant state with respect to the ELF when a prolonged insulin infusion rate of  $0.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  was used (21). In vivo models of longer hyperinsulinemic states in humans are less clear. Nondiabetic, obese patients with fasting hyperinsulinemia revealed a certain degree of insulin resistance with

respect to the antiproteolytic effect of insulin (6). Also, chronic endogenous hyperinsulinemia induced by benign insulinoma (22) or by total lipotrophic diabetes (23) caused a defect in insulin action that was generalized to both the antiproteolytic effects of insulin and on the stimulation of glucose metabolism. Therefore, we cannot exclude the possibility that a mild hyperinsulinemic state in the offspring of type 2 diabetic patients may be the cause of insulin resistance with respect to leucine turnover, even if hyperinsulinemic patients with kidney-pancreas transplants did not show any impairment of the antiproteolytic effects of insulin in vivo when studied in similar experimental conditions (24). In the present study, we observed a trend for lower fasting plasma glucagon concentration in the offspring of type 2 diabetic parents (Table 1). Hyperglucagonemia seems to modulate leucine metabolism only in conditions of insulin deficiency (25), when plasma leucine concentration is in excess (26), and in patients with glucagonoma (27) increasing the proteolytic rates (28). To our knowledge, no studies were performed to assess the role of reduced plasma glucagon on protein metabolism, and in the present set of experiments we could not find a correlative association between the lower levels of hormones and parameters of protein metabolism. Therefore, it is difficult to speculate about a possible role of the slightly lower glucagon levels observed in our offspring of type 2 diabetic parents, but we cannot exclude the possibility that it might have implications for protein/amino acid metabolism. Adiponectin is a peptide secreted by the adipocytes, and lower plasma concentrations have been associated with impairment of the insulin action with respect to carbohydrate and lipid metabolism; however, to our knowledge there are no data with respect to the effects of adiponectin or an association with amino acid metabolism in vivo in humans. Based on our data, we could not detect any relationship in the offspring of type 2 diabetic parents and normal subjects, separately or together ( $R^2 = 0.08$ ;  $P = 0.69$ ), using linear regression analysis.  $\alpha$ -TNF-R2 has been reported to be higher in lean nondiabetic offspring of type 2 diabetic subjects (29) and because cytokines such as  $\alpha$ -TNF may be associated with increased protein turnover, we also measured the plasma concentration

of this circulating receptor but could not detect any difference with respect to the normal subjects.

Among the nutritional factors, higher plasma FFA concentrations were found in offspring of type 2 diabetic parents. Tessari et al. (30) showed that increased FFA availability may affect whole-body protein metabolism, reducing a leucine flux; more recently, it was confirmed that, especially in the fasting condition, FFAs may play a protein-sparing effect (31). Based on these reports, it may be speculated that in the fasting state, the ELF was not different between study groups because of a sparing effect of the increased FFA availability in the offspring of type 2 diabetic parents; in contrast, during the clamp the sparing effect of increased FFA concentration vanished because of the profound insulin-dependent suppression of lipolysis, revealing a certain degree of insulin resistance with respect to protein degradation. It must be emphasized that the protocol was not designed to assess the impact of FFA on protein degradation, but we cannot exclude the possibility that the higher plasma concentration in the offspring of type 2 diabetic parents may have affected the results.

Despite the fact that in this study we demonstrated a number of abnormalities involving glucose, fatty acid, and amino acid metabolism, whole-body energy homeostasis appeared to be spared in the offspring of type 2 diabetic parents in the postabsorptive and insulin-stimulated conditions (Table 1). This is in contrast to the lower REE reported in the offspring of type 2 diabetic parents in the Botnia study (32). This contrast is probably caused by the fact that we compared two groups of individuals with similar anthropometric features and, therefore, with a similar degree of abdominal fat accumulation. Abdominal obesity has been claimed as the factor responsible for the lower REE in the offspring of type 2 diabetic parents in the Botnia study.

In conclusion, insulin-resistant offspring of type 2 diabetic patients were characterized by an impairment of insulin action on protein breakdown, which was proportional to the insulin resistance with respect to glucose metabolism. These results demonstrate that in vivo in humans, a primary impairment of insulin action is not only limited to glucose and fatty acid metabolism, as previously shown, but also extended to protein/amino acid me-

tabolism, suggesting that abnormality of the signal system of insulin involved in the pathogenesis of type 2 diabetes must be upstream to the key steps common to the control of muscle glycogen synthesis, lipolysis, and proteolysis.

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