Insulin Resistance of Protein Metabolism in Type 2 Diabetes

Sandra Pereira, Errol B. Marliss, José A. Morais, Stéphanie Chevalier, and Réjeanne Gougeon

OBJECTIVE—We previously demonstrated that *1*) obesity impairs and *2*) sex influences insulin sensitivity of protein metabolism, while *3*) poor glycemic control in type 2 diabetes accelerates protein turnover in daily fed-fasted states. We hypothesized that type 2 diabetes alters the insulin sensitivity of protein metabolism and that sex modulates it.

RESEARCH DESIGN AND METHODS—Hyperinsulinemic (~570 pmol/l), euglycemic (5.5 mmol/l), and isoaminoacidemic (kept at postabsorptive concentrations) clamps were performed in 17 hyperglycemic type 2 diabetic subjects and 23 subjects without diabetes matched for age and body composition, after 7 days on a inpatient, protein-controlled, isoenergetic diet. Glucose and leucine kinetics were determined using tracers.

RESULTS—In type 2 diabetes, postabsorptive (baseline) glycemia was 8–9 mmol/l, glucose production (R_a) and disposal (R_d) were elevated, and once clamped, endogenous glucose R_a remained greater and R_d was less ($P < 0.05$) than in control subjects. Baseline leucine kinetics did not differ despite higher insulin levels. The latter was an independent predictor of leucine flux within each sex. With clamp, total flux increased less ($P = 0.016$) in type 2 diabetic men, although protein breakdown decreased equally (~20%) in male groups but less in female groups. Whereas protein synthesis increased in male control subjects and in both female groups, it did not in male subjects with type 2 diabetes. In men, homeostasis model assessment of insulin resistance predicted 44%, and, in women, waist-to-hip ratio predicted 40% of the change in synthesis.

CONCLUSIONS—During our clamp, men with type 2 diabetes have greater insulin resistance of protein metabolism than that conferred by excess adiposity itself, whereas women do not. These results may have implications for dietary protein requirements. ***Diabetes*** **57:56–63, 2008**

There is clear evidence for altered protein metabolism in type 1 diabetes (1–5), but in type 2 diabetes, results have been inconsistent. That protein metabolism in type 2 diabetes has been reported to be both unaffected and altered may stem from differences in study design: tracer method, adiposity, and sex of subjects; prevailing glycemia; normalization of data; and types of statistical analyses. We reported accelerated integrated fed-fasted kinetics of whole-body protein metabolism (using [^{15}N]glycine) in obese type 2 diabetic subjects with hyperglycemia (6–9) compared with obese control subjects (6,7). Such studies required adjusting data for fat-free mass (FFM), sex, and age (6,8,9) and had precise control of protein and energy intake. When glycemic control was normalized with insulin (7), improved with oral antihyperglycemic agents (6), or normalized with oral agents and energy restriction (6), protein turnover was either improved or not different from that of obese control subjects.

Most reports showing no alterations in type 2 diabetes (10–14) assessed postabsorptive and postinsulin states using amino acid tracers. However, one reported elevated postabsorptive catabolism in hyperglycemic type 2 diabetic patients, not corrected by prior insulin treatment (15). Another showed elevated rates of leucine transamination that decreased with better glycemic control, without altering leucine oxidation (16). During the hyperinsulinemic-euglycemic clamp, suppression of breakdown was shown to be both blunted (15) and unaffected (12,13). Any decrease in catabolism decreases endogenous amino acid concentrations and availability, thereby producing "paradoxical" decreases in synthesis (17,18).

Therefore, defining the roles of insulin on synthesis and catabolism requires that plasma amino acids be maintained constant. We have used an hyperinsulinemic-euglycemic clamp with circulating amino acids clamped at fasting levels. This also avoids raising plasma amino acids, which themselves stimulate anabolism. Both the conventional clamp (19) and our approach are experimental models rather than simulation of the fed state after mixed meals that cause hyperaminoacidemia and hyperglycemia. With this method, we found increased protein synthesis in lean men (20), a blunted response in obese women (21), and less net protein accretion in healthy women compared with men (22).

The hypotheses of the present study are that type 2 diabetes affects the protein anabolic responses to hyperinsulinemia and that sex modifies these alterations. Hyperinsulinemic, euglycemic, and isoaminoacidemic clamps with leucine and glucose tracers were performed in overweight and obese men and women with or without type 2 diabetes. Groups of the same sex were matched for body composition and age. Data from some of the control subjects have been previously published (20–23).

RESEARCH DESIGN AND METHODS

Seventeen type 2 diabetic patients (7 women, 10 men) and 23 control subjects (12 women, 11 men) were admitted to the Clinical Investigation Unit of the MUHC/Royal Victoria Hospital (Table 1). Consent was obtained according to the institutional research ethics board. Subjects were screened by medical

From the McGill Nutrition and Food Science Centre, McGill University Health Centre/Royal Victoria Hospital, Montreal, Quebec, Canada.

Address correspondence and reprint requests to Réjeanne Gougeon, McGill Nutrition and Food Science Centre, MUHC/Royal Victoria Hospital, 687 Pine Ave. West, H6.61, Montreal, QC H3A 1A1, Canada. E-mail: rejeanne.gougeon@muhc.mcgill.ca.

Received for publication 22 July 2007 and accepted in revised form 11 October 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 16 October 2007. DOI: 10.2337/db07-0887.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0887.

α-KIC, α-ketoisocaproic acid; BCAA, branched-chain amino acid; FFA, free fatty acid; FFM, fat-free mass; HOMA-IR, homeostasis model assessment of insulin resistance; REE, resting energy expenditure.

TABLE 1
Subject characteristics

	Male control subjects	Male type 2 diabetic	Female control subjects	Female type 2 diabetic
n	11	10	12	7
Age (years)	46.3 ± 4.6	54.7 ± 1.5	51.0 ± 4.1	58.6 ± 3.4
Height (cm)	174.7 ± 1.8	175.1 ± 2.2	160.6 ± 1.9	163.1 ± 3.2
Weight (kg)	93.2 ± 5.0	90.5 ± 5.5	84.7 ± 6.2	92.8 ± 8.3
BMI (kg/m^2)	30.6 ± 1.7	29.3 ± 1.2	32.5 ± 1.8	34.9 ± 2.8
FFM (kg)	63.5 ± 2.1	64.6 ± 3.1	46.9 ± 2.2*	48.8 ± 2.1*
Body fat (%)	31.0 ± 1.7	28.0 ± 1.5	44.6 ± 1.5*	45.2 ± 2.5*
Waist circumference (cm)	103.4 ± 3.0	104.2 ± 3.9	99.5 ± 4.3	109.7 ± 6.2
Hip circumference (cm)	107.5 ± 2.5	105.2 ± 2.9	115.3 ± 3.9*	118.6 ± 7.1*
Waist-to-hip ratio	0.96 ± 0.01	0.99 ± 0.01	0.86 ± 0.02*	0.93 ± 0.04*†
HOMA-IR	2.7 ± 0.4	6.6 ± 0.9†	3.1 ± 0.3	7.4 ± 1.2†
Triglycerides (mmol/l)	1.8 ± 0.2	2.3 ± 0.4	1.8 ± 0.3	3.3 ± 0.7

Data are means ± SE. *$P < 0.01$ vs. corresponding male subjects. Type 2 diabetic vs. control subjects were analyzed for each sex separately by unpaired *t* test; †$P < 0.01$ vs. control subjects. Men vs. women were analyzed for type 2 diabetic and control subjects separately by unpaired *t* test. HOMA-IR: [insulin (μU/ml) · glucose (mmol/l)]/22.5.

and dietary history, physical examination, and laboratory evaluation to assure the absence of hepatic, hematologic, renal, pulmonary, thyroid, and cardiovascular dysfunction. Inclusion criteria were that subjects be nonsmokers and have stable weight for 6 months and protein intakes within the Dietary Reference Intakes (24). Control subjects took no medications that affected metabolism and underwent a 75-g oral glucose tolerance test (25). The groups within each sex were matched for anthropometric variables. Diabetes medications were stopped for 1 week and lipid-lowering medications upon admission, but antihypertensive agents were continued in four subjects.

Subjects consumed an isoenergetic, protein-controlled liquid formula (Ensure; Ross Laboratories, Montreal, QC, Canada) for 7 days in control and 8 days in type 2 diabetic subjects, with or without additional energy as canola oil and a glucose polymer (20). Total energy intakes were 1.5 times resting energy expenditure (REE), by indirect calorimetry (Deltatrac; SensorMedics, Yorba Linda, CA), with 60% from carbohydrate, 25% from fat, and 15% (1.7 g · kg FFM^{-1} · day^{-1}) from protein.

Premenopausal women were studied during the follicular phase. Twenty-four–hour urine was collected daily for determination of nitrogen balance (9). Subjects were weighed daily. Physical activity was limited to walks in and around the hospital. Premeal capillary glucose determinations (Accu-Chek Advantage; Roche Diagnostics, Laval, QC, Canada) were done in type 2 diabetic subjects. If hyperglycemia was >15 mmol/l, insulin (Humulin-R; Eli Lilly Canada, Toronto, ON, Canada) was administered but not for 15 h before the clamp. Energy in glycosuria was added daily (50% glucose polymer and 50% canola oil). Waist and hip circumferences were measured according to World Health Organization criteria (26). Body composition was assessed by bioelectrical impedance analysis (RJL-101A; RJL Systems, Detroit, MI) using equations validated for lean (27), overweight (28), obese (28), and older (29) subjects.

Hyperinsulinemic, euglycemic, and isoaminoacidemic clamp protocol. On the last day, the clamp was performed, as detailed (20), with glycemia at 5.5 mmol/l and total branched-chain amino acids (BCAAs) maintained at each individual subject's postabsorptive concentrations (online appendix 1 [available at http://dx.doi.org/10.2337/db07-0887]). Briefly, catheters were inserted into an antecubital vein for infusions and a contralateral dorsal hand vein for sampling arterialized venous blood with the hand in a warming box at 65°C. At 8:00 A.M., an oral bolus of 0.1 mg/kg NaH$^{13}CO_2$ (MassTrace, Woburn, MA) in water was ingested and a primed L-[1-^{13}C]leucine (0.5 mg/kg, Isotec; Sigma-Aldrich, St. Louis, MO) constant infusion at 0.008 mg · kg^{-1} · min^{-1} was started. D-[3-^{3}H]glucose (PerkinElmer, Life and Analytical Sciences, Boston, MA) was used for glucose kinetics, with prime of 22 μCi (814 kBq) in control subjects and 30 μCi (1,110 kBq) in type 2 diabetic patients and continuous infusion of 0.22 μCi/min (8.14 kBq/min). A primed infusion of human insulin (Humulin-R; Eli Lilly Canada, Toronto, ON, Canada) was started 180 min later and then maintained at 40 mU/m^2 per min. At 184 min, the amino acid infusion (10% TrophAmine without electrolytes; B Braun Medical, Irvine, CA) was started and rates adjusted based on plasma BCAA, determined every 5 min. Potato starch–derived glucose (20% [wt/vol]; Avebe, Foxhol, Netherlands) with added D-[3-^{3}H]glucose ("hot ginf" [30]) was infused with rates adjusted every 5 min.

Postabsorptive and clamp physiological and isotopic steady states were attained. Blood was collected for substrates, hormones, and glucose and leucine kinetic determinations at baseline, every 10 min for 40 min before insulin, every 30 min until the last 40 min, and then every 10 min. Expired air samples were collected and then stored in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Indirect calorimetry was performed for 20 min before and during the last 30 min of the clamp, and data used for calculation of leucine oxidation, nonprotein respiratory quotient, and glucose, protein, and fat oxidation.

Leucine kinetics were calculated according to ref. 31, using plasma [^{13}C]α-ketoisocaproic acid (α-KIC) enrichment (reciprocal model), providing leucine total Ra (flux), oxidation, endogenous rates of appearance (R_a) (protein breakdown), nonoxidative rates of disappearance (R_d) (protein synthesis), and net endogenous balance (synthesis minus breakdown). Recovery factors of ^{13}C from the bicarbonate pool for the calculation of postabsorptive and clamp leucine oxidation were 0.671 and 0.799, respectively (20). Glucose turnover was calculated using OOPSEG (32). In subjects with type 2 diabetes, 20% glucose was started when euglycemia was reached during insulin infusion. Postabsorptive and clamp isotopic steady states for glucose specific activity and α-KIC enrichment were designated based on a slope not different from zero for the last 30 min of each period. In the calculation of leucine oxidation rates, correction was made for the dilution effect of $^{13}CO_2$ due to the low ^{13}C content of the glucose infusion, as previously described (20). The correction factor in additional clamp studies without ^{13}C leucine in some type 2 diabetic and obese control subjects was 7% in both groups. Postabsorptive and clamp $^{13}CO_2$ recovery factors used also took this dilution effect into account.

Assays. The ^{13}C enrichment of plasma α-KIC was analyzed by gas chromatography–mass spectrometry with electron-impact ionization (GCMS 5988A; Hewlett-Packard, Palo Alto, CA), after derivatization with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (Regis Technologies, Morton Grove, IL) (21,23). Expired air was analyzed for $^{13}CO_2$ enrichment by isotope ratio mass spectrometry (Micromass 903D; Vacuum Generators, Winsforce, U.K.) (20). Glucose was determined by the glucose oxidase method (GM7 Micro-Stat; Analox Instruments USA, Lunenberg, MA) and BCAA by an enzymic fluorometric assay (20). The radioimmunoassays for insulin and glucagon and analysis of glucose specific activity were previously documented (30,33). Free fatty acids (FFAs) were measured using the NEFA C kit (Wako Chemicals USA, Richmond, VA). Reverse-phase high-performance liquid chromatography (Beckman Coulter Canada, Mississauga, ON, Canada) with automated precolumn *o*-phthaldialdehyde derivatization was used to determine plasma amino acids.

Statistical analyses. Results are presented as means ± SE. Subject characteristics of both sexes were compared separately by unpaired *t* test, and steady-state baseline and clamp hormone and substrate concentrations were compared using ANOVA. Kinetics were compared between control and type 2 diabetic groups separately by sex, by ANCOVA, with FFM as a covariate when found to have a predictive value from prior regression analysis (23,34). Responses to the clamp were analyzed using repeated-measures ANOVA (clamp as within-subject factor and type 2 diabetes as between-subject factor) for each sex. Unpaired *t* tests were used to compare the percent change in leucine kinetics in subjects with and without type 2 diabetes. Homeostasis model assessment of insulin resistance (HOMA-IR) and fasting insulin values were log transformed to yield a normal distribution. Stepwise linear regression analysis was conducted to assess for independent predictors of flux and synthesis among those found by simple correlations. Significance was set at

TABLE 2
Substrate and hormone concentrations at baseline and during clamp

	Male control subjects	Male type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)	Female control subjects	Female type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)
Plasma glucose (mmol/l)*						
Baseline	5.09 ± 0.08	9.11 ± 0.55†		4.97 ± 0.08	8.29 ± 0.77†	
Clamp	5.50 ± 0.02	5.53 ± 0.03	<0.001	5.53 ± 0.02	5.52 ± 0.02	0.002
BCAA (μmol/l)						
Baseline	438 ± 24	461 ± 17		390 ± 11	395 ± 14	
Clamp	436 ± 23	455 ± 17	—	397 ± 8	400 ± 20	—
AA infusion rate (mg/min)	43 ± 2	40 ± 2		33 ± 1	33 ± 2	
IAAs (μmol/l)‡						
Baseline	933 ± 36	1,024 ± 27		868 ± 24	876 ± 53	
Clamp	956 ± 36	1,031 ± 31	—	928 ± 21	913 ± 57	—
Percent change	2.7 ± 1.5	0.7 ± 1.6		7.3 ± 1.6	4.3 ± 2.1	
Total AAs (μmol/l)§						
Baseline	2,399 ± 59	2,667 ± 57†		2,375 ± 31	2,367 ± 99	
Clamp	2,344 ± 61	2,556 ± 48†	—	2,405 ± 29	2,324 ± 109	—
Percent change	–2.2 ± 1.5	–3.9 ± 1.5		1.3 ± 1.1	–1.9 ± 0.9	
FFA (μmol/l)*						
Baseline	536 ± 61	610 ± 42		777 ± 34	854 ± 63	
Clamp	103 ± 4	166 ± 21†	—	118 ± 17	184 ± 18†	—
Insulin (pmol/l)*						
Baseline	71 ± 10	98 ± 13		83 ± 9	121 ± 16†	
Clamp	605 ± 59	525 ± 19	—	583 ± 33	555 ± 28	—
Glucagon (pmol/l)*						
Baseline	27 ± 3	27 ± 4		22 ± 1	22 ± 2	
Clamp	19 ± 2	28 ± 4†	<0.001	16 ± 1	21 ± 1†	0.007

Data are means ± SE. Diabetes effect was sought at baseline and clamp steady states by ANOVA. The response to the clamp was analyzed by repeated-measures ANOVA; *P* values are stated where significant interaction is found (i.e., response to clamp is different between control and type 2 diabetes groups). *$P < 0.05$, clamp effect in all groups. †$P < 0.05$, type 2 diabetic vs. control subjects of the same sex. ‡$P < 0.05$, clamp effect in female subjects. §$P < 0.05$, clamp effect in male subjects. AA, amino acid; IAA, indispensable amino acid.

0.05 and power at 80%. A minimum sample size of seven per group was estimated with change in net leucine balance as end point and SDs from our previous studies. Analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL).

RESULTS

All subjects were overweight or obese (Table 1). Within each sex, age, body composition, and body fat distribution did not differ between type 2 diabetic and control subjects. HOMA-IR was higher in type 2 diabetic subjects. Two-hour oral glucose tolerance test glycemia was 7.1 ± 0.3 mmol/l in control subjects. Diabetes duration was 6 ± 1 years. Substrates and hormones are presented in Table 2. Baseline hyperglycemia in type 2 diabetic subjects was by design, but total BCAA, indispensable amino acids, and FFA were not different. In men, dispensable amino acids being higher (data not shown), total amino acid levels were higher in type 2 diabetic subjects. Results of individual amino acids are presented in online appendix 2. Insulin was significantly higher in female type 2 diabetic subjects. Baseline glucagon was not different. Sex differences included higher FFA and lower BCAA in women ($P < 0.05$).

The clamp glycemic goal of 5.5 mmol/l was reached in all groups, but it took 76 ± 11 min in the type 2 diabetic subjects; BCAA were clamped at baseline levels with amino acid infusion rates that did not differ in type 2 diabetes but were lower in women ($P < 0.05$), even when adjusted for FFM. Small but significant changes in indispensable amino acids and total amino acids were not different between control and type 2 diabetic subjects. FFAs were suppressed equally but to higher clamp levels in type 2 diabetes. Insulin reached the same typical postprandial concentrations among groups. Glucagon declined in control subjects but not in type 2 diabetic subjects.

Results of plasma [^{13}C]α-KIC and expired $^{13}CO_2$ enrichments are shown in online appendix 3. Coefficients of variation of enrichment at each plateau were less than 3% in all groups. Leucine kinetics (Table 3) did not correlate with either clamp insulin or its change from baseline when controlled for FFM. Thus, no adjustments for insulin were necessary. At baseline, within each sex, there was no type 2 diabetes effect in any kinetic variable, whether as absolute values or adjusted for FFM (data not shown), despite the presence of hyperinsulinemia and hyperglycemia. In contrast, the clamp increase in flux (total R_a) was smaller in male type 2 diabetic subjects. Protein breakdown (endogenous R_a) decreased equally in type 2 diabetic and control subjects, but percent change was less in women ($P < 0.001$). Leucine infusion rates were not different in type 2 diabetic versus control subjects. There was a significant increase in protein synthesis (nonoxidative R_d) in all groups except male type 2 diabetic subjects. Leucine oxidation increased comparably in all groups, but percent increase was greater in women ($P = 0.039$). These kinetic responses resulted in an increase in net endogenous balance, which did not differ between type 2 diabetic and control subjects but was positive in control men and greater in men than women ($P < 0.001$).

Endogenous glucose R_a was higher at baseline in type 2 diabetic subjects and decreased by a comparable amount with control subjects to higher clamp rates (Table 4). Glucose infusion rates were much lower in type 2 diabetic

TABLE 3
Postabsorptive and clamp whole-body leucine kinetics

Leucine kinetics (μmol/min)	Male control subjects	Male type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)	Female control subjects	Female type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)
Total R_a*						
Baseline	164 ± 6	169 ± 10		126 ± 8	140 ± 10	
Clamp	177 ± 6	173 ± 9	0.016	141 ± 9	152 ± 11	—
Endogenous R_a*						
Baseline	164 ± 6	169 ± 10		126 ± 8	140 ± 10	
Clamp	131 ± 6	131 ± 8	—	106 ± 8	118 ± 10	—
Leucine infusion rate						
Clamp	46 ± 2	43 ± 2		35 ± 1	35 ± 2	
Nonoxidative R_d*†						
Baseline	129 ± 4	134 ± 8		102 ± 6	112 ± 7	
Clamp	136 ± 4	130 ± 7	0.001	105 ± 6	116 ± 8	—
Oxidation*						
Baseline	35 ± 3	36 ± 3		24 ± 2	28 ± 3	
Clamp	41 ± 2	43 ± 2	—	36 ± 3	36 ± 3	—
Net endogenous balance*						
Baseline	–35 ± 3	–36 ± 3		–24 ± 2	–28 ± 3	
Clamp	5 ± 2	0 ± 2	—	0 ± 3	–2 ± 2	—

Data are means ± SE. Each sex is analyzed separately. Diabetes effect was sought at baseline and clamp steady states by one-way ANCOVA with FFM as covariate. The response to clamp was analyzed by repeated-measures ANOVA; *P* values are stated where significant interaction is found between control and type 2 diabetic groups. Total R_a, total leucine flux, including exogenous leucine infusion during the clamp period; Endogenous R_a, index of protein breakdown; Nonoxidative R_d, index of protein synthesis; Net balance, protein synthesis minus breakdown. *$P < 0.05$, clamp effect in all groups. †$P < 0.05$, no clamp effect in male type 2 diabetic subjects.

subjects. Total R_d in type 2 diabetes was higher at baseline and increased less to lower clamp levels. Oxidative R_d did not differ at baseline and increased to lower values in female type 2 diabetic patients. Nonoxidative R_d in type 2 diabetic subjects was higher at baseline but decreased to lower clamp values, whereas it increased in control subjects. Fat oxidation decreased equally during the clamp in all groups but was higher in female type 2 diabetic subjects both at baseline and during the clamp—even when adjusted for FFM. There was no type 2 diabetes effect on REE at baseline or during the clamp. It increased during the clamp only in men. Glucose infusion rates and clamp nonoxidative R_d adjusted for FFM were higher in male than in female control subjects ($P < 0.05$).

Controlled for FFM, postabsorptive leucine flux and protein synthesis correlated positively with fasting plasma insulin in men ($r = 0.506$, $P = 0.023$ and $r = 0.528$, $P = 0.017$, respectively) and leucine flux correlated with fast-

TABLE 4
Postabsorptive and clamp glucose kinetics and energetics

	Male control subjects	Male type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)	Female control subjects	Female type 2 diabetic	Type 2 diabetes effect on clamp response (*P*)
Endogenous R_a (mg/min)*						
Baseline	152 ± 7	188 ± 12†		121 ± 3	168 ± 13†	
Clamp	12 ± 10	45 ± 12†	—	3 ± 6	35 ± 7†	—
Infusion rate (mg/min)	418 ± 31	185 ± 25†		320 ± 22	164 ± 17†	
Total R_d (mg/min)*						
Baseline	152 ± 7	202 ± 11†		121 ± 5	175 ± 16†	
Clamp	430 ± 31	232 ± 19†	< 0.001	326 ± 21	202 ± 14†	<0.001
Oxidative R_d (mg/min)*						
Baseline	84 ± 16	60 ± 19		47 ± 7	34 ± 7	
Clamp	154 ± 15	132 ± 15	—	135 ± 8	89 ± 9†	0.019
Nonoxidative R_d (mg/min)*						
Baseline	68 ± 15	143 ± 20†		74 ± 8	141 ± 14†	
Clamp	276 ± 30	100 ± 15†	<0.001	191 ± 22	112 ± 12†	0.001
Fat oxidation (mg/min)*						
Baseline	61 ± 6	75 ± 7		66 ± 5	86 ± 6†	
Clamp	36 ± 6	46 ± 9	—	29 ± 5	59 ± 7†	—
REE (kcal/day)‡						
Baseline	1,782 ± 60	1,836 ± 74		1,516 ± 78	1,741 ± 102	
Clamp	1,856 ± 47	1,878 ± 83	—	1,539 ± 83	1,707 ± 97	—

Data are means ± SE. Each sex is analyzed separately. One-way ANCOVA, with type 2 diabetes as main effect and FFM as covariate, was used at baseline and clamp steady states. The response to clamp was analyzed by repeated-measures one-way ANOVA, with type 2 diabetes as main effect. *P* values are stated where significant interaction was found. *$P < 0.05$, clamp effect in all groups. †$P < 0.05$, type 2 diabetic vs. control subjects of the same sex adjusted for FFM. ‡$P < 0.05$, clamp effect in male subjects. EGP, endogenous glucose production.

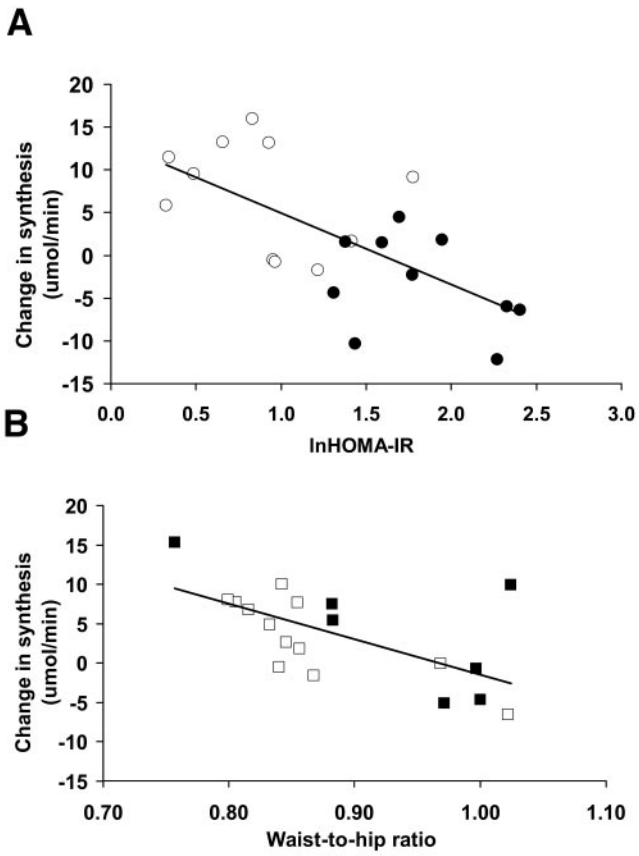

FIG. 1. Simple linear correlations between change in synthesis in μmol/min and lnHOMA-IR in men ($r = -0.665$, $P = 0.001$) (*A*) and waist-to-hip ratio in women ($r = -0.629$, $P = 0.004$) (*B*). ○, control subjects; ●, type 2 diabetic subjects.

ing insulin ($r = 0.541$, $P = 0.020$) and waist circumference ($r = 0.579$, $P = 0.012$) in women. Stepwise regression analysis showed that FFM and log of fasting insulin were the two significant independent variables that predicted 74% of the variance in postabsorptive flux in men and 87% of that in women.

In men, the change in synthesis correlated with markers of insulin resistance of glucose: lnHOMA-IR (Fig. 1*A*), fasting lninsulin ($r = -0.566$, $P = 0.008$), and fasting plasma glucose ($r = -0.576$, $P = 0.006$), as well as with markers of insulin sensitivity: *M* (glucose infusion rate · $kg^{-1} \cdot min^{-1}$) ($r = 0.557$, $P = 0.009$) and insulin sensitivity index (*M*/clamp insulin, $r = 0.508$, $P = 0.019$). Lesser suppression of protein breakdown correlated with higher fasting lninsulin ($r = -0.450$, $P = 0.041$).

Unlike men, in women, the increase in net leucine balance did not correlate with the increase in protein synthesis—only with that in breakdown ($r = -0.701$, $P = 0.001$) and in oxidation ($r = -0.497$, $P = 0.030$). Changes in synthesis related to body fat distribution, with a significant correlation between waist-to-hip ratio and the change in synthesis (Fig. 1*B*).

It is noteworthy that lnHOMA-IR correlated strongly with lnM and insulin sensitivity index ($r = -0.816$, $P < 0.001$ and $r = -0.786$, $P < 0.001$, respectively) in men and ($r = -0.889$, $P < 0.001$ and $r = -0.813$, $P < 0.001$) in women.

Data for percent changes in leucine kinetics during the clamp are compared with our previous results from lean subjects (nine men: BMI 21.3 ± 0.4 kg/m^2, age 26 ± 1 years FFM 59 ± 1 kg and eight women: BMI 20.8 ± 0.3 kg/m^2, age 24 ± 1 years, FFM 41 ± 1 kg) using the identical protocol, in Fig. 2*A* for men and Fig. 2*B* for women. This emphasizes that the abnormalities in type 2 diabetes, although relatively modest when compared with matched subjects without diabetes, are extremely large compared with lean subjects (20,21).

Stepwise regression analysis of the present study showed that in men, lnHOMA-IR predicted 37% of the variance in the increase in flux and 44% of that in synthesis; in women, waist-to-hip ratio and hip circumference predicted 66% of the variance in the increase in flux, and waist-to-hip ratio predicted 40% of that in synthesis.

DISCUSSION

This study demonstrates that insulin-resistant overweight and obese individuals with type 2 diabetes also have insulin resistance of protein metabolism that is expressed differently in men than in women. While in the postabsorptive state, in both sexes elevated plasma insulin, a marker of insulin resistance, is associated with elevated leucine flux, the response to the clamp is sex determined. The increase in total flux in male type 2 diabetic subjects is

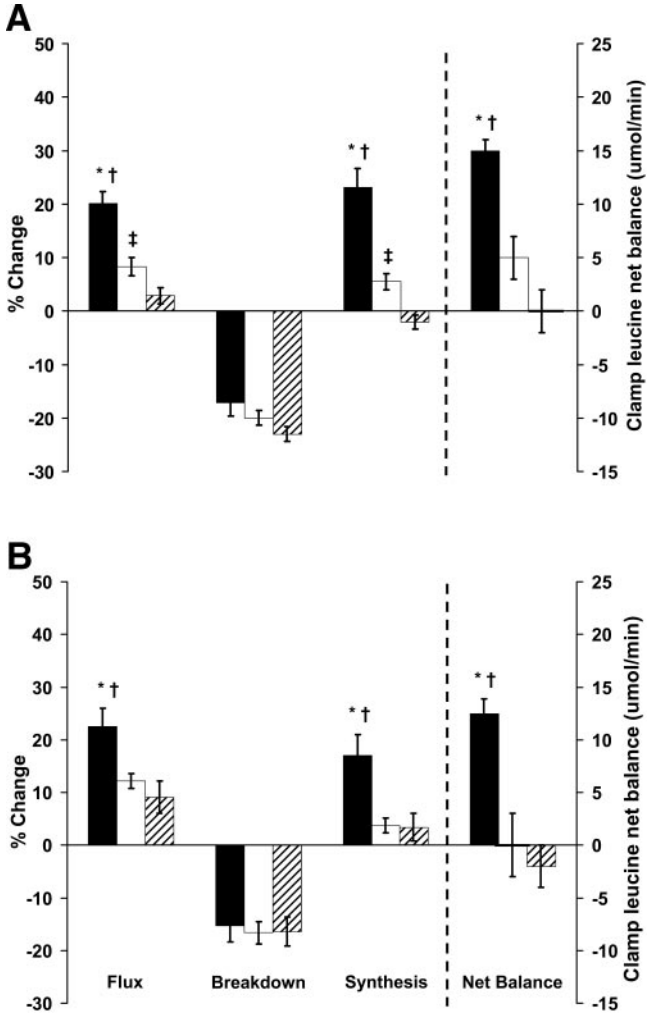

FIG. 2. Percent change in protein flux, breakdown, and synthesis from baseline to clamp and clamp net leucine balance: ■, lean subjects; □, control subjects; ▨, type 2 diabetic subjects. *$P < 0.05$ type 2 diabetic vs. lean subjects, †$P < 0.05$ control vs. lean subjects, ‡$P < 0.05$ type 2 diabetic vs. control subjects. Unpaired *t* tests were used for these comparisons.

blunted, and this difference is driven by failure of stimulation of protein synthesis (Fig. 2*A*). This abnormal response to hyperinsulinemia is predicted by multiple recognized markers of insulin resistance of glucose, indicating concurrent resistance of both. In women, type 2 diabetes does not have an additive effect on the already blunted responses in flux and synthesis of body composition–matched control women (Fig. 2*B*).

Our postabsorptive leucine kinetic results are consistent with most studies comparing type 2 diabetic subjects with weight, BMI, percentage of ideal body weight, or percent body fat–matched control subjects (10–13,16). Elevated fasting rates of leucine oxidation in type 2 diabetes in one study is likely because only the type 2 diabetic subjects were on a hypoenergetic diet (35). The study of type 2 diabetes with highest A1C showing augmented postabsorptive protein breakdown (or flux) suggests that poor diabetes control is an important factor (15). That study and ours found positive correlations of breakdown with fasting insulin. Furthermore, we found a negative correlation between fasting insulin and net balance (all subjects included, $P < 0.02$). Therefore, given their greater hyperinsulinemia, our results support the presence of insulin resistance of postabsorptive protein metabolism in type 2 diabetes, as previously implied (12,13).

By clamping amino acids at fasting levels and preventing the hypoaminoacidemia of conventional clamps, we observe smaller increases in total leucine flux and no response of protein synthesis in type 2 diabetic men. This is due primarily to resistance during hyperinsulinemia at clinically relevant peak concentrations reached by similar subjects after a mixed meal (data not shown). Our protocol maintained euglycemia, constant baseline BCAA, and total indispensable and total amino acids within ranges of interindividual variability and reference postabsorptive concentrations (36). The small changes in individual amino acids observed are unlikely to be of physiological significance compared with hypoaminoacidemia systematically observed in conventional clamp studies that do not report a type 2 diabetes effect (12,13,15).

A sustained glycemia of 5.5 mmol/l was a prerequisite for comparison of leucine and glucose kinetics to those of control subjects. Although this glycemic normalization was brief, it may have attenuated even greater abnormalities in type 2 diabetes. Despite this, there is a significant negative correlation between fasting glycemia and change in synthesis in the men. The smaller insulin-induced increase in REE in diabetes (Table 4) is consistent with the lack of stimulation of protein synthesis, an energy-requiring process (37).

Our fed-fasted ^{15}N-glycine studies showed that protein flux, breakdown, and synthesis are elevated in hyperglycemic type 2 diabetic subjects, while net balance is diminished (6–8). We now show that higher fasting insulin correlates with higher postabsorptive flux and more negative net balance. There is evidence that the accelerated fed-fasted whole-body protein turnover rates in type 2 diabetes likely lie in amino acid and insulin effects captured during the postprandial states. First, amino acid–induced protein anabolism could be impaired in type 2 diabetes, as has been found in cirrhosis, another insulin-resistant disorder (38). Second is the lower postprandial insulin response in type 2 diabetes (39). Third, improving glucose control with insulin or antihyperglycemic agents improves protein metabolism (6,7), and the rates of protein breakdown are proportional to glycemia (40). Hence, lesser postprandial insulin response, greater postprandial hyperglycemia, and impaired amino acid action could elevate proteolysis, thereby increasing the availability of substrates for protein synthesis. Increased amino acid availability also downregulates the insulin signaling pathway (41), thereby creating a cycle of insulin resistance of glucose and protein metabolism that would worsen as metabolic control deteriorates in type 2 diabetes.

The overweight-obese control groups have insulin resistance when compared with lean normal subjects, as previously shown for obesity (21). Male type 2 diabetic subjects have a markedly smaller increment of flux and synthesis than lean subjects and a somewhat smaller increment than control subjects (Fig. 2*A*). Clamp net balance is less than in lean men. Female type 2 diabetic and nondiabetic control subjects both have impaired stimulation of flux and synthesis compared with lean subjects, and clamp net balance is less (Fig. 2*B*). The percent increment in oxidation is not different among groups within each sex, but it is more in women than in men (data not shown). That breakdown inhibition is not different among groups, within each sex, indicates that the level of hyperinsulinemia (that was the same in lean subjects) was sufficient for optimal suppression; therefore, the processes involved may have different insulin dose-response relationships than for synthesis.

Sex differences in leucine kinetics are only present during the clamp. The women have significantly less suppression of protein breakdown than men (16 vs. 22%) (Table 3 and Fig. 2), as previously published (22). This is reflected in lower amino acid infusion rates, also consistent with less insulin sensitivity of protein metabolism. The increase in net balance in men results from the magnitudes in the decrease in breakdown, increase in synthesis, and lower rates of oxidation. In women, the increase in net balance is due to the magnitude of suppression of protein breakdown concurrent with lesser increases in oxidation. Unlike in men, rates of synthesis did not relate to net balance. The fates of amino acids from infusion and from protein breakdown differ between male and female control subjects: percent increases in rates of synthesis are comparable, but those of oxidation are higher in women (40 vs. 18%, $P = 0.039$). This could be interpreted as "glucose sparing," suggested by their lower rates of glucose infusion and insulin-mediated nonoxidative R_d.

Of note is that the sex differences in clamp glucose R_d in control subjects are not observed in type 2 diabetic subjects. In the latter, clamp FFA concentrations are higher, fat oxidation is higher (significantly so in women), and endogenous glucose production is less suppressed, such that glucose infusion rates and disposal are substantially less in both male and female type 2 diabetic versus control subjects. This coexistence of insulin resistance of glucose and protein metabolism is reinforced by HOMA-IR as a predictor of the changes in flux and synthesis and the negative correlation between the change in synthesis and HOMA-IR in men (Fig. 1*A*). The negative correlation between the change in protein synthesis and waist-to-hip ratio in women (Fig. 1*B*) suggests that body fat distribution has a greater impact on protein metabolism than in men. This is consistent with a study showing that larger hip circumferences have a "protective" effect on insulin sensitivity of glucose (42).

The relative importance of insulin versus amino acids in stimulating protein synthesis and anabolism in humans

depends on their concentrations and availability. In skeletal muscle, where most protein turnover occurs in the postprandial state, both insulin and amino acids stimulate synthesis, but their complementary effects vary according to their concentrations (18). It has been suggested that insulin does not have additional effects on protein synthesis during hyperaminoacidemia (17). However, increasing amino acid concentrations with insulin at 500–600 pmol/l increased whole-body protein synthesis stepwise (17). Since we maintained isoaminoacidemia at comparable amino acid infusion rates, it is probable that the differences are due to insulin resistance.

Our results suggest a change is required in the prevailing view that protein metabolism is "normal" in type 2 diabetes. Prior studies included *1*) study groups having both sexes (15) or only women (10,11), *2*) differing adjustments for body composition (10–16,35), *3*) no prior diet control (12,35), *4*) presence of comorbidities (11,35), *5*) insulin therapy overlapping the kinetic study (11,12) attenuating possible differences, *6*) pharmacologic hyperinsulinemia (15), *7*) lean type 2 diabetic subjects (13,35), and *8*) use of conventional hyperinsulinemic-euglycemic clamps (12,13, 15). To our knowledge, no prior clamp study in type 2 diabetes was isoaminoacidemic.

Thus, using a clamp that maintains postabsorptive amino acids and glycemia and postprandial-level hyperinsulinemia helps resolve prior controversies regarding insulin resistance of protein metabolism in overweight and obese individuals with type 2 diabetes. The magnitude of this defect is considerable when compared with lean normal subjects, but much of it is due to adiposity itself (21). In addition, the sex influence on the protein anabolic action of insulin (22) contributes to differences in responses in type 2 diabetes. These findings have implications for dietary protein requirements, which are likely to be influenced by diabetes control, concurrent obesity or overweight, energy restriction, and sex.

ACKNOWLEDGMENTS

This study was supported by grants from the Canadian Institutes of Health Research (MOP-94059 and MOP-77562 to R.G.) and salary awards to R.G. and S.P. from the McGill University Health Centre Research Institute, to S.C. from the Canadian Diabetes Association, and to J.A.M. from the Fonds de recherche en santé du Québec.

We thank Mary Shingler, Josie Plescia, Karen French, Marie Lamarche, Ginette Sabourin, Concettina Nardolillo, Madeleine Giroux, and Paul Meillon for their assistance.

REFERENCES

1. Bennet WM, Connacher AA, Jung RT, Stehle P, Rennie MJ: Effects of insulin and amino acids on leg protein turnover in IDDM patients. *Diabetes* 40:499–508, 1991
2. Tessari P, Nosadini R, Trevisan R, De Kreutzenberg SV, Inchiostro S, Duner E, Biolo G, Marescotti MC, Tiengo A, Crepaldi G: Defective suppression by insulin of leucine-carbon appearance and oxidation in type 1, insulin-dependent diabetes mellitus: evidence for insulin resistance involving glucose and amino acid metabolism. *J Clin Invest* 77:1797–1804, 1986
3. Nair KS, Ford GC, Halliday D: Effect of intravenous insulin treatment on in vivo whole body leucine kinetics and oxygen consumption in insulin-deprived type I diabetic patients. *Metabolism* 36:491–495, 1987
4. Luzi L, Castellino P, Simonson DC, Petrides AS, DeFronzo RA: Leucine metabolism in IDDM: role of insulin and substrate availability. *Diabetes* 39:38–48, 1990
5. Umpleby AM, Boroujerdi MA, Brown PM, Carson ER, Sonksen PH: The effect of metabolic control on leucine metabolism in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 29:131–141, 1986
6. Gougeon R, Styhler K, Morais JA, Jones PJ, Marliss EB: Effects of oral hypoglycemic agents and diet on protein metabolism in type 2 diabetes. *Diabetes Care* 23:1–8, 2000
7. Gougeon R, Marliss EB, Jones PJ, Pencharz PB, Morais JA: Effect of exogenous insulin on protein metabolism with differing nonprotein energy intakes in type 2 diabetes mellitus. *Int J Obes Relat Metab Disord* 22:250–261, 1998
8. Gougeon R, Pencharz PB, Marliss EB: Effect of NIDDM on the kinetics of whole-body protein metabolism. *Diabetes* 43:318–328, 1994
9. Gougeon R, Pencharz PB, Sigal RJ: Effect of glycemic control on the kinetics of whole-body protein metabolism in obese subjects with non-insulin-dependent diabetes mellitus during iso- and hypoenergetic feeding. *Am J Clin Nutr* 65:861–870, 1997
10. Welle S, Nair KS: Failure of glyburide and insulin treatment to decrease leucine flux in obese type II diabetic patients. *Int J Obes* 14:701–710, 1990
11. Staten MA, Matthews DE, Bier DM: Leucine metabolism in type II diabetes mellitus. *Diabetes* 35:1249–1253, 1986
12. Halvatsiotis PG, Turk D, Alzaid A, Dinneen S, Rizza RA, Nair KS: Insulin effect on leucine kinetics in type 2 diabetes mellitus. *Diabetes Nutr Metab* 15:136–142, 2002
13. Luzi L, Petrides AS, De Fronzo RA: Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. *Diabetes* 42:1868–1877, 1993
14. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Sabadin L, Fongher C, Panebianco G, Fratton MG, Tiengo A: Fasting and postmeal phenylalanine metabolism in mild type 2 diabetes. *Am J Physiol* 263:E877–E883, 1992
15. Denne SC, Brechtel G, Johnson A, Liechty EA, Baron AD: Skeletal muscle proteolysis is reduced in noninsulin-dependent diabetes mellitus and is unaltered by euglycemic hyperinsulinemia or intensive insulin therapy. *J Clin Endocrinol Metab* 80:2371–2377, 1995
16. Halvatsiotis P, Short KR, Bigelow M, Nair KS: Synthesis rate of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes. *Diabetes* 51:2395–2404, 2002
17. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of insulin and plasma amino acid concentrations on leucine metabolism in man: role of substrate availability on estimates of whole body protein synthesis. *J Clin Invest* 80:1784–1793, 1987
18. Nygren J, Nair KS: Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. *Diabetes* 52:1377–1385, 2003
19. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D: Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia* 25:400–403, 1983
20. Chevalier S, Gougeon R, Kreisman SH, Cassis C, Morais JA: The hyperinsulinemic amino acid clamp increases whole-body protein synthesis in young subjects. *Metabolism* 53:388–396, 2004
21. Chevalier S, Marliss EB, Morais JA, Lamarche M, Gougeon R: Whole-body protein anabolic response is resistant to the action of insulin in obese women. *Am J Clin Nutr* 82:355–365, 2005
22. Chevalier S, Marliss EB, Morais JA, Lamarche M, Gougeon R: The influence of sex on the protein anabolic response to insulin. *Metabolism* 54:1529–1535, 2005
23. Chevalier S, Gougeon R, Choong N, Lamarche M, Morais JA: Influence of adiposity in the blunted whole-body protein anabolic response to insulin with aging. *J Gerontol A Biol Sci Med Sci* 61:156–164, 2006
24. Otten JJ, Pitzi Hellwig J, Myers LD: *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC, The National Academies Press, 2006, p. 144–155
25. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee: Canadian Diabetes Association 2003 Clinical Practice Guidelines for the prevention and management of diabetes in Canada. *Canadian J Diabetes* 27 (Suppl. 2):S1–S152, 2003
26. World Health Organization: *Physical Status: The Use and Interpretation of Anthropometry: Report of a WHO Expert Committee*. Geneva, World Health Org., 1995
27. Goran MI, Poehlman ET, Danforth E Jr, Nair KS: Comparison of body fat estimates derived from underwater weight and total body water. *Int J Obes Relat Metab Disord* 18:622–626, 1994
28. Kushner RF, Kunigk A, Alspaugh M, Andronis PT, Leitch CA, Schoeller DA: Validation of bioelectrical-impedance analysis as a measurement of change in body composition in obesity. *Am J Clin Nutr* 52:219–223, 1990 [See comment]
29. Roubenoff R, Baumgartner RN, Harris TB, Dallal GE, Hannan MT, Economos CD, Stauber PM, Wilson PW, Kiel DP: Application of bioelectrical impedance analysis to elderly populations. *J Gerontol A Biol Sci Med Sci* 52:M129–M136, 1997
30. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: compar-

ison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
31. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM: Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1–3C]leucine. *Am J Physiol* 238:E473–E479, 1980
32. Bradley DC, Steil GM, Bergman RN: Quantitation of measurement error with Optimal Segments: basis for adaptive time course smoothing. *Am J Physiol* 264:E902–E911, 1993
33. Sigal RJ, Purdon C, Fisher SJ, Halter JB, Vranic M, Marliss EB: Hyperinsulinemia prevents prolonged hyperglycemia after intense exercise in insulin-dependent diabetic subjects. *J Clin Endocrinol Metab* 79:1049–1057, 1994
34. Bogardus C, Ravussin E, Robbins DC, Wolfe RR, Horton ES, Sims EA: Effects of physical training and diet therapy on carbohydrate metabolism in patients with glucose intolerance and non-insulin-dependent diabetes mellitus. *Diabetes* 33:311–318, 1984
35. Umpleby AM, Scobie IN, Boroujerdi MA, Carson ER, Sonksen PH: Diurnal variation in glucose and leucine metabolism in non-insulin-dependent diabetes. *Diabetes Res Clin Pract* 9:89–96, 1990
36. Luzi L, Castellino P, DeFronzo RA: Insulin and hyperaminoacidemia regulate by a different mechanism leucine turnover and oxidation in obesity. *Am J Physiol* 270:E273–E281, 1996
37. Welle S, Nair KS: Relationship of resting metabolic rate to body composition and protein turnover. *Am J Physiol* 258:E990–E998, 1990
38. McCullough AJ, Mullen KD, Kalhan SC: Defective nonoxidative leucine degradation and endogenous leucine flux in cirrhosis during an amino acid infusion. *Hepatology* 28:1357–1364, 1998
39. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50:609–613, 2001
40. Flakoll PJ, Hill JO, Abumrad NN: Acute hyperglycemia enhances proteolysis in normal man. *Am J Physiol* 265:E715–E721, 1993
41. Tremblay F, Marette A: Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway: a negative feedback mechanism leading to insulin resistance in skeletal muscle cells. *J Biol Chem* 276:38052–38060, 2001
42. Buemann B, Sorensen TIA, Pedersen O, Black E, Holst C, Toubro S, Echwald S, Holst JJ, Rasmussen C, Astrup A: Lower-body fat mass as an independent marker of insulin sensitivity–the role of adiponectin. *Int J Obes* 29:624–631, 2005