

# Synthesis Rate of Muscle Proteins, Muscle Functions, and Amino Acid Kinetics in Type 2 Diabetes

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**Improvement of glycemic status by insulin is associated with profound changes in amino acid metabolism in type 1 diabetes. In contrast, a dissociation of insulin effect on glucose and amino acid metabolism has been reported in type 2 diabetes. Type 2 diabetic patients are reported to have reduced muscle oxidative enzymes and  $VO_{2max}$ . We investigated the effect of 11 days of intensive insulin treatment ( $T_2D+$ ) on whole-body amino acid kinetics, muscle protein synthesis rates, and muscle functions in eight type 2 diabetic subjects after withdrawing all treatments for 2 weeks ( $T_2D-$ ) and compared the results with those of weight-matched lean control subjects using stable isotopes of the amino acids. Whole-body leucine, phenylalanine and tyrosine fluxes, leucine oxidation, and plasma amino acid levels were similar in all groups, although plasma glucose levels were significantly higher in  $T_2D-$ . Insulin treatment reduced leucine nitrogen flux and transamination rates in subjects with type 2 diabetes. Synthesis rates of muscle mitochondrial, sarcoplasmic, and mixed muscle proteins were not affected by glycemic status or insulin treatment in subjects with type 2 diabetes. Muscle strength was also unaffected by diabetes or glycemic status. In contrast, the diabetic patients showed increased tendency for muscle fatigability. Insulin treatment also failed to stimulate muscle cytochrome C oxidase activity in the diabetic patients, although it modestly elevated citrate synthase. In conclusion, improvement of glycemic status by insulin treatment did not alter whole-body amino acid turnover in type 2 diabetic subjects, but leucine nitrogen flux, transamination rates, and plasma ketoisocaproate level were decreased. Insulin treatments in subjects with type 2 diabetes had no effect on muscle mitochondrial protein synthesis and cytochrome C oxidase, a key enzyme for ATP production. *Diabetes* 51:2395–2404, 2002**

**A** metabolic abnormality common to type 1 and type 2 diabetic patients is altered glucose metabolism. Increased endogenous glucose production and hyperglycemia are characteristic when untreated in these two types of diabetic patients. Type 1 diabetic subjects are C-peptide negative and insulin deficient, whereas type 2 diabetic subjects have high to low levels of circulating insulin (1–4) and are relatively resistant to insulin effect on glucose disposal (5). Transient withdrawal of insulin treatment in patients with type 1 diabetes is associated with a substantial increase in circulating amino acid concentrations (especially of those of branched-chain amino acids), urinary nitrogen loss, and whole-body protein turnover (1,2,6). In contrast, in type 2 diabetic subjects, withdrawal or intensifying insulin treatment has little impact on leucine kinetics (7,8), although glucose metabolism is substantially altered (7). It has, however, been reported that during hyperinsulinemic-euglycemic clamp, the decline in leucine flux in patients with type 2 diabetes is identical to that of nondiabetic control subjects, whereas the insulin-induced glucose disposal in type 2 diabetes is lower than in control subjects (9). One potential explanation is that the maximal effect on leucine kinetics is achieved at a level of circulating insulin that is insufficient to inhibit glucose production. However, studies performed using the urea end product method indicated that in type 2 diabetic patients, even when hyperinsulinemic, urinary nitrogen loss and net body protein loss are enhanced (10–12). The latter studies were performed using [ $^{15}N$ ]glycine as a tracer, whereas the amino acid kinetic studies (7–9,13) were conducted using carboxy-labeled leucine or labeled phenylalanine as tracers. Except for one study involving lean type 2 diabetic patients (13), no studies have demonstrated an increased leucine oxidation or net increase in protein breakdown in people with type 2 diabetes. It is possible that leucine oxidation may not truly reflect net urinary nitrogen loss in all physiological and pathological states (14,15). For example, there is an increased gluconeogenesis in type 2 diabetic subjects (16) when the circulating glucose levels are high, although their leucine flux is usually normal in this situation (7). Increased gluconeogenesis involves consumption of glucogenic amino acids that could theoretically increase urinary nitrogen loss. It is possible that levels of essential amino acids, such as leucine, are maintained by increasing transamination rates without necessarily increasing oxidation of ketoisocaproate, the deamination product of leucine. It has also been noted that leucine transamination rate increases in type 1 diabetic

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AI $_g$ , acute insulin response to glucose; FFM, fat-free mass; FSR, fractional synthetic rate; GCRC, General Clinical Research Center; HPLC, high-performance liquid chromatography; IRMA, immunoradiometric assay;  $S_I$ , insulin sensitivity;  $T_2D+$ , intensive insulin treatment group;  $T_2D-$ , treatment withdrawn group.

TABLE 1  
Physical characteristics of the subjects

Variable	Lean control subjects	Weight-matched control subjects	Type 2 diabetic subjects	ANOVA <i>P</i> value
Age (years)	58 ± 2	56 ± 2	56 ± 3	0.901
Height (cm)	174 ± 4	170 ± 3	170 ± 4	0.696
Body weight (kg)	76 ± 4	87 ± 4	86 ± 4	0.099
BMI (kg/m <sup>2</sup> )	25.0 ± 0.5	30.1 ± 1.0*	29.7 ± 0.9*	0.001
FFM (kg)	49.3 ± 4.5	51.2 ± 3.7	52.8 ± 4.6	0.850
Fat mass (kg)	21.4 ± 1.8	29.6 ± 3.1	27.8 ± 2.4	0.073
Total abdominal fat (cm <sup>2</sup> )	265 ± 31	412 ± 51*	417 ± 35*	0.017
Visceral fat (cm <sup>2</sup> )	91 ± 18	158 ± 21*	185 ± 21*	0.008
Muscle area per leg (cm <sup>2</sup> )	116 ± 8	135 ± 9	116 ± 9	0.242
Fat area per leg (cm <sup>2</sup> )	70 ± 13	85 ± 14	91 ± 23	0.674

Data are means ± SE for eight subjects (four women and four men) per group. Fat mass and FFM determined by dual X-ray absorptiometry. Abdominal and visceral fat and muscle and fat area per leg were determined from single-slice computed tomography scans of the abdomen and mid-thigh, respectively. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and type 2 diabetic patients. \*Greater than lean, *P* < 0.05.

subjects during insulin deprivation and that this effect on leucine transamination is substantially higher than insulin effect of leucine carbon flux (2). Although insulin treatment or withdrawal of hypoglycemic treatment has no effect on leucine carbon flux in type 2 diabetic patients (7), the effect of withdrawal of treatment on leucine transamination rate in type 2 diabetic patients has not been investigated. We therefore conducted a study in which leucine kinetics, including leucine transamination and kinetics of phenylalanine and tyrosine, were also measured in type 2 diabetic subjects to further determine the impact of withdrawal of treatment and BMI on protein turnover in humans.

We also measured muscle functions,  $VO_{2max}$ , and muscle mitochondrial protein synthesis in these subjects to test a hypothesis that people with type 2 diabetes have diminished muscle mitochondrial protein synthesis. An association between habitual levels of physical activity and the incidence of type 2 diabetes has been well established (17,18). It has also been reported that the first-degree relatives of people with type 2 diabetes (19) and diabetic patients (20–23) have reduced  $VO_{2max}$ . In addition, it has been reported that oxidative mitochondrial enzymes are reduced in the skeletal muscle of type 2 diabetic subjects (24), suggesting a potential reduction in muscle mitochondrial functions. Recently it was reported that muscle mitochondrial DNA copy numbers were reduced in a group of type 2 diabetic patients, although mitochondrial gene transcript levels were high (25). The ultimate gene expression is synthesis of protein. The effect of diabetes on muscle mitochondrial protein synthesis has not been investigated. A reduced mitochondrial protein synthesis rate (as occurs in aging) (26) may contribute to muscle weakness and decrease in  $VO_{2max}$ . Insulin has been shown to stimulate muscle mitochondrial protein synthesis in a swine model (27). In this study, we also investigated whether muscle mitochondrial, sarcoplasmic, and mixed muscle protein synthesis are altered in people with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

**Materials.** L-[1-<sup>13</sup>C,<sup>15</sup>N]leucine (99 atom percent excess), L-[<sup>15</sup>N]phenylalanine (99), sodium [<sup>13</sup>C]bicarbonate (98), and [<sup>2</sup>H<sub>4</sub>]tyrosine (99) were purchased from Mass Trace (Woburn MA); L-[<sup>2</sup>H<sub>4</sub>] tyrosine (99) was from Cambridge

Isotope Laboratories (Woburn, MA), and L-[<sup>15</sup>N]tyrosine (99) was from Isotec (Miami, OH). Isotopes were tested before use for their isotopic and chemical purity. The isotope solutions were prepared under sterile conditions and determined to be bacterial and pyrogen free before being administered to humans. The protocol was approved by the Institutional Review Board of the Mayo Foundation, and informed consent was obtained from every volunteer before their participation in the study.

**Subjects.** A group of eight people with type 2 diabetes, whose clinical characteristics are shown in Table 1, were studied. Average diabetes duration was 8.5 years (range 3–19). Six of the diabetic patients were on oral hypoglycemic agents, whereas the other two were on diet therapy alone. Two control groups of eight each were also studied. The first group (weight matched) was well matched to the diabetic population in terms of sex, age, and body composition (see Table 1 for demographic data). In the second control group (lean) (BMI 19–25 kg/m<sup>2</sup>), healthy nondiabetic people were matched to the diabetic group for sex and age only but not for BMI and body composition. Members of both control groups did not have any first-degree relatives with diabetes.

**Study protocol.** All female volunteers were studied during the luteal phase of their menstrual cycle. Two separate studies were performed in the diabetic patients, one after a period when their antidiabetic treatments were withdrawn for 2 weeks (T<sub>2</sub>D<sup>-</sup>) and another after an intensive insulin treatment for 11 days (T<sub>2</sub>D<sup>+</sup>). Half of the diabetic patients were studied first during the T<sub>2</sub>D<sup>-</sup> phase, while the other half were studied first during T<sub>2</sub>D<sup>+</sup> phase. For the T<sub>2</sub>D<sup>-</sup> phase, the diabetic patients stopped taking their medication for 2 weeks before the study. For the T<sub>2</sub>D<sup>+</sup> phase, they were treated with daily injections of regular human insulin four times per day during the 11 days before the study. The insulin dose was determined based on blood glucose values monitored before each meal and at bedtime. The target glucose was 80–120 mg/dl, and insulin doses were adjusted to achieve this target. Both the diabetic and nondiabetic patients were on a weight-maintaining diet (provided from the General Clinical Research Center [GCRC] dietetics section) for 3 days before the study and stayed in the General Clinic Research Center overnight before the evening meal. The last subcutaneous injection of insulin was given to the diabetic subjects (T<sub>2</sub>D<sup>+</sup>) at 6:00 P.M. before their supper. At 9:00 P.M., an intravenous insulin infusion was started to maintain euglycemia overnight (80–100 mg/dl) according to a protocol previously described (28). Control subjects and the subjects on T<sub>2</sub>D<sup>-</sup> phase were given constant intravenous normal saline infusion at a rate of 30 mg/h from 9:00 P.M. Participants were given a weighed snack at 10:00 P.M. and then fasted overnight. At 7:00 A.M. the next morning, insulin administration was discontinued and an intravenous glucose tolerance test was performed (glucose infusion = 300 mg/kg) for the determination of insulin sensitivity (S<sub>I</sub>) and acute insulin response to glucose (AIR<sub>g</sub>) (29,30). Body composition was measured on this same day. In all subjects, blood samples were collected for measuring natural abundance of amino acids and CO<sub>2</sub> isotopic enrichment. That evening after the ingestion of a standard meal (6:00 P.M.) and the last subcutaneous insulin administration (postmeal), diabetic patients on T<sub>2</sub>D<sup>+</sup> phase were maintained on a continuous intravenous insulin infusion as during the previous evening. During the T<sub>2</sub>D<sup>-</sup> phase, control and diabetic subjects were on continuous intravenous normal saline infusion at a rate of 30 ml/h. After their 10:00 P.M. weighed snack, all subjects fasted, except for water, until the study was completed. At 9:00 P.M., an indwelling intravenous catheter was

placed in a brachial vein for infusion and kept patent by saline administration. At 2:00 A.M., a bolus injection of ( $^{13}\text{C}$ ) sodium bicarbonate at 0.35 mg/kg fat-free mass (FFM), L-[1- $^{13}\text{C}$ ,  $^{15}\text{N}$ ]leucine at 12.9  $\mu\text{mol/kg}$  FFM, L-[ $^{15}\text{N}$ ]phenylalanine at 7.5  $\mu\text{mol/kg}$  FFM, L-[ $^{15}\text{N}$ ]tyrosine at 2.8  $\mu\text{mol/kg}$  FFM, and L-[ $^2\text{H}_4$ ] tyrosine at 3.5  $\mu\text{mol/kg}$  FFM were given to achieve an early isotopic plateau. A continuous infusion of L-[1- $^{13}\text{C}$ ]leucine (12.9  $\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$ ), Leu[ $^{15}\text{N}$ ]phenylalanine (7.5  $\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$ ), and L-[ $^2\text{H}_4$ ]tyrosine (3.5  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) was started immediately after until 12:00 P.M. (2,31). At 6:00 A.M., a retrograde catheter was inserted in a dorsal hand vein for blood sampling, the hand was kept in a hot box (49°C), and arterialized venous blood samples were collected as previously described (32). From 5 h after the initiation of tracer infusion (at 7:00 A.M.) and for the next 5 h, blood samples were drawn hourly for determination of isotopic enrichment, substrates, and hormonal determination. At 5 and 10 h of isotope infusion, percutaneous muscle biopsy specimens from the vastus lateralis were collected under local anesthesia (33). Samples were rapidly dissected of blood, fat, and connective tissue and then frozen in liquid nitrogen and stored at  $-80^\circ$  until analysis. Expired air samples for  $^{13}\text{CO}_2$  analysis were collected at the same intervals. Indirect calorimetry was performed to measure  $\text{CO}_2$  production and  $\text{O}_2$  consumption for 40 min using Deltrac equipment and software (Sensor-Medics, Yorba Linda, CA), with the last 30 min used for calculation of energy expenditure. The study was completed at 12:00 P.M., and all subjects were discharged from the GCRC after a general meal.

**Body composition.** Fat and FFM were determined using dual X-ray absorptiometry (Lunar DPX-L; Lunar, Madison, WI). A single-slice (6-mm thick) computed tomography scan (Imatron C-150; Imatron, San Francisco, CA) at the level of the L4–L5 intervertebral space was used to measure abdominal fat. Total and subcutaneous abdominal fat areas were estimated by manual planimetry using custom software (34). Visceral fat area was calculated as the difference between total and subcutaneous fat areas.

**Peak aerobic capacity (peak  $\dot{V}\text{O}_2$ ).** At the time of initial screening, a standard incremental treadmill walking test was performed to verify the absence of cardiorespiratory abnormalities. Continuous monitoring of 12-lead electrocardiogram, blood pressure, and expired gases were performed. All subjects were encouraged to give a maximal effort during the test to increase their familiarity with the testing environment and procedures. At least 2 weeks separated the treadmill test from the subsequent test of peak aerobic capacity. Peak  $\dot{V}\text{O}_2$  was measured using an incremental protocol on an electronically braked cycle ergometer. After an initial 3-min stage at 25–50 W, workload was increased by 10–25 W every minute until volitional fatigue was achieved. Expired gases were analyzed using a Perkin Elmer mass spectrometer and MedGraphics software (35).

**Muscle strength testing.** Strength of the knee extensor muscle group was measured using a Kin-Com dynamometer (Chattanooga Group, Hixson, TN). After a warm-up (10 min cycling or walking, plus stretching) and familiarization (10–15 min) period, peak voluntary isometric torque was measured at a knee angle of  $60^\circ$  of flexion. The best of five or six trials, each separated by 1 min rest, was used for analysis. After an additional 5-min familiarization period and a 5-min rest, the rate of muscle fatigue was measured during a series of 30 maximal voluntary contractions at a test speed of  $180^\circ/\text{s}$  (3.14 rad/s). The fatigue rate was calculated from the line of best fit through the data after transformation of torque data to relative values (where peak torque = 100%). The maximal torque recorded within the first three contractions and the fatigue rate were used for comparisons among groups.

**Isotopic enrichment.** The enrichment levels of [1- $^{13}\text{C}$ ,  $^{15}\text{N}$ ] and total [ $^{13}\text{C}$ ]leucine in plasma were determined using an HP5988 gas chromatograph/mass spectrometer by multiple ion monitoring under positive ion methane chemical ionization conditions as previously described (2).  $^{15}\text{N}$ -phenylalanine,  $^{15}\text{N}$ -tyrosine, and  $^2\text{H}_4$ -tyrosine were measured as their *t*-butyldimethylsilyl ester derivatives under electron ionization conditions by monitoring the [M-57] $^+$  fragment ions at  $m/z$  337/336 for phenylalanine and  $m/z$  470/467/466 for tyrosine.  $^{13}\text{C}$ -ketoisocaproate in plasma was determined as its quinoxalino-TMS derivative under electron ionization conditions using an HP5988 gas chromatograph/mass spectrometer (36), and KIC concentration was measured using ketoisovalerate acid as an internal standard (31).  $^{13}\text{CO}_2$  enrichment in the breath samples was measured with isotope ratio mass spectrometry as previously described (37).

**Substrate concentration.** Plasma levels of amino acids were measured by a high-performance liquid chromatography (HPLC) system (HP 1090, 1046 fluorescence detector and cooling system) with precolumn *O*-phthalaldehyde derivatization (38). Glucose was analyzed on site by an analyzer using an enzymatic technique (Beckman Instruments, Fullerton, CA).

**Hormonal assays.** Plasma concentration of insulin was measured using a two-site immunoenzymatic assay, and plasma glucagon levels were measured by a direct double-antibody radioimmunoassay (Linco Research, St. Louis, MO).

Norepinephrine and epinephrine in plasma were measured by reverse-phase HPLC with electrochemical detection after extraction with activated alumina. Cortisol was measured by a competitive binding immunoenzymatic assay on the Access automated immunoassay system (Beckman Instruments, Chaska, MN). Human growth hormone was measured with a two-site immunoenzymatic assay that was also performed on the Access system.

Concentrations of total IGF-I and -II were measured by two-site immunoradiometric assays (IRMAs) after separation from their binding proteins with a simple organic solvent extraction (Diagnostic Systems Laboratories, Webster, TX). IGFBP-1 and -3 were also measured by two-site IRMAs, whereas IGFBP-2 was measured by a double-antibody radioimmunoassay (Diagnostic Systems Laboratories).

DHEA (dehydroepiandrosterone) sulfate was measured by a competitive chemiluminescent immunoassay on the Immulite automated immunoassay system (Diagnostic Products, Los Angeles, CA).

**Muscle protein analysis.** A 150-mg portion of each muscle sample was used for the isolation of mitochondrial and sarcoplasmic protein fractions by differential centrifugation as previously described (26,39,40). Aliquots of the muscle homogenate were used to measure the activities of citrate synthase and cytochrome C oxidase, two representative mitochondrial enzymes (26). A separate 20- to 30-mg piece of muscle was used to prepare total mixed muscle proteins and to isolate free tissue fluid amino acids as previously described (41).

The muscle protein fractions were hydrolyzed overnight in 0.6 mol/l HCl in the presence of cation exchange resin (AG-50; BioRad, Richmond, CA) and purified the next day using a column of the same resin. The amino acids were dried (SpeedVac; Savant Instruments) and then derivatized as their trimethyl acetyl methyl ester. [ $^{13}\text{C}$ ]leucine enrichments in muscle proteins were determined using a gas chromatograph–combustion isotope ratio mass spectrometer (GC-c-IRMS; Finigan MAT, Bremen, Germany) as described (37). Tissue fluid amino acids were derivatized as their *t*-butyldimethylsilyl ester derivatives and analyzed for [ $^{13}\text{C}$ ]leucine enrichments using a gas chromatograph–mass spectrometer (GC-MS; Hewlett-Packard Engine, Avondale, CA) under electron ionization conditions (37,42).

**Calculations.** The fractional synthetic rate (FSR) of mitochondrial and sarcoplasmic proteins was calculated using the equation (43):

$$\text{FSR (\% / h)} = 100 \times (E_{10h} - E_{5h}) / (E_{\text{TF}} \times t)$$

where  $(E_{10h} - E_{5h})$  represents the increment in [ $^{13}\text{C}$ ]leucine enrichment in muscle proteins between 5 and 10 h of infusion,  $E_{\text{TF}}$  is the average enrichment of [ $^{13}\text{C}$ ]leucine in muscle tissue fluid taken from the 5- and 10-h biopsies, and  $t$  is the time of incorporation between the two biopsies, which in this case is 5 h. Previous experiments demonstrated that muscle tissue fluid is the best surrogate measure of enrichment of muscle leucyl tRNA, which is the obligate precursor of protein synthesis (41).

**Leucine kinetics.** For amino acid kinetics calculations, mean values of the isotopic enrichment at all five time points from 5 to 10 h of infusion were used. Leucine carbon flux ( $Q_c$ ) and leucine nitrogen flux ( $Q_n$ ) were estimated by tracer dilution techniques (43) with the equation:  $Q = I[(E_i/E_p) - 1]$ , where  $I$  represents the rate of tracer infusion in  $\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$  and  $E_i$  and  $E_p$  are the enrichment of the tracer in the infusate and the plasma at the isotopic plateau, respectively. Leucine oxidation ( $C$ ) was assessed by using [ $^{13}\text{C}$ ]KIC as the precursor pool (14). Nonoxidative leucine flux, an index of whole-body protein synthesis, was calculated by subtracting leucine oxidation from leucine flux. For  $Q_c$  calculations, the plasma levels of [ $^{13}\text{C}$ ]KIC were used because this measurement better represents the intracellular leucine environment than that of plasma leucine (36,44), whereas for leucine nitrogen flux ( $Q_n$  calculation), the isotopic enrichment of [1- $^{13}\text{C}$ ,  $^{15}\text{N}$ ]leucine at plateau was used (2,45,46). The reversible transamination of leucine, deamination into KIC ( $X_o$ ), and reamination of KIC ( $X_n$ ) back to leucine molecules was calculated with the following formula:  $Q_n - Q_c = X_n = X_o - C$ . Based on this equation, we estimated  $X_n$  and  $X_o$ , since we had measured  $Q_n$ ,  $Q_c$ , and  $C$ .

**Phenylalanine kinetics.** Phenylalanine and tyrosine flux values were calculated based on the same dilution principles as for leucine. The mean plasma enrichment of [ $^{15}\text{N}$ ]phenylalanine from 5 to 10 h was used for phenylalanine flux ( $Q_p$ ), and tyrosine enrichment was used for tyrosine flux [ $^2\text{H}_4$ ]. The phenylalanine conversion to tyrosine (IPT) was calculated as previously reported (2,47). The phenylalanine incorporation into protein for whole body is calculated by subtracting IPT from  $Q_p$  because phenylalanine is either irreversibly converted into tyrosine or incorporated into protein (2).

**Statistics.** Values are expressed as means  $\pm$  SE. We used ANOVA for multiple comparisons among groups. Two-tailed paired and unpaired  $t$  tests were performed for comparisons within or between the groups. The significance of the observed differences from the parametric analysis of the data did not differ from that of the nonparametric analysis.

Multiple comparison procedures also were adopted to answer specific

TABLE 2  
Plasma glucose and insulin levels,  $S_I$ , and  $AIR_g$

Variable	Lean control subjects	Weight-matched control subjects	T <sub>2</sub> D-	T <sub>2</sub> D+	ANOVA <i>P</i> values
Glucose (mmol/l)	5.1 ± 0.1	5.1 ± 0.1	12.6 ± 1.0*†‡	5.5 ± 0.2	0.001, 0.928
Insulin (pmol/l)	28 ± 4	41 ± 12	29 ± 4‡	77 ± 11*†	0.454, 0.005
Insulin sensitivity ( $\times 10^{-5}$ /pmol/l)	7.64 ± 1.54	6.14 ± 1.04	2.03 ± 0.38*†	4.14 ± 1.12	0.019, 0.186
$AIR_g$ (pmol/l)	350.9 ± 53.1	332.3 ± 55.7	27.8 ± 2.3*†‡	86.1 ± 16.3*†	0.001, 0.001

Data are means ± SE. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D- subjects and among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D+ subjects, respectively. \*Different from lean, †different from weight-matched, ‡different from T<sub>2</sub>D+ (*P* < 0.05).

questions of whether one group is different from another specific group (48). Paired *t* tests were performed to determine whether the insulin treatment had any effect on various parameters in people with type 2 diabetes.

## RESULTS

**Physical characteristics.** By design, the lean control subjects had lower weight, BMI, and total abdominal and visceral fat than the type 2 diabetic patients and the weight-matched control subjects, whereas the latter two groups had similar body composition and were similar in all other respects (Table 1).

**Hormones.** Plasma glucose, insulin levels, and  $S_I$  are given in Table 2. The main effect of group was significant (*P* < 0.05) with ANOVA for each of these variables.  $S_I$  was significantly lower in T<sub>2</sub>D- than in lean and weight-matched control subjects.  $AIR_g$  was significantly lower in the diabetic patients than in both control groups in either study phase (T<sub>2</sub>D- and T<sub>2</sub>D+). The  $AIR_g$  was significantly higher in the T<sub>2</sub>D+ phase than the T<sub>2</sub>D- phase.

Levels of selected hormones are shown in Table 3. Only IGF-2, IGFBP-2, IGFBP-3, and C-peptide showed significant group effects (*P* < 0.05) with ANOVA. C-peptide levels were lower in T<sub>2</sub>D+ than in the lean and weight-matched control subjects as well as T<sub>2</sub>D-. IGF-2 levels were significantly higher in T<sub>2</sub>D- than in the lean group. IGFBP-2 and -3 were significantly lower in T<sub>2</sub>D- than in T<sub>2</sub>D+.

**Energy expenditure and physical performance.** Resting energy expenditure, peak workload, and heart rate during the peak  $V_{O_2}$  measurements were similar in all

three groups (Table 4). With ANOVA, a significant main effect was noted only for peak  $V_{O_2}$ . Peak  $V_{O_2}$  was significantly lower (*P* < 0.02) in T<sub>2</sub>D- than in weight-matched control subjects. Muscle strength (peak isometric torque and peak isokinetic torque) was similar in all groups.

**Amino acid concentrations and kinetics.** All plasma amino acid levels except glycine were similar among the groups. Glycine levels were lower in T<sub>2</sub>D- than in T<sub>2</sub>D+ (Table 5). The plasma isotope enrichment values used for calculations demonstrated isotopic plateaus (Fig. 1). The slopes of any of the isotopic enrichment values were not significantly different from zero when plotted against time. Kinetics of leucine carbon, phenylalanine, and tyrosine are given in Table 6. In general, the average flux values of leucine, phenylalanine, and tyrosine tended to be lower in the lean control subjects. ANOVA showed nonsignificant differences. Multiple comparison by unpaired *t* test showed that leucine carbon flux was higher in T<sub>2</sub>D- than in the lean control subjects (*P* = 0.032). ANOVA showed no significant differences among the groups for leucine nitrogen kinetics (Fig. 2). However, unpaired *t* test showed that leucine nitrogen flux (*P* = 0.024), KIC to leucine (*P* = 0.043), and leucine in KIC (*P* = 0.032) were higher in T<sub>2</sub>D- than in lean control subjects. Paired *t* test showed T<sub>2</sub>D- had higher leucine nitrogen flux (*P* = 0.028), KIC to leucine (*P* = 0.011), and leucine to KIC (*P* = 0.016). Plasma concentration of KIC was significantly lower in T<sub>2</sub>D+ than in T<sub>2</sub>D- (Fig. 2).

**Muscle protein synthesis.** Table 7 gives the enrichment

TABLE 3  
Circulating hormone levels

Variable	Lean control subjects	Weight-matched control subjects	T <sub>2</sub> D-	T <sub>2</sub> D+	ANOVA <i>P</i> values
C-peptide (nmol/l)	0.148 ± 0.018	0.182 ± 0.024	0.183 ± 0.016‡	0.088 ± 0.014*†	0.369, 0.007
Cortisol (nmol/l)	348 ± 37	334 ± 41	336 ± 33	260 ± 36	0.958, 0.234
DHEA-S (μmol/l)	1.9 ± 0.4	1.9 ± 0.5	2.4 ± 0.4	2.1 ± 0.3	0.712, 0.930
Epinephrine (pmol/l)	131 ± 24	102 ± 15	91 ± 16	128 ± 28	0.331, 0.626
Glucagon (ng/l)	45 ± 4	51 ± 4	51 ± 5	50 ± 5	0.456, 0.529
Growth hormone (μg/l)	1.4 ± 0.5	0.5 ± 0.1	0.4 ± 0.2‡	0.9 ± 0.3	0.054, 0.152
IGF-1 total (μg/l)	157.7 ± 25.6	132.7 ± 17.6	148.1 ± 19.4	153.0 ± 27.4	0.704, 0.737
IGF-1 free (μg/l)	1.0 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.230, 0.235
IGF-2 (μg/l)	557 ± 52	635 ± 35	801 ± 80*	713 ± 60	0.025, 0.257
IGFBP-1 (μg/l)	47.9 ± 10.1	28.2 ± 8.2	33.0 ± 4.8	44.3 ± 7.6	0.217, 0.257
IGFBP-2 (μg/l)	399 ± 49	403 ± 61	290 ± 14‡	469 ± 34	0.164, 0.538
IGFBP-3 (μg/l)	3289 ± 202	3324 ± 150	3801 ± 369‡	3512 ± 385	0.312, 0.817
Norepinephrine (nmol/l)	1.000 ± 0.157	0.938 ± 0.095	0.733 ± 0.069	0.855 ± 0.069	0.239, 0.663

Data are means ± SE. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D- subjects and among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D+ subjects, respectively. \*Different from lean, †different from weight-matched, ‡different from T<sub>2</sub>D+ (*P* < 0.05).

TABLE 4  
Resting energy expenditure, peak exercise responses during cycling, and knee extensor strength

Variable	Lean control subjects	Weight-matched control subjects	T <sub>2</sub> D-	T <sub>2</sub> D+	ANOVA <i>P</i> values
REE (kcal · day <sup>-1</sup> · kg FFM <sup>-1</sup> )	31.7 ± 1.4	33.2 ± 1.3	35.5 ± 1.4	33.8 ± 1.8	0.307, 0.888
Peak workload (w)	161 ± 28	186 ± 24	156 ± 22	163 ± 24	0.832, 0.837
Peak heart rate (beats/min)	162 ± 7	162 ± 5	165 ± 8	163 ± 6	0.409, 0.893
Peak VO <sub>2</sub> (ml · min <sup>-1</sup> · kg FFM <sup>-1</sup> )	36.3 ± 2.9	44.4 ± 2.9	34.7 ± 2.0*	36.6 ± 3.1	0.048, 0.112
Peak isometric torque (Nm)	133 ± 25	140 ± 23	122 ± 16	128 ± 17	0.698, 0.866
Peak isokinetic torque (Nm)	79 ± 18	102 ± 18	67 ± 9	78 ± 11	0.345, 0.624

Data are means ± SE. Isometric torque was measured at 60° of knee flexion. Isokinetic torque was measured at 180°/s. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D- subjects and among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D+ subjects, respectively. \*Different from weight matched (*P* < 0.05). REE, resting energy expenditure.

values of [<sup>13</sup>C]leucine in muscle tissue fluid, mixed muscle protein, mitochondrial protein, and sarcoplasmic protein used for the calculation of fractional synthesis rates. There were no significant time-related changes for any of the isotopes.

Figure 3 shows the fractional synthesis rates of mixed muscle, mitochondrial, and sarcoplasmic proteins. No differences among the groups were noted for mixed muscle or sarcoplasmic proteins. Fractional synthesis rate of mitochondrial protein was lower in the lean control subjects than in T<sub>2</sub>D- and weight-matched control subjects. T<sub>2</sub>D+ did not significantly differ in FSR of mitochondrial protein from T<sub>2</sub>D-.

**Mitochondrial enzymes.** Citrate synthase activity was slightly but significantly higher (*P* < 0.05) in T<sub>2</sub>D+ than in T<sub>2</sub>D-, whereas cytochrome C oxidase did not differ among groups or between treatment phases in diabetic patients (Fig. 4).

**Muscle fatigue.** The rate of muscle fatigue, determined as the line of best fit through the data points, tended to be greater in diabetic patients (T<sub>2</sub>D-, 53 ± 7%; T<sub>2</sub>D+, 52 ± 7%) than in control subjects (lean, 41 ± 7%; weight matched, 43 ± 3%) but did not reach statistical significance. Because there was no difference between the two control groups, the results from lean and weight-matched

control subjects were pooled. Compared with the pooled control group, the relative contraction forces produced by the subjects with type 2 diabetes were significantly lower during the last third of the test (Fig. 5).

## DISCUSSION

The current study demonstrated that people with type 2 diabetes on 2 weeks of poor glycemic control had a whole-body protein turnover, as measured by leucine carbon flux, phenylalanine flux, and tyrosine flux, similar to weight-matched nondiabetic control subjects. However, withdrawal of treatment increased leucine nitrogen flux and transamination rates of leucine (deamination of leucine to KIC and reamination of KIC to leucine) in association with an increase in plasma leucine concentration. In addition, we did not find any change in synthesis rate of mixed muscle, mitochondrial, and sarcoplasmic protein with insulin treatment in comparison with poor glycemic control in the same subjects, nor were there differences compared with weight-matched control subjects. The peak oxygen uptake during aerobic exercise was higher in weight-matched control subjects than in type 2 diabetic subjects with poor glycemic control. In addition, the test of muscle fatigability demonstrated that

TABLE 5  
Plasma amino acid concentrations (μmol/l)

Amino acid	Lean control subjects	Weight-matched control subjects	T <sub>2</sub> D-	T <sub>2</sub> D+	ANOVA <i>P</i> values
GLU	45.3 ± 9.7	46.3 ± 12.2	43.1 ± 7.4	39.5 ± 6.7	0.914, 0.968
SER	64.0 ± 7.1	64.6 ± 5.8	61.8 ± 5.8	60.5 ± 5.5	0.530, 0.700
GLN	376.4 ± 34.0	414.9 ± 34.1	319.4 ± 26.2	336.8 ± 18.0	0.791, 0.558
HIS	53.9 ± 4.2	52.3 ± 4.9	43.9 ± 5.3	43.3 ± 4.0	0.795, 0.294
GLY	189.3 ± 17.4	179.1 ± 19.2	156.2 ± 9.4*	185.1 ± 12.6	0.681, 0.628
THR	73.0 ± 10.1	70.9 ± 7.8	58.7 ± 9.7	62.1 ± 9.0	0.808, 0.737
ALA	196.0 ± 29.4	252.5 ± 26.0	205.0 ± 32.4	191.4 ± 13.7	0.251, 0.522
ARG	72.8 ± 5.6	75.2 ± 4.5	71.4 ± 5.2	73.3 ± 6.6	0.998, 0.863
TYR	54.6 ± 6.4	55.8 ± 5.8	46.8 ± 6.7	47.0 ± 8.1	0.897, 0.728
VAL	114.7 ± 15.0	124.8 ± 10.6	128.0 ± 18.1	109.8 ± 11.0	0.494, 0.746
MET	36.6 ± 4.3	32.9 ± 4.2	30.3 ± 5.2	28.6 ± 4.2	0.709, 0.382
PHE	62.9 ± 7.2	62.2 ± 5.2	50.6 ± 4.8	50.5 ± 6.1	0.850, 0.434
ILE	40.1 ± 7.8	34.2 ± 9.2	39.8 ± 8.4	32.3 ± 6.6	0.934, 0.851
LEU	102.1 ± 9.1	114.3 ± 7.6	116.4 ± 13.7	99.3 ± 8.2	0.390, 0.689
LYS	187.3 ± 11.2	187.8 ± 8.5	181.7 ± 8.5	168.3 ± 9.1	0.961, 0.293

Data are means ± SE. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D- subjects and among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D+ subjects, respectively. \*Different from T<sub>2</sub>D+, *P* < 0.05.

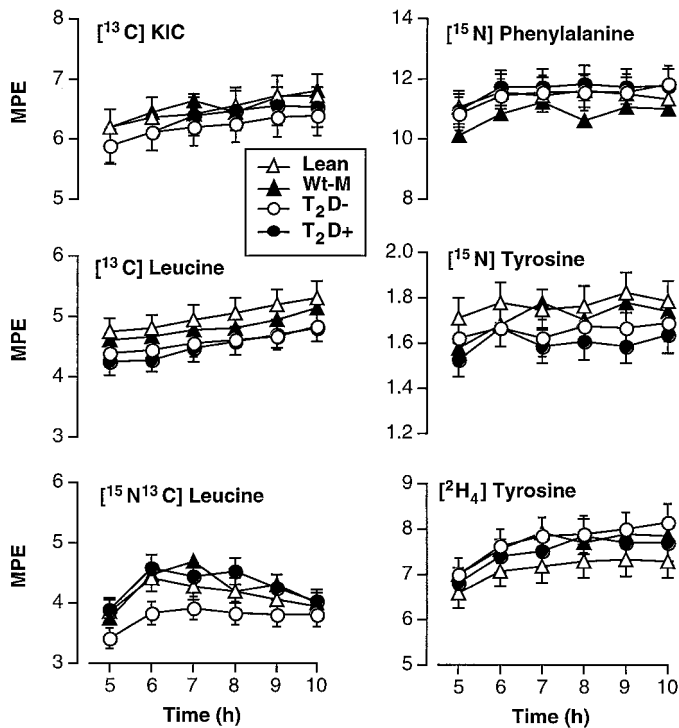


FIG. 1. Plasma isotopic enrichment values at plateau. Lean, lean control subjects; T<sub>2</sub>D<sup>-</sup> and T<sub>2</sub>D<sup>+</sup>, type 2 diabetic patients without and with insulin therapy, respectively; Wt-M, weight-matched control subjects. Calculations of amino acid kinetics used averaged values from 5 to 10 h.

in type 2 diabetic patients, irrespective of their treatment status, there is increased tendency for muscle to become fatigued.

The current study demonstrated that, whereas the 2 weeks of withdrawal of all treatments in people with type 2 diabetes had no effect on amino acid kinetics and plasma amino acid concentrations, there was a substantial increase in plasma glucose levels. The increased circulating insulin levels on 11 days of insulin treatment substantially reduced fasting glucose levels but had little impact on circulating amino acid concentrations and amino acid kinetics, reflecting protein turnover. Previous studies in nondiabetic people demonstrated that leucine carbon flux declined progressively in a dose-dependent manner on

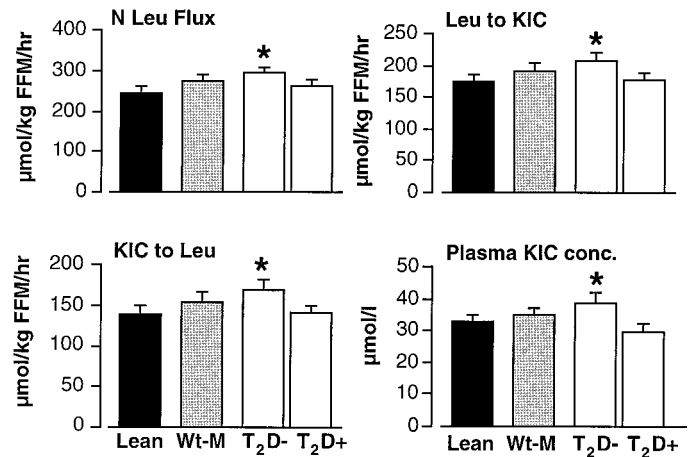


FIG. 2. Leucine nitrogen flux (N Leu Flux), leucine carbon flux (KIC to Leu), and leucine transamination to KIC (Leu to KIC) and plasma KIC concentration. Group abbreviations are the same as in Fig. 1. *P* values for ANOVA tests were 0.159 and 0.535 (N Leu Flux), 0.206 and 0.561 (KIC to Leu), 0.158 and 0.532 (Leu to KIC), and 0.307 and 0.303 (KIC concentration) for comparisons among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D<sup>-</sup> and lean control subjects, weight-matched control subjects, and T<sub>2</sub>D<sup>+</sup>, respectively. Paired analysis showed significant difference in Leu N flux (*P* = 0.028), KIC to Leu (*P* = 0.011), and Leu to KIC (*P* < 0.021) between T<sub>2</sub>D<sup>+</sup> and T<sub>2</sub>D<sup>-</sup>. \*Greater than T<sub>2</sub>D<sup>+</sup>, *P* < 0.03; representing paired *t* test of T<sub>2</sub>D<sup>-</sup> and T<sub>2</sub>D<sup>+</sup>.

intravenous insulin infusion (31,49,50). However, these dose-dependent effects clearly showed that the near-maximal insulin effect was achieved when insulin was infused at a dose of 1 mU · kg<sup>-1</sup> · min<sup>-1</sup> (31). The insulin levels achieved by a previous study in nondiabetic subjects (31) were substantially higher than what has been achieved in the type 2 diabetic patients on insulin treatment in the current study. The above fact suggests a relative resistance to insulin action on leucine, phenylalanine, and tyrosine fluxes in type 2 diabetes. These essential amino acid fluxes represent the rate of appearance of unlabelled amino acid from protein breakdown. The current findings, therefore, suggest that in people with type 2 diabetes, insulin treatment had no effect on protein breakdown, unlike in type 1 diabetes and in nondiabetic people receiving intravenous insulin administration. However, a previous study (9) demonstrated that insulin acutely reduced leucine flux type 2 diabetic patients in a manner similar to

TABLE 6  
Amino acid kinetics (μmol · kg FFM<sup>-1</sup> · h<sup>-1</sup>)

Measurements	Lean control subjects	Weight-matched control subjects	T <sub>2</sub> D <sup>-</sup>	T <sub>2</sub> D <sup>+</sup>	ANOVA <i>P</i> values
Leucine carbon flux	154.1 ± 4.1	166.7 ± 6.2	172.1 ± 6.2	172.6 ± 10.2	0.089, 0.210
Leucine oxidation	35.7 ± 1.7	37.9 ± 1.1	39.0 ± 1.3	36.6 ± 2.8	0.266, 0.751
Nonoxidative leucine flux	118.4 ± 5.2	128.9 ± 6.0	133.1 ± 7.3	136.0 ± 8.1	0.249, 0.184
Phenylalanine flux	53.5 ± 1.1	55.3 ± 1.2	52.0 ± 1.0	54.1 ± 2.8	0.121, 0.803
Tyrosine flux	37.7 ± 1.6	43.4 ± 2.8	37.8 ± 1.5	41.2 ± 2.7	0.105, 0.263
Phenylalanine to tyrosine	5.3 ± 0.1	5.9 ± 0.5	4.8 ± 0.3	5.0 ± 0.4	0.081, 0.217
Phenylalanine to protein	48.3 ± 1.0	49.3 ± 0.8	47.1 ± 0.9	49.0 ± 2.6	0.247, 0.899

Data are means ± SE. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D<sup>-</sup> subjects and among lean control subjects, weight-matched control subjects, and T<sub>2</sub>D<sup>+</sup> subjects, respectively.

TABLE 7  
 $[^{13}\text{C}]$ leucine enrichments in muscle tissue fluid and protein fractions

Variable	Lean control subjects	Weight-matched control subjects	$T_2D-$	$T_2D+$	ANOVA <i>P</i> values
Tissue fluid (MPE)	$7.168 \pm 0.259$	$6.789 \pm 0.345$	$6.203 \pm 0.321$	$6.184 \pm 0.389$	0.111, 0.137
Mixed protein (APE)	$0.0213 \pm 0.0064$	$0.0206 \pm 0.0031$	$0.0189 \pm 0.0029$	$0.0223 \pm 0.0026$	0.941, 0.965
Mitochondrial protein (APE)	$0.0175 \pm 0.0019$	$0.0257 \pm 0.0030$	$0.0222 \pm 0.0024$	$0.0192 \pm 0.0022$	0.075, 0.058
Sarcoplasmic protein (APE)	$0.0204 \pm 0.0025$	$0.0223 \pm 0.0024$	$0.0174 \pm 0.0031$	$0.0175 \pm 0.0031$	0.477, 0.364

Data are means  $\pm$  SE. *P* values for ANOVAs are shown for comparison among lean control subjects, weight-matched control subjects, and  $T_2D-$  subjects and among lean control subjects, weight-matched control subjects, and  $T_2D+$  subjects, respectively. APE, atoms percent excess; MPE, molar percent excess.

that in nondiabetic subjects. This previous study represented acute effects of insulin and may be more comparable to the studies performed in nondiabetic individuals (31,49,50), in which insulin was infused during a short

period of time. In the current study, the diabetic patients received insulin treatment over an 11-day period, although they received an insulin intravenous infusion for several hours only. The differences in amino acid and kinetics between the current study and those of previous studies in type 2 diabetic patients (9) and nondiabetic subjects may represent the acute versus chronic effect of insulin treatment. The current study is relevant to the condition existing in people with type 2 diabetes on chronic insulin treatment.

Of note, the studies in type 1 diabetic people demonstrated pronounced alterations in protein breakdown on insulin withdrawal (51) or decrease in protein breakdown on insulin replacement (3,52–54). These studies represent the effect of insulin replacement and cannot be compared with the current study in type 2 diabetes, in which the circulating insulin levels were already similar to those of

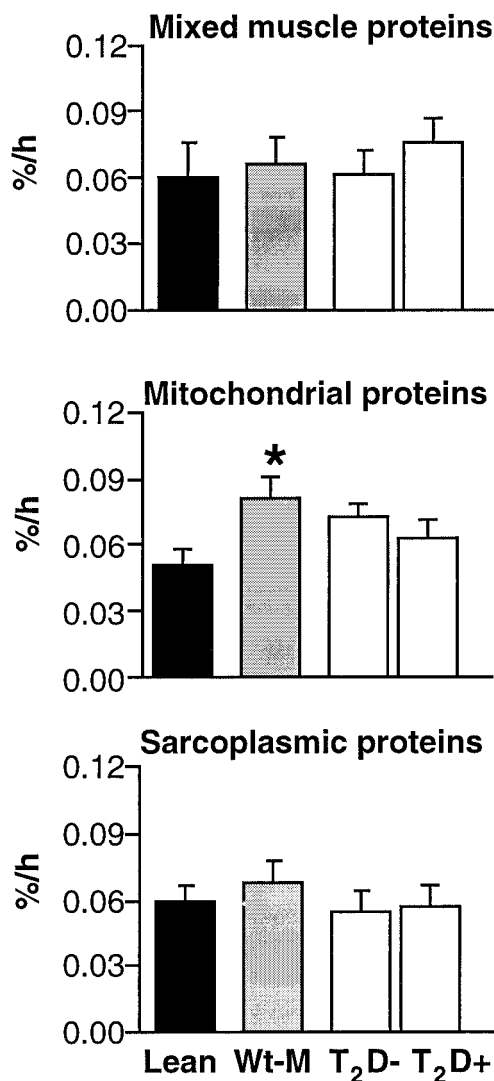


FIG. 3. Fractional synthesis rate of total mixed muscle, mitochondrial, and sarcoplasmic protein in skeletal muscle. Calculations are based on the incorporation of  $[^{13}\text{C}]$ leucine into protein using tissue fluid enrichment as the precursor pool. Group abbreviations are the same as Fig. 1. *P* values for ANOVA were 0.728 and 0.697 (mixed), 0.045 and 0.062 (mitochondrial), and 0.388 and 0.378 (sarcoplasmic) for comparisons among lean control subjects, weight-matched control subjects, and  $T_2D-$  and lean control subjects, weight-matched control subjects, and  $T_2D+$ , respectively. \*Greater than lean,  $P < 0.05$ .

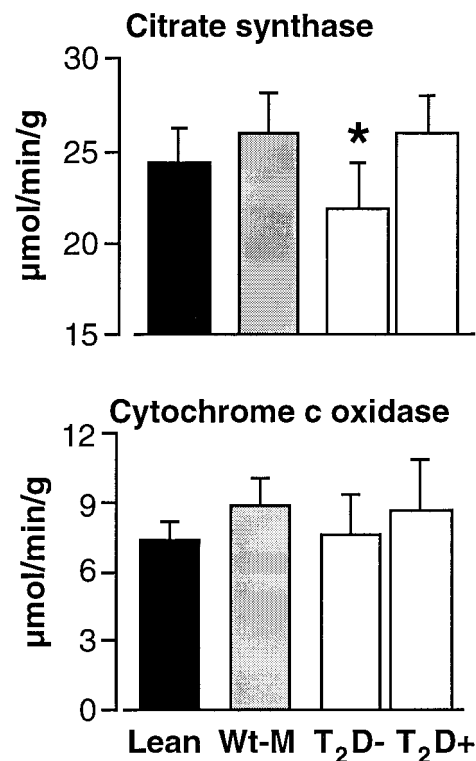


FIG. 4. Enzymatic activities of citrate synthase and cytochrome C oxidase in skeletal muscle. Group abbreviations are the same as Fig. 1. *P* values for ANOVA were 0.539 and 0.837 (citrate synthase) and 0.662 and 0.738 (cytochrome C oxidase) for comparisons among lean control subjects, weight-matched control subjects, and  $T_2D-$  and among lean control subjects, weight-matched control subjects, and  $T_2D+$ , respectively. \*Less than  $T_2D+$ ,  $P < 0.05$ ; on paired *t* test.

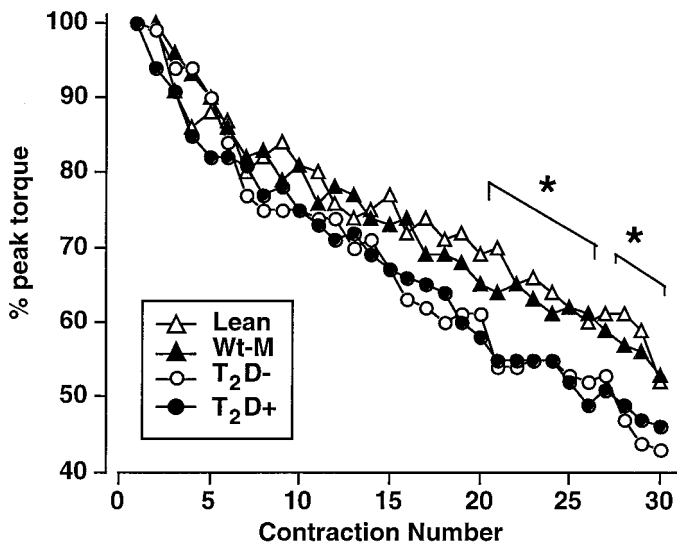


FIG. 5. Progression of muscle fatigue during repeated maximal contractions of the knee extensors. Group abbreviations are the same as Fig. 1. There were no differences between lean and weight-matched control subjects or between  $T_2D^-$  and  $T_2D^+$ . Pooled value from lean and weight-matched control subjects were higher than diabetic patients at the points denoted by the asterisk (\* $P < 0.05$ ).

nondiabetic control subjects during the phase of poor glycemic control.

The current study demonstrated a modest increase in leucine nitrogen flux and transamination rate in people with type 2 diabetes when their glycemic control was poor. Insulin treatment reduced leucine transamination rate and plasma leucine concentration, which is consistent with what has been reported in people with type 1 diabetes (2). In type 1 diabetic patients, the increased leucine transamination is associated with increased leucine oxidation (2), thus providing a basis for increased protein loss (6). In the current study, type 2 diabetic patients have only shown changes in leucine transamination but not for leucine oxidation or leucine kinetics. The increased leucine transamination observed in type 2 diabetic patients is a much lesser magnitude than what has been reported in people with type 1 diabetes (2). This increased leucine transamination may represent the transfer of amino group for synthesis of glutamine and alanine, which are key substrates for gluconeogenesis. Increased gluconeogenesis is consistent with the current observation. Although gluconeogenesis has not been measured in the current study, increased glucose production and increased gluconeogenesis are reported to occur in type 2 diabetic patients when they are not treated (16). In the current study, the circulating amino acid concentrations remained unchanged, including alanine and glutamine. In the presence of an increased gluconeogenesis, the unaltered concentrations of alanine and glutamine suggest increased production of these glucogenic precursors.

A previous study (8) examined the effect of intensive insulin treatment versus conventional insulin treatment in type 2 diabetes and reported no effect on both leucine carbon flux and leucine nitrogen flux. In contrast, the current study was undertaken to determine the effect of intensive insulin treatment on leucine kinetics in type 2 diabetic patients compared with nondiabetic control sub-

jects who were not treated for 2 weeks. Based on a previous study (8) and the current study, it appears that leucine transamination is more sensitive to insulin action than glucose metabolism. Studies using  $^{15}N$ -labeled glycine demonstrated increased nitrogen flux in people with type 2 diabetes when they are in poor glycemic control (11). Consistent with a previous study (11), the current study showed that nitrogen flux and transamination rate of leucine are increased during poor glycemic control in type 2 diabetic subjects. However, increased leucine transamination did not result in increased irreversible catabolism of the carboxyl group (KIC) of leucine and increasing KIC levels. These results suggest that increased leucine oxidation does not always occur in association with increased leucine transamination.

Muscle protein synthesis has not been evaluated in people with type 2 diabetes. The current study demonstrates that in people with type 2 diabetes, synthesis rates of mixed muscle, sarcoplasmic, and mitochondrial protein are unaffected by insulin treatment. These results are consistent with what has been reported in type 1 diabetes, in which both mixed muscle protein (55,56) and myosin heavy chain synthesis rate (56) are not affected by acute changes in glycemic control. Recent studies demonstrated that in a swine model, insulin selectively stimulates muscle mitochondrial protein synthesis without any significant effect on myosin heavy chain or sarcoplasmic proteins (27). Intensive insulin treatment for 11 days and intravenous infusion of insulin for several hours had no significant effect on muscle mitochondrial protein synthesis and cytochrome oxidase in people with type 2 diabetes. This observation is important because  $T_2D^+$  patients had higher circulating insulin than all other subjects in the current study, but this increased insulin level had no effect on mitochondrial protein synthesis. Interestingly, we observed that—unlike the diabetic patients—weight-matched nondiabetic subjects have a higher mitochondrial protein synthesis than lean nondiabetic subjects.

Previous studies have shown that muscle citrate synthase is lower in type 2 diabetic people than in lean (57) and obese nondiabetic control subjects (58). In contrast, another study found no differences in muscle oxidative enzyme activity among overweight type 2 diabetic people and nondiabetic control groups (23,24,59). However, a clear association between muscle oxidative enzyme activities and glucose disposal rate has been noted (24). The current study demonstrated that insulin treatment had no effect on muscle cytochrome C oxidase, although a modest elevation of citrate synthase occurred. The lack of increase in cytochrome C oxidase is consistent with a lack of increase in muscle mitochondrial protein synthesis in type 2 diabetes. Previous studies demonstrated a significant correlation between cytochrome C oxidase and muscle mitochondrial protein synthesis (26).

It is believed that inefficient ATP production in response to continued high demand is a determinant of muscle performance at a high level on a continuous basis. There will be decline in the muscle performance if ATP production cannot be sustained during a continuous activity. Such a decline is often described as muscle fatigue, and the current study demonstrates that in people with type 2 diabetes, there is evidence of increased muscle fatigue.

This may suggest a reduced mitochondrial function. Other factors, such as vascular factors, inability to mobilize glycogen stores, and phosphocreatinine at the start of exercise, may contribute to the muscle fatigability. Lack of motivation also could be a factor. It remains to be determined whether lack of stimulation of muscle mitochondrial protein synthesis by insulin in type 2 diabetic patients has an impact on muscle mitochondrial function and muscle function.

There are reports about reduced  $VO_{2max}$  in people with type 2 diabetes and their first-degree relatives (19). Studies also show lower  $VO_{2max}$  in type 2 diabetic patients compared with age-matched nondiabetic control subjects (22,60,61). The current study showed that the peak  $VO_{2max}$  in type 2 diabetic subjects with poor glycemic control was lower than weight-matched control subjects, although insulin treatment did not significantly alter this. The reduced peak  $VO_2$  in people with diabetes observed in the current study is consistent with increased muscle fatigability also noted in this group. However, we did not find any change in the muscle strength or any other parameters of fitness after 11 days of insulin treatment.

In summary, the current study demonstrated that in people with type 2 diabetes, poor glycemic control by withdrawal of treatment had no impact on whole-body protein breakdown. We also did not observe any change in synthesis rate of muscle mitochondrial, mixed muscle, or sarcoplasmic protein in type 2 diabetic patients. Insulin treatment normalized glucose levels but had no effect on amino acid concentrations. This may represent either a relative resistance to insulin action on chronic treatment or a lack of insulin effect once a certain level of circulating insulin level is achieved in these people. Withdrawal of treatment caused increased leucine nitrogen flux, transamination rates, and KIC concentration, indicating that leucine transamination and oxidation of leucine carboxyl group are differently affected in type 2 diabetes. The study also demonstrated some evidence of increased muscle fatigability in type 2 diabetic patients irrespective of their treatment. In addition, a reduced peak  $VO_2$  in type 2 diabetic patients on poor glycemic control may represent either reduced perfusion of the tissue related to cardiovascular factors and/or reduced mitochondrial functions. The lack of insulin-induced stimulation of muscle mitochondrial protein synthesis suggests the possibility of insulin resistance to mitochondrial protein synthesis, and this needs further investigation.

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