Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Skeletal Muscle Protein Anabolic Response to Increased Energy and Insulin Is Preserved in Poorly Controlled Type 2 Diabetes\(^1,2\)

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ABSTRACT Type 2 diabetes (T2DM) subjects failing diet treatment are characterized by hyperinsulinemia and insulin resistance leading to fasting and postprandial hyperglycemia and hyperlipidemia. Energy is essential for allowing the process of protein synthesis to proceed. Additionally, insulin can stimulate protein synthesis in human muscle. The aims of this study were to determine if poorly controlled T2DM affects postabsorptive muscle protein anabolism, and if the muscle anabolic response to hyperinsulinemia with high energy availability is maintained. Control (\(n = 6\)) and T2DM subjects (\(n = 6\)) were studied in the postabsorptive state and during an isoenergetic high nutritional energy clamp (relative to postabsorptive state). Muscle protein synthesis and breakdown (nmol \(\cdot \text{min}^{-1} \cdot 100 \text{ g leg muscle}^{-1}\)) were assessed using stable isotope methodology, femoral arterio-venous sampling, muscle biopsies, and a three-pool model to calculate protein turnover. Postabsorptive phenylalanine net balance and whole body rate of appearance (Ra) were not different between groups; however, basal muscle protein breakdown was higher in T2DM (94 ± 9) than in controls (58 ± 12) \((P < 0.05)\) and muscle protein synthesis tended \((P = 0.07)\) to be elevated in T2DM (66 ± 14) compared with controls (39 ± 6). During the clamp, net balance increased, whole body Ra and muscle protein breakdown decreased \((P < 0.05)\), and muscle protein synthesis tended to decrease \((P = 0.08)\) to a similar extent in both groups. We conclude that postabsorptive muscle protein turnover is elevated in poorly controlled T2DM, however, there is no excessive loss of muscle protein because net balance is not different from controls. Moreover, the anabolic response to increased insulin and energy availability is maintained in T2DM.

KEY WORDS: • type 2 diabetes • protein metabolism • muscle protein synthesis • protein turnover

Individuals with type 2 diabetes (T2DM)\(^4\) are characterized by hyperinsulinemia and insulin resistance leading to fasting and postprandial hyperglycemia and abnormal fatty acid metabolism (1,2). In addition, T2DM is associated with metabolic abnormalities in glucose utilization, including impaired glucose tolerance and insulin resistance (3,4) that might also extend to muscle protein metabolism if insulin signaling is compromised (5). Although insulin is a potent stimulator of skeletal muscle protein anabolism, the specific mechanism by which insulin exerts its effects in human skeletal muscle is controversial. For example, several studies have reported insulin to have an inhibitory effect on muscle protein breakdown (6–10), whereas others have shown insulin to have a direct stimulatory effect on muscle protein synthesis (11–14). The importance of insulin in regulating muscle protein turnover is highlighted by the estimated contribution of skeletal muscle (30–50%) to whole body protein breakdown (6,15). Furthermore, muscle protein turnover is an energy-requiring process, and several studies have shown that when additional energy is supplied in the form of either glucose or lipids, the rate of protein breakdown is also subsequently reduced (16–20). However, the interaction between an increase in insulin and nutritional energy on muscle protein turnover in T2DM has not been well described.

Few studies have addressed skeletal muscle amino acid kinetics in T2DM. Most have reported that whole body protein turnover in insulin resistant obese individuals and individuals with T2DM is not impaired (21–25). However, it has also been shown that when additional energy is supplied in the form of either glucose or lipids, the rate of protein breakdown is also subsequently reduced (16–20). However, the interaction between an increase in insulin and nutritional energy on muscle protein turnover in T2DM has not been well described.
two-pool model to calculate labeled phenylalanine kinetics across the leg, one study found that, even though whole body protein synthesis was elevated in T2DM, the rate of phenylalanine release across the leg (an index of leg muscle protein synthesis) was reduced (15). In addition, a recent study reports no differences in muscle fractional synthetic rates between the T2DM group and healthy controls (24). The traditional arteriovenous two-pool model, which is often used to calculate amino acid kinetics across the leg, actually measures the net kinetics of plasma amino acids across the leg while not offering any insight into the intracellular amino acid kinetics. Therefore, it remains unclear whether differences in muscle intracellular amino acid kinetics are present in the insulin resistant state of T2DM.

The first aim of this study was to determine if poorly controlled T2DM (i.e., hemoglobin A1c >7%) affects postabsorptive muscle protein anabolism. Our second aim was to determine if hyperinsulinemia, in combination with high nutritional energy availability, stimulates muscle protein anabolism to the same extent as healthy controls. To address our aims we studied whole body and regional phenylalanine kinetics following an overnight fast and during a hyperinsulinemic-isocaloric nutritional energy clamp in controls and in subjects with poorly controlled T2DM.

METHODS

Subjects. We recruited 12 subjects (9 men and 3 women) to participate in the study. The characteristics of the 6 controls and the 6 T2DM subjects are shown in Table 1. A thorough physical examination with clinical history was performed on all potential subjects, along with laboratory tests including complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, and an oral glucose tolerance test. Patients with T2DM were eligible for participation if either fasting blood glucose levels were ≥7 mmol/L and/or 2-h blood glucose levels were ≥11mmol/L (200 mg/dl) following the oral glucose tolerance test and if they were on diet treatment alone and not taking any medications. As a result of elevated blood glucose levels (8.3 ± 0.2 mmol/L), the T2DM subjects also demonstrated elevated hemoglobin A1c (HbA1c), or nonenzymatically glycated hemoglobin, levels (8.7 ± 1.5%), which is an index of blood glucose control over the past 2–3 months. According to the World Health Organization and the American Diabetes Association (28), HbA1c levels >7% require pharmacological treatment; thus, T2DM subjects enrolled in the study were considered poorly controlled. Controls had a normal oral glucose tolerance test response and both groups were exposed to hyperglycemia (relative to their baseline value). Although the glucose infusion varied in order to monitor the plasma glucose concentration. A 20% dextrose infusion was also initiated and varied to increase blood glucose by 2.2 mmol/L above the baseline value in both healthy controls and T2DM subjects, and thus both groups were exposed to hyperglycemia (relative to their baseline value). Although the glucose infusion varied in order to increase blood glucose levels to a similar increase above baseline, Intralipid® (0.7 mL · kg⁻¹ · min⁻¹) and heparin (7 U · kg⁻¹ · min⁻¹) (Baxter, Deerfield) were also infused in an effort to prevent the expected decrease in plasma FFA and which was achieved with a constant infusion. In addition, during the 5-h hyperglycemic-hyperinsulinemic-hyperlipidemic (high energy clamp), the total amount of nutritional energy from glucose (controls, 410 ± 75 kJ · h⁻¹; T2DM subjects, 318 ± 63 kJ · h⁻¹) and lipid (controls, 264 ± 17 kJ · h⁻¹; T2DM subjects, 247 ± 17 kJ · h⁻¹) was similar in the 2 groups. At the end of the insulin infusion period, ICG was infused to measure leg blood flow.

Study design. The study protocol (Fig. 1) was designed to assess amino acid kinetics in T2DM subjects under postabsorptive (basal) conditions and during a 5-h hyperglycemic-hyperinsulinemic-hyperlipidemic (high energy relative to basal) clamp. Therefore, blood glucose levels were increased to a similar extent above baseline in both T2DM and control groups during the clamp so that the total amount of nutritional energy was similar in the 2 groups. The subjects arrived at the USC General Clinical Research Center the evening before the study. Following admission, volunteers underwent a Dual-Energy X-Ray Absorptiometry (DEXA) scan (Hologic QDR 4500W) to measure body composition and muscle mass and then consumed a standard meal (42 kJ/kg of body weight) before sleeping the night.

The study began the next morning at ~0700 with the insertion of polyethylene catheters into a forearm vein for the systemic infusions, in a contralateral wrist vein, which was heated for arterialized blood sampling, and in the femoral artery and vein of the right leg for blood sampling. The arterial catheter was also used for the infusion of indocyanine green (ICG, Akorn) to measure blood flow (30). Background blood samples were obtained and a primed (2 μmol/kg), continuous (0.05 μmol · kg⁻¹ · min⁻¹) infusion of L-[ring-²H₅]phenylalanine, (Cambridge Isotope Laboratories) was initiated and maintained at a constant rate until the end of the experiment. Following steady state amino acid enrichments in the blood (~120 min), infusion of ICG was started and blood samples were obtained for measuring amino acid concentrations and enrichments, ICG concentration, and plasma glucose and insulin concentrations at 10-min intervals (Fig. 1). Using a 5 mm Bergström biopsy needle, a first muscle biopsy was taken immediately after the lateral portion of the vastus lateralis about 20 cm above the knee, using local anesthesia with 1% lidocaine injected subcutaneously and on the fascia. The muscle sample was rinsed with ice-cold saline, blotted, and any visible fat or connective tissue was quickly removed, immediately before the tissue was frozen in liquid nitrogen and stored at −80°C until analysis.

Immediately following the first skeletal muscle biopsy, plasma insulin concentrations were elevated with a systemic insulin infusion (0.3 μU · kg⁻¹ · min⁻¹) into a forearm vein. After the start of the insulin infusion, blood samples (0.5 mL) were taken every 5–10 min to monitor the plasma glucose concentration. A 20% dextrose infusion was also initiated and varied to increase blood glucose by 2.2 mmol/L above the baseline value in both healthy controls and T2DM subjects, and thus both groups were exposed to hyperglycemia (relative to their baseline value). Although the glucose infusion varied in order to increase blood glucose levels to a similar increase above baseline, Intralipid® (0.7 mL · kg⁻¹ · min⁻¹) and heparin (7 U · kg⁻¹ · min⁻¹) (Baxter, Deerfield) were also infused in an effort to prevent the expected decrease in plasma FFA and which was achieved with a constant infusion. In addition, during the 5-h hyperglycemic-hyperinsulinemic-hyperlipidemic (high energy clamp), the total amount of nutritional energy from glucose (controls, 410 ± 75 kJ · h⁻¹; T2DM subjects, 318 ± 63 kJ · h⁻¹) and lipid (controls, 264 ± 17 kJ · h⁻¹; T2DM subjects, 247 ± 17 kJ · h⁻¹) was similar in the 2 groups. At the end of the insulin infusion period, ICG was infused to measure leg blood flow.

![Diagram](image-url)

**FIGURE 1** Study design consisting of a basal postabsorptive period and a high energy-hyperinsulinemic clamp period. ICG, indocyanine green.
Plasma insulin concentrations were determined by radioimmunoassay (Diagnostic Products). Plasma glucose was determined by the glucose oxidase method using a YSI 2700 analyzer (Yellow Springs Instruments), immediately after each blood draw. Plasma free fatty acid concentration (Wako NEFA; Wako Chemicals) was determined enzymatically. Concentrations of plasma C-reactive protein (CRP) and cortisol were determined using a commercially available solid-phase chemiluminescent assay kits (Diagnostic Products Corporation) and a high throughput automated immunoassay hormone analyzer (Immulite 2000, DPC). Serum ICG concentration for determining leg blood flow was measured spectrophotometrically (Beckman Coulter) at λ = 805 nm (30).

Sulfinpyrazone (15%) was used to precipitate and separate plasma amino acids and the supernatant was eluted through a cation exchange column with 4 mol/L NH₄OH (31). Muscle free concentrations and enrichments of phenylalanine were determined by gas chromatography-mass spectrometry (GCMS) using an appropriate internal standard (31). Mixed muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction (31), using the external standard curve approach (32).

Calculations. Two models were used to assess phenylalanine kinetics: a two-pool arteriovenous (A-V) model (31) and a three-pool A-V model that also incorporates phenylalanine from the muscle free amino acid pool for the calculation of the intracellular amino acid parameters (33). We have previously utilized both model systems to assess phenylalanine kinetics and the methodology is detailed elsewhere (29). The differences between the two-pool and the three-pool models are summarized below (Fig. 2). Leg blood flow was calculated from the steady state ICG concentration values in the femoral and popliteal veins, as previously described (30). Data are expressed per 100 g of muscle (i.e., leg fat-free mass was assumed to be mostly skeletal muscle).

Additionally, we calculated the amino acid availability (see Fig. 2) as the sum of transport into the muscle F₀M, and the rate of appearance from the muscle FₚM₀, and the efficiency of amino acid utilization for muscle protein synthesis as follows:

\[
\text{Efficiency of amino acid utilization for protein synthesis} = \frac{F_{OM}}{F_{MA} + F_{MO}}.
\]

To calculate the FSR (fractional synthetic rate) of mixed muscle proteins during the clamp, we measured the rate of incorporation of the phenylalanine tracer into the proteins (ΔEp/εt) and then used the precursor-product model to calculate the synthesis rate:

\[
\text{FSR} = \frac{\Delta E_p/\epsilon_t}{(\Delta ME_M + \Delta ME_D)/2} \cdot 60 \cdot 100.
\]

ΔEp is the increment in protein-bound phenylalanine enrichment between 2 sequential biopsies, t is the time between the 2 sequential biopsies, EM₁ and EM₂ are the phenylalanine enrichments in the free intracellular pool in the 2 sequential biopsies. Data are expressed as percent per hour.

To determine whole body phenylalanine rate of appearance (Ra) from protein breakdown, the following equation was used:

\[
\text{Whole body Ra} = \left(\frac{1}{E_A}\right) \cdot \text{BW}.
\]

where I is the infusion rate of the phenylalanine tracer, E₀ is the enrichment in the artery, and BW is the body weight of the subjects in kilograms.

Leg glucose utilization was calculated as net glucose uptake across the leg:

\[
\text{Leg glucose uptake} = \left(\text{C}_A - \text{C}_V\right) \cdot \text{BF},
\]

where CA and CV are the phenylalanine concentrations in the femoral artery and vein, respectively, and BF is leg blood flow.

Statistical analysis. All values are expressed as means ± SEM. Group (controls vs. T2DM subjects) and period (basal vs. clamp) comparisons were performed using a 2-way ANOVA with repeated measures. Post-hoc testing was performed using a Student’s t test when there was a significant interaction. To compare subject characteristics, fractional synthetic rate, and energy infused during the high energy clamp between groups, the Student’s t test for independent samples was used. Differences were considered significant at P < 0.05.

RESULTS

Subject characteristics. The healthy controls and T2DM subjects were matched for both age and BMI (Table 1). Although total body fat was significantly greater in T2DM subjects, the composition of the legs in the 2 groups did not differ (Table 1).

Energy provided. The high energy infusion during the clamp period was isoenergetic because the control (3382 ± 435 kJ) and T2DM (2837 ± 331 kJ) groups did not differ in the total amount of nutritional energy provided during the clamp. In addition, the total amount of fat energy (3114 ± 94 vs. 1238 ± 88 kJ; P = 0.229) and carbohydrate energy (2071 ± 381 vs. 1598 ± 310 kJ; P = 0.204) provided during the clamp did not differ between the control and T2DM groups. The total energy given to the control group was slightly higher than that given to the T2DM subjects because the latter were insulin resistant and required less glucose to elevate their baseline glucose concentrations to the same extent as the control group.

Blood flow. Basal leg blood flow (mL · min⁻¹ · 100 mL leg⁻¹) did not differ between controls (3.0 ± 0.4) and T2DM subjects (4.5 ± 0.5). During the clamp, blood flow did not change significantly in either the control (3.9 ± 0.5) or T2DM (4.6 ± 0.7) group.

Substrates and hormones. Basal and clamp arterial glucose was greater in T2DM subjects than in controls and increased in both groups during the clamp (P < 0.01) (Table 2). Basal glucose uptake was not different and increased to a similar extent in both groups during the clamp (P < 0.01). Plasma FFA was not different at baseline and increased in both groups during the clamp due to the infusion of intralipids (P < 0.01). Basal plasma insulin concentrations did not differ between

![FIGURE 2](https://example.com/figure2.png)

Shown are 2- and 3-pool compartment models of leg phenylalanine (phe) kinetics. Free phe pools in femoral artery (A), femoral vein (V) and muscle (M) are connected by arrows indicating unidirectional phe flow in each compartment. With both models, phe enters the leg via femoral artery (F₀A) and leaves the leg via femoral vein (F₀M). For the 2-pool model, Rd is the rate of phe disappearance (estimate of protein synthesis) and Ra is the rate of phe appearance from breakdown. For the 3-pool model, Fₐ is direct phenylalanine flow from artery to vein without entering intracellular fluid. FₚM and FₚM₀ are inward and outward transport from artery to muscle and from muscle to vein, respectively. FₚM₀, intracellular phenylalanine appearance (breakdown). FₚM is intracellular phenylalanine disappearance (protein synthesis). FₜM / (FₚM₀ + FₚM) is protein synthesis efficiency.
groups. However, during the clamp plasma insulin concentration, increased significantly in both groups, with the final plasma insulin concentration being higher in the control group than in the T2DM subjects. Plasma CRP and cortisol concentrations did not differ between groups under basal or clamp conditions.

**Amino acid enrichments and concentrations.** Phenylalanine concentrations in the femoral artery and vein, and in the muscle tissue, did not differ between groups under basal or clamp conditions (Table 3). However, phenylalanine arterial concentrations decreased \(P < 0.05\) during the clamp, in both the controls \((27 \pm 3\%\) and T2DM subjects \((18 \pm 2\%)\). Muscle phenylalanine concentrations were lower during the clamp in both groups \((P < 0.01\), time effect).

Phenylalanine enrichment in the femoral artery, vein, and muscle did not differ between groups in the basal or clamp periods (Table 3). During the clamp, phenylalanine enrichment in the artery, vein, and muscle increased in both groups \((P < 0.01)\).

Net phenylalanine balance across the leg, an anabolic index of protein metabolism, did not differ between groups under basal or clamp conditions. During the clamp, net balance improved in both groups \((P < 0.05\), time effect) (Table 4). Whole body phenylalanine Ra from protein breakdown did not differ between the controls and T2DM subjects under basal \((60 \pm 2\) vs. \(54 \pm 5\) \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) or clamp \((36 \pm 3\) vs. \(46 \pm 4\) \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\)) conditions, respectively. During the high energy clamp period, whole body Ra was reduced in both groups \((P < 0.05)\).

**Amino acid kinetics.** Using the two-pool model, the Ra of phenylalanine across the leg, an index of leg muscle protein breakdown, was greater under basal and clamp conditions in the T2DM group \((P < 0.05\), group effect) (Table 4). During the clamp, Leg Ra decreased in both groups \((P < 0.01\), time effect). The rate of phenylalanine disappearance across the leg (Leg Rd), an index of leg muscle protein synthesis, tended to be higher in the T2DM group \((P = 0.06\), group effect) and tended to decrease during the clamp \((P = 0.07\), time effect).

Using the 3-pool model, basal phenylalanine delivery to \((F_{in})\) and from \((F_{out})\) the muscle did not differ between groups. During the clamp, \(F_{in}\) was unchanged, however, \(F_{out}\) was reduced in both groups \((P < 0.05\), time effect). Phenylalanine transport into the muscle \((F_{Ma})\) and out of the muscle \((F_{Va})\) did not differ at baseline and did not change during the clamp in either group. Arteriovenous shunting \((F_{Va})\) tended to be higher in the T2DM group \((P = 0.06\), group effect). Efficiency of amino acid utilization for protein synthesis did not differ between groups at baseline or during the clamp. The amino acid availability in the muscle free pool did not differ between

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**TABLE 2**

Plasma glucose, FFA, glucose uptake, and hormones in control and T2DM subjects at baseline and during high energy-hyperglycemic clamp

<table>
<thead>
<tr>
<th>Glucose, mmol/L</th>
<th>Control</th>
<th>T2DM</th>
<th>Control</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5.4 ± 0.2</td>
<td>7.6 ± 1.3(^2)</td>
<td>8.3 ± 0.2(^2)</td>
<td>10.7 ± 0.3(^2, 3)</td>
</tr>
<tr>
<td>Glucose uptake, mmol/min · leg</td>
<td>0.36 ± 0.04</td>
<td>0.31 ± 0.11</td>
<td>2.5 ± 0.94(^2)</td>
<td>1.9 ± 0.5(^2)</td>
</tr>
<tr>
<td>Plasma FFA, mmol/L</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.2(^2)</td>
<td>1.0 ± 0.1(^2)</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>33 ± 7</td>
<td>63 ± 12</td>
<td>240 ± 24(^4)</td>
<td>144 ± 36(^2, 3)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.4 ± 1.0</td>
<td>8.5 ± 6.0</td>
<td>2.0 ± 0.8</td>
<td>9.0 ± 6.8</td>
</tr>
<tr>
<td>Cortisol, µg/L</td>
<td>130 ± 30</td>
<td>90 ± 10</td>
<td>140 ± 30</td>
<td>90 ± 10</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \(n = 6\).
2 Different from basal, \(P < 0.05\).
3 Different from controls, \(P < 0.05\).

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**TABLE 3**

Free phenylalanine concentrations and enrichments in femoral artery and vein and in muscle tissue at baseline and during the high energy-hyperinsulinemic clamp in control and T2DM subjects

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Control</th>
<th>T2DM</th>
<th>Control</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine, µmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>56 ± 3</td>
<td>60 ± 5</td>
<td>41 ± 2(^*)</td>
<td>49 ± 5(^*)</td>
</tr>
<tr>
<td>Vein</td>
<td>63 ± 3</td>
<td>67 ± 4</td>
<td>42 ± 3(^*)</td>
<td>50 ± 4(^*)</td>
</tr>
<tr>
<td>Muscle</td>
<td>86 ± 5</td>
<td>88 ± 6</td>
<td>73 ± 3(^*)</td>
<td>73 ± 3(^*)</td>
</tr>
<tr>
<td>Enrichments, tracer/tracee</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>0.068 ± 0.004</td>
<td>0.074 ± 0.003</td>
<td>0.085 ± 0.005(^*)</td>
<td>0.086 ± 0.002(^*)</td>
</tr>
<tr>
<td>Vein</td>
<td>0.051 ± 0.004</td>
<td>0.055 ± 0.003</td>
<td>0.068 ± 0.004(^*)</td>
<td>0.069 ± 0.003(^*)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.049 ± 0.005</td>
<td>0.050 ± 0.003</td>
<td>0.066 ± 0.006(^*)</td>
<td>0.064 ± 0.003(^*)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \(n = 6\).
* Different from basal, \(P < 0.05\).
groups, however, it was reduced (P < 0.05, time effect) in both groups during the clamp (Table 4). Phenylalanine F_{O,M}, a measure of phenylalanine utilization for muscle protein synthesis, tended to be higher in the T2DM group (P = 0.07, group effect) and tended to be reduced during the clamp in both groups (P = 0.08, time effect) (Table 4). Basal phenylalanine release from protein breakdown (F_{M,O}) was greater in the T2DM group than in the healthy controls under basal conditions (P = 0.044) (Fig. 3). During the clamp, F_{M,O} was significantly reduced to a similar extent in both groups (P < 0.05).

Fractional synthetic rate of mixed muscle proteins during the clamp was significantly higher (0.07 ± 0.01 vs. 0.05 ± 0.01% per h, P = 0.04) in the T2DM group than in the control group, respectively. The higher fractional synthetic rate in the T2DM group during the clamp was consistent with the trend for F_{O,M} to be higher in the T2DM group.

**DISCUSSION**

The current study demonstrates significantly higher postabsorptive skeletal muscle protein breakdown rates in T2DM subjects than in healthy controls. However, postabsorptive phenylalanine net balance across the leg was comparable in both groups, indicating there was not an excessive loss of skeletal muscle protein in T2DM subjects due to a concomitant increase in muscle protein synthesis in T2DM. Further, we also found that postabsorptive whole body protein breakdown was not different between controls and T2DM subjects, and both whole body protein breakdown and leg muscle protein breakdown was suppressed to a similar extent in both groups during the insulin and high energy clamp. Furthermore, in agreement with other groups, our findings also indicate that measures of whole body amino acid kinetics are not appropriate for assessing regional muscle protein metabolism.

The 3-pool model provided a direct measurement of the amino acid intracellular utilization for protein synthesis, and release from protein breakdown. With this model, postabsorptive skeletal muscle protein breakdown was significantly greater in T2DM subjects than in controls. The systemic infusion of insulin resulted in a significant reduction in muscle proteolysis in T2DM subjects to rates comparable with healthy controls. The availability of amino acids during an insulin infusion is an important factor in determining skeletal muscle protein turnover rates (29). For example, if blood amino acid concentrations and/or delivery to the muscle are maintained or increased during an insulin infusion, then muscle protein synthesis will be stimulated with little change in muscle protein breakdown (11–14). On the other hand, blood amino acid concentrations decrease during a systemic insulin infusion, muscle protein breakdown is inhibited, and muscle protein synthesis is unchanged (6–10). In our study, we reported similar postabsorptive blood phenylalanine concentrations in the healthy controls and T2DM subjects, and a significant reduction during the clamp period by 27% and 18%, respectively. Therefore, it was not possible to ascertain the precise mechanism by which insulin inhibited muscle protein breakdown in our study because the decline could have been due to a direct insulin effect and/or an indirect effect via a decrease in blood amino acid availability. However, our results provide more information about the role of energy availability during sufficient or insufficient amino acid availability. For example, previous studies that show a positive effect of insulin on muscle protein synthesis in human subjects also show no change or a slight increase in amino acid availability (11–14). On the other hand, those studies reporting an inhibition of muscle protein breakdown with no change, or a decrease in muscle protein synthesis in human subjects exposed to insulin, also report a decrease in amino acid availability (6–10). Therefore, our findings provide additional evidence that amino acid availability is a major regulator of muscle protein synthesis and that an increase in energy availability cannot override the inhibition of muscle protein synthesis. In addition, it appears that the response of T2DM subjects to insulin and energy availability (when amino acid concentrations are reduced) does not differ from healthy control subjects.

Our findings also agree with previous investigations that report no differences in whole body proteolysis between T2DM subjects and healthy controls (21–25). In addition, when insulin was infused at a high rate to achieve postprandial plasma insulin concentrations, whole body protein breakdown was reduced in healthy controls and T2DM subjects, with no significant differences between groups. The current data agree with other studies of obese individuals (34,35) and show that the effect of systemic hyperinsulinemia on whole protein utilization is normal in T2DM subjects. Moreover, net phenylalanine balance across the leg, an index of muscle protein anabolism, was similar in both groups under postabsorptive conditions and significantly improved in the healthy controls (73%) and
T2DM subjects (65%) during the clamp. Therefore, the net anabolic effect of insulin and high energy availability on protein metabolism at the whole body level and across the leg is preserved in T2DM. However, we cannot rule out the potential effect of elevated FFAs during the clamp influencing our results because Tessari et al. (36) show that increasing FFA availability, with a triglyceride and heparin infusion in dogs, can inhibit whole body protein breakdown.

Our results differ from one study that found decreased leg Ra in T2DM subjects (26). The most likely reason for the difference with our study was the extremely high insulin and HbA1c values in the prior study (26). Although the T2DM subjects in our study were poorly controlled (as shown by their elevated HbA1c values), their insulin values were significantly lower. Therefore, as the severity of T2DM progresses toward complete β-cell failure, there is the possibility that our finding of elevated muscle protein turnover may be different.

Recent evidence seems to suggest that inflammation may also be involved in the pathogenesis of T2DM. For instance, current research findings have revealed a direct link between increased CRP levels to obesity and insulin resistance (37,38). Moreover, a linear relationship between CRP levels and T2DM controls and T2DM subjects, although CRP levels tended to be higher in the T2DM group under both basal and clamp conditions. It remains to be determined whether inflammation is responsible for the increase in postabsorptive muscle protein breakdown in T2DM subjects.

Another interesting finding was the significantly higher arteriovenous shunting of phenylalanine in the T2DM group. Therefore, it appears that nutritive blood flow to the muscle (i.e., Fmir) has also been reported (39–41). In the current study, we did not find a significant difference in CRP levels between the healthy controls and T2DM subjects, although CRP levels tended to be higher in the T2DM group under both basal and clamp conditions. Specifically, we conclude that the role of blood flow as a mediator of insulin's effect on muscle metabolism at the whole body level and across the leg is preserved in T2DM subjects and controls. Therefore, we also conclude that the muscle protein net anabolic response to increased insulin and energy availability is maintained in T2DM subjects. However, future studies are required to determine if the inhibition of muscle protein breakdown was due entirely to the combined effect of insulin and energy availability and/or to the insulin-induced decrease in blood amino acid availability.

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LITERATURE CITED


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