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# Increased muscle protein breakdown in chronic hemodialysis patients with type 2 diabetes mellitus

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**Increased muscle protein breakdown in chronic hemodialysis patients with type 2 diabetes mellitus.**

*Background.* The presence of diabetes mellitus (DM) in chronic hemodialysis (CHD) patients has potential to increase body protein losses and muscle wasting.

*Methods.* In this study, we examined whole-body and skeletal muscle protein metabolism in 6 CHD patients with type 2 (T2) DM (2 male, 44.4 ± 6.1 years old, 2 white/4 African American $HbA_1C = 9.5 \pm 1.1\%$), and 6 non-DM CHD patients (2 male, 43.3 ± 6.7 years old, 2 white/4 African American) in a fasting state, using a primed-constant infusion of L-(1-$^{13}$C) leucine and L-(ring-$^2H_5$) phenylalanine.

*Results.* CHD patients with T2DM had significantly increased (83%) skeletal muscle protein breakdown (137 ± 27 vs. 75 ± 25 μg/100 mL/min). There was no significant difference in muscle protein synthesis between groups (78 ± 27 vs. 66 ± 21 μg/100 mL/min, for DM and non-DM respectively), resulting in significantly more negative net protein balance in the muscle compartment in the DM group (−59 ± 4 vs. −9 ± 6 μg/100 mL/min, $P < 0.05$). A similar trend was observed in whole-body protein synthesis and breakdown. Plasma glucose levels were 113 ± 16 and 71 ± 2 mg/dL, $P < 0.05$, and insulin levels were 25.3 ± 9.6 and 7.3 ± 1.0 uU/mL, for DM versus non-DM, respectively, $P < 0.05$. No significant differences between DM and non-DM were found in other metabolic hormones.

*Conclusion.* The results of this study demonstrate that CHD patients with T2DM under a suboptimal metabolic control display accelerated muscle protein loss compared with a matched group of non-DM CHD patients.

Over the last 2 decades, the proportion of end-stage renal disease (ESRD) patients initiating chronic hemodialysis (CHD) with a diagnosis of diabetes mellitus (DM) has increased more than 3 times, reaching an alarming incidence of about 60% [1, 2]. CHD patients with DM have the highest morbidity and mortality rates. They also display increased incidence of body protein loss, as assessed by low concentrations of serum biomarkers of nutritional status, when compared to CHD patients without DM [3]. Interestingly, the high prevalence of protein depletion in CHD patients with DM seems to be independent of its commonly recognized causes, such as inadequate dialysis dose and insufficient protein intake [3, 4].

Several studies have shown that there is increased protein breakdown in poorly controlled DM [4–9]. In insulin-depleted type 1 diabetic patients, Biolo et al showed 30% greater whole-body protein breakdown compared to controls [10]. However, the impact of insulin resistance on protein metabolism is less certain. While changes in protein breakdown due to insulin infusion in type 2 (T2) DM patients have been demonstrated to be similar to that in control subjects [9], synthesis of muscle proteins does not appear to be responsive to insulin treatment [11].

Studies in ESRD patients suggest that at stable conditions whole-body protein balance is preserved. On the other hand, during stressful conditions such as severe metabolic acidosis and the hemodialysis procedure, there is significantly increased net protein breakdown. While epidemiologic data suggest that diabetic ESRD patients tend to have worse nutritional status compared to nondiabetic ESRD patients, it is not clear if DM alone and/or in combination with uremia-related factors would act synergistically to promote whole-body or skeletal muscle protein breakdown. In order to explore the mechanism(s) by which CHD patients with T2DM are predisposed to increased protein depletion, we studied 6 CHD patients without DM and 6 CHD patients with DM matched for age, gender, and race in a fasting state, using primed-constant infusion of L-(1-$^{13}$C) leucine and L-(ring-$^2H_5$) phenylalanine. T2DM subjects were studied during glycemic and insulinemic states that were not

**Key words:** dialysis, diabetes, metabolism, nutrition.

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necessarily optimally controlled, but rather with the insulin regimens maintained at the discretion of their physician.

## METHODS

### Patients

Patients were recruited from the Vanderbilt University Outpatient Dialysis Unit. Inclusion criteria for the study included patients who had been on CHD therapy for at least 6 months, with less than 100 mL/min of urine output, using a biocompatible hemodialysis membrane (Fresenius F80, Fresenius USA, Lexington, MA, USA), receiving an adequate dose of dialysis (double pool Kt/V $\geq$1.4), on a thrice-weekly CHD program, for 6 to 108 months (median 24 months). Patients with active infectious or inflammatory disease (i.e., vascular access infections and overt periodontal disease), patients hospitalized within the last 3 months prior to the study, patients with recirculation in the vascular access and/or vascular access blood flow less than 750 mL/min detected on the arterioveous (AV) shunt, and those receiving steroids and/or immunosuppressive agents were excluded from the study. The Institutional Review Board of Vanderbilt University approved the study protocol and written informed consent was obtained from all study patients. Patient characteristics are shown in Table 1.

**Table 1.** Demographic, nutritional characteristics, and biochemical parameters of the study population ($N = 6$)

| Demographics | DM | Non-DM |
|---|---|---|
| Gender *M/F* | 2/4 | 2/4 |
| Race (White/Black) | 2/4 | 2/4 |
| Age *years* | 44.4 ± 2.5 | 43.3 ± 6.7 |
| Etiology of ESRD | 3 Type 2 DM<br>2 HTN<br>1 Sickle cell | 4 HTN<br>2 ADPKD |
| Body composition | DM | Non-DM |
| BMI $kg/m^2$ | 32.1 ± 3.2 | 22.7 ± 1.4 |
| Body weight *kg* | 92.8 ± 7.4 | 64.8 ± 5.6[a] |
| Fat mass by DEXA *kg* | 39.1 ± 5.8 | 16.9 ± 3.3[a] |
| Fat-free mass by DEXA *kg* | 53.7 ± 5.0 | 47.9 ± 5.8 |
| Laboratory values | | |
| Serum albumin *g/dL* | 3.33 ± 0.1 | 3.37 ± 0.2 |
| Serum prealbumin *mg/dL* | 32.3 ± 5.9 | 32.2 ± 3.9 |
| Serum creatinine *mg/dL* | 9.2 ± 1.0 | 9.8 ± 1.2 |
| Serum transferrin *mg/dL* | 163 ± 15 | 150 ± 17 |
| Blood urea nitrogen *mg/dL* | 55.2 ± 5.2 | 47.5 ± 5.8 |
| Serum CRP *mg/dL* | 0.87 ± 0.6<br>(0.3; 0.3–3.7) | 0.53 ± 0.2<br>(0.3; 0.3–1.3) |
| Plasma glucose *mg/dL* | 113 ± 16 | 71 ± 2[a] |
| Plasma c-peptide *ng/mL* | 4.0 ± 0.7 | 6.6 ± 1.3 |
| $HbA_1C$% | 9.5 ± 1.1 | N/A |
| HOMA-R | 2.92 ± 0.13 | 1.38 ± 0.17[a] |
| Hemoglobin *g/dL* | 11.8 ± 0.6 | 12.5 ± 1.1 |
| Plasma bicarbonate *mg/dL* | 25.3 ± 0.9 | 26.3 ± 1.1 |

Abbreviations are: M, male; F, female; ESRD, end-stage renal disease; ADPKD, autosomal dominant polycystic kidney disease; DEXA, dual-energy x-ray absorptiometry; CRP, C-reactive protein; $HbA_1C$, glycosylated hemoglobin; N/A, not available; HOMA-R, homeostasis model assessment. Values are absolute numbers and percentage or mean ± SEM for each study group (DM and non-DM), except for serum CRP (mean, SEM, median, and range).

[a]Denotes significant difference vs. T2DM.

### Design

After reviewing the inclusion and exclusion criteria, 6 CHD patients with T2DM were recruited and studied along with 6 age-, gender-, and race-matched nondiabetic CHD patients. Presence of DM was determined by the American Diabetes Association guidelines [12], by one of the following: (*1*) Symptoms of diabetes plus casual plasma glucose concentration equal or greater than 200 mg/dL (11.1 mmol/L), where casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss. (*2*) Fasting plasma glucose levels greater than 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours. (*3*) A 2-hour postload glucose level greater than 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test. The test should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Five out the 6 patients with T2DM required insulin administration, and 1 was maintained on a low carbohydrate diet as the glucose-lowering treatment. Insulin treatments were designed to bring fasting plasma glucose between 90 and 120 mg/dL. Long-acting insulin was maintained at regular regimen for all study patients. The study subjects received their maintenance dose long-acting insulin as prescribed at the outpatient setting (15 units, 18 units, 20 units, 50 units for 4 subjects and 20 units twice a day for 1 subject). Any additional short-acting insulin was withheld after the last meal prior to the study. Estimation of insulin resistance was performed by the homeostasis assessment model (HOMA-R), as follows: [plasma insulin (μU/mL) × plasma glucose (mmol/L)]/22.5. Within a week prior to each study, dual-energy x-ray absorptiometry (DEXA) was performed to estimate lean and fat body masses, and resting metabolic rate was measured via indirect calorimetry to establish energy requirements prior to experimentation. Patients were admitted to the General Clinical Research Center (GCRC) at approximately 7 PM on a nondialysis day, received a balanced meal from the GCRC bionutrition service, and remained fasting after that.

A schematic diagram of the metabolic study day protocol is depicted in Figure 1. Each metabolic study consisted of a 2-hour equilibration phase followed by a 1-hour sampling phase. A dialysis catheter was placed at the venous site of the AV shunt of the forearm at 8 AM to collect a baseline blood sample (to assess baseline biochemical nutritional markers and isotopic backgrounds) and then

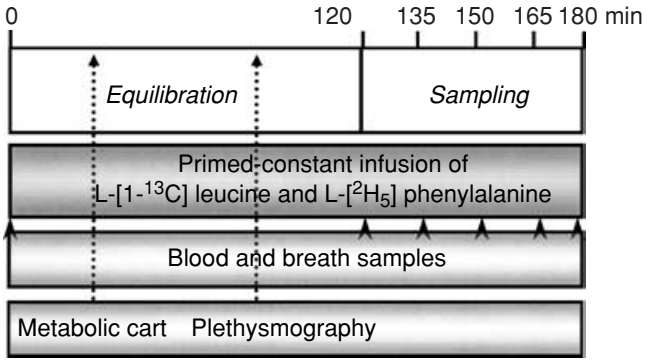


**Fig. 1. Schematic diagram of the metabolic study day protocol.** Arrowheads denote time points for blood draws and breath sample collections. Metabolic cart and plethysmography measurements were performed once during the equilibration phase. A primed-constant infusion of L-(1-$^{13}$C) leucine and L-(ring-$^{2}H_5$) phenylalanine was maintained throughout the entire study (360 min).

to initiate the isotope infusion. The arterial side of the AV shunt was the site of choice used for sampling arterial blood. The only occasion that would affect the arterial purity of the samples would be if there were stenoses in the AV shunt, causing the venous blood to mix with arterial blood (recirculation). Therefore, recirculation of the AV shunt as well as vascular access blood flow to assess stenoses in the AV shunts was checked in every patient prior to each study utilizing the ultrasound dilution technique (Transonic Systems, Inc., Ithaca, NY, USA). The venous site of the AV shunt was used to infuse the isotopes. Another catheter was placed in a deep vein (on a retrograde insertion) of the contralateral forearm to sample blood draining from the forearm muscle bed. At the start of the infusion, subjects received a bolus injection of $NaH^{13}CO_3$ (0.12 mg/kg), L-(1-$^{13}$C) leucine (7.2 μmol/kg), and L- (ring-$^{2}H_5$) phenylalanine (7.2 μmol/kg) to prime the $CO_2$, leucine, and phenylalanine pools, respectively. A continuous infusion of leucine (0.12 μmol/kg/min) and phenylalanine (0.12 μmol/kg/min) isotopes was then started and continued throughout the remainder of the study.

Simultaneously with each blood sampling, breath samples were collected from the subjects via a Douglas bag with duplicate 20 mL samples placed into nonsiliconized glass vacutainer tubes for measurement of breath $^{13}CO_2$ enrichment. Subjects were asked to breathe through a mask for 1 minute each time blood was collected. Forearm blood flow was estimated using capacitance plethysmography (Model 2560 with URI/CP software version 3.0; Moro Bay, CA, USA) during the equilibration phase. Specifically, 2 blood pressure cuffs were placed on the subject's forearm contralateral to the forearm with the AV shunt: 1 cuff 3 cm above the antecubital fossa and another cuff on the wrist. The technique involves abrupt interruption of venous outflow with a proximal cuff inflated to above venous pressure, but below arterial pressure (approximately 50 mm Hg), which causes the volume of the limb to increase due to arterial inflow. A mercury strain gauge plethysmograph is used to measure the volume change in the limb. The rate of change of the volume, in percent per minute, is the arterial flow rate at the moment of venous occlusion. This procedure was repeated for at least 5 times or until 5 similar measurements were obtained. Energy expenditure and respiratory quotient were also determined during the equilibration period by indirect calorimetry using a metabolic cart (Sensormedics 2900; Palo Alto, CA, USA) to measure ventilation rates, $CO_2$ production, and $O_2$ consumption. Metabolic cart measurements were performed for a period of 20 to 30 minutes. Patients had been resting in bed for at least 1 hour before measurements, and were instructed to stay still, breath normally, and not to talk during the measurement. All study patients had been acclimated to the metabolic cart procedure within a week before the study, as described earlier. In addition, we allowed the initial 5 to 10 minutes of measurements on the study day for additional acclimation and stability of oxygen consumption, and averaged measurements thereafter to obtain a single value for each patient. Simultaneous blood and breath samples were collected once prior to the start of the study and 5 times during the sampling phase. Once the study was finished, patients were given a meal, received their usual hemodialysis treatment, and were observed at the GCRC until stable, upon which they were discharged.

### Analytic procedures

Blood samples were collected into Venoject tubes containing 15 mg $Na_2$EDTA (Terumo Medical Corp., Elkton, MD, USA). A 3-mL aliquot of blood was transferred to a tube containing EDTA and reduced glutathione with the plasma stored at −80°C for later measurement of plasma epinephrine and norepinephrine concentrations by high-performance liquid chromatography [13]. The remaining blood was spun in a refrigerated (4°C) centrifuge (Beckman Instruments, Fullerton, CA, USA) at 3000 rpm for 10 minutes and plasma was extracted and stored at −80°C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method (Model II Glucose Analyzer; Beckman Instruments).

Nutritional biochemical parameters were done at a specialized ESRD clinical and special chemistry laboratory (Spectra Laboratories, San Juan, CA, USA). Serum albumin was analyzed using bromcresol green technique. Serum prealbumin was analyzed by an antigen-antibody complex assay and serum transferrin was analyzed by turbidimetric reading (Hitachi 717; Boehringer Mannheim, Indianapolis, IN, USA). C-reactive protein (CRP) was measured using nephelometric analysis at the Vanderbilt University Medical Center Clinical Chemistry laboratory.

Plasma c-peptide and insulin were measured by a double-antibody radioimmunoassay (Linco Research, Inc., St. Charles, MO, USA). Immunoreactive insulin was determined in plasma with a double-antibody system. Plasma aliquots for glucagon determination were placed in tubes containing 25 kallikrein-inhibitor units of aprotinin (Trasylol; FBA Pharmaceutical, New York, NY, USA) and were later measured by established radioimmunoassay with a double-antibody system modified from the method of Morgan and Lazarow for insulin [14]. Insulin and glucagon antisera and standards, as well as ($^{125}$I)-labeled hormones, were obtained from RL Gingerich (Linco Research, Inc.). Clinical Assays Gammacoat Radioimmunoassay kit (Travenol-GenTech, Cambridge, MA, USA) was used to measure plasma cortisol concentrations. Plasma insulin-like growth factor-I (IGF-I) concentrations were determined by a radioimmunoassay acid-extraction procedure (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Plasma amino acid concentrations were determined by reverse-phase high-performance liquid chromatography after derivatization with phenyliosthiocynate [15]. Individual amino acids were also placed into groups for analysis purposes. These groups included branched chain amino acids (BCAA–the sum of leucine, isoleucine, and valine); essential amino acids (EAA–the sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, theonine, tryptophan, and valine); total amino acids (TAA–the sum of all individual amino acids); and nonessential amino acids (NEAA–the difference between TAA and EAA).

Plasma enrichments of ($^{13}$C) leucine, ($^{13}$C) ketoisocaproate (KIC), and (ring-$^{2}H_5$) phenylalanine were determined using gas chromatography/mass spectrometry (GC/MS, Hewlett-Packard 5890a GC and 5970 MS; Hewlett-Packard, San Fernando, CA, USA). Plasma was deproteinized with 4% perchloric acid, and the supernatant was passed over a cation exchange resin to separate the keto and amino acids. The keto acids were further extracted with methylene chloride and 0.5 mol/L ammonium hydroxide [16]. After drying under nitrogen gas, keto and amino acids fractions were derivatized [17] with N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide containing 1% t-butyldimethylchlorosilane (MtBSTFA + 1% t-BDMCS; Regis Technologies, Inc., Morton Grove, IL, USA). The derivatized samples were then analyzed with GC/MS for plasma leucine, phenylalanine, and KIC enrichments using selected ion monitoring. The major fragments analyzed for the tBDMCS derivative of KIC and $^{13}$C-KIC were the (M-57) ion fragments 301 m/z and 302 m/z, respectively. The enrichment was quantified in plasma as the ratio of $^{13}$C-KIC:KIC (ion abundance of 301/302 m/z). Enrichment measurements were made in duplicate, and duplicates had a coefficient of variation <3%. Breath $^{13}CO_2$ was measured by isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH, USA) [18].

## Calculations

Net skeletal muscle protein balance (synthesis-breakdown) was determined by dilution and enrichment of phenylalanine across the forearm as described by Gelfand and Barrett [19]. Because phenylalanine is neither synthesized de novo, nor metabolized by skeletal muscle, rate of appearance (Ra) of unlabeled phenylalanine reflects muscle protein breakdown, whereas the rate of disappearance (Rd) of labeled phenylalanine estimates muscle protein synthesis [19]. Phenylalanine Rd was calculated by multiplying the fractional extraction of the labeled phenylalanine (based on plasma arterial and venous phenylalanine enrichments and concentrations) by the arterial phenylalanine concentration and normalized to forearm blood flow measured by plethysmography (expressed as 100 mL/min). Net phenylalanine Ra was calculated by subtracting the net AV balance of phenylalanine across the extremity from the phenylalanine Rd [19, 20]. Rates of skeletal muscle protein breakdown and net synthesis were determined from the phenylalanine Rd and Ra, assuming that 3.8% of skeletal muscle protein is composed of phenylalanine.

The steady-state rates of total whole-body leucine Ra were calculated by dividing the ($^{13}$C)leucine infusion rate by the plasma ($^{13}$C)KIC enrichment [20]. Plasma KIC provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment due to the fact that KIC is derived from intracellular leucine metabolism [20]. Steady-state conditions for KIC, phenylalanine (Fig. 2), and $CO_2$ (Fig. 3) enrichments were achieved as evidenced by slopes within each phase not significantly different than zero. Breath $^{13}CO_2$ production was determined by multiplying the total $CO_2$ production rate by the breath $^{13}CO_2$ enrichment [20]. The rate of whole-body leucine oxidation was calculated by dividing breath $^{13}CO_2$ production by 0.8 (correction factor for the retention of $^{13}CO_2$ in the bicarbonate pool) [21] and by the plasma KIC enrichment. The leucine Rd, an estimate of whole-body protein synthesis, was determined indirectly by subtracting leucine oxidation from total leucine Rd. Rates of whole-body protein breakdown, amino acid oxidation, and protein synthesis were calculated from the endogenous leucine Ra, the leucine oxidation rate, and the nonoxidative leucine Rd, respectively, assuming that 7.8% of whole-body protein is composed of leucine [22].

Rates of whole-body amino acid, carbohydrate, and lipid oxidation were determined from indirect calorimetry in combination with the leucine oxidation data. The energy expended due to amino acid oxidation was subtracted from the total energy expenditure (TEE), and

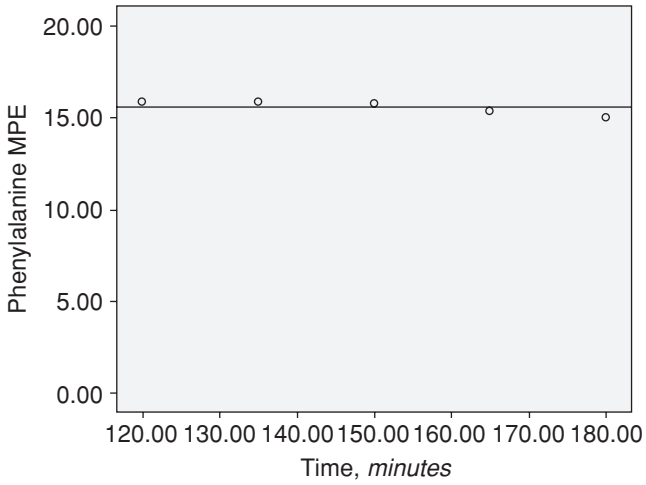


**Fig. 2. Plasma enrichment of expired $CO_2$ at study time points.**

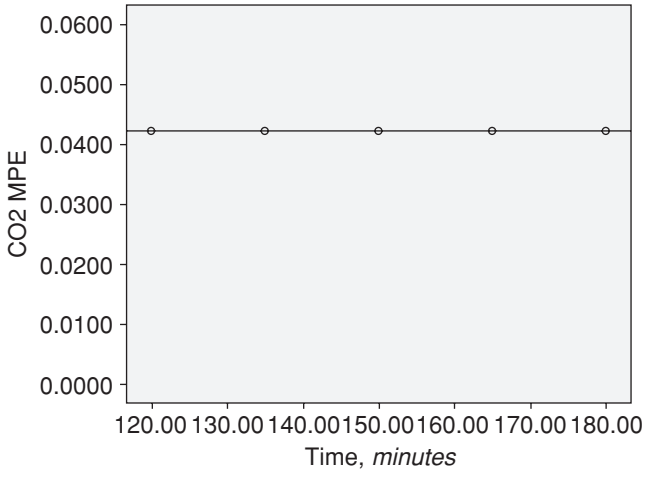


**Fig. 3. Plasma enrichment of KIC at study time points.**

the net rates of carbohydrate and lipid oxidation were calculated based on the nonprotein respiratory quotient [23]. The assumptions and limitations of calculating net substrate oxidation based on indirect calorimetry measurements have been previously reviewed [23].

### Statistical analysis

All values of each variable measured during the sampling period were averaged for comparisons. Values presented in the text and figures are mean ± SEM. For comparisons between the matched groups (DM vs. non-DM), a paired *t* test was used for parametric distribution and Wilcoxon signed-rank test for nonparametric distribution. The statistical software package SPSS 12.0 (Chicago, IL, USA) was used for analyses, and two-sided *P* values of less than 0.05 were required to reject the null hypothesis of no difference between the means.

**Table 2.** Metabolic hormones and glucose balance

| | DM | Non-DM |
|---|---|---|
| Insulin μ*U/mL* | 25.3 ± 10.3 | 7.3 ± 1.0[a] |
| Glucagon *ng/L* | 136 ± 15 | 142 ± 13 |
| Growth hormone *ng/mL* | 1.9 ± 1.1 | 0.6 ± 0.2 |
| IGF-I *ng/mL* | 273 ± 25 | 243 ± 39 |
| Cortisol μ*g/dL* | 15.3 ± 2.3 | 14.9 ± 2.4 |
| Epinephrine *pg/mL* | 45 ± 7 | 61 ± 16 |
| Norepinephrine *pg/mL* | 228 ± 18 | 314 ± 57 |

Abbreviations are: IGF-I, insulin-like growth factor-1; FM, forearm muscle. Values reported as mean ± SEM.
[a]Denotes significant difference vs. DM.

## RESULTS

### Demographics, body composition, and laboratory values

Table 1 depicts demographic characteristics, body composition, and baseline laboratory values of the study population. Subjects were well-matched for age, gender, and race. As expected, body weight and fat mass were significantly higher in patients with T2DM. Markers of nutritional and inflammatory statuses were not significantly different between patient groups. Both groups had less than adequate values for serum biomarkers of nutritional status. Glucose levels were significantly higher in patients with T2DM compared to non-DM CHD patients (113 ± 16 mg/dL vs. 71 ± 2 mg/dL, $P < 0.05$). Diabetic patients had on average $HbA_1C$ concentrations of 9.5 ± 1.0%. C-peptide concentrations were 4.0 ± 0.7 ng/mL and 6.6 ± 3.2 ng/mL, in DM and non-DM patients, respectively, $P =$ NS. HOMA-R was significantly higher in DM patients (2.92 ± 0.13 vs. 1.38 ± 0.17, $P = 0.034$)

### Metabolic parameters

Table 2 shows the results for plasma metabolic hormones in the 2 study groups. Whereas insulin concentrations were significantly higher in T2DM patients compared with non-DM CHD patients (25.3 ± 10.3 vs. 7.3 ± 1.0 uU/mL, $P < 0.05$), the other metabolic hormones did not show statistically significant differences between the 2 groups. Glucagon and cortisol levels were higher in both study groups compared to values in healthy subjects (data comparison not shown).

### Plasma amino acids

The arterial plasma amino acid (AA) concentrations during the studies are graphed in Figure 4 by groups (Total AA–TAA, Nonessential AA–NEAA, Essential AA–EAA, Branched chain AA–BCAA). In patients with T2DM, the plasma concentrations of EAA and BCAA were significantly higher than in non-DM patients. Specifically, EAA concentrations were 855 ± 26 μmol/L and 714 ± 50 μmol/L, and BCAA concentrations were 371 ± 14 μmol/L and 281 ± 20 μmol/L, for DM and non-DM patients, respectively ($P < 0.05$ for both

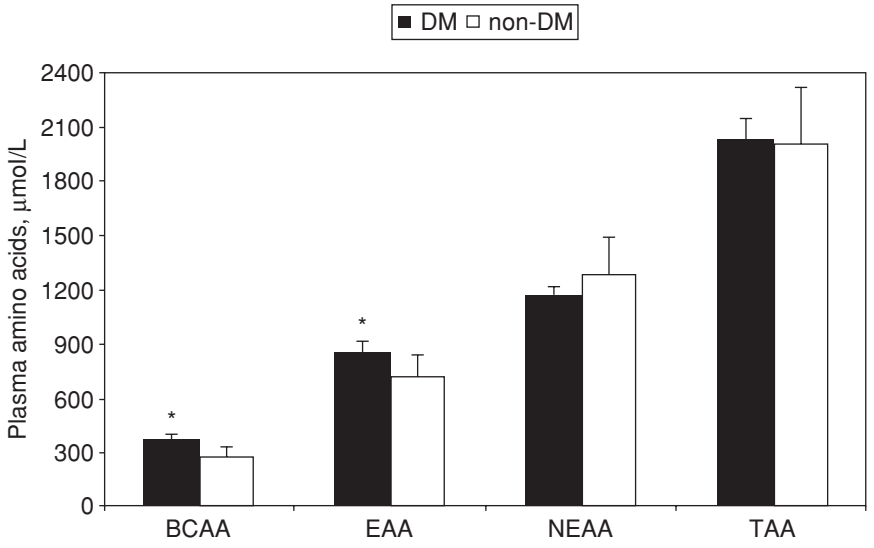


**Fig. 4. Plasma amino acids concentrations comparing DM (■) with non-DM (□) patients.** TAA, amino acids; EAA, essential amino acids; NEAA, nonessential amino acids; BCAA, branched-chain amino acids. ∗ Denotes significant difference between type 2 DM versus non-DM.

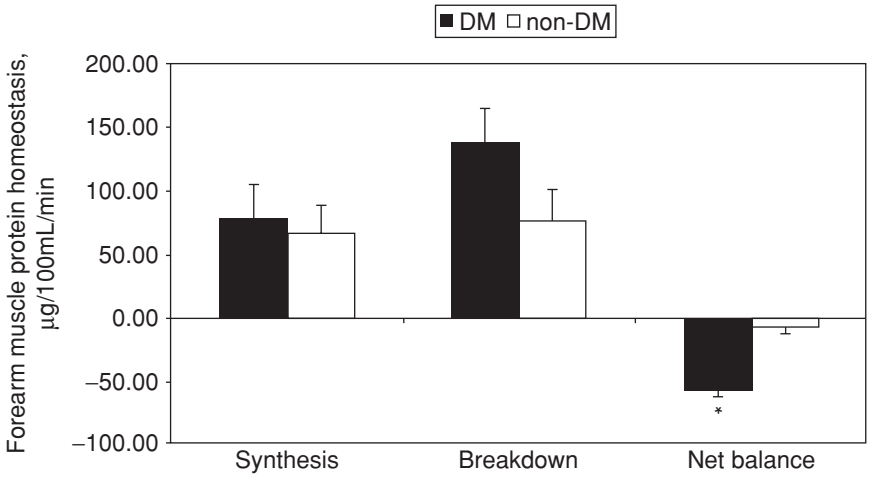


**Fig. 5. Forearm muscle protein components comparing DM (■) with non-DM (□) patients.** *Denotes significant difference between T2DM versus non-DM.

comparisons). Plasma concentrations of all 3 individual BCAA amino acids were higher in patients with T2DM but only statistically significantly higher for isoleucine (isoleucine: 63.1 ± 9.8 vs. 39.7 ± 3.8 μmol/L, $P < 0.05$; leucine: 136.0 ± 4.2 vs. 104.6 ± 11.7 μmol/L, and valine: 172.3 ± 13.5 vs. 136.3 ± 9.4 μmol/L, for DM vs. non-DM, respectively).

## Forearm muscle protein metabolism

The results for forearm muscle protein metabolism components are included in Figure 5. Forearm muscle protein breakdown was numerically higher (83%) in CHD patients with T2DM compared to those without DM ($P = 0.144$). Forearm muscle protein synthesis was only 19% higher ($P =$ NS) in DM compared to non-DM. The net protein balance across the forearm was significantly more negative (reflecting more net muscle protein loss) in T2DM patients compared to patients without DM (−59 ± 4 μg/100 mL/min vs. −9 ± 6 μg/100 mL/min, $P < 0.05$).

## Whole-body protein metabolism

Figure 6 depicts the results for whole body protein metabolism components expressed per fat-free mass (FFM). Chronic hemodialysis patients with T2DM were in an overall higher protein turnover state in the whole-body compartment when compared to non-DM CHD patients, although the differences did not reach statistical significance. Specifically, whole-body protein synthesis was 20% higher in patients with T2DM patients compared to those without diabetes. Whole-body protein breakdown was 14% higher in DM versus non-DM patients (Fig. 6). The net whole-body protein balance, a reflection of leucine oxidation, was not different between DM and non-DM subjects (−0.62 ± 0.19 and −0.69 ± 0.07 mg/kg FFM/min, respectively, $P =$ NS).

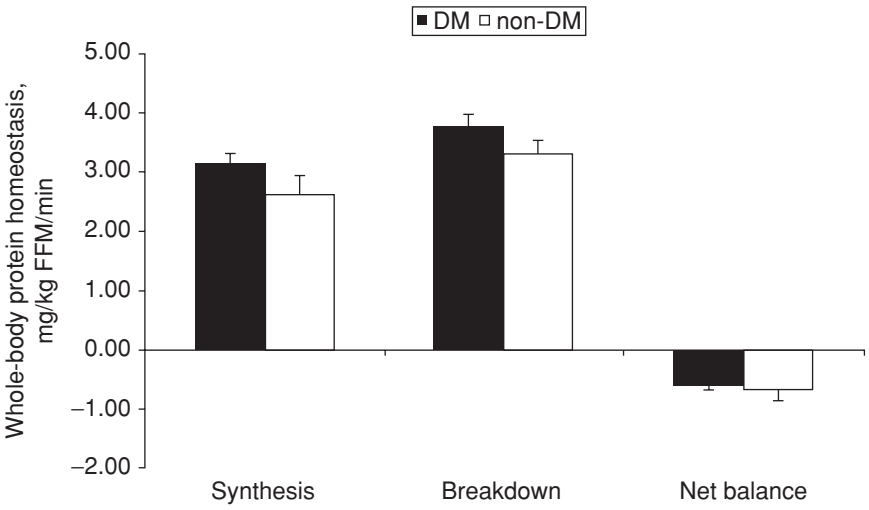


**Fig. 6. Whole-body protein components comparing DM (■) with non-DM (□) patients.**

### Energy metabolism and substrate oxidation

Table 3 shows the components of energy metabolism and substrate oxidation. Unadjusted resting energy expenditure (REE) was significantly higher in patients with T2DM compared with non-DM patients (67.2 ± 4.0 kcal/hr vs. 60.3 ± 5.1 kcal/hr, $P < 0.05$). This significant difference disappeared after adjusting the values for FFM, although it still remained numerically higher in DM patients (1.46 ± 0.08 kcal/kg FFM/hr vs. 1.29 ± 0.07 kcal/kg FFM/hr, $P$ = NS). The proportion of calories coming from carbohydrate oxidation was significantly lower in diabetics (40 ± 10% vs. 53 ± 3%, $P < 0.05$). Consequently, more energy was derived from lipid oxidation in DM compared to non-DM patients (50 ± 10% vs. 35 ± 5%, $P < 0.05$). Finally, there was also a small, but significantly, higher portion of calories coming from protein oxidation in non-DM CHD patients compared to DM CHD patients (12 ± 3% vs. 10 ± 1%, respectively, $P < 0.05$).

**Table 3.** Components of energy metabolism and substrate oxidation

| | DM | Non-DM |
|---|---|---|
| Energy expenditure *kcal/hr* | 67.2 ± 4.0 | 60.3 ± 5.1[a] |
| Energy expenditure *kcal/kg FFM/hr* | 1.46 ± 0.08 | 1.29 ± 0.07 |
| Respiratory quotient | 0.83 ± 0.03 | 0.86 ± 0.01[a] |
| Amino acid oxidation *% of energy expenditure* | 10 ± 1 | 12 ± 3[a] |
| Carbohydrate oxidation *% of energy expenditure* | 40 ± 10 | 53 ± 3[a] |
| Lipid oxidation *% of energy expenditure* | 50 ± 10 | 35 ± 5[a] |

Abbreviations are: BW, body weight, FFM, fat-free mass. Values reported as mean ± SEM.
[a]Denotes significant difference vs. DM.

## DISCUSSION

Body protein loss and muscle wasting are highly prevalent and are associated with increased morbidity and mortality in CHD patients [1]. The presence of DM in CHD patients is known to increase the risk of developing protein depletion and loss of lean body mass [3]. In a recent analysis of 142 ESRD patients starting renal replacement therapy, we found that presence of DM is the strongest independent predictor of loss of lean body mass over 12 months (Pupim et al, in press, *Kidney Int* 2005). In the current study, we further provide evidence that CHD patients with T2DM have increased muscle protein breakdown, a mechanistic explanation for the high prevalence of protein depletion and muscle wasting in this patient population.

The results presented herein are consistent with in vitro data and animal studies examining muscle protein homeostasis in catabolic states such as diabetes and metabolic acidosis, and further extends these findings to CHD patients with T2DM, an observation not previously reported [24–26]. Mitch et al have elegantly shown that in insulin-deprived animals, muscle protein breakdown is significantly increased and that this process is mediated by the proteasome-ubiquitin pathway. They have also recently reported that the modulation of phosphatidylinositol 3-kinase is a rate-limiting step in this process, all suggesting that a critical role may exist for insulin resistance as the primary mediator of the accelerated protein breakdown in CHD patients [24].

Indeed, a plethora of research has clearly demonstrated insulin's role as an anabolic hormone, both at skeletal muscle and whole-body levels. Insulin deprivation elevates protein breakdown, while insulin elevation blunts protein breakdown, and insulin interacts with amino acid availability to regulate protein synthesis [27]. In type 1 DM patients, the anabolic effects of insulin on protein homeostasis appear to be defective [4] and, thus,

it is reasonable to expect similar defects under conditions of insulin resistance.

However, similar studies examining insulin-mediated protein metabolism are less clear in patients with T2DM and nonexistent in CHD patients with DM**.** Several investigators have demonstrated a discrepancy between resistance to insulin-mediated glucose versus protein homeostasis in T2DM patients [9, 11, 28, 29]. Biolo et al examined protein metabolism in 8 non–insulin-dependent T2DM without CHD compared to 6 healthy subjects and found no differences in protein turnover in fasting or fed state between the 2 groups [10]. Luzi et al found no difference in protein turnover changes in response to euglycemic insulin clamp, with and without hyperaminoacidemia, in 6 non–insulin-dependent DM and 7 healthy subjects [9]. However, the fact that muscle protein synthesis does not appear to be responsive to insulin treatment in T2DM patients and protein breakdown was not different at basal in T2DM patients in spite of 4-fold greater insulin concentrations suggests that anomalies in protein homeostasis may exist in T2DM.

Data from the present study demonstrate that protein breakdown is increased in CHD patients with T2DM who presented with significantly higher insulin resistance as assessed by HOMA-R index. This suggests that either the effects of insulin resistance/deficiency are additive to those of uremia-related factors or vice versa. Specifically, uremia per se may further substantiate the rate of increased muscle protein breakdown, primarily by worsening the level of insulin resistance [24–26]. While a comparative analysis of protein homeostasis in T2DM patients without renal disease would provide the appropriate answer to this question, such an experiment was not the primary goal of this particular study and should be explored with further research, which would allow us to delineate these various mechanisms, especially to better understand the degree of insulin resistance and to control glycemic and insulinemic levels by using more precise techniques such as euglycemic hyperinsulinemic clamp. Nevertheless, the data presented herein are important because they demonstrate accelerated rate of proteolysis by muscle tissues as a mechanism for enhanced loss of protein stores in CHD patients with T2DM.

In addition to insulin resistance, there are several other potential factors that may lead to accelerated muscle protein breakdown in DM CHD patients. A recent study by Cano et al [3] suggested that commonly recognizable causes of protein depletion, such as inadequate dialysis dose and metabolic acidosis, are not in effect in this process. In addition, diabetic gastroparesis, which could lead to poor nutritional state, is more likely to affect (decrease) meal-related protein synthesis. In the present study, dietary protein intake and, more importantly, protein synthesis rates were similar in DM and non-DM patients, suggesting that diabetic gastroparesis is an unlikely cause for the differences observed between study groups. Glucagon, glucocorticoids, and catecholamines are counterregulatory hormones whose metabolic role may also counteract protein anabolism [30]. Studies have speculated that these hormones contribute further to already existing insulin resistance [31]. The separate effects of each of these hormones on muscle proteins under stressful situations are not well-defined because they are generally concomitantly altered in such conditions. Nonetheless, in the present study there were no significant differences in these hormones between the study groups. With regards to energy expenditure, in the present study, REE was significantly higher in CHD patients with T2DM compared to non-DM but the statistical significance disappeared after adjusting for FFM, although the numerical difference remained.

An interesting finding in this study was that the difference between the diabetic and nondiabetic groups was only present for the muscle compartment but was not significant for whole-body protein turnover. While skeletal muscle comprises approximately 50% of the whole-body protein pool, and skeletal muscle breakdown changes were in a similar direction as the whole-body protein pool, the possibility of changes in protein breakdown by other tissues needs to be raised [32]. One potential speculation is that DM patients may have a compensatory decrease in breakdown of proteins other than the skeletal muscle proteins. Conversely, there may be increases in the synthetic rates of acute phase proteins in the liver [33]. Future studies examining synthetic and catabolic rates of individual proteins representative of specific metabolic pathways may provide insight into this finding.

It should be noted that our study population for both groups were stable and specifically did not have evidence of inflammation, as estimated by CRP serum concentrations (except for 1 T2DM patient who had serum CRP of 3.7 mg/dL). This allows us to exclude metabolic abnormalities like inflammation that may influence protein homeostasis when interpreting our results. Similar studies in patient populations with more diverse nutritional and inflammatory status would be useful to further examine the factors that predispose CHD patients to protein depletion. Furthermore, the importance of the difference in body fat between the DM and non-DM groups cannot be evaluated from the design of the present study. Finally, while the techniques used in this study are associated with a margin of error, these are small and the techniques are well accepted and validated. In addition, we have used these techniques in several study populations and have found them to be reliable and reproducible.

## CONCLUSION

CHD patients with T2DM have significantly increased skeletal muscle protein breakdown as compared to

matched non-DM CHD patients. There is no significant difference in protein synthesis rates, leading to net muscle protein breakdown with amino acid release to the bloodstream. These findings could provide a mechanistic explanation as to why CHD patients with T2DM are more prone to protein depletion than CHD patients without diabetes. Further studies with controlled insulin and glucose concentrations are warranted to further define the mechanisms of impaired protein metabolism in CHD patients with T2DM.

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